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Jeanette Brun Larsen

Psychiatric symptoms and signs in relation to immune markers

Doctoral thesis

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Norwegian University of Science and Technology
Thesis for the Degree of
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NORSK SAMMENDRAG

Norsk tittel: Immunmarkører og deres relasjon til enkelte symptomuttrykk i psykiatri

Tidligere mente man at hjernen var immunologisk privilegert, altså at den var beskyttet fra påvirkning av immunsystemet. Forskning gjennom de siste 20 årene har kartlagt flere mekanismer for hvordan immuncellens signalproteiner (f.eks. cytokiner) kan påvirke hjernen, som igjen kan gi endret atferd. Det er videre godt dokumentert at pasienter med ulike psykiske lidelse har endrede nivåer av cytokiner på gruppenivå sammenlignet med friske kontroller. En utfordring med denne typen forskning er at psykiske lidelser er heterogene, og pasienter med samme lidelse kan ha ulikt symptomuttrykk. Innenfor forskningsfeltet som omhandler immunologi og psykiatri (immunopsykiatri) har derfor flere etterspurt studier som ser på immunmarkører i relasjon til spesifikke symptomer og tegn.

Hovedmålet med denne avhandlingen var å utforske sammenhengen mellom cytokiner og enkelte psykiatriske symptomer og tegn. Vi fant at høye nivåer av cytokinet tumor nekrose faktor alfa (TNF-α) var assosiert med agitasjon i en akuttpsykiatrisk populasjon. I samme populasjon fant vi også en ikke-signifikant sammenheng mellom lavere nivåer av cytokinet interferon gamma (IFN-γ) og økt motorisk aktivitet. IFN-γ var også assosiert med psykomotorisk tempo over tid i en psykosepopulasjon som ble fulgt opp i et år. De fleste cytokinene som vi målte, hadde ingen signifikant sammenheng med noen av de inkluderte tegnene eller symptomene.

Samlet sett har ikke denne avhandlingen identifisert undergrupper av symptomer hos pasienter med psykiske lidelser som er sterkt assosiert med cytokiner. Noen tendenser er likevel identifisert, og det er viktig at disse utforskes nærmere. Dette kan gjøres på flere måter, bl.a. ved å gjennomføre flere longitudinelle studier som kan øke forståelse av underliggende mekanismer.

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ACKNOWLEDGMENTS

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LIST OF PAPERS

Paper 1

Larsen JB, Stunes AK, Vaaler A, Reitan SK. Cytokines in agitated and non-agitated patients admitted to an acute psychiatric department: A cross-sectional study. PLoS One. 2019;14(9):e0222242.

Paper 2

Larsen JB, Stunes AK, Iversen VC, Vaaler AE, Reitan SK. Cytokines in Relation to Motor Activity in an Acute Psychiatric Population. Frontiers in psychiatry. 2019;10(920).

Paper 3

Larsen JB, Reitan SK, Løberg EM, Rettenbacher M, Bruserud Ø, Larsen TK, Anda L, Bartz-Johannessen C, Johnsen E, Kroken RA. The association between cytokines and psychomotor speed in a spectrum of psychotic disorders: A longitudinal study. Brain, Behavior, & Immunity - Health. 2021;18:100392.

SUMMARY

Introduction and aims:

The causes of mental disorders are complex and, to date, not fully understood. During the last two decades, research has gained increased insight into how the immune system affects the psyche, and vice versa. It is widely confirmed that patients with mental disorders have altered blood levels of immune markers such as cytokines compared to healthy controls. However, the phenomenon is largely transdiagnostic. As mental disorders define by symptoms and signs that typically co-occur, there is a possibility for a stronger relation to cytokine changes within subgroups of patients with certain symptoms.

Overall, the aim of this thesis was to assess the association between psychiatric symptoms or signs, and peripheral cytokines across various mental disorders, using both cross-sectional and longitudinal designs. In the cross-sectional study we aimed to explore potential associations between symptoms or signs and cytokines. The purpose with the longitudinal study was to investigate how cytokines vary over time in relation to symptoms. This might indicate that cytokines affect symptoms. We chose symptoms and signs that are common within several mental disorders, such as agitation, altered gross motor activity, psychomotor retardation, and psychomotor processing speed. These phenomena are to some degree objectively observable, and they are therefore clinically useful. Cytokines were selected to represent different Th-responses and based on previous findings in the field.

We hypothesized that peripheral cytokines would be related to and affect certain symptoms, both across various mental disorders and within specific disorders where the particular symptom is more common. The hypothesis in paper 1 is that agitated patients have higher levels of Th1 cytokines compared to patients without agitation. In paper 2, we hypothesized that increased motor activity would be associated with Th1 cytokines, whereas motor retardation would be related to cytokines from other immune responses. The hypothesis of

paper 3 is that Th1 cytokines would affect psychomotor speed performance over time in patients with psychotic disorders.

Methods:

Paper 1 and 2 withholds data from an acute psychiatric inpatient population with various mental disorders and have a cross-sectional design (study 1). After excluding patients with infection or immune related disease, a total of 318 were available for statistical analyses. We measured serum levels of the following cytokines: interleukin (IL) -1β, IL-4, IL-6, IL-10, tumor necrosis factor (TNF) -α, interferon (IFN) -γ and transforming growth factor (TGF) -β. The first paper investigated the relation between cytokines and agitation assessed by Positive and Negative Syndrome Scale, Excitement Component (PANSS-EC). We stratified patients into two groups based on the presence or absence of agitation and compared cytokine levels between the two. In paper 2, we explored the relation between the degree of increased or decreased motor activity and cytokines using correlation analyses and multiple linear regression. We corrected for multiple testing in both papers with the Bonferroni correction.

Paper 3 (study 2) is a prospective cohort study with data from a multicenter pragmatic, randomised controlled trial (RCT) comparing three different atypical antipsychotics in patients with a spectrum of psychotic disorders. Importantly, we do not compare treatment groups in this paper, but investigate the relation between psychomotor speed and cytokines analyzing all patients collectively. The total sample consisted of 144 patients. Psychomotor processing speed was assessed using the neuropsychological tests trail making test (TMT) –A and B, and symbol coding. Serum concentration of the following cytokines were measured: IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 p70, IL-17a, IFN-γ and TNF-α. Study 2 has a longitudinal design, with eight visits during a total period of 52 weeks. We analysed the effect of cytokine levels on psychomotor speed over time in linear mixed effect models correcting for several potential confounders. No correction for multiple testing was applied.

Results:

In the acute psychiatric population, serum levels of TNF-α were significantly higher in patients with agitation compared to those without, both when all patients were included in the analyses ($d = 0.16$, $p = 0.004$), and within a subgroup of non-affective psychosis ($d = 0.35$, $p = 0.027$) and after this subgroup was excluded from the total population ($d = 0.13$, $p = 0.025$). Only the finding regarding agitation in the total sample remained significant after correction for multiple testing. After adjusting for confounders or multiple testing, we found no significant association between deviations in motor activity and cytokines in this study. In the unadjusted model, there was a non-significant trend towards an association between increased motor activity and IFN-γ.

Within patients with psychosis in study 2, IFN-γ had a statistically significant negative effect on TMT-A (model estimate = -2.923, $p = 0.011$) and symbol coding performance (model estimate = -2.564, $p = 0.038$) over time in our linear mixed effects models controlling for several possible confounders. Overall psychomotor speed performance increased significantly across the study period while serum cytokine levels remained stable.

Conclusions:

Considering the overall aim, we did not identify strong associations between psychiatric symptoms and peripheral cytokines. Still, TNF-α was related to agitation in a cross-sectional study including patients admitted to an acute psychiatric ward. This could indicate a relation between agitation and a Th1-response. IFN-γ is another Th1 cytokine, which in study 2 was significantly associated with psychomotor speed over time in a population with psychotic disorders. Although this finding would not have sustained after correction for multiple testing, it might indicate that a Th1-response affects psychomotor speed. However, most of the measured cytokines were not associated with any of the included symptoms or signs. Still, we

hope that the findings from this thesis will inspire future research. More studies on larger materials including several well-defined symptoms would help to elaborate the field further.

ABBREVIATIONS

ADHD: Attention deficit hyperactivity disorder

BBB: Blood-brain barrier

BMI: Body mass index

CNS: Central nervous system

CRP: C-reactive protein

CSF: Cerebrospinal fluid

DSM: The Diagnostic and Statistical Manual of Mental Disorders

ELISA: Enzyme-linked immunosorbent assay

FDA: US Food and Drug Administration Center for Drug Evaluation and Research

HIV: Human immunodeficiency virus

hs-CRP: High-sensitivity C-reactive protein

ICD: International Classification of Diseases

IL: Interleukin

LME: linear mixed effects

MDD: Major depressive disorder

MS: Multiple sclerosis

NSAID: Non-steroidal anti-inflammatory drug

PANSS: Positive and Negative Syndrome Scale

PANSS-EC Positive and Negative Syndrome Scale, Excited Component

PICU: Psychiatric Intensive Care Unit

RA: Rheumatoid arthritis

RCT: Randomised controlled trial

SCID-I: Structured Clinical Interview for DSM-IV Axis I Disorders

SLE: Systematic lupus erythematosus

Tfh: follicular helper T cells

TGF: Transforming growth factor

Th cells: T helper cells

TMT: Trail making test

TNF: Tumor necrosis factor

Treg: T regulatory

WHO: World Health Organization

1 INTRODUCTION

"I had a frightful cold, and was exceedingly depressed and miserable. Not that I had any reason but illness for being so."

(Charles Dickens, December 26th, 1867).

As this vivid description by Charles Dickens illustrates (1), coming down with a common cold does not only produces somatic symptoms, but also influences the mood. This blunted mood during a cold is something that most would recognize and is therefore often used to exemplify how the immune system influences the brain, resulting in psychiatric symptoms.

With antecedents dating back to Hippocrates times, the idea of a link between the immune system and psychiatry, is far from being new. Although Hippocrates did not know that the immune system existed, he observed that malaria could cure patients suffering from epilepsy (2). Later on, in the 19th century, the well-known psychiatrist Emil Kraepelin investigated the association between psychiatric disorders and acute inflammatory diseases during his days as a medicine student (3). In fact, Kraepelin hypothesized that fever could cause mental illnesses through effects on the nerves conductivity and metabolism (3). Kraepelin continued to follow the path of relations between immune diseases and mental health when he defined dementia praecox, the precursor to schizophrenia. He stated that dementia praecox was caused by an "autointoxication" from a local somatic infection (4).

In 1920-1940, a few more published studies indicated a possible link between the immune system and psychosis (5). Notably, Lehmann-Facius described a presence of circulating antibodies in sera of schizophrenic patients in 1939 (6). This was followed by a couple of studies in the 1950s and 1960s suggesting that psychological stress could aggravate autoimmune diseases such as systematic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (7, 8). Later on, in the 1970s and 1980s, one often referred to this field with the term

psychoneuroimmunology (9), indicating that the psyche affects the immune system. The notion that psychological phenomena influenced the immune system, and not the other way around, was a leading view until the beginning of the 21st century. However, during the last two decades increased insight has been gained indicating that the immune system also affects the psyche, creating the term *immunopsychiatry* (10).

Today, we know several mechanisms regarding how the immune system influences the brain, and vice versa. It is also well-established that patients with severe mental disorders have higher levels of inflammatory markers compared to healthy controls (11, 12), even though the causality is not clear. A common critique of these previous studies is the heterogeneity of mental disorders where patients classified with the same disorder can have very different symptoms. This is troublesome when investigating biological mechanisms related to behavior, as it possible that each symptom within a mental disorder is a pathological expression of various biological mechanisms.

The main aim of this thesis was to assess the association between single psychiatric symptoms or signs, and immune markers across various mental disorders. We chose symptoms based on several aspects; the clinical knowledge that these symptoms are prominent during known immune related diseases, animal studies on immunology and behavior, and a few previous studies on symptoms in psychiatric patients and immune markers. Also, the symptoms and signs included in this thesis are important in a clinical setting and possible to observe objectively. In addition, all symptoms we chose are clinically challenging causing a lot of suffering to the patients and can be difficult to alleviate.

1.1 MENTAL ILLNESS

Since the beginning of humankind, mental illness has existed along with an attempt to treat the people suffering from it. For instance, the earliest evidence for trephination exists from skulls and cave art rating to 6500 B.C. (13). Among the hypothetical reasons why ancient

humans performed this high risk procedure, one is treating mental illness by releasing evil spirts (14). Not all ancient treatments for mental illness were this brutal. Ancient Egyptians around 1900 B.C. believed mental illness in women resulted from a wandering uterus and treated it with smelling substances that should lure the uterus back in place (15). Still, theories of demonic possession were the leading understanding of mental illness until Hippocrates described imbalance in the four body fluids as the main reason of all diseases (somatic and mental) around 400 B.C (16). After Hippocrates, the explanations for mental illness have shifted between spiritual, psychological, social and somatic, leading up to today's biopsychosocial understanding.

Along with the development of modern psychiatry, the classification of mental suffering into disorders have emerged. Severe depression is probably the mental disorder first described, which was characterized in ancient Greece as *melancholia*, a word meaning "black bile" in Greek (17). Among several psychiatrist describing mental disorders in the 19th century, Emil Kraepelin is the most well-known (18). However, it was not until the middle of the 20th century that the first two systems of diagnostic criteria for mental disorders got published: The Diagnostic and Statistical Manual of Mental Disorders (DSM) of the American Psychiatric Association and the International Classification of Diseases (ICD) organized by the World Health Organization (WHO) (19, 20).

1.1.1 Definition of mental disorders, diagnosis, symptoms and signs

The term disease refers to a condition with known underlying pathophysiological mechanisms or causes, i.e. infectious disease. The term disorder has many similarities with disease, although a structural underlying change does not need to be fully understood. The Oxford Medical Dictionary describes disorder as a "condition that disrupts normal physical or mental functions" (21), which emphasizes the functional outcome more than the underlying cause. Further, a mental disorder is defined as a clinically significant disturbance in cognition,

emotion or behavior, which can be due to psychological, biological or developmental processes (22). A mental disorder is diagnosed after considering a set of diagnostic criteria, which constitutes of various symptoms and signs that often co-occur. A symptom may be defined as subjective (i.e. depressive thoughts), whereas a sign is objective (i.e. restlessness) (23, 24). This separation between symptom and sign might be somewhat arbitrary, as for instance both the patient and the clinician might note that the patient is restless.

1.1.2 Burden of mental disorders

Overall, mental disorders cause substantial burden to both the individual suffering from the disease, relatives, and the society. Over the last three decades, estimates indicate that mental disorders constitute more than 14% of years lived with disability throughout the world (25). Further, people with severe mental disorders have a life expectancy that on average is 10-20 years shorter than the general population (26, 27). According to the World Health Organization (WHO) studies on global burden of diseases, treatment and prevention of mental disorders are important in order to reduce the total burden of chronic diseases (25). To achieve this goal, better knowledge about the pathophysiology behind severe mental disorders is necessary. A potential link between the immune system and mental illness, is one possibility for better understanding and subsequently treatment and prevention of mental disorders.

1.1.3 Symptoms and signs in mental disorders

Several symptoms and signs are common amongst a number of mental disorders (22). Examples of such symptoms are loss of interest, hallucinations, psychomotor symptoms, muscle pain, agitation, and loss of energy. As mental disorders are constitutions of symptoms and signs that often occur together, different symptoms can be prominent for two patients fulfilling diagnostic criteria for the same disorder (22). Vice versa, the same symptom may be

present in two otherwise very different disorders. For the individual patient, it is often specific symptoms that causes the most suffering.

1.1.3.1 Agitation and aggression

Agitation is a constellation of symptoms and signs that occurs in several mental disorders, including schizophrenia, bipolar disorder, personality disorders, (agitated) depression and substance use disorders (28, 29). Often agitation is defined as a syndrome of behaviors such as increased psychomotor activity, irritability, hostility, threatening gestures and lack of cooperativeness (30). However, there are discrepancies in the definition of agitation (31). DSM fifth edition (DSM-5) uses a wider definition, describing agitation as "excessive motor activity associated with a feeling of inner tension" (22). As highlighted by the US Food and Drug Administration Center for Drug Evaluation and Research (FDA), there are various definitions of agitation. The FDA also concluded that the definitions of agitation often include exceeding restlessness and increased motor activity associated with inner tensions (32). In the literature, aggression is sometimes used as a synonym to agitation, but these terms do not describe the exact same phenomenon. More specifically, agitation can lead to aggression if being untreated (30). Aggression on the other hand, describes the observed hostility or feeling of anger leading to either threating gestures or violent behavior (33).

It is extensively confirmed in research that agitation is commonly seen in patients admitted to both acute and non-acute inpatient psychiatric wards (28, 34). Unmanaged, agitation might escalate into violence and may result in use of coercive means such as involuntarily medication, restraint, and seclusion (34, 35). Interestingly, agitation is seen in several somatic disorders involving inflammatory processes such as encephalitis, delirium and stroke (36). Delirium is an organic brain disorder that can be caused by several underlying somatic conditions, including infection and other inflammatory diseases (37). As many as one third of

patients with delirium demonstrate agitation (38), and agitation in delirium might accordingly have immunological underpinnings.

1.1.3.2 Deviations in motor activity

In mental disorders, motor activity can be both increased and decreased. There are several synonyms to increased motor activity, including hyperactivity, overactivity, and restlessness. Decreased motor activity is commonly referred to as psychomotor retardation. As for agitation, deviations in motor activity are seen in several mental disorders (22, 39). Importantly, altered motor activity is a prominent feature of attention deficit hyperactivity disorder (ADHD) (40), schizophrenia (41), tic disorders (42), all mood states of bipolar disorder (43, 44), and unipolar depression (45). In unipolar depression, psychomotor retardation often indicates that the patient is more severely ill (46). Alterations in motor activity is often measured with clinical rating scales containing 1-2 items for motor changes within research (47). However, research in recent years has also included actigraphy, which is a more objective measure of deviations in motor activity (41, 45).

1.1.3.3 Cognition and its subdomains psychomotor and processing speed

Cognition is defined as "the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses" (48, 49). In the literature, cognition has been variously categorized into different subdomains (50). The DSM-5 operates with six cognitive domains: complex attention, executive function, learning and memory, language, perceptual–motor function, and social cognition (22). In this thesis, we focus on perceptual-motor function and complex attention.

The term dementia praecox by Kraepelin indicated that he regarded cognitive deficits to be associated to the disorder. Over several decades, intensive research has yielded more knowledge about this connection between cognition and mental disorders. Specifically, we know that cognitive deficits are a core feature of many organic brain disorders including

dementia and traumatic brain injury (22, 39). Such deficits are also a common feature of several mental disorders, including schizophrenia, bipolar disorders, depression and obsessive-compulsive disorder (50, 51). Particularly, schizophrenia and bipolar disorder are associated with a broad spectrum of cognitive impairments, being most severe for patients with schizophrenia (51). Cognitive impairment also predicts functional outcome in bipolar disorder and schizophrenia (52, 53). In this context, processing psychomotor speed and working memory might be of particular importance (54, 55).

Psychomotor speed can be defined as the ability to identify and respond to rapid changes when given a task (48). In other words, the term is separated from gross motor activity by also describing how the brain processes and coordinates a psychomotor task. It is measured by various neuropsychological tests where subjects are required to perform simple tasks, often after reacting to a certain change in the environment. The raw scores from these tests are often given in seconds to completing the task correctly (56, 57). Processing speed is a cognitive domain that has many similarities with psychomotor speed and within the literature the two terms are often used as synonyms (58). However, in processing speed the timed response to a stimulus can be either motor or oral (59). This means that processing speed is a wider term. Several have argued that psychomotor speed is a subcategory of the broader term processing speed (58, 60).

1.1.4 The acute psychiatric setting

Since the 1950s, the deinstitutionalization of psychiatry has led to marked changes in the acute psychiatric inpatient services (61). Central psychiatric hospitals have been downsized in favor of more outpatient- and community based services (62). As a result of this reorganization, it is claimed, acute inpatient departments give more intensive care for patients in acute crisis (63).

In Norway, acute psychiatric inpatient wards are organized in geographic catchment areas and the departments are publicly funded. All adults (≥ 18 years old) in need for an acute admission in each catchment area will be admitted to the same department. The admissions can be both voluntary and involuntary according to The Norwegian Mental Health Care Act (64). It is common to organize the acute psychiatric departments in ordinary wards and Psychiatric Intensive Care Units (PICUs) (65). PICUs are inpatient facilities with higher staff to patient ratio, giving care to patients with high suicidal risk, violence, threatening gestures or other deviant behavior needing close attention (65). Finally, as acute psychiatric inpatient wards are publicly funded, patients from all socioeconomic classes are admitted to the facility.

The acute psychiatric population is very heterogeneous. For instance, patients present with various mental disorders, where the most common are psychosis, substance abuse, personality disorders and affective disorders (66). Further, a large proportion of the patients have comorbid somatic diseases (67). In addition, suicidal ideation, aggression, and violence are common problems within the population (68, 69).

1.1.5 Non-affective psychotic disorders

Non-affective psychosis, also sometimes referred to as schizophrenia spectrum disorders, are a group of disorders consisting of schizophrenia, delusional disorder, schizotypal disorder, acute and transient psychosis, schizoaffective disorder and other unspecified nonorganic psychotic disorders (22, 39). For simplicity, I hereby refer to this group of disorders by the term psychotic disorders. Like other mental disorders, the diagnostic criteria for psychotic disorders consists of several symptoms and signs with a defined minimum duration to fulfill the specific diagnosis (22, 39). For instance, among the diagnostic characteristics for schizophrenia, are delusion, hallucinations, thought disorder, catatonic behavior and negative symptoms (i.e. apathy, blunting of emotional response) (22, 39). Although schizophrenia is associated with a broad spectrum of cognitive impairments (70, 71), they are only described

in the DSM-5 and not included in the diagnostic criteria (22). In addition, negative symptoms categorized as deficit schizophrenia might be related to more severe cognitive impairment (71). Furthermore, neurological soft signs (i.e. sensory integration, motor coordination and motor sequencing of complex movements) are often seen in schizophrenia patients as a stable feature independent of medication (72, 73), indicating neurological correlates of the disorder.

The median prevalence for schizophrenia ranges from 0.2-1.0% (74). Contrary to previous assumptions, the prevalence varies among different geographic areas, being higher in urban regions and northern latitudes (74). There are several risk factors for developing schizophrenia, especially migration (74), drug abuse (75), obstetric complications (75) and prenatal infections . In addition, twin studies indicate a heritability of schizophrenia (76). From genome-wide association studies we also know that genes relevant for adaptive immunity is associated with the risk for developing schizophrenia (77).

1.2 THE IMMUNE SYSTEM

Human biology is complex and with advances in technology our understanding of it increases constantly. We puzzle the parts together and construct a system that makes meaning to us. However, we always must be aware that the description we have may not be correct. With this limitation in mind, I will describe essential parts of the immune system based on today's knowledge.

1.2.1 Innate and adaptive immune system

One way to describe the immune system, is to divide it into innate and adaptive immunity. The innate immune system is part of our first-line defense against intruders, consisting of cells that respond immediately after recognizing parts of a pathogen or foreigner (78). This system protects us from several pathogens before severe infectious disease develops (78). On the other hand, the adaptive immune system consists of lymphocytes with the capacity to distinguish between various pathogens and molecules with a high specificity (79). The

adaptive immune system's main task is to clear intra- and extracellular organisms when the innate immune system fails (80). It takes approximately 4-6 days for the adaptive immune response to respond the first time it is exposed to a new pathogen (81). Specific engaged cells of the adaptive immune system multiply in a response, leading to a large "army" defending against later attack with the same pathogen.

All described mechanisms involve and are coordinated by a set of small signaling proteins called cytokines. The cytokines are not only important for the immune cells to communicate with each other, but also for the immune cells to modulate multiple physiological functions after binding to various cells throughout the body (82, 83).

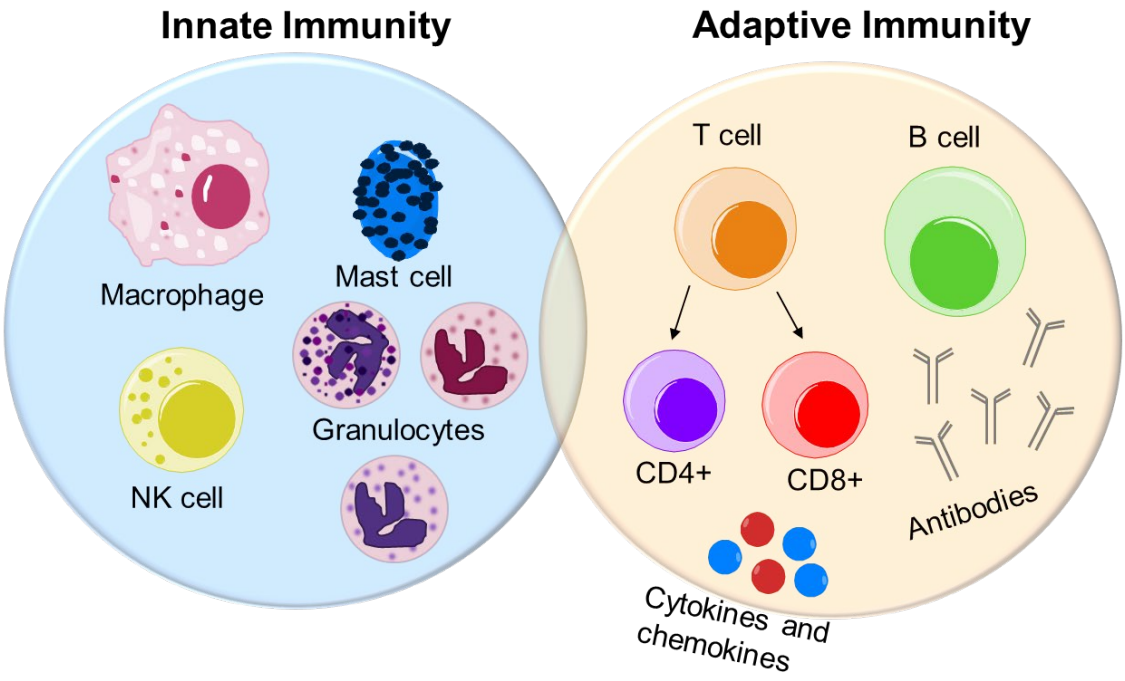

Figure 1. An overview of the immune system illustrating the different parts of innate and adaptive immunity. Illustration: Jeanette Brun Larsen

1.2.2 Cytokines and other immune markers

Cytokines are small signaling proteins that are secreted by various types of cells throughout the body, including immune cells, neurons and tissue cells (80). Cytokines bind specific receptors on target cells, leading to a response in the target cell. Chemokines are cytokine-like proteins that work as chemo attractants, meaning that cells with receptors for chemokines migrate towards an increasing chemokine gradient (80). Cytokines and chemokines concentration can be detected in blood (serum or plasma), cerebrospinal fluid (CSF), saliva etc. In general, different types of cytokines promote a specific type of immune response (84). Because of this, cytokines are often assessed in research to reveal type and strength of immune activity in the body.

Another immune marker frequently measured in serum or plasma in immunopsychiatry, is C-reactive protein (CRP). CRP is also widely used in the clinic because analyses are cheap, accessible and give a quick answer on the general inflammatory status in the body. CRP is classified as an acute-phase protein, meaning a protein produced by hepatocytes in the acute phase inflammatory response, mediated by cytokines such as interleukin (IL) – 6, tumor necrosis factor (TNF) – α and IL-1β (85). A major role of CRP in inflammation, is opsonization of pathogens and activation of certain molecules of the complement system (86, 87). Opsonization means that a pathogen is coated by proteins (antibodies, CRP or complement) that enhances destruction by phagocytic cells (80). Increase in low levels of CRP (measured as high-sensitivity CRP, hs-CRP) is also gaining attention as a marker of cardiovascular risk (88).

1.2.3 Cells of the immune system

Leukocytes are a collective term for all of the immune system's cells. Macrophages, dendritic cells, granulocytes and natural killer cells are the major groups of cells of the innate immune system. The leukocytes of the adaptive immune system are classified as lymphocytes, which

are further subdivided into lymphocytes produced in the bone marrow (B-cells) or lymphocytes matured in the thymus (T-cells). Mainly, B-cells mature into antibody-secreting plasma cells or memory B-cells. The subtypes of T-cells are more diverse. Based on our present knowledge, we classify T-cells into CD4+ and CD8+ T-cells. The main task of CD8+ T-cells, also called cytotoxic T-cells, is to kill damaged cells (virus infected cells, cancer cells or cells that are damaged in other ways). CD4+ T-cells, or T-helper (Th) cells, are cytokine secreting cells that help the immune system to develop into an accurate response most suitable to disarm the actual pathological threat (i.e. virus, bacteria or fungus). CD4+ T-cells consist of several subclasses depending on which effector mechanism they promote and each of these subsets are characterized by a set of cytokines (Figure 2).

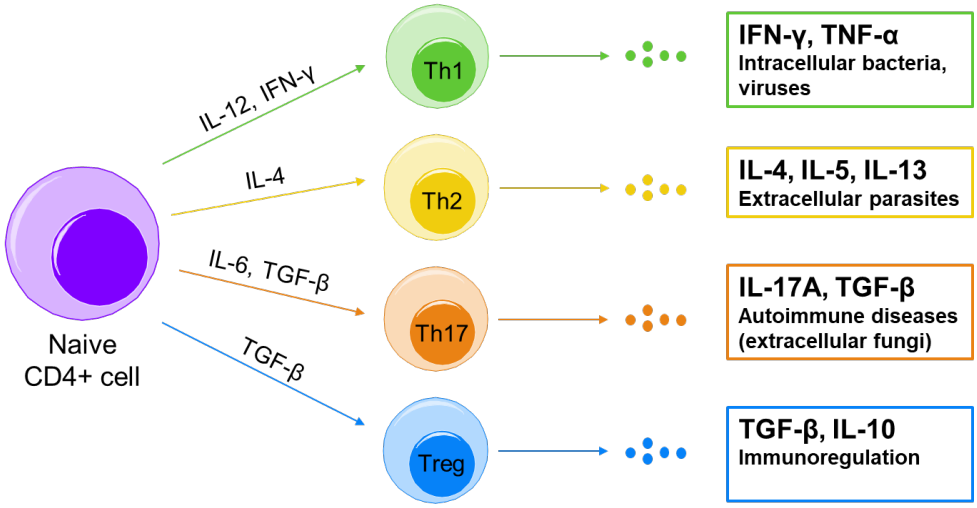

Figure 2. T helper cell subtypes including the common cytokines inducing development of each subtype. The main tasks and cytokines produced by the mature cells are given to the right, the inductor cytokines to the left. Illustration: Jeanette Brun Larsen

A naïve CD4+ cell matures into a Th1 cell when cytokines such as IL-12 and interferon (IFN) – γ are present (84). The Th1 response is particularly important in our defense against intracellular pathogens (viruses and intracellular bacteria). Predominantly, Th1 response is

characterized by the production of the cytokines IFN-γ and TNF-α. Although being a simplification, Th2 response is often viewed as contrary to the Th1 response. Th2 cells are essential in our defense against extracellular pathogens like helminths, producing cytokines such as IL-4 and IL-13 (89). Th17 is hallmarked by cytokines such as IL-17 and transforming growth factor (TGF) – β (90). Th17 response is known to be associated with autoimmune diseases such as inflammatory bowel disease and thyroid diseases (90, 91). Finally, we have T regulatory (Treg) cells limiting the immune response from becoming vigorous. Treg response is both induced and hallmarked by the cytokine TGF-β (84).

In addition to these four widely described subtypes of Th cells, a couple more have been described in the recent years, including Th9, Th22 and Tfh (92-94). Th9 cells produce the cytokine IL-9 and is involved in several inflammatory diseases, both autoimmune and allergic (92). Th22 cells are important in immune responses of epithelium and tissue and have IL-22 as its hallmark cytokine (95). A Th22 response might be important in inflammatory skin diseases (93). As quite newly described cells, the effector mechanisms and physiological role of Th9 and Th22 are still largely unknown (92, 93). At last, we have follicular helper CD4+ T cells (Tfh cells), which might be of importance for differentiation of B cells through production of the cytokine IL-21 (94, 96). Tfh cells are particularly important in our immune response -including response to vaccine, and might also be involved in autoimmune diseases such as SLE (96).

Although being a simplification of the immune system's complex biology, this subdivision in various immune responses is useful when trying to understand which are most active at a given time. Each response is hallmarked by a set of cytokines and measuring cytokines in blood is therefore helpful when determining which part of the immune system that is most active. Therefore, measuring cytokines in blood is a widely used method in immunopsychiatric research.

1.3 IMMUNE-TO-BRAIN COMMUNICATION

For a long time, the brain was considered to be "immunologically privileged", meaning that it is protected from influence of the immune system. Today, we know that the immune system interacts with the brain through several pathways (97). A common example of one such interaction, is the human behavioral changes seen during an infection. These behavioral changes, referred to as *sickness behavior*, include lethargy, depressed mood, loss of appetite, sleepiness and hyperalgesia (98). Notably, the same symptoms seen in *sickness behavior* are also common in various mental disorders, such as MDD, bipolar disorder and schizophrenia (39). *Sickness behavior* may be triggered by the circulating cytokines' effect upon the brain (99).

There are several pathways making it possible for peripheral cytokines to communicate with the central nervous system (CNS). Cytokines can cross the blood-brain barrier (BBB) (100), bind to their respective receptors on neurons or glial cells (101), and induce an effect (102). Additionally, cytokines can bind to afferent peripheral nerves (e.g. the vagus nerve) and influence signaling (103). Receptors for several cytokines are also found on cerebral endothelial cells (100). When binding to cerebral endothelial cells, some cytokines induce production of free radicals and other messengers with known effects upon the CNS (104). Finally, certain cytokines influence the tryptophan/kynurenine metabolism, inducing production of the neurotoxic substances' kynurenine and quinolinic acid (105, 106). L-kynurenine and precursors to quinolinic acid might cross the BBB. In addition, this dysregulation of the tryptophan/kynurenine metabolism results in a consumption of tryptophan, making less tryptophan available for the production of the important neurotransmitter serotonin (107).

In summary, there are several mechanisms making it possible for peripheral cytokines to influence the brain. This immune-to-brain interaction is important both for brain homeostasis, which might influence the progression of psychiatric disorders (108).

1.4 THE ROLE OF CYTOKINES WITHIN THE CNS

When entering the brain, circulating cytokines can mediate several potential effects, one being enhancement of the brain's own cytokine production (109). Microglia are a type of glial cells which are often referred to as the immune cells of the CNS. More specifically, microglia are macrophages that in a healthy brain is key for maintaining homeostasis around the neurons (110). Microglial cells are the main producer of several cytokines within the CNS (111, 112). This microglial cytokine production is most pronounced when the microglial cells are activated, which can occur as a response to peripheral inflammation (113, 114). Interestingly, this activation of microglia can also occur after psychological stressors (114). However, microglial cells are not the only CNS cells producing cytokines. Astrocytes, another subtype of glial cells, have receptors for cytokines and can synthesize cytokines when activated (115). Further, the endothelial cells of the BBB and ependymal cells of the blood-CSF barrier, can produce several cytokines that are secreted into the CNS (116, 117). Additionally, although not being the largest contribution to cytokine production in the CNS, neurons can also produce cytokines in response to injury (118).

When present in the CNS, cytokines and inflammation can be either neuroprotective and necessary, or damaging (119). Especially, chronic inflammation with the presence of cytokines over time, is harmful to neurons (119). The mechanism and function of cytokines have many similarities with neurotransmitters and hormones (120, 121), opening the possibility for a more diverse function of cytokines.

1.5 PSYCHIATRIC SYMPTOMS AND INFLAMMATORY MARKERS

1.5.1 Animal studies

Animal studies are not only important when understanding basic mechanisms for interactions between the CNS and cytokines. These studies have yielded information on behavioral changes caused by inflammation and specific cytokines. For instance, we know from animal studies on cats that the two cytokines IL-1β and IL-2 bind to certain sites in the brain (e.g. periaqueductal grey matter) and are important modulators of aggression (33). The importance of cytokines for aggression and anxiety like behavior, has been further demonstrated in mice, where deletion (knock out) of genes encoding the TNF receptors 1 and 2 resulted in a striking absence of these behaviors (122). The linkage between inflammation and behavior in animals, is not only limited to aggression and anxiety. In mice, prenatal inflammation is shown to give cognitive and behavioral abnormalities in the offspring (123, 124). These abnormalities might be related to negative symptoms in schizophrenia (123).

1.5.2 Findings within inflammatory somatic diseases

Several studies have demonstrated an association between psychiatric symptoms and inflammatory markers in somatic diseases. In patients with diabetes mellitus (type 1 and 2), serum levels of hs-CRP and IL-1 receptor antagonist were associated with depressive symptoms (125). The same relation between inflammatory markers and depressive symptoms, is identified in patients with chronic heart disease and autoimmune diseases (i.e. SLE and RA) (126-128) . Interestingly, low-grade inflammation, measured as hs-CRP, might be a predictor of whether patients with known coronary heart disease develop depressive symptoms (129). Further, a previous study by Uguz *et al*. demonstrated that patients with RA who received anti-TNF-α treatment, developed symptoms of anxiety and depression less frequently compared to RA patients under other, less potent anti-inflammatory treatment regimens (128).

The fact that cytokines are given as treatment for somatic diseases, gives us the possibility to study what effect administrated cytokines have upon psychiatric symptoms. This is the case for hepatitis C, where the inflammatory cytokine IFN-α is given together with the antiviral agent ribavirin (130). It is well known, that after IFN-α treatment in hepatitis C, several patients develop depressive symptoms, fatigue and mood disorders (131, 132). Patients with cytokine-induced depression seem to have a slightly different symptom profile compared to somatically healthy depressed patients (133). More specifically, cytokine-induced depression is associated with more somatic symptoms and reduced psychomotor speed (47, 133). This supports that we need to investigate specific symptoms to understand the relation between inflammation and mental health.

1.5.3 Studies on inflammation related to psychiatric symptoms in patients with mental disorders

Over the past 5-10 years, there has been an increasing number of studies examining the association between inflammatory markers and specific symptoms in patients with mental disorders. In schizophrenia, several have found an association between cytokines and severity of psychosis symptoms measured by the Positive and Negative Syndrome Scale (PANSS) (134-136). However, others found that cytokines were only associated with negative psychotic symptoms and not the total symptom burden of psychosis (137, 138). For patients with MDD, there are indications for an association between immune markers and severity of depressive symptoms (139, 140). Certain depressive symptoms such as altered cognition, depressed mood and suicidality, have been identified as potential subtypes of depression associated with inflammation (140). Supplementing the field, studies from our group on patients with fibromyalgia and chronic fatigue syndrome have shown higher levels of immune markers (141), not necessarily related to depressive symptoms (142).

1.5.3.1 Aggression and agitation

There are also some studies finding an association between inflammatory markers and psychiatric symptoms that are less specific to a diagnosis. In patients with personality disorders, aggression and hostility scores were higher in the patients with increased hsCRP levels (> 1.0 mg/L) (143). The association between aggressive behavior and inflammation measured by cytokines, is also found in patients with schizophrenia (144, 145). However, these studies are not directly comparable with each other as different cytokines were investigated. Li *et al*. included only Th17 related cytokines and found them to be significantly associated with aggression (145). Das *et al*. analysed the two cytokines IFN-γ and IL-10, finding that both cytokines were related to aggression (144). As previously explained, IFN-γ is characterized as a Th1 cytokine, whereas IL-10 has more anti-inflammatory properties (84, 89). Although this is a simplification and the function of cytokines in the brain may differ from other parts of the body, it is in any case difficult to compare two studies including cytokines that represent different arms of the immune system.

Agitation is related to aggression. A couple of studies have found agitation to be associated with CRP in patients with schizophrenia (146, 147). The syndrome of agitation has also been related to the cytokines IL-17 and IL-23 in schizophrenia (145). To my knowledge, no other studies have examined the association between agitation and cytokines, and the question on whether this syndrome is related to changes in the immune system, remains largely unanswered. As mentioned earlier, agitation is a symptom that could be related to inflammation due to the presence in somatic, inflammatory diseases.

1.5.3.2 Cognition

As introduced before, cognitive impairments are reported in several mental disorders (51), and are important for functional outcome of bipolar disorder and schizophrenia (52, 53). In fact, cognitive impairments are also described in several diseases affecting the immune

system (148, 149). In individuals infected with human immunodeficiency virus (HIV), the proportion of patients with severe cognitive deficits have declined after the introduction of combination antiretroviral therapies (CARTs) in 1996 (149). There are many potential causes for this association, although it might indicate a relation between inflammation and cognitive symptoms. Further, studies have found an association between cognitive performance and inflammatory markers in severe mental disorders such as schizophrenia, bipolar disorder and MDD (150-152). Accordingly, this has led to some systematic reviews on the subject (153, 154). Misiak and colleagues concluded in their systematic review of all studies published before 2017, that the evidence is strongest for an association between CRP and cognition in patients with schizophrenia, whereas the involvement of cytokines is more uncertain due to few studies (153). Further, it was stressed that previous studies in the field are heterogeneous, regarding both measurements of immune markers and instruments assessing cognition (153).

1.5.3.3 Deviations in psychomotor speed

Patients with cytokine-induced depression (i.e. after treatment for hepatitis C) have at group level more psychomotor retardation compared to medically healthy depressed patients (133). In addition, treatment with IFN-α induces reduced performance on neuropsychological tests for psychomotor speed (155). In the immune-mediated disease multiple sclerosis (MS), reduction in psychomotor processing speed is the most common cognitive impairment (156, 157), suggesting that this cognitive domain might be under influence of the immune system. The possible link between psychomotor speed and cytokines, might be mediated through alterations in basal ganglia and white matter integrity (158, 159).

To date, a couple of studies have identified an association between performance on tests for psychomotor speed and inflammatory markers (CRP and cytokines) in patients with mental disorders (160-163). Only two of these studies were hypothesis-driven with the specific aim to investigate psychomotor speed and immune markers (and not cognition in general) (161,

162). Additionally, two previous studies did not find an association between immune markers and psychomotor speed (164, 165). Moreover, previous studies are limited by small samples and cross-sectional design. It is also difficult to determine whether the relation between psychomotor speed and immune markers is related to motor activity specifically, or other cognitive functions that are demanded to execute neuropsychological test (i.e. processing speed and working memory). To my knowledge, no study has investigated the association between cytokines and gross motor function in patients with mental disorders.

In summary, symptoms that transcend traditional diagnostic boundaries within psychiatry, might be of importance to identify subgroups of psychiatric patients with relation to changes in the immune system. Recently, more studies in the field of immunopsychiatry have focused on psychiatric symptoms (phenotypes) rather than specific disorders. Still, the number of studies regarding psychiatric symptoms and cytokines are quite few and more studies are needed to elaborate the field.

1.6 STATUS AND CHALLENGES OF THE FIELD IMMUNOPSYCHIATRY

In summary, there is increasing evidence for a linkage between mental disorders and inflammation (166). This evidence ranges from epidemiological studies (167, 168), to genetic studies (77, 169), to clinical studies of inflammation in patients with mental disorders (170, 171). In epidemiology, Eaton and colleagues found that the presence of autoimmune diseases was associated with an increased risk for developing schizophrenia, non-affective psychosis or bipolar disorder later in life (167). Similarly, higher levels of cytokines in childhood and adolescence increase the risk of developing depressive and psychotic symptoms in adulthood (172). In genetics, schizophrenia is associated with genes involved in adaptive immunity (77). Finally, giving certain cytokines (e.g. IFN-α) to mentally healthy individuals lead to symptoms of depression, anxiety and cognitive dysfunction (173).

1.6.1 Clinical studies including patients with mental disorders

A majority of clinical studies within the field of immunopsychiatry have focused on psychotic disorders (schizophrenia and first episode psychosis) and affective disorders (MDD and bipolar disorder). Both patients with psychotic and affective disorders have altered levels of certain cytokines and CRP in peripheral blood when compared to healthy controls (174-176). Although less studied, increased inflammatory markers have also been demonstrated in patients with anxiety disorders (177), post-traumatic stress disorder (178) and autism (179). Inflammatory markers normalize after treatment for patients with depression and partly for patients with schizophrenia (180, 181). Interestingly, this normalization of cytokines might only be present in patients that respond to the given treatment (170).

An increasing number of studies have investigated the effect of anti-inflammatory treatment for patients with depression and psychosis, both as add-on and as sole treatment (182, 183). Most of these studies include non-steroidal anti-inflammatory drugs (NSAIDs) (182, 183). Recently, more studies on more potent anti-inflammatory agents (e.g. cytokine inhibitors) have also emerged (184). Possibly, these treatments are only effective for patients with evidence of inflammation (185). A few studies have also found that anti-inflammatory agents improve negative symptoms and cognitive function in schizophrenia (186, 187). These are symptoms that are difficult to treat with today's antipsychotic drugs. Indeed, this raises the question of whether certain symptoms are more associated with inflammation and therefore more likely to respond to anti-inflammatory treatment.

1.6.2 Challenges of the field

The phenomenon of altered levels of inflammatory markers in psychiatric patients is largely transdiagnostic (174). A meta-analysis on peripheral cytokines in schizophrenia showed that some, but not all, of the elevated cytokines normalized during treatment (175, 188). This indicates that some cytokines are trait markers, whereas others are state markers (175).

However, it is unclear if the difference in cytokine profile between acute and chronic states of diseases, are related to the acute state itself, symptoms that are typically more prominent in acute states or other common features in acute psychiatry settings (infections, increased substance use, poor self-care etc.). The relation between inflammatory markers and mental disorders, are further complicated by the fact that only a proportion of psychiatric patients have indications of inflammation (10, 189).

Several have argued that the field of immunopsychiatry needs to shift its focus from syndrome to symptom (10, 190). As stated in the beginning of this thesis, mental disorders constitute of several symptoms and signs that often appear together, constituting the criteria for mental disorders. There is a substantial heterogeneity within the disorders. Also, it is the symptoms, not diagnostic categories, that bother the patient and needs treatment. Therefore, clinical studies in the recent years have focused on symptoms that typically transcends traditional diagnostic boundaries (150, 191). However, more studies on such symptoms are requested with the purpose of selecting the patients that is more likely to have inflammation and effect of anti-inflammatory treatment (10).

When selecting relevant symptoms, one can consider known behavioral changes during immune diseases, results from animal studies or a few previous studies within patients with mental disorders. Simultaneously, the phenomena studied should be of clinical relevance. As introduced previously, both agitation and psychomotor deviations are related to these pieces of evidence, i.e. the occurrence of psychomotor retardation in autoimmune disease. Also, these symptoms are clinically challenging and difficult to treat, causing the patients substantial suffering. It is therefore important to investigate the cause of these symptoms, which might result in new treatments.

1.7 STUDY AIMS AND HYPOTHESES

The overall aim of this thesis was to assess the association between psychiatric symptoms or signs, and peripheral cytokines across various mental disorders, using both cross-sectional and longitudinal designs. In the cross-sectional study we aimed to explore potential associations between symptoms or signs and cytokines. We then wanted to perform a longitudinal study with the aim to investigate certain associations more thoroughly, identifying if cytokines vary over time in relation to symptoms. This could indicate if cytokines affect these symptoms. We hypothesized that peripheral cytokines would be related to and affect certain symptoms, both across various mental disorders and within specific disorders where the particular symptom is more common.

Study 1:

This study examines levels of peripheral cytokines in relation to symptoms in an acute psychiatric population with a broad spectrum of mental disorders. Associations were investigated in the total sample and diagnostic subgroups. We chose these subgroups because the symptom of interest is more frequent within these disorders. In addition, the population in study 2 is patients with psychotic disorders, which means that selecting patients with these disorders in study 1 would make the results more comparable between the two studies.

Paper 1: The primary aim of paper 1 was to assess the association between levels of peripheral cytokines and agitation in an acute psychiatric population. A secondary aim was to investigate if a potential association was driven by psychosis, resulting in two subgroup analyses: one within patients with non-affective psychosis and a second within all patients after exclusion of the non-affective psychosis group. We hypothesized that agitated patients would have higher levels of Th1 cytokines compared to patients without agitation. For the secondary aim, we hypothesized that the association would not be driven by psychosis, meaning that the finding would be reproduced in all subgroups.

Paper 2: The primary aim of paper 2 was to assess the relation between peripheral cytokines, motor retardation and increased motor activity in an acute psychiatric population with various mental disorders. A secondary aim was to investigate if the association would be reproduced in diagnostic subgroups where deviations in motor activity are common. We hypothesized that increased motor activity would be associated with Th1 cytokines (same immune response as the hypothesis for agitation), whereas motor retardation would be related to cytokines from other immune responses. For the secondary aim, we hypothesized that a potential association would be identified in all subgroups, indicating a transdiagnostic phenomenon.

Study 2:

This study withholds data from a population of patients with psychotic disorders with several measurements over time (total follow up period 52 weeks).

Paper 3: The aim of paper 3 was to investigate whether peripheral cytokine levels affected performance on neuropsychological tests for psychomotor speed over time in a study including patients with a spectrum of psychotic disorders. We hypothesized that Th1 cytokines would affect psychomotor speed performance over time in patients with psychotic disorders.

2 MATERIALS AND METHODS

2.1 STUDY DESIGN

All three papers in this thesis are studies of peripheral cytokines in relation to symptoms or signs in patients with mental disorders. The first two papers (study 1, paper 1 and 2) withhold data from patients admitted to an acute psychiatric department with a broad spectrum of mental disorders. The third paper (study 2, paper 3) includes data from a population of patients with a spectrum of psychotic disorders. This study is part of a multicenter pragmatic, randomised controlled trial (RCT) comparing three different atypical antipsychotics. Importantly, we do not compare treatment groups in this paper. Study 1 has a cross-sectional study design including patients from a single center, whereas study 2 is a multi-center study including 4 centers with 8 visits over a total follow-up period of 52 weeks. Figure 3 gives an overview of the study design for all three papers in the thesis.

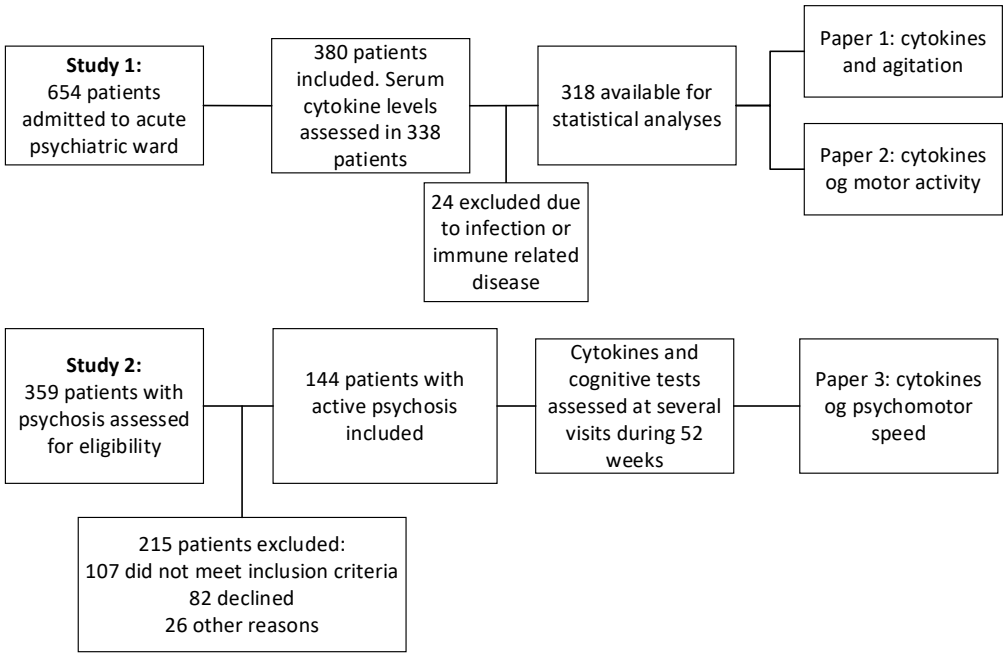

Figure 3: Flow chart illustrating the study design of all three papers included in the thesis.

2.2 SETTING AND PARTICIPANTS

2.2.1 Study 1

This study was conducted at the acute psychiatric inpatient ward of St. Olav's University hospital, Trondheim, Norway. All eligible inpatients acutely admitted between September 2011 and March 2012 were asked to participate. At the time of inclusion, the psychiatric department served a catchment area of 228.000 inhabitants (≥ 18 years) and was the only psychiatric acute ward in the catchment area. In this region of Norway, all acute psychiatric services are public and free of charge. The ward consisted of both ordinary, closed ward areas and PICUs, and we included patients from both. Admitted patients suffered from a variety of mental disorders. Of the total 654 admitted patients in the inclusion period, 382 (58.4%) patients were included.

All patients able to give informed consent were asked to participate. Ability to give informed consent to participate in the study was evaluated by an experienced psychiatrist / specialist in clinical psychology the first day after admission. For the two papers in this thesis, we chose to exclude patients with possible immune related diseases. More specifically, the following exclusion criteria were applied for paper 1 and 2: (1) chronic or ongoing infections, (2) comorbid autoimmune diseases, (3) CRP levels above 35 mg/L (level based on clinical experience). When patients had multiple admissions, we only included the first admission in our analyses. Patients could be admitted voluntarily or by coercion.

2.2.2 Study 2

This study is part of a pragmatic multicenter RCT including patients with active psychosis. Elective patients were above the age of 18, having a psychotic disorder in the ICD-10 F20 – 29 group and about to start or change antipsychotic treatment. The primary results from comparison of treatment groups are previously published (192). In paper 3 for this thesis, patients from all treatment groups were analysed collectively. The follow-up period was 52

weeks with visits at baseline as well as after 1, 3, 6, 12, 26, 39 and 52 weeks. Blood samples were collected at all these visits, neuropsychological testing was conducted at baseline, and at week 6, 12, 26 and 52. Three treatment centers in Norway (Bergen, Stavanger and Trondheim) and one in Austria (Innsbruck) participated in collecting the data. The recruitment period was from October 2011 until December 2016. All patients ≥ 18 years old were eligible for inclusion if they had a psychotic disorder (ICD-10 diagnosis F20-29) with active psychosis. The definition of active psychosis was a score ≥ 4 on at least one of the following PANSS items: "*P1 Delusions*", "*P3 Hallucinations*", "*P5 Grandiosity*", "*P6 Suspiciousness/persecution*" or "*G9 Unusual thought content*", corresponding to positive symptoms of psychosis. Exclusion criteria were inability to understand the native language (Norwegian or German), pregnancy, breastfeeding, limbic encephalitis, hypersensitivity to any of three antipsychotic drugs tested in the main study, or somatic disorders known as precautions for the three antipsychotics. Patients might be receiving treatment voluntarily or by coercion.

2.3 DIAGNOSTIC EVALUATION

In study 1, patients were diagnosed according to the ICD-10 Criteria for Research (39). The diagnoses were set in a consensus meeting always including at least two senior psychiatrists or experienced clinical psychologists of whom at least one had personally examined the patient. All clinical data from patient files were available. For subgroup analyses in paper 1, patients were divided into non-affective psychosis group (ICD-10 F20–29), and a group of all patients without the psychosis group. In paper 2, patients were divided into unipolar depression (ICD-10 F32 and F33) and non-affective psychosis (ICD-10 F20–29) for subgroup analyses. If patients had multiple diagnoses, we chose the main diagnosis for the current admission. We selected these diagnostic subgroups because agitation and/or psychomotor deviation are known to be frequent and clinically important within these disorders.

In study 2, diagnoses were set according to the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), which were converted to ICD-10 diagnoses.

2.4 CLINICAL ASSESSMENTS

2.4.1 Study 1

Sociodemographic data, smoking status and the history of somatic diseases were recorded by a staff member. Height and weight were measured to calculate body mass index (BMI, kg/m^2). The patients underwent clinical interviews regarding substance use. In addition, urine samples were screened for drugs. Patients were also screened in a general medical examination and with routine blood tests, including CRP.

2.4.1.1 Agitation

In paper 1, agitation was assessed by experienced psychiatrists or clinical psychologist using the Positive and Negative Syndrome Scale, Excited Component (PANSS-EC). The PANSS-EC is a validated and commonly used scale assessing agitation in acute psychiatry (193). The interrater reliability of PANSS-EC is previously demonstrated to be very good (193). For this study, clinicians received training in the entire PANSS and for the subscale PANSS-EC. However, we did not perform any interrater reliability score. It is calculated as the sum of the following PANSS items: "*P4 Excitement*", "*P7 Hostility*", "*G4 Tension*", "*G8 Uncooperativeness"* and "*G14 Poor impulse control*" giving a total scoring range of 5 to 35. Clinically significant agitation is considered with PANSS-EC score ≥ 14 (194, 195). For statistical analyses, patients were divided into two groups: agitated patients (PANSS-EC ≥ 14) and non-agitated patients (PANSS-EC < 14).

2.4.1.2 Deviations in motor activity

For recordings of motor activity in paper 2, an experienced psychiatrist or clinical psychologist scored the patients using the Symptomatic Organic Mental Disorder Assessment Scale (SOMAS). The clinicians were given training in SOMAS before the study. Unfortunately, there are no studies on interrater reliability score. SOMAS is a 5-item scale developed to assess atypical depressive symptoms. Item B rates the degree of motor retardation, and item C rates the degree of increased motor activity, both when the patient was most depressed during the previous 24 hours (196). The two items are given in Table 1. Both items are modified from the Positive and Negative Syndrome Scale (PANSS), where item B was modified from PANSS item Motor retardation (G7), and item C from Hyperactivity (P4). Although there are no published validity studies on SOMAS, a previous study found that these two categories correspond well with activity levels measured by a wrist-worn actigraph in patients with unipolar depression (45).

Table 1: The Symptomatic Organic Mental Disorder Assessment Scale (SOMAS), item A and B.

B: Degree of motor retardation, rated during the period or periods of the previous 24 hours in which the patient was most depressed.	
1	The patient has been almost completely immobile and virtually unresponsive to external stimuli.
2	Movements are extremely slow, resulting in a minimum of activity and speech. The patient is mostly sitting idly or lying down.
3	The patient has slow movements, and speech may be characterized by poor productivity, including long response latency, extended pauses, or slow pace.
4	Slight diminution in rate of movements and speech.
5	No motor retardation.
C: Degree of increased motor activity, rated during the period or periods the previous 24 hours when the patient was most depressed.	
1	No increased motor activity.
2	The patient is slightly agitated with hypervigilance or has a tendency towards mild overarousal. The speech is slightly pressured.
3	The patient is clearly agitated and over aroused with affected speech and motor activity.
4	Marked excitement dominates the period and restricts attention and vital functions such as eating and sleeping.
5	The excitement is so extreme that interpersonal interaction is virtually impossible. The patient has acceleration of speech and motor activity resulting in incoherence and exhaustion.

For statistical analyses, patients were subdivided into two groups: with or without increased motor activity according to SOMAS item C. If the patients were scored as ≥ 2 on SOMAS item C, they were grouped as motor active. Similarly, patients were separated into the two groups with or without motor retardation according to SOMAS item B. A score on SOMAS item B ≤ 3 was set to group the patients as motor retarded. In order to simplify the interpretation of findings on SOMAS item B, it was reverse-coded, resulting in that a higher score on both items would be interpreted as more severe symptoms. The cut-off scores for dichotomizing the SOMAS items were chosen based on a clinical evaluation of the descriptions within the scale. We evaluated that SOMAS item B had minimal symptoms at score 2, whereas item C had more prominent symptoms at score 2, which resulted in slightly

different cut-off scores for the two items. After this dichotomizing, 115 patients were grouped as motor retarded and 93 patients as motor active. In addition, the SOMAS scale was designed with scores ranging from 1-10, but with only 5 options within each item, meaning that it is an ordinal scale with 5 ranks. For statistical analyses, we therefore adjusted the scale ranging from 1-5. SOMAS is described with a 1-5 score in Table 1.

2.4.2 Study 2

Sociodemographic data such as ethnicity, smoking status, gender and age, were recorded at baseline. Alcohol and drug abuse were assessed with the Clinical Alcohol Use Scale and the Clinical Drug Use Scale (197). Participants were interviewed with PANSS at each visit. Height at baseline, and weight were also measured throughout the entire study period to calculate BMI.

2.4.2.1 Neuropsychological testing

We included the following neuropsychological tests in this paper: trail making test (TMT) –A and -B, and symbol coding. Trail making test (TMT) –A and -B, were conducted at baseline and visits at week 6, 12, 26 and 52. Symbol coding was conducted at baseline and weeks 6, 26 and 52. All raw scores from the cognitive tests were converted to t-scores according to best available norms and corresponding manuals. Clinicians who performed the neuropsychological tests went to a thorough training program at University of Bergen.

TMT consists of two parts, TMT-A and -B. In TMT-A, subjects are instructed to draw a line between encircled numbers in numerical order. In TMT-B, subjects must alternate between encircled numbers and letters. Both tests yield a raw score of mean seconds required to complete the tasks. TMT-A is a test of psychomotor speed and visuospatial abilities, whereas part B also demands higher cognitive abilities such as working memory (57, 198). The interrater reliability of the TMT is previously demonstrated to be very good (57).

The symbol coding test is a subtest of the Brief Assessment of Cognition in Schizophrenia (56, 199). Here, subjects are instructed to write numbers (1-9) under their corresponding symbols according to the symbol-number combination that are given on top of the response sheet (199). The score is based on number of correct items within the time frame of 90 seconds. The symbol coding test is measuring psychomotor processing speed and attention (199). Although the interrater reliability for the specific symbol coding test is not known, the test-retest reliability is good, and it has moderate to strong correlations with other cognitive tests of the same domains (56). All the neuropsychological tests used in this study had a manual with strict instructions. The final raw scores were in seconds, meaning that we had an objective measure which could be less sensitive to interrater errors.

We chose to include all three tests as measures of psychomotor speed. As the tests differ in complexity, including all three could help to differentiate if a possible association was related to more pure psychomotor speed or other higher cognitive demands such as attention and working memory. In addition, all three tests are easily accessible and already widely used in clinical practice. This is of importance because a potential finding would be easier to implicate in a daily clinical setting.

2.5 IMMUNE MARKERS

In study 1, blood samples were collected between 08:00 and 13:00 (median at 10:00) at the first working day after admission. Strict instructions regarding fasting were not given, though most patients would be fasting overnight. Samples were immediately cooled on ice, protected from daylight, and centrifuged within 30 minutes (15 min, 1500 g, 4ºC). In study 2, blood samples were collected at all 8 visits in a fasting state between 08.00 and 10.00. Before centrifugation at 3300 rpm for 10 minutes, the blood was let to clot at room temperature for 20-120 minutes. Serum samples were stored at -80ºC in both studies. Cytokines were chosen

based on previous studies within the field, the availability of commercial assays and with an attempt to represent main pathways of the immune system according to present knowledge.

Multiplex immunoassays were used in both studies. In study 1, the following cytokines were analysed by Miliplex MAP assays: IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p40, IL-12 p70, TNF-α, and IFN-γ (Millipore Corporation, Billerica, MA, US). Additionally, TGF-β1, TGF- β2 and TGF- β3 were measured by a Bio-Plex Pro TGF-β Assay (Biorad Hercules, CA, US). All cytokine analyses in study 1 were performed according to the manufacturers' protocol. In study 2, cytokines were analysed with the immunoassay High Sensitivity 9-Plex Human ProcartaPlex™ Panel including the following cytokines: IL-1β, IL-2, IL-4, IL-6, IL-10, IL12 p70, IL-17a, IFN-γ and TNF-α (ThermoFisher Scientific, Waltham, MA, USA). This panel was also performed in accordance with manufacturer's protocol, but the universal assay buffer was substituted with 1X phosphate buffer saline (PBS) with 0.10% tween to improve fluorescence intensities. In both studies, the detection limit was defined as the lowest detected value within the standard curve. The range of detected values and number of samples under the detection limit are given in Table 2.

Table 2: range of detected values and number under detection limit for cytokines in both studies.

	Range of values		Under detection limit, n (%)	
	Study 1	Study 2	Study 1	Study 2
IL-1α (pg/ml)	4.70-2430.72	-	196 (61.6)	-
IL-1β (pg/ml)	0.06 - 198.72	0.02 - 22.74	236 (74.2)	229 (30.1)
IL-2 (pg/ml)	0.13-844.40	0.06 - 217.47	-	41 (5.4)
IL-4 (pg/ml)	0.92 – 286.01	0.10 - 227.41	242 (76.1)	162 (21.3)
IL-6 (pg/ml)	0.10 – 576.42	0.07 - 15.19	177 (55.7)	239 (31.4)
IL-8 (pg/ml)	0.08-329,80	-	21 (6.6)	-
IL-10 (pg/ml)	0.10 – 1125-33	0.02 - 22.10	177 (55.7)	74 (9.7)
IL-12 p40 (pg/ml)	0.43-444.85	-	227 (71.4)	-
IL-12 p70 (pg/ml)	0.10-999.26	0.12 – 80.82	220 (69.4)	171 (26.3)
IL-17a (pg/ml)	-	0.02 - 279.43	-	40 (6.1)
IFN-γ (pg/ml)	0.04 – 1529.70	0.13 - 70.87	68 (21.4)	158 (20.8)
TNF-α (pg/ml)	0.70 – 268.89	0.11 - 445.08	11 (3.5)	146 (19.2)
TGF-β1 (ng/ml)	13.07 – 415.61	-	0	-
TGF-β2 (ng/ml)	0.68-7.44	-	0	-
TGF-β3 (ng/ml)	0.11-3.22	-	0	-

Data from study 2 includes cytokine levels from all visits collectively.
IL: Interleukin, IFN: Interferon, TGF: Transforming growth factor, TNF: Tumor necrosis factor.

In study 1, we chose to exclude the following cytokines before statistical analyses to limit problems with multiple testing: IL-1α, IL-2, IL-8, IL-12 p40, IL-12 p70. The cytokines were excluded if they were not known to be altered in mental disorders and if they had a high portion (> 60%) below the detection limit. IL-1β was included despite a high portion below the detection limit because of its known relevance within mental disorders. For the TGF-β cytokines, we chose to only include TGF-β1 as its functions within the immune system are more well-known. Also, within the literature, it is often TGF- β1 researcher are referring to when mentioning TGF-β. For simplicity, we also used the term TGF-β in the papers.

2.6 ETHICS

In both studies, patients gave written informed consent prior to inclusion, and they were conducted according to the Declaration of Helsinki. Study 1 was approved by the Regional Committee for Medical and Health Research ethics in Norway (REC South East number 2011/137). Study 2 was approved by the ethical committees in both countries; REC in

Norway (REC west number 2010/3387-6), and the Ethikkommission der Medizinische Universität Innsbruck in Austria. Both studies were registered at ClinicalTrials.gov (Study 1: NCT01415323, Study 2: NCT01446328). The funding source had no role in design or conduction of either study.

2.7 STATISTICAL ANALYSES

Statistical analyses were performed using SPSS in paper 1 and 2, whereas R was used in paper 3. The level of significance was set at $p \leq 0.05$ and all analyses were two-tailed. Descriptive statistics were calculated by chi-square tests for categorical variables and student's independent samples t-tests or Mann-Whitney U test (depending on distribution) for continuous variables.

In study 1, significant findings were adjusted for multiple testing with the Bonferroni correction (α/k where k= the seven tested cytokines giving $\alpha/k = 0.007$). Because correction for multiple testing most likely would make the one significant finding insignificant, we chose not to correct for multiple testing in study 2. Instead, we had this limitation in mind when evaluating our results, being more cautious when concluding. Data normality was assessed by normally tests (Kolmogorov-Smirnov and Shapiro-Wilk), and evaluation of QQ-plot. Only TGF-β of the cytokines analysed in study 1 was normally distributed. The cytokines in this study remained skewed after logarithmic transformation. In study 2, all cytokine values were heavily right skewed, and we used log transformed cytokine values in the statistical analyses. TMT-A, TMT-B and symbol coding t-scores were all close to normally distributed.

Cytokine levels were compared between agitated and non-agitated patients using a Mann-Whitney U test or student's independent samples t-test, depending on the distribution. The same methodology was applied when comparing cytokine levels between patients with and without motor deviations (increased motor activity and motor retardation). In addition, the association between cytokines and motor deviations was assessed with Spearman's

correlation between each cytokine and SOMAS. Further, the association between SOMAS and cytokines, was assess in a multiple-linear-regression model correcting for age, gender and BMI. As it was not possible to correct for these possible confounders in Paper 1 when comparing two groups of patients, the relation between cytokines and confounders was assessed individually. In these analyses of confounders, the level of significance was set at $p \leq 0.05$. More specifically, Spearman correlation coefficient was calculated for the relationship between cytokines, BMI and age, and cytokines levels were compared between males and females using Mann-Whitney U test or student's independent samples t-test.

In study 2, we used linear mixed effect (LME) models to see if log transformed cytokines, TMT-A, TMT-B or symbol coding changed over time alone without adjusting for the effect of each other. A random intercept for each patient was included to account for dependencies in the data due to repeated measures from the same participants. Secondly, the Pearson correlation coefficient was calculated for the relationship between log transformed cytokines, and TMT-A, TMT-B or symbol coding at baseline and at end point. Finally, we used LME models to assess the relationship between log transformed cytokines and tests for psychomotor speed. We made one model for each test, including the test as dependent variable, and cytokines and visit as independent variables. Again, a random intercept for each patient was included in order to account for dependencies in the data. The following possible confounders were included in the model as independent variables: age, gender, BMI, ethnicity, smoking, study site, antipsychotic drug and PANSS positive subscale score. Due to the possible confounding effect of including patients with various psychotic disorders, we conducted sensitivity analyses where patients with F22 Delusional disorder and F23 Acute and transient psychosis were excluded. After excluding these patients, we conducted the same LME models investigating the association between log transformed cytokines and tests for psychomotor speed correcting for possible confounders.

3 RESULTS

3.1 DEMOGRAPHIC DATA OF THE TWO STUDIES

Table 3: Demographic and clinical variables at baseline in study 1 and 2

	Study 1 N = 358	Study 2 N = 144
Age (years), mean ± SD	38.9 ± 14.8	31.7 ± 12.7
Gender (female), N (%)	174 (49)	51 (35.4)
Smoking, N (%)	175 (49)[a]	84 (66.1)[b]
BMI (kg/m^2), mean ± SD	25.5 ± 5.9[c]	25.5 ± 6.0[d]
Higher education (above high school), N (%)	50 (14)	25 (19)[e]
Unemployment (incl. sick leave), N (%)	255 (71)	100 (73.5)[f]
Alcohol misuse or dependence, N (%)	99 (28)	13 (9.6)[e]
Drug misuse or dependence, N (%)	78 (22)	27 (19.9)[f]
Number with blood samples, N (%)	318 (89)	140 (97)

[a]Missing 60
[b]Missing 17
[c]Missing 89
[d]Missing 22
[e]Missing 9
[f]Missing 8
Abbreviations: SD: standard deviation, BMI: body mass index

3.2 SUMMARY OF PAPER 1

Cytokines in agitated and non-agitated patients admitted to an acute psychiatric department: A cross-sectional study.

Larsen J.B., Stunes A.K., Vaaler A., Reitan S.K.

PLoS One. 2019;14(9):e0222242.

The aim of this paper was to assess the levels of peripheral cytokines related to agitation in an acute psychiatric inpatient population. We used data from 316 patients with various mental disorders. Patients were stratified into two groups depending on their PANSS-EC scores, resulting in 65 agitated patients and 249 non-agitated patients. The serum cytokine levels of IL-1β, IL-4, IL-6, IL-10, TNF-α, IFN-γ and TGF-β were compared between the two groups.

MAIN FINDINGS:

- Levels of TNF-α were significantly higher in patients with agitation compared to those without. This finding was significant with a small effect size when including all patients in the analyses, and in the two subgroups of patients with and without non-affective psychosis (with respectively medium and small effect sizes).
- After correcting for multiple testing, only the finding of higher TNF-α in the total sample remained significant.
- No other differences in cytokines levels between agitated and non-agitated patients, reached the level of significance.
- We did not correct for potential confounders, but in exploratory analyses, few cytokines were associated with age, gender, BMI or smoking. None of confounders were significantly associated with TNF-α.

Table 4: comparison of serum cytokine levels based on the presence or absence of agitation.

	All patients			Non-affective psychosis group			Patients without non-affective psychosis		
	Agitated (n = 67)	**Non-agitated (n = 249)**	***P***	**Agitated (n = 13)**	**Non-agitated (n = 26)**	***P***	**Agitated (n = 54)**	**Non-agitated (n = 223)**	***p***
IL-1β (pg/mL)	0.03 (0.03-0.03)	0.03 (0.03-0.12)	0.724[b]	0.03 (0.03-0.03)	0.03 (0.03-1.00)	0.415[b]	0.03 (0.03-0.14)	0.03 (0.03-0.09)	0.981[b]
IL-6 (pg/mL)	0.05 (0.05-45.38)	0.05 (0.05-17.58)	0.220[b]	0.05 (0.05-133.20)	5.66 (0.05-20.71)	0.547[b]	0.05 (0.05-31.43)	0.05 (0.05-15.65)	0.319[b]
TNF-α (pg/mL)	21.87 (11.83-29.79)	14.94 (7.26-26.12)	**0.004[b]**	21.71 (16.89-43.89)	12.84 (7.36-25.50)	**0.027[b]**	22.80 (11.33-29.56)	15.31 (7.13-26.35)	**0.025[b]**
IFN-γ (pg/mL)	7.05 (0.02-20.59)	5.12 (0.59-16.91)	0.907[b]	20.90 (1.48-39.62)	3.49 (0.08-9.24)	0.055[b]	3.62 (0.02-17.81)	5.20 (0.67-19.49)	0.477[b]
IL-10 (pg/mL)	0.05 (0.05-37.03)	0.05 (0.05-22.00)	0.423[b]	0.05 (0.05-85.73)	3.49 (0.05-54.48)	0.885[b]	0.05 (0.05-34.20)	0.05 (0.05-18.27)	0.405[b]
IL-4 (pg/mL)	0.46 (0.46-20.35)	0.46 (0.46-0.46)	0.110[b]	0.46 (0.46-39.47)	0.46 (0.46-7.52)	0.478[b]	0.46 (0.46-12.33)	0.46 (0.46-0.46)	0.193[b]
TGF-β (ng/mL)	78.41 (63.46-95.72)	80.47 (62.13-97.42)	0.924[c]	69.58 (60.31-95.21)	80.21 (63.57-93.51)	0.789[c]	78.68 (63.72-96.10)	80.47 (61.21-98.13)	0.687[c]

All data are presented as median with interquartile range.

[a]Agitation was classified by Positive and Negative Syndrome Scale, Excited Component (PANSS-EC) score ≥ 14.

[b]Mann-Whitney U test

[c]Independent students' samples t-test

Abbreviations: IL: interleukin, TNF: tumor necrosis factor, IFN: interferon, TGF: transforming growth factor.

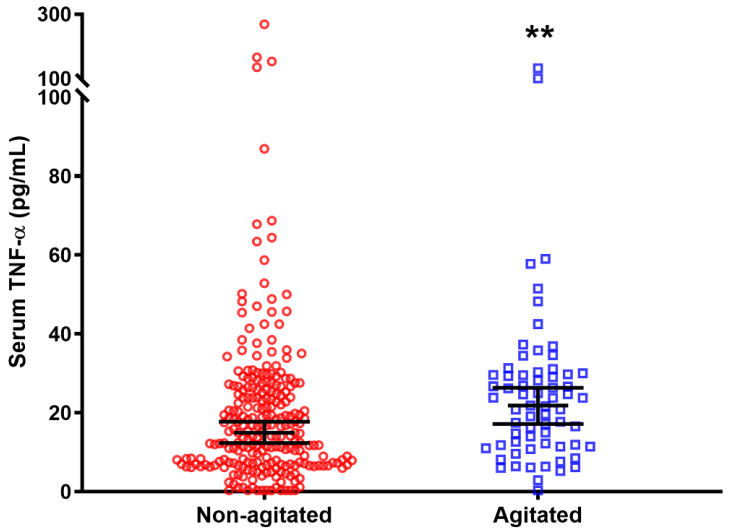

Figure 4. TNF-α levels in all acutely admitted patients. Serum TNF-α levels were significantly higher in agitated patients compared to non-agitated patients (21.87 ± 17.96 vs 14.94 ± 18.86 pg/mL). Data are expressed as median ± 95% confidence interval. **p = 0.004.

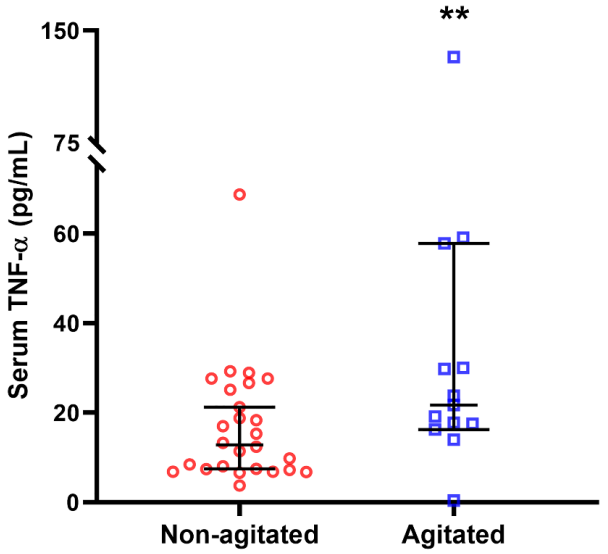

Figure 5. TNF-α levels in psychosis group. Agitated psychosis patients had significantly higher serum levels of TNF-α compared to non-agitated psychosis patients (21.71 ± 27.00 vs 12.84 ± 18.15 pg/ml). Data are expressed as median ± 95% confidence interval. **p = 0.027. This finding did not remain after correction for multiple testing.

3.3 SUMMARY OF PAPER 2

Cytokines in Relation to Motor Activity in an Acute Psychiatric Population.

Larsen J.B., Stunes A.K., Iversen V.C., Vaaler A.E., Reitan S.K.

Frontiers in psychiatry. 2019;10(920).

The purpose of this paper was to assess the relation between peripheral cytokines, motor retardation and increased motor activity in an acute psychiatric population. In this paper, we used the same sample as in paper 1. Motor activity was assessed with items from SOMAS, scoring the degree of increased motor activity and psychomotor retardation. Data on motor activity and cytokines were available in 318 patients.

MAIN FINDINGS:

- After adjusting for the potential confounder age, gender and BMI in a multiple-linear regression model, we did not find any significant associations between motor activity and cytokines.
- There was a trend towards an association between motor activity and the cytokines TGF-β and IFN-γ when including all patients in the analyses, but this was not reproduced in the groups non-affective psychosis and unipolar depression.
- No significant correlations between motor retardation and circulating cytokines were found.

Table 5: Correlation coefficients (rho) between serum cytokines, motor retardation and motor activity[a]

	IL-1β	IL-6	TNF-α	IFN-γ	IL-10	IL-4	TGF-β
All patients							
- Motor retardation	0.112	0.066	-0.027	0.090	0.061	-0.043	-0.042
- Motor activity	0.001	-0.010	-0.034	-0.128*	-0.002	0.057	0.118
Non-affective psychosis							
- Motor retardation	0.154	0.165	0.002	0.191	0.194	-0.181	-0.153
- Motor activity	0.052	0.251	0.029	0.000	0.085	0.080	-0.054
Unipolar depression							
- Motor retardation	-0.089	-0.003	-0.047	-0.007	-0.056	0.059	0.173
- Motor activity	-0.046	-0.133	-0.181	-0.173	-0.239	-0.002	0.107

*$p \leq 0.05$.

[a]Motor retardation and motor activity were scored on a scale from 1-5.

Abbreviations: IL: interleukin, TNF: tumor necrosis factor, IFN: interferon, TGF: transforming growth factor.

3.4 SUMMARY OF PAPER 3

The association between cytokines and psychomotor speed in a spectrum of psychotic disorders: a longitudinal study.

Larsen JB, Reitan SK, Løberg EM, Rettenbacher M, Bruserud Ø, Larsen TK, Anda L, Bartz-Johannessen C, Johnsen E, Kroken RA

Brain, Behavior, & Immunity - Health. 2021;18:100392.

The aim of paper 3 was to investigate if serum cytokine levels affected the performance on neuropsychological tests for psychomotor speed over time. This study is part of a pragmatic, randomised controlled trial comparing three atypical antipsychotics including 144 patients with a spectrum of psychotic disorders. Patients underwent neuropsychological testing and blood sampling at several visits over the total follow-up period of 52 weeks. The neuropsychological tests TMT-A, TMT-B and symbol coding were included as measures of psychomotor speed. The following cytokines were analysed: IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 p70, IL-17a, IFN-γ and TNF-α.

MAIN FINDINGS:

- In LME models controlling for possible confounders, IFN-γ had a significant negative effect on TMT-A (model estimate = -2.923, p = 0.011) and symbol coding performance (model estimate = 2.564, p = 0.038).
- None of the other tested cytokines were significantly associated with the tests for psychomotor speed in the total sample.
- The performance on tests for psychomotor speed, improved significantly during the study period, while serum cytokine levels remained stable.
- When excluding patients with delusional disorder and acute transient psychosis (ICD-10 diagnoses F22 and F23), IFN-γ was still significantly associated with TMT-A

(model estimate = -3.059, p = 0.042), but not symbol coding (model estimate = -1.882, p = 0.212), in LME models. In addition, IL-4 was associated with TMT-A (model estimate = 2.506, p = 0.030) and TMT-B (model estimate = 2.417, p = 0.046).

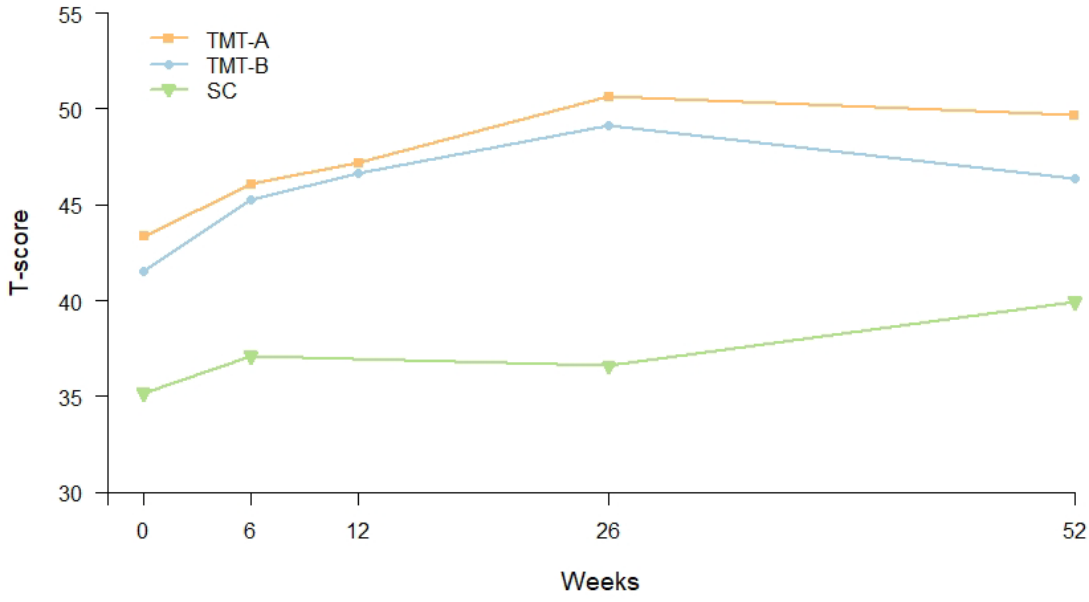

Figure 6. Estimated mean t-score of psychomotor speed over time. Data estimated in linear mixed effect models including three different tests for psychomotor speed. TMT-A changed significantly from baseline to week 6, and from week 12 to 26. TMT-B changed significantly from baseline to week 6. SC changed significantly from week 26 to 52. Abbreviations: TMT; trail making test, SC; symbol coding

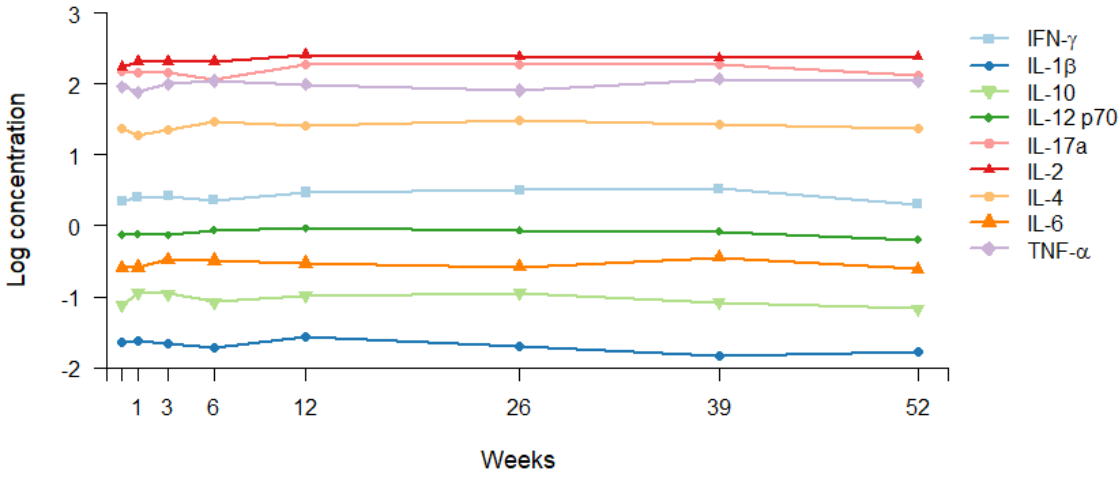

Figure 7: Estimated mean level and change in cytokines over time.

Data estimated in linear mixed effect models including log transformed cytokine values. None of the cytokines changed significantly from baseline to end point, or between any of the visits. Abbreviations: IFN; interferon, IL; interleukin, TNF; tumor necrosis factor.

Table 6: Estimated effect of log-transformed cytokines on t-scores from test of psychomotor speed

	TMT-A			TMT-B			Symbol coding		
	Model estimate	SE	*p*	Model estimate	SE	*p*	Model estimate	SE	*p*
IFN-γ	**-2.923**	**1.14**	**0.011**	-1.131	1.07	0.294	**-2.564**	**1.22**	**0.038**
IL-1β	0.161	0.55	0.768	-0.199	0.55	0.716	0.444	0.60	0.463
IL-10	0.835	0.58	0.149	0.550	0.56	0.330	-0.039	0.59	0.948
IL-12 p70	0.959	1.27	0.451	-0.867	1.26	0.493	1.044	1.31	0.427
IL-17a	-0.264	0.96	0.782	0.849	0.94	0.370	-1.602	1.15	0.166
IL-2	0.951	1.30	0.465	-0.607	1.27	0.635	0.248	1.346	0.854
IL-4	0.648	0.77	0.399	1.297	0.73	0.079	0.668	0.92	0.468
IL-6	-0.327	0.61	0.594	0.220	0.61	0.719	-0.576	0.68	0.399
TNF-α	-0.007	0.83	0.993	-0.217	0.83	0.793	1.516	0.92	0.103

Estimates from linear mixed effect (LME) models. The models included age, gender, BMI, ethnicity, smoking, study site, antipsychotic drug and PANSS positive score as independent variables.

Abbreviations: IFN; interferon, IL; interleukin, TMT; trail making test, TNF; transforming growth factor

4 DISCUSSION

In the two papers regarding cytokines and symptoms in an acute psychiatric sample with various psychiatric diagnoses (study 1), the findings can be summarized as follows: agitation was significantly associated with increased TNF-α levels, whereas increased motor activity was significantly associated with lower levels of IFN-γ. However, the association between IFN-γ and increased motor activity did not remain statistically significant after correction for age, BMI and gender. We also found a non-significant trend towards an association between TGF-β and increased motor activity. Reduced motor activity was not associated with any of the tested cytokines in our material. In the paper including patients with a spectrum of psychotic disorders (study 2), worse performance on tests for psychomotor speed (TMT-A and symbol coding) was associated with the cytokine IFN-γ over time. In this population, cytokines remained stable over time whereas patients improved significantly on tests for psychomotor speed.

4.1 DISCUSSION OF MATERIALS AND METHODS

4.1.1 Samples

In Norway, health care is primarily publicly funded, meaning that we had access to patients from all sociodemographic classes at the Norwegian clinics. Inhabitants may have a private or employer-financed health assurance, but we assume this is to our knowledge rarely activated for severe acute psychiatric problems, and hardly for inpatient services. Austria has a publicly funded care, but individuals also have the option to purchase supplementary health insurance gaining access to additional services. In both study 1 and 2, patients did not receive any financial support if they participated, eliminating the potential ethical dilemma of whether a participant was bought into contributing to a study conflicting to their personal opinions and believes. However, lack of financial support or other benefits from participating, could reduce the percentage accepting to be included and thereby affecting the representativeness.

In study 1, we included patients from an acute psychiatric inpatient ward. For all practical purposes, the public system is the only provider of acute psychiatric inpatient services in this region of Norway. It is therefore likely that the patients in study 1 represent the general inpatient acute psychiatric population. The catchment area for the sample is typical for Norway regarding number of beds, percentage with drug/alcohol abuse and the distribution between central and rural areas (200). Most patients who declined were admitted less than 24 hours and were discharged before inclusion was possible (200). It is also demonstrated that non-participants and participants from this study were similar in age and gender, but the diagnostic distribution variated among the two groups (201). For instance, bipolar disorder, MDD and neurotic disorders were more frequent among the participants (201).

The sample in study 2 is more strictly selected based on several inclusion and exclusion criteria being a pragmatic RCT. The purpose with a pragmatic RCT, is to investigate treatment effectiveness under normal clinical circumstances with a more heterogeneous sample, compared to a stricter RCT of efficacy. One could argue that the inclusion criteria are quite broad for an RCT, hence the pragmatic design, including patients with comorbid mental disorders, additional psychotropic drug usage, drug/alcohol abuse and suicidal ideation. Although this increases the representativeness of the population, the sample is still by definition not representative for a naturalistic, general population with psychotic disorders as they are selected by certain inclusion criteria.

4.1.2 Potential selection bias in study 1

Among the potentially eligible patients, 52% were originally included in study 1. As we chose to exclude patients with evidence of immune related disease or infection, and blood samples were missing for some patients, 48% of the total number of patients admitted in the study period were included in our two papers. In other words, a large proportion of the patients declined to participate. Here, a potential selection bias could be that the most severely ill

patients were unable to participate. However, as already mentioned, most patients who declined were admitted at night and discharged the following day. This could indicate that these patients were not among the most severely ill, i.e. a state of acute psychosis or a severe depression usually result in an admission lasting longer than one night. It is also of importance that the participants deviate from the non-participants in diagnosis, indicating a selection bias towards certain mental disorders in this study. Due to the setting of inclusion of patients admitted to an acute psychiatric ward, we do not know whether there are any association between symptoms and cytokines in other patient groups with acute psychiatric symptoms. Several patients with acute psychiatric symptomatology might be in outpatient care, and these patients are by definition not included in study 1.

An important remark about participation in this study, is that data were registered on all patients that were admitted, and consent to participate was collected the following day. For the patients who lacked capacity to consent at that time, for instance due to an acute psychosis, consent and participation would be evaluated later when the patient had improved. If the patient then had capacity to consent and accepted participation, the patient would be included. If the patient had capacity to consent and declined, the data registered on this patient would not be included in the study. This was a practice that enabled us to include and explore patients when the symptoms were at its most profound, and could help to limit the selection bias towards the patients with less serious symptomatology,

In addition, we were missing blood samples on 42 patients of the total 380. It is possible that the patients missing blood samples are different from patients who underwent blood sampling. This could be due to lack of cooperation, which again is related to the severity of other psychiatric symptoms such as agitation, psychomotor retardation (and general lack of motivation to participate) and increased motor activity.

4.1.3 Potential selection and attritional bias in study 2

Of the total patients assessed for eligibility, 40% were included in study 2. In other words, it was a quite large proportion of the patients that declined to participate or that did not meet the inclusion criteria. In addition, there is a significant selection bias in the study design itself regarding which patients that were assessed for eligibility. As for study 1, patients had to be under care by the current specialist health services, missing the opportunity to include patients from other services. Also, patients had to be under consideration for antipsychotic drugs, missing patients that refused this treatment or where only a few, specific antipsychotic drugs would be relevant. Further, patients had to return for repeated measures over a year, months after discharge from the hospital. It is also possible that the most severely ill patients never were considered eligible for inclusion, for instance due to low treatment adherence.

Considering the clinical and demographic factors of this particular population, one might argue that the average age and proportion of women, are lower than expected (202). Still, it is difficult to estimate what an expected average age should be in a population with psychosis including subjects from various clinics and in different states of the disease (both first episode and chronic). Further, only 26% of the included patients had problems with alcohol or drug abuse. In comparison, a large meta-analysis found that 42% of patients with schizophrenia spectrum disorders had a co-occurring substance use disorder (203). However, frequencies of substance use disorders vary among countries and numbers from psychosis patients in Nordic countries are more similar to the percentage in study 2 (202, 204, 205). Finally, 63.2% of the patients were recruited from hospital admissions and the rest from outpatient clinics (192). This is important, because the symptomatology differs between inpatients and outpatients with schizophrenia (206). More specifically, psychotic symptoms were more prominent in hospitalized patients, whereas depressive symptoms were higher in outpatients (206). When

most patients in this study were hospitalized, the risk of underestimating the importance of certain symptoms increases.

In this study, few patients were missing blood samples. However, we were missing data on TMT-A from 43 (30%) participants, TMT-B from 55 (38%) participants and symbol coding from 52 (36%) participants. As the study included 144 participants, the frequency of missing data on tests for psychomotor speed ranges from 30% to 38%. Neuropsychological tests are time consuming, demands some motivation and the ability to concentrate. These are aspects that could be difficult when being psychotic and under influence of psychotropic drugs. Further, a patient who has been through this testing before and performed badly, might lack the motivation for repeating it. In summary, these are arguments for a selection bias towards that the patients who underwent neuropsychological testing, were those performing best on these tests.

Another important aspect regarding study 2, is the drop-out throughout the observational period of 52 weeks. This kind of drop-out is an object for attritional bias (207). Importantly, analyses indicate that the drop-out data was missing at random after evaluating total PANSS scores in several models (192). Analyses thus support the notion that the sample at endpoint is representative of the population at baseline (192). Therefore, attritional bias is less likely.

4.1.4 General considerations about selection bias

In all types of studies, the potential of selection bias needs to be addressed as it is likely that non-included subjects differ from the included ones (208). In a study investigating clinical outcomes among hospitalized psychiatric patients, Thomas *et al*. found that participants differed from non-participants regarding diagnoses, rates of readmission and education (209). This difference was found both between participants and patients who declined, and between participants and patients that were never asked to be included (209). Importantly, this was a

study with broad inclusion criteria, suggesting that most hospitalized patients should have been included.

Trying to explain why some patients are never asked to participate in studies, we can look for reasons within staff members, researchers, and patients. As previously discussed, symptoms of the mental disorders itself can influence the patients' consent competence. In addition, patients with high anxiety are more likely to perceive actions of health care staff as threating, resulting in aggression (210). The nature of the psychotic disorders with potential ambivalence, paranoid ideas and poor insight into illness might have influenced participation. This increased hostility among anxious patients, could also influence their willingness to participate in clinical studies, as well as the staff's decision to ask them to participate.

Although psychiatrists tend to reject negative attributes about psychiatric patients such as dangerousness and individual responsibility for the disease (211), psychiatrist as a group still have some prejudices (212, 213). Accordingly, psychiatrists might want to keep some social distance to psychiatric patients, representing tendencies towards a "not in my backyard" phenomenon (212). The psychiatrists' attitudes of social distancing might be more pronounced towards patients with schizophrenia (213). Among psychiatric nurses, studies have identified negative attitudes towards psychiatric patients, especially for patients with borderline personality disorders (214, 215). Theoretically, these attitudes could influence whether health care professionals ask a patient to participate in a study, causing a selection bias.

4.1.5 Study design

4.1.5.1 Secondary data and aims

Neither of the two studies were designed for the particular study aims of this thesis. This especially applies for study 2, where the primary purpose was to investigate the effects of

three different antipsychotics. Study 1 was planned with the aim to assess different clinical presentations of agitation at admittance and to explore the consequences of these. The aims of paper 1 and 2 were both related to symptoms of agitation and are therefore more in line with the general aims of the study. Even so, all the papers were written on secondary outcomes, which is an important limitation increasing the chance of both type 1 and 2 errors. This also means that none of the aims in the papers were subject to power calculations before the data collection. Still, cytokines lack a well-defined reference range, making power estimations more uncertain.

Despite the limitations using secondary outcomes, there are also some advantages. First, it is cost effective (216). Secondly, it gives researchers the opportunity to access data sets of high quality, for instance with a large number of included subjects and long follow-up period (216). Thirdly, findings from secondary outcomes can be hypothesis-generating, proposing hypotheses that subsequent research can explore. Finally, it might be ethically right to use data already collected, especially when considering the participants that invested time or even took a risk to contribute to medical research. One can imagine that these participants would appreciate that their contributions came to greater use than just answering one narrow research question. On the contrary, the secondary aim can be incompatible with the informed consent, raising other ethical issues (217). We took this issue into account when constructing the aims for the thesis, and all our aims were covered by the consent given by the participants and approvals from the ethics committees.

As already mentioned, there are several disadvantages of using secondary outcomes. However, it is possible to account for many of these concerns. For instance, it is important that researchers evaluate if the data set is appropriate for the outcome, choose the right statistical methods and are transparent about analytic methods in the paper (216, 218). We followed these advices in our papers.

4.1.5.2 Healthy controls

None of the studies in this thesis used a healthy control group for comparisons. Although this is not strictly necessary for any of the study aims in the thesis, healthy controls could extend the interpretation of our findings. Cytokines are markers with no certain reference intervals, making healthy controls useful to interpret if the values are high or low. In addition, healthy controls can increase the comparability with other studies in field.

Leaving healthy controls out of our analyses might also have helped us to avoid some biases. The difference between a population of psychiatric patients and healthy controls could extend over several areas (i.e. lifestyle, somatic diseases, socioeconomic class, general mental stress), meaning that it is difficult to know the exact cause of a potential difference between the two. Further, we would expect a major difference between psychopathology in patients and healthy, increasing the risk of overestimating the strength of a finding. In other words, if agitated patients had higher levels of cytokines compared to healthy controls, we do not know if this is due to agitation or other aspects of being a psychiatric patient. Finally, the inclusion of healthy controls can be troublesome as we do not know for sure if the controls are mentally healthy. In a study screening healthy volunteers for mental disorders, Bunce *et al*. found that 44% of the volunteers had a personality disorder (219). As most healthy controls are screened for only DSM Axis I disorders, the prevalence of unknown mental disorders can bias findings in research (220).

4.1.5.3 Comparison of study design in study 1 and 2

A specific limitation considering study 1, is the cross-sectional design. Therefore, interpretations about causality in paper 1 and 2 are impossible. Further, data in study 1 is collected from one single center, making the sample more vulnerable to biases due to different clinical practices between hospitals (221, 222).

On the other hand, study 2 is a longitudinal, multicenter study, which is a more robust study design regarding geographical variations and the ability to detect associations over time. Still, the longitudinal study design is susceptible for attritional bias due to drop-out. A multicenter study could also be more challenging during data collection. When more people are involved in the data collection, it increases the risk of errors. Especially, there is a risk for differences in practices around data collection between various study sites. Importantly, we corrected for study sites in our main analyses without it having any effect on the finding's strength.

4.1.6 Diagnostic procedures

In study 1, diagnoses were set at discharge in a consensus meeting after clinical evaluation by a psychiatrist or senior psychologist without using systematic structured diagnostic interviews. Although following diagnostic guidelines, this procedure increases the possibility for the influence of the clinician's personal interpretations and opinions on diagnostics. A large scale example of variations in diagnostic practices, is illustrated by the different prevalence of adolescent bipolar disorder between USA and European countries (223). The different prevalence can partly be explained by variations between the ICD-10 and DSM-IV, albeit cultural differences in diagnostic practices could also contribute (223). In clinical practice, it is demonstrated substantial discrepancies in the diagnoses yielded from routine clinical interviews and the ones from structured diagnostic interviews (224). Several have argued that the diagnoses made during routine examination are the least accurate, favoring the use of structured clinical interviews (224, 225).

On the contrary, an important pitfall with structured clinical interviews is the risk of false negatives. A structured interview will only be able to detect a selection of mental disorders, which could be detected by a routine evaluation by an experienced clinician. In addition, structured clinical interviews might be less valid detecting mental disorders in patients with substance abuse (226). There is also a risk of false positives with interviews, as patients might

answer to questions in accordance with a disorder they have read about. While opinions about clinical practice vary, the objectivity of structured interviews might be particularly useful in research.

In study 2, patients already clinically considered to have a psychotic disorder underwent SCID-I interviews and the results were converted to ICD-10 diagnoses. SCID-I is considered the gold standard of semi-structured interviews (227), albeit there are weaknesses with structured interviews as mentioned above. In addition, we did not consider inter-rater reliability and used only one instrument. In other studies, inter-rater reliability of SCID-I is often considered as good (227, 228).

4.1.7 Assessment of symptoms

When assessing symptoms, there are several potential sources of bias. In general, the bias could be located in the assessor (rater bias), the assessed (response bias) or in the instrument itself. A study by Söderberg and colleagues illustrates an example of rater bias, finding that the Global Assessment of Functioning (GAF) varied in reliability depending on the raters attitude and motivation towards the scale (229). When considering a rating scale, important phenomena are how much random error the scale gives (reliability) and if the score measure the characteristic exactly (validity) (230, 231). For instance, the General Health Questionnaire, a measure of anxiety and depression, yields an excessive number of false positives when compared to more thorough assessments (232).

4.1.7.1 Assessment of agitation and deviations in motor activity in study 1

The PANSS-EC is frequently used as a measurement of agitation, both in clinical practice and in pharmacological treatment studies (31, 233). It is a clinician or staff member scoring the patients on each item based on their behavior the last 24 hours. This means that the scale is prone to rater bias, whereas response bias is less of a concern. It is validated against two other agitation scales in an observational, prospective study on patients with acute psychosis (193).

Although the scale has strong correlation with other agitation scales, this is only tested in patients with psychosis and not in the general acute psychiatric population. The lack of validation within other patient groups than those with psychosis, must be considered a weakness. For instance, we might evaluate agitated psychosis patients different than a patient with agitated depression. Further, PANSS-EC has no items assessing the patients' feeling of inner tension, which is a phenomenon emphasized in most attempts of defining agitation (32).

In paper 2, we used two items from SOMAS to assess the levels of increased motor activity and psychomotor retardation. Importantly, this scale is not validated, which makes our results in paper 2 more uncertain. When a scale is not validated, the risk of false positive or false negative results is more uncertain. In addition, there is uncertainty linked to whether the scale measures the actual symptom of interest. A particular challenge in SOMAS is the clinician's interpretation of the questions. In SOMAS there is a formulation indicating that the patients should be scored when they were at their most depressed. This was also the case when the clinician should evaluate psychomotor activity. It is possible that an association between SOMAS items and cytokines is related to depressed mood rather than altered motor activity. Further, patients might have altered motor activity without being in a depressed mood, which we could have missed with this formulation. Still, the two items motor retardation and increased motor activity are based on the validated and widely used PANSS (234). Further, the motor deviation items on SOMAS are in line with the objective findings in two actigraphy studies (45, 235). This indicate that SOMAS identifies clinically observable motor retardation and increased motor activity.

4.1.7.2 Neuropsychological tests of psychomotor speed in study 2

All of the included neuropsychological tests in paper 3 measure psychomotor speed to some extent, albeit they differentiate in whether they assess higher cognitive domains as well. As mentioned in the methods section, TMT-A is the purest measure of psychomotor speed of the

three, whilst TMT-B and symbol coding demands more attention and working memory (57, 198, 199). In addition, TMT-A tests other cognitive abilities, such as visuospatial abilities and mental flexibility (57, 198, 236). Scores from TMT-A, TMT-B and symbol coding correlate strongly with overall intelligence (237), which can bias our interpretation of findings. Still, it is possible to avoid this bias by being aware of the common and distinctive features of each test.

Both TMT and symbol coding are well-known, frequently used in the clinic and easy to administrate. They have normative scores correcting for age and education (199, 236), and have good reliability (56, 238). For TMT, excellent interrater reliability has been demonstrated (57). Neuropsychological tests are vulnerable to practice effects, and this is also true for TMT-A, TMT-B and symbol coding (56, 239). Symbol coding is demonstrated to have minimal practice effects within schizophrenia patients (56). The TMT subsets have large practice effects after 6 weeks (239), which is less evident after 6 months and not significant after 1 year (240, 241). This means that practice effects are of most concern for the TMT subsets. Even so, studies conclude that the test-retest reliability is sufficient for longitudinal studies (240, 241).

4.1.8 Immune markers

4.1.8.1 Analytic considerations

Cytokines were analysed using multianalyte profiling in both studies. Several cytokines had a large degree of values below the detection limit, which is a challenge when handling the cytokine data in a statistical model. One way around this, is extending the standard curve and extrapolating values that are below the lowest detected values (242). Although this could increase the number of cytokines with a value in the statistical analyses, the extrapolated values are just an estimate and thereby uncertain. Another approach in order to account for the large spread in cytokine values and skewness in the data, is doing cluster analyses (243) or

composing an inflammatory composite score (244). In cluster analyses, patients are divided into subgroups depending on cytokine profiles, making it possible to compare characteristics between these subgroups (245). Although this could help to elaborate mechanisms between co-occurring cytokines, there is a risk of underestimating the contribution of each cytokine and this approach is to a large degree experimental. An inflammatory composite score means that all analysed cytokines are converted into a common z-score. Here, the risk of undermining potential biological mechanisms is even larger, as cytokines have various effector mechanisms, all are not necessarily elevated at the same time.

Multianalyte profiling, or multiplex assay, is a technique with the same principles as enzyme-linked immunosorbent assay (ELISA), using capture and labelled detection antibodies to measure cytokines. In multiplex assays, beads are precoated with a capture antibody specific for each cytokine (246). After washing to remove unbound proteins, detection antibodies (also specific for each cytokine) is added and results read, i.e. with fluorescent signaling (246). The technique is designed to detect cytokines, but like any laboratory method it is not perfect. For instance, there is a risk for false positives if the capture antibody detects a protein with a structure partly similar to the cytokine of interest (247). Likewise, false negative results can occur if the binding site is blocked, i.e. by an antibody or other protein (247). In addition, cytokines often bind to their soluble receptors, which might result in an underestimation of their concentration (248). An alternative, but time-consuming and therefore more costly approach is to analyse cytokines is growing cells dependent on each cytokine, where only biologically active cytokine of the wanted type would be detected. It is important to keep in mind, when using immunoassays, the risks of false positives and negatives as well as the inability to differentiate between biologically active and inactive cytokines.

4.1.9 Statistical considerations

4.1.9.1 Distribution of data

In both study 1 and 2, the cytokines were heavily right skewed, which limited the selection of statistical tests. Although this generally means that non-parametric tests are preferred, some parametric tests are most robust against violations of normal distribution (249, 250). Pearson's correlation tests the strengths of a linear association, and thereby assumes a linear association of the data and that both variables are continuous (251).At least one of the two variables should be normally distributed for this test (252). In study 2 (paper 3), cytokines were skewed whereas all variables for psychomotor speed were close to normally distributed, meaning that Pearson's correlation could be used to assess the association between the two. In paper 1, both the scale PANSS-EC and the cytokines were right skewed, meaning that Pearson's correlation could not be performed. As a solution to this challenge, we chose to divide patients into agitated and non-agitated group and compare the cytokine levels between the two with the non-parametric Mann Whitney U test.

The rational for grouping the patients in paper 1, is that PANSS-EC has a commonly used cut-off. Converting a continuous scale into a dichotomous variable reduces the statistical power (253). An alternative solution would have been to use the Spearman's correlation, which we did in paper 2 regarding SOMAS and cytokines. In addition, we chose to group patients on the presence or absence of increased motor activity in paper 2, but this did not change the main finding. However, the power loss due to dichotomizing may be less prominent if the variables are highly skewed or if we expect the association to be non-linear (254). As already mentioned, both dependent and independent variables in study 1 were highly right skewed. It is also possible that the association between agitation and inflammations is a non-linear one, meaning that the inflammation is more strongly related to the presence or absence of the symptom and not the increasing level of it.

The student's independent samples t-test might be robust to violations of the normal distribution assumption under some conditions (250), although violating this assumption increases the risk of type I error (255). Therefore, we chose Mann-Whitney U test when comparing a continuous, non-normally distributed variable between two groups. For LME models, it is only the outcome (dependent) variable that should be normally distributed (256). As this was the case for all psychomotor speed variables in study 2, LME-models were used. Psychomotor speed tests were defined as dependent variables and cytokines as independent variables based on the hypothesis, and not to be suitable in a statistical test.

4.1.9.2 Multiple testing and confounders

We corrected for multiple testing in paper 1 and 2, but not in paper 3. This increased the risk of type I error in paper 3. However, the Bonferroni correction is highly conservative (218), increasing the risk of type II error in paper 1 and 2. Alternatively, we could have used a less conservative multiple comparison procedure such as Least Significant Differences test. However, with so many tests as in paper 3 (9 cytokines and 3 tests for psychomotor speed), it is evident that the results would not remain significant after correction with less conservative methods as well. Lacking correction for multiple testing is an important limitation of this paper.

Further, we did not correct for potential confounders in all papers. In study 1, few cytokines were significantly associated with potential confounders such as age, gender, smoking and BMI. We did therefore not continue with correction of these in paper 1, albeit this was done in paper 2 (paper 2 was written before paper 1). When correcting for possible confounders in paper 2, the p-value increased to above 0.05. In this linear regression model, neither age, gender nor BMI was significantly associated with any of the tested cytokines. This suggests that the loss of a significant p-value could be due to changes the statistical test and not the actual correction of possible confounders. In paper 3, we corrected for several potential

confounders (age, gender, BMI, ethnicity, smoking, study site, antipsychotic drug and PANSS positive subscale score), without it having any effects on the results.

4.2 DISCUSSION OF FINDINGS

In general, the findings are thoroughly discussed in the papers. This paragraph aims to give a summary of the discussion in the papers in addition to elaborate on certain aspects.

4.2.1 Agitation and cytokines (study 1)

Serum levels of TNF-α were significantly higher in agitated patients compared to non-agitated patients (defined by PANSS-EC). We found this association in a general acute psychiatric inpatient sample, and within the subgroup non-affective psychosis, and after excluding non-affective psychosis from the total sample. Schizophrenia is the most common mental disorder associated with agitation (28), and the association between TNF-α and agitation could therefore be mediated by psychosis. However, the finding was replicated in all three groups, suggesting TNF-α relating to agitation in itself. Finally, the finding remained significant after controlling for multiple testing only in the general acute psychiatric sample. Although the exact reason for the loss of statistical significance after controlling for multiple testing is uncertain, one possibility is power loss in groups with lower number of patients.

To our knowledge, no previous studies have investigated the association between agitation and inflammation in a general acute psychiatric population. A few studies explore the association within schizophrenia patients (145-147, 257), where most studies included CRP as the only immune marker. As mentioned in the introduction section, CRP is an acute-phase protein whose production is stimulated by several cytokines including TNF-α. Therefore, the study by Barzilay and colleagues finding that PANSS-EC scores were significantly higher in schizophrenia patients with elevated CRP levels (> 1 mg/dL) compared to the ones with normal CRP, is in line with our study (146). In addition, a recent French study demonstrated that agitated schizophrenia patients had significantly higher levels of CRP than non-agitated

patients (257) (only abstract available in English). The third study regarding CRP and agitation, found that hs-CRP levels were significantly higher in agitated patients with schizophrenia compared to healthy controls (147). In this study by Pan *et al.*, it is more uncertain if the finding of higher hs-CRP in agitated patients is related to agitation, schizophrenia or other aspects of being a psychiatric patient.

One previous study, published by Li et al., have investigated agitation measured by PANSS-EC and cytokines in schizophrenia patients, finding a correlation between IL-17, IL-23 and TGF-β and PANSS-EC score (145). This study is not in line with our findings, as they found an association between agitation and Th17 cytokines. However, Li et al. did not measure TNF-α or other Th1 cytokines, and it is therefore unknown if their findings would have been more in line with ours if this was done. As will be discussed below, we found a non-significant trend towards an association between TGF-β and increased motor activity (paper 2). Increased motor activity is among the signs of agitation, which makes it possible that the association between agitation and Th17 cytokines is mediated by increased motor activity. Importantly, this is only hypothetical, as neither ours nor Li and colleagues' studies provide an answer to this exact research question.

4.2.1.1 The term agitation – is it more specific than a mental disorder?

The rationale for studying cytokines in relation to psychiatric symptoms or signs, was that they might be more specific and closer to basal biology (i.e. brain circuits) than mental disorders. Still, the specificity of certain symptoms and signs might also be discussed. As mentioned in the introduction, there are no consensus on the definition of agitation (31). It is proposed that agitation might be part of a continuum, ranging from anxiety to agitation to aggression (30, 31). Most commonly, agitation is defined as a syndrome constituting of several symptoms and signs. Therefore, it is difficult to know if a potential finding is related to agitation solely or related signs, i.e. hostility or increased motor activity. Likewise, an

association between agitation and cytokines, could also be mediated by the other symptoms in the continuum (anxiety or aggression). As we were unable to control for these symptoms in our study, this aspect is still unknown.

As already discussed, another important remark regarding agitation, is the assessment. The vast majority of the scales are observation-based (31). Although this is practical when assessing uncooperative patients, it diminishes the opportunity to rate the patients feeling of inner tension, which is a core feature of agitation (22). In daily clinical practice, the patients' feeling of inner tension can be difficult to catch and might not be something that patients report spontaneously. Therefore, the patient is more often classified as agitated based on the clinician's discretion, rather than a symptom scale. An experienced clinician's intuition can be correct regarding both mental and somatic states of the patients (258, 259). Still, a phenomenon that is largely based on discretion in clinical practice (i.e. agitation), is of course difficult to translate into accurate research.

4.2.2 Increased motor activity and cytokines (study 1)

Overall, we did not find a significant association between increased motor activity and cytokines as none of the findings remained significant after correction for confounders, or multiple testing. In an unadjusted model, we found a trend towards an association between increased motor activity assessed by SOMAS and lower serum levels of IFN-γ and higher levels of TGF-β in patients admitted to an acute psychiatric ward. This trend was reproduced when dividing the patients based on the presence or absence of increased motor activity. Although this finding is merely a trend due to the loss of significance after corrections, it is still worth a brief discussion. Considering immunological mechanisms, it makes sense that IFN-γ is low when TGF-β is high, as they represent arms of the immune system that might be opposed to each other (Th1 and Treg). Although this is a simplification, it is the most appropriate interpretation using today's knowledge about the immune system.

To our knowledge, no previous studies have investigated the association between increased gross motor activity and immune markers in a psychiatric population. A potential parallel is one study who investigated the effect of a maximal-workload exercise on cytokine levels in depressed patients and controls, finding that IL-8, IL-6 and TNF-α increased in both groups (260). In the same study, they found that IFN-γ decreased in the healthy control group (260). This finding could be in line with the trend of lower IFN- γ and increased motor activity in our study, suggesting that it is the psychical activity part of the sign that causes cytokine changes. Still keeping in mind, that we cannot say anything about causality.

4.2.2.1 Increased motor activity and agitation

Another way to interpret our findings regarding increased motor activity and cytokines, is in the context of the syndrome agitation. Increased motor activity is among the signs associated with agitation, and we therefore expected agitation and increased motor activity to be related to the same immune responses. We found agitation to be associated with higher levels of TNF-α, which in most cases represent a pro-inflammatory Th1 response (84). This contradicts our finding of a possible Treg response in motor active patients, as these two responses are often interpreted as opposites. The finding is also contradictory to the other previously mentioned studies, finding a relation between higher levels of CRP and acute agitation (146, 257). Still, the categorization of the immune system based on cytokines and T-helper cells is a simplification and based on the limited knowledge we have so far. This means that although TNF-α and TGF-β might be opposed to each other, they could work together under certain conditions (261). The same coherence could be stated about IFN-γ and TNF-α, which are Th1 cytokines and expected to be increased at the same time but might be opposed to each other under certain conditions.

Considering the scores PANSS-EC and SOMAS, it is theoretically possible to categorize a patient as agitated although having no increased motor activity. However, this also means that

a patient must have a high score on items such as hostility, uncooperativeness and poor impulse control. Albeit it is clinically possible for a patient to be uncooperative and hostile without having increased motor activity, this is less likely when considering the wording of the PANSS-EC items. For instance, high scores on the items uncooperativeness and hostility involve extensive physical violence. It is also a possibility that the raters misinterpreted the scales (rater bias), yielding false results. An alternative approach could have been to retrieve data from the charts. The chart data also has its limitations, the most important one being that it is not systematic research data and that the clinician often only write what is most relevant at that exact point.

In summary, the findings regarding motor activity and agitation could be interpreted as contradictory considering the cytokines. The functions of cytokines are complex, and our knowledge about their function both in the immune system and in the CNS is limited. Thus, our findings should be further explored.

4.2.3 Psychomotor retardation and cytokines (study 1)

In study 1, we found no association between the degree of psychomotor retardation and serum levels of cytokines. This lack of association was found both when psychomotor retardation was included as a continuous variable and when subdividing patients based on the presence or absence of the sign.

Based on previous studies on the subject finding an association between psychomotor retardation and cytokines among patients with depression and schizophrenia (161, 162, 262), we expected to reproduce these findings in our acute psychiatric population. This hypothesis has gained additional support from a study demonstrating that patients with cytokine-induced depression have more psychomotor retardation compared to somatically healthy depressed patients (133). In addition, psychomotor retardation is demonstrated to be a predictor for whether patients with hepatitis C treated with IFN-α actually do develop depression (47).

Still, none of these studies investigated the association within a general acute psychiatric population. Several of the studies also assessed psychomotor retardation with neuropsychological tests measuring psychomotor speed, and not by a clinical scale (161, 162). There might be other factors that drive the inflammation in acute psychiatry (i.e. infections, traumas before admittance, increased substance use). In addition, the association between psychomotor retardation and inflammation might only be prominent within certain mental disorders. Finally, we might have underestimated the effect of psychomotor speed on cytokines in study 1 due to uncertain measurements. In order to control for some of these weaknesses, we investigated the association between psychomotor speed (measured by neuropsychological tests) and cytokines in the sample with a spectrum of psychotic disorders in paper 3.

4.2.4 Psychomotor processing speed and cytokines (study 2)

In study 2, we found a significant association between levels of IFN-γ and worse psychomotor speed performance (assessed by TMT-A and symbol coding) in patients with a spectrum of psychotic disorders over time. This finding remained significant after correcting for several possible confounders. No other cytokines were significantly associated with psychomotor speed in the group with a spectrum of psychotic disorders. Of the included tests for psychomotor speed, only TMT-A and symbol coding were significantly associated with a cytokine. TMT-A is the purest measure of psychomotor speed of the three tests, whereas symbol coding measures psychomotor speed and attention. In other words, our finding might indicate that IFN-γ is related to psychomotor speed and attention.

To our knowledge, no studies investigating the association between cytokines and psychomotor speed within mental disorders have included the cytokine IFN-γ. However, previous cross-sectional studies have found an association between psychomotor speed and other cytokines, e.g. IL-6, TNF-α and IL-10, in patients with psychotic disorders (160, 161).

Bulzacka et al. also found that patients with schizophrenia grouped by higher levels of hs-CRP (> 3 mg/ml) performed significantly worse on TMT-A and TMT-B compared to those with normal hs-CRP levels (263). As both CRP, TNF-α, IFN-γ and to some extent IL-6 represent a Th1 response (84, 264, 265), these previous studies are in line with our finding. On the contrary, most of the tested cytokines were not associated with psychomotor speed in our study, whereas Goldsmith and colleagues found several cytokines to be associated with various neuropsychological tests for psychomotor speed (161).

Other studies have also found no association between cytokines and psychomotor speed within groups of patients with schizophrenia, bipolar disorder and major depressive disorder (151, 165, 266). Importantly, all these negative studies included only a few cytokines, none analysed IFN-γ, and merely one study was longitudinal. Notably, the study by Hori et al. found no significant correlation between any of the tested cognitive domains (including psychomotor speed) and the cytokines IL-6 and TNF-α in a cross-section study including 146 patients with chronic schizophrenia (165). In neither paper 2 nor paper 3 we found an association between psychomotor speed and these two cytokines, meaning that the study from Hori and colleagues supports our findings.

After excluding patients with delusional disorder and acute transient psychosis, IFN-γ was still associated with TMT-A, but not symbol coding. The loss of a significant association between IFN-γ and symbol coding might be explained by loss of statistical power. In addition, we found an association between IL-4 and the two tests TMT-A and TMT-B. Although false positive findings are more probable when performing additional statistical tests, the spectrum of schizophrenia patients might represent a different entity. Regarding Th-responses, IL-4 is classified as a Th2-cytokine, which might be oppose to the Th1-cytokine IFN-γ (89). In our study, the association between IL-4 and IFN-γ is inverse, which makes sense considering the

underlying known biological function of these cytokines. More specifically, IFN-γ had a negative effect on psychomotor speed, whereas IL-4 had a positive effect.

The cytokine group interferons might be particularly important for the development of psychomotor speed. The basis for this hypothesis, is that when given IFN-α as treatment for chronic hepatitis C, patients develop decreased psychomotor speed and depressive symptoms (131). The decreased psychomotor speed is also more pronounced in depression induced by interferons compared to somatically healthy patients with major depression (267). Further, IFN-γ was associated with reduced white matter integrity in a study of patients with bipolar disorder (159), a neurobiological finding that might be related to psychomotor speed (268). Finally, IFN-γ has effects on myelin that are important in the pathology of MS (269), a disease where reduced psychomotor speed is the most common cognitive impairment (156, 270).

4.2.4.1 Psychomotor speed in study 1 and 2

Although assessed with different methods (rating scales and neuropsychological tests), psychomotor speed was investigated in relation to cytokines in both study 1 and 2. In study 1, no association was found, whereas IFN-γ was related to psychomotor speed in study 2. There are several explanations for this discrepancy between the two studies. First, the difference in assessment of psychomotor speed is important. Scoring a patient as clinically psychomotor retarded on SOMAS and preforming worse on neuropsychological tests might yield different results. For instance, a neuropsychological test can capture deviations that are less obvious and not detectable by a clinical rating scale. Also, a high score on SOMAS indicate that the psychomotor retardation is so profound that it would be difficult to even complete a neuropsychological test. Secondly, the two studies have different designs with study 1 being cross-sectional and study 2 longitudinal. It is possible that association between IFN-γ and psychomotor speed is due to a covariation over time. Supporting this theory, is the fact the

cross-sectional correlation analyses also yielded no significant associations between psychomotor speed and cytokines in study 2. Finally, the finding regarding IFN-γ and TMT-A might simply be a result of multiple testing, yielding an incidental significant result (type I error).

4.2.5 Changes in serum cytokine levels over time (study 2)

In study 2, cytokines remained stable throughout the entire observational period of 52 weeks. Several studies have shown that cytokines change from acute states of psychosis to more chronic phases (162, 188, 271), whereas other cytokines remain unchanged (181, 272). This could indicate that certain cytokines mark a state, whilst others are trait markers. Still, the patients in paper 3 were in both acute and more chronic phases of psychotic disorders. Therefore, the mixture of chronic and acute states in this sample might contribute into masking changes of cytokine levels over time. Furthermore, type of antipsychotic medication and treatment response, are factors associated with altered cytokine levels over time in patients with psychotic disorders (181, 189, 271). We did not distinguish between these factors when analysing the cytokines over time alone (without including the effect of psychomotor speed). Although including these variables would help to elaborate the finding regarding cytokine levels over time in more detail, these analyses were not covered by the aim of paper 3 and were therefore not included.

4.2.6 Cytokines in relation to potential confounders

We investigated the effect of potential confounders on cytokines as exploratory analyses in study 1. Here, IFN-γ was significantly correlated with age, and levels of IL-6 and IL-10 were significantly higher in male patients. No cytokines were significantly associated with BMI or smoking status. In summary, few cytokines were associated with possible confounders in this study.

It may be surprising that BMI was not associated to any cytokine, as it is highly associated to CRP (141, 273). This knowledge is also supported by findings from animal studies and epidemiological studies, indicating an association between BMI and low-grade inflammation (274). A meta-analysis on immune markers in patients with schizophrenia also concluded that CRP levels were associated with BMI independently of antipsychotic treatment (275). One explanation for this absence of an association, could be that the patients in our sample are severely ill where other factors have a stronger effect on cytokines than BMI. It also possible that cytokines have a weaker association to BMI than CRP.

The findings regarding age in study 2 are in line with several other studies demonstrating that immune markers are associated with higher age (276). Because of previous studies on the subject, we expected to find the same association within the acute psychiatric population of study 1. However, other studies within acute psychiatry have also found no association between immune markers and age (147, 277). In addition, Dunjic-Kostic *et al*. found the same lack of an association between smoking status and cytokines in their acute psychiatric population as we did in our study (277).

Regarding gender differences the results are more conflicting (278, 279). Although most studies conclude with different levels of immune markers between the genders, the direction of the difference is more uncertain. It might be that the distinction in immune responses between genders are more complex than high versus low immune markers (280). Therefore, it is uncertain if most previous studies are in line with study 1, finding higher levels within male patients.

Several medications (i.e. clozapine and other neuroleptics) are known to affect the immune system (281). In study 1, we did not adjust for use of medications, which is a weakness. In study 2, medication was recorded, and we therefore adjusted for antipsychotic drug. Although

this is a strength, we did not adjust for other psychotropic drugs or immune modulating medications.

In conclusion, few of the previously known confounders were significantly associated with cytokines in study 1 including acute psychiatric patients. A hypothetical explanation for this finding might be that other factors are important for inflammation in this population, masking the potential effect of confounders.

4.3 IMPLICATIONS AND FUTURE DIRECTIONS

Overall, we did not identify subtypes of psychiatric patients based on symptoms or signs that were strongly associated with changes in cytokine levels. Although certain cytokines might be related to agitation, increased motor activity and decreased psychomotor processing speed, the results are uncertain.

As already mentioned, few studies have investigated the relation between agitation or increased gross motor activity and cytokines in patients with mental disorders. Our study might indicate a relation between these signs and cytokines, but more studies are needed to confirm the results. Considering the lacking consensus on a definition of agitation, investigating its related, more well-defined sub-symptoms could be helpful.

Furthermore, we do not know from this study if there are neurobiological mechanisms making it possible for peripheral cytokines to change behavior. For instance, we can hypothesize that the effect of IFN-γ upon psychomotor speed is due to changes in white matter integrity or basal ganglia.

Another challenge is that psychiatric symptoms are closely connected to each other. Although agitation is seen in several mental disorders, the sign is generally related to psychosis, substance use or underlying somatic causes. Consequently, we cannot know from our study if the finding regarding agitation and TNF-α is mediated by for instance substance use or

psychosis. Including symptoms that often co-occur and/or interfere with each other, would therefore bring progress to the field.

Among the arguments for the overall aim of this thesis, we argued that certain symptoms and signs would be more specific than a mental disorder and thereby helpful to elaborate the field. Seen in retrospect, all of the investigated symptoms and signs in this thesis are closely related to other traits. Certain sings also lack a consensus on definition and one of the scales (SOMAS) is not validated. Therefore, it is possible that some of the included signs are more precise compared to mental disorders. In order to increase specificity, future studies could only include instruments that are validated and frequently used.

Finally, finding a biological correlate to specific symptoms withholds largely the same challenges as the same approach with mental disorders. Agitation might be caused by several underlying somatic diseases, i.e. urinary infection, head trauma, stroke and electrolyte abnormalities. Considering the many possible causes for agitation in somatic medicine, this might also be the case for psychiatric patients. Maybe the field of immunopsychiatry needs to explore the association between immune markers and symptoms the other way round, investigating if there are any common features for patients with high levels of cytokines.

4.4 CONCLUSIONS

Despite uncertainty in results due to limitations of the studies, some conclusions can be drawn from this thesis. The finding of higher serum TNF-α levels in patients with acute agitation compared to those without agitation, indicates a relation between a Th1 response and agitation in acute psychiatry. Regarding gross motor activity and cytokines, we only found a non-significant trend towards an association between increased motor activity and lower levels of IFN-γ and higher levels of TGF-β. Although merely a trend, the finding regarding IFN-γ and motor activity might be contrary to the association between agitation and TNF-α due to the close relation between these signs. However, the mechanisms of the immune system are more

intricate than a strict subdivision of T-helper cell responses. Finally, IFN-γ had a negative effect on psychomotor speed in a sample with active psychosis, indicating a covariation between Th1 response and psychomotor speed. All of these associations had small effect sizes, which limits the conclusions of the thesis.

Overall, we did not identify symptoms or signs that are strongly associated with peripheral cytokines, or that cytokines had a strong effect on certain symptoms over time. Future studies may help to understand the field better.

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APPENDIX

Paper I

PLOS ONE

RESEARCH ARTICLE

Cytokines in agitated and non-agitated patients admitted to an acute psychiatric department: A cross-sectional study

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Abstract

Background

Different psychiatric diagnostic groups have been reported to have cytokine levels deviating from healthy controls. In acute clinical settings however, the specific challenging symptoms and signs are more important than a diagnostic group. Thus, exploration of cytokines and immune activity and their role in specific symptoms is important. Reports in this field so far are sparse.

Objective

In the present study, we aimed to examine the association between immune activity measured as levels of cytokines and agitation (independent of diagnostic group) in patients admitted to an acute psychiatric inpatient department.

Methods

A total of 316 patients admitted to an acute psychiatric inpatient department were included. Thirty-nine patients with psychosis were subject to subgroup analyses. Agitation was assessed by the Positive and Negative Syndrome Scale, Excitement Component (PANSS-EC). Based on PANNS-EC patients were stratified into two groups: 67 agitated patients and 249 non-agitated patients. Serum concentrations of the following immune markers were measured: interleukin (IL) -1β, IL-4, IL-6, IL-10, tumor necrosis factor (TNF) -α, interferon (IFN) -γ and transforming growth factor (TGF) -β.

Results

Serum levels of TNF-α were significantly higher in patients with agitation compared to those without, both when all patients were included in the analyses ($p = 0.004$) and in the

Competing interests: The authors have declared that no competing interests exist.

psychosis group (p = 0.027). After correcting for multiple testing, only the findings in the total population remained significant.

Conclusions

Our findings suggest an association between TNF-α and agitation in an acute psychiatric population. A similar trend was reproduced to the psychosis subgroup. This suggests that agitation might be an independent entity associated with cytokines across different diagnostic groups.

Introduction

Different psychiatric diagnostic groups have been reported to have immune cytokine levels deviating from those seen in healthy controls [1, 2]. In clinical settings, the specific challenging symptoms and signs are more important than diagnostic groups. Thus, exploration of cytokines and immune activity and its role in specific symptoms and signs is important. In experiments, immune factors such as cytokines might influence the brain and mediate behavioral changes seen in psychiatric disorders [3, 4]. Clinical reports in this field so far are however sparse. Level of cytokines has been associated with severity of depressive symptoms in major depression and with negative symptoms in schizophrenia [5, 6].

Agitation is a challenging clinical sign. The term agitation may be defined as a syndrome of behaviors such as increased psychomotor activity, irritability, hostility, threatening gestures and lack of cooperativeness [7]. Acute agitation is particularly frequent in acute psychiatric services, and is associated with several psychiatric disorders, most commonly schizophrenia, drug intoxication and bipolar disorder [8, 9]. Agitation is causing suffering and may result in use of coercive means (involuntary medication, restraint, and seclusion) [10]. Untreated agitation may escalate to violence and adverse outcomes for patient, family, society and staff [10]. Thus, knowledge on mechanisms behind agitation is essential.

A few recent studies have shown a possible association between acute agitation in schizophrenia and the inflammatory marker C-reactive protein (CRP) [11, 12]. To our knowledge, only one previous study has investigated the association between cytokines and agitation, finding IL-17 and IL-23 to be associated with agitation [13]. However, this study was limited by a small study population and that it only included three different cytokines. It is also not known if the association between immunological markers (CRP or cytokines) and agitation is present in other diagnostic group than psychosis or schizophrenia.

There are also some studies on the association between aggression and cytokines. A study on inpatients diagnosed with schizophrenia, demonstrated a possible association between interferon (IFN) -γ and interleukin (IL) -10 and aggression [14]. However, the results are conflicting and another study found aggression to be associated with IL-17, IL-3, and transforming growth factor (TGF) -β [13]. Although aggression and agitation have several symptoms and traits in common, they are not completely the same. Therefore, we would state the association between cytokines and agitation is largely unknown.

The aim of the present study was to assess the levels of cytokines related to agitation in an acute inpatient setting. Levels of cytokines were compared between patients with or without symptoms of agitation measured with the Positive and Negative Syndrome Scale, Excitement Component (PANSS-EC). Agitation is known to be more frequent in certain patients groups, such as those with psychosis. Also, most previous studies have selected patients with psychosis

when investigating the association between immune markers and agitation. As these reports are conflicting, separate analyses were done on the group non-affective psychoses. We hypothesized that the agitated patients would have higher levels of pro-inflammatory cytokines when compared to patients without agitation.

Materials and methods

Setting and participants

This was a cross-sectional study conducted in the acute psychiatric department of St. Olav's University Hospital, Trondheim, Norway. All eligible inpatients acutely admitted between September 2011 and March 2012 were asked to participate. At the time of inclusion, the psychiatric department served a catchment area of 228.000 inhabitants ($\geq$ 18 years). Of the total 654 admitted patients in the inclusion period, 382 (58.4%) patients gave written informed consent prior to inclusion. The study was approved by the regional committee for ethics (REC South East number 2011/137) and registered at ClinicalTrials.gov (NCT01415323). The study was conducted according to the Declaration of Helsinki.

Inclusion and exclusion criteria

All patients evaluated to be able to give informed consent were asked to participate, independently of diagnostic group. Ability to give informed consent was evaluated by an experienced psychiatrist / clinical psychologist the first day after admission and in accordance with national and international regulations. Exclusion criteria were as follows: (1) chronic or ongoing infections, (2) comorbid autoimmune diseases, (3) CRP levels above 35 mg/L (level based on clinical experience), or (4) lack of patient consent. When patients had multiple admissions, we only included the first admission in our analyses.

Diagnostic evaluation

Patients were diagnosed according to the International Classification of Diseases-10 (ICD-10) Criteria for Research [15]. The diagnoses were set in a consensus meeting always including at least two senior psychiatrists or clinical psychologist of whom at least one had personally examined the patient. All clinical data from patient files were available. For subgroup analyses, patients with non-affective psychosis (ICD-10 F20–29, psychosis group) and a group of all patients when excluding the psychosis group were studied.

Assessments

Sociodemographic history, smoking status and the history of somatic diseases were recorded after an interview by a member of staff. Height and weight were measured in order to calculate body mass index (BMI, kg/m^2). The patients were interviewed regarding substance use in addition to screening for drugs in the urine. In addition, participants were screened in a general medical examination and with routine blood tests, including CRP and leukocytes.

Agitation was assessed by experienced psychiatrists or clinical psychologist using the Positive and Negative Syndrome Scale, Excited Component (PANSS-EC). The PANSS-EC is a validated and commonly used scale assessing agitation in acute- and emergency psychiatry [16]. It is calculated as the sum of the following PANSS items: Excitement (P4), Hostility (P7), Tension (G4), Uncooperativeness (G8) and Poor impulse control (G14) giving a total scoring range 5 to 35. Clinically significant agitation is considered with PANSS-EC score $\geq$ 14 [17, 18]. For analyses, patients were divided into two groups: agitated patients (PANSS-EC $\geq$ 14) and non-agitated patients (PANSS-EC $<$ 14).

Immune markers

Blood samples were collected on 9 ml serum tubes with SiO2 without gel between 08:00 and 13:00 (median at 10:00) at the first working day after admission. Strict instructions regarding fasting were not given, though most patients would be fasting overnight. Samples were immediately cooled on ice, protected from daylight, and centrifuged within 30 minutes (15 min, 1500 g, 4˚C). Serum samples were stored at -80˚C in a registered Biobank (Biobank1, St. Olav's University Hospital, Trondheim, Norway) until further analysis. The cytokines were chosen based on previous studies in our group as well as multiple reports from other groups in the field [19]. Also, the availability of commercial assays and general experience in the immune lab affected the cytokines chosen for analyses. An attempt was made to represent all the main assumed pathways of the immune system. The following parameters were analysed by multianalyte profiling Milliplex MAP assays: IL-1β, IL-4, IL-6, IL-10, TNF-α, and IFN-γ (Millipore Corporation, Billerica, MA, US). TGF-β was measured by a Bio-Plex Pro TGF-β Assay (Biorad Hercules, CA, US). All analyses were performed according to the manufacturers' protocol. The range of detected values was as following: IL-1β: 0.06–198.72 pg/ml; IL-4: 0.92–286.01 pg/ml; IL-6: 0.10–576.42 pg/ml; IL-10 0.10-1125-33 pg/ml; TNF-α: 0.70–268.89 pg/ml; IFN-y: 0.04–1529.70 pg/ml; and TGF-β: 13.07–415.61 ng/ml. The number of samples and percentage under the detection limit was as follows: IL-1β 236 (74.2%), IL-4: 242 (76.1%), IL-6: 177 (55.7%), IL-10:177 (55.7%), TNF-α: 11 (3.5%), IFN-y: 68 (21.4%) and TGF-β: 0.

Statistical analyses

Statistical analyses were performed using SPSS version 24.0 for Windows. The level of significance was set at $p \leq 0.05$, and all analyses were two-tailed. Significant findings were adjusted for multiple testing with the Bonferroni correction (α/k where k = the seven tested cytokines giving $\alpha/k = 0.007$). Data normality was assessed by using a Kolmogorov-Smirnov test. Only TGF-β was normally distributed and all the other cytokine values were skewed. Descriptive statistics were calculated by chi-square tests for categorical variables and student's independent samples t-tests or Mann-Whitney U test (depending on distribution) for continuous variables. When comparing cytokine levels between two groups, such as agitated and non-agitated, we used a Mann-Whitney U test or a student's independent samples t-test, depending on the distribution. Additionally, we examined the association between cytokines and possible confounders that are well known for influencing the immune system such as age, BMI and gender. The Spearman correlation coefficient was calculated for the relationship between cytokines, BMI and age. Patients were also stratified according to gender and cytokine levels were compared between female and male patients using a Mann-Whitney U test or a student's independent samples t-test.

Results

Sociodemographic and clinical characteristics of the population

Of 382 patients initially included, 24 were excluded due to infection or autoimmune disease. For the remaining 358 patients, serum samples were available for cytokine analyses in 318 of these patients. Two of these patients were missing PANSS-EC scores and were therefore not included in the statistical analyses, leaving us with 316 patients in the analyses. The agitated group and the non-agitated group were similar in the demographic and clinical characteristics recorded in our study, including age, gender, smoking status and BMI (Table 1).

The percentage of main ICD-10 diagnostic categories in the 316 patients were as follows: F32 depressive episode or F33 recurrent depressive disorder (n = 68, 21.5%)), F10-F19 Mental

Table 1. Demographic and clinical parameters.

	All patients n = 316	Agitated patients n = 67	Non-agitated patients n = 249	p-value[a]
Age (years)	39.0 (26.2–49.0)	39.0 (27.0–56.0)	39.0 (26.0–49.0)	0.535[b]
Gender (female)	156 (49)	37 (55)	119 (48)	0.280[c]
Smoking	157 (50)[d]	38 (69)	119 (55)	0.056[c]
BMI (kg/m^2)	24.4 (21.7–28.2)[e]	24.5 (22.0–27.6)	24.4 (21.6-28-4)	0.959[b]
Higher education (above high school)	48 (15)	7 (10)	41 (16)	0.223[c]
Unemployment (incl. sick leave)	224 (71)	53 (79)	171 (69)	0.104[c]
Alcohol use upon admission	87 (28)	19 (29)	68 (27)	0.811[c]
Substance abuse	64 (20)	15 (23)	49 (20)	0.584[c]

Data are presented as median with interquartile range or n (%)

[a]Comparison between agitated and non-agitated patients. Agitation was classified by Positive and Negative Syndrome Scale, Excited Component (PANSS-EC) score ≥ 14.

[b]Mann-Whitney U test

[c]Chi-square test

[d]Missing 44

[e]Missing 68

Abbreviations: BMI: body mass index, PANSS-EC: Positive and Negative Syndrome Scale, Excited Component, SD: standard deviation

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and behavioral disorders due to psychoactive substance use (n = 53, 16.8), F20-F29 schizophrenia, schizotypal and delusional disorders (n = 39, 12.3%)), F31 bipolar affective disorder (n = 42, 13,2%), F40-F48 neurotic, stress-related, and somatoform disorders (n = 32, 10,1%), and F60-F69 disorders of adult personality and behavior (n = 29, 9.2%).

Comparisons of serum cytokine levels between agitated and non-agitated patients

When all 316 participants were included in the analyses, levels of TNF-α were significantly higher in agitated patients compared to non-agitated patients (21.87 ± 17.96 vs 14.94 ± 18.86, Mann-Whitney U = 6434.0, d = 0.16, p = 0.004) (Table 2 and Fig 1). This finding was

Table 2. Comparisons of cytokine levels based on the presence or absence of agitation [a]. All data are presented as median with interquartile range.

	All patients			Psychosis group			Patients without psychosis		
	Agitated (n = 67)	Non-agitated (n = 249)	p-value	Agitated (n = 13)	Non-agitated (n = 26)	p-value	Agitated (n = 54)	Non-agitated (n = 223)	p-value
IL-1β (pg/mL)	0.03 (0.03–0.03)	0.03 (0.03–0.12)	0.724[b]	0.03 (0.03–0.03	0.03 (0.03–1.00)	0.415[b]	0.03 (0.03–0.14)	0.03 (0.03–0.09)	0.981[b]
IL-6 (pg/mL)	0.05 (0.05–45.38)	0.05 (0.05–17.58)	0.220[b]	0.05 (0.05–133.20)	5.66 (0.05–20.71)	0.547[b]	0.05 (0.05–31.43)	0.05 (0.05–15.65)	0.319[b]
TNF-α (pg/mL)	21.87 (11.83–29.79)	14.94 (7.26–26.12)	**0.004**[b]	21.71 (16.89–43.89)	12.84 (7.36–25.50)	**0.027**[b]	22.80 (11.33–29.56)	15.31 (7.13–26.35)	**0.025**[b]
IFN-γ (pg/mL)	7.05 (0.02–20.59)	5.12 (0.59–16.91)	0.907[b]	20.90 (1.48–39.62)	3.49 (0.08–9.24)	0.055[b]	3.62 (0.02–17.81)	5.20 (0.67–19.49)	0.477[b]
IL-10 (pg/mL)	0.05 (0.05–37.03)	0.05 (0.05–22.00)	0.423[b]	0.05 (0.05–85.73)	3.49 (0.05–54.48)	0.885[b]	0.05 (0.05–34.20)	0.05 (0.05–18.27)	0.405[b]
IL-4 (pg/mL)	0.46 (0.46–20.35)	0.46 (0.46–0.46)	0.110[b]	0.46 (0.46–39.47)	0.46 (0.46–7.52)	0.478[b]	0.46 (0.46–12.33)	0.46 (0.46–0.46)	0.193[b]
TGF-β (ng/mL)	78.41 (63.46–95.72)	80.47 (62.13–97.42)	0.924[c]	69.58 (60.31–95.21)	80.21 (63.57–93.51)	0.789[c]	78.68 (63.72–96.10)	80.47 (61.21–98.13)	0.687[c]

[a]Agitation was classified by Positive and Negative Syndrome Scale, Excited Component (PANSS-EC) score ≥ 14.

[b]Mann-Whitney U test

[c]Independent students' samples t-test

Abbreviations: IL: interleukin, TNF: tumor necrosis factor, IFN: interferon, TGF: transforming growth factor.

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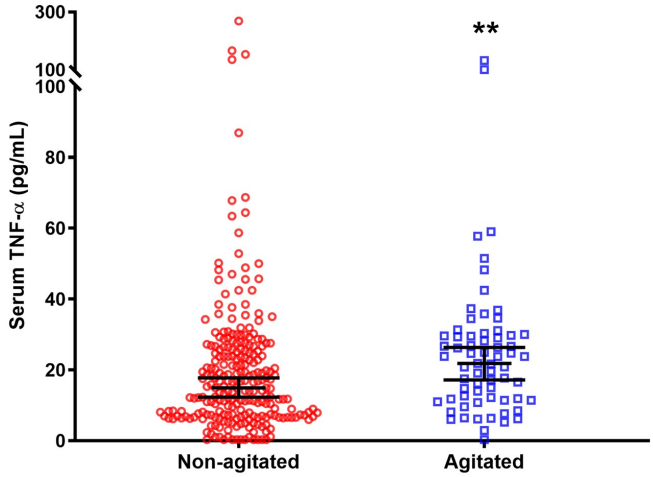

Fig 1. TNF-α levels in all acutely admitted patients. Serum TNF-α levels were significantly higher in agitated patients compared to non-agitated patients (21.87 ± 17.96 vs 14.94 ± 18.86 pg/mL). Data are expressed as median ± 95% confidence interval. **p = 0.004.

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replicated in the subgroup with psychosis, where agitated psychosis patients had significantly higher levels of TNF-α compared to non-agitated psychosis patients (21.71 ± 27.00 vs 12.84 ± 18.15, Mann-Whitney U = 95.0, d = 0.35, p = 0.027) (Fig 2). The finding of significantly higher levels of TNF-α in agitated patients when compared to non-agitated patients was also replicated in a subgroup analysis where the psychosis group was excluded (22.84 ± 18.23 vs 15.31 ± 19.22, Mann-Whitney U = 4841.0, d = 0.13, p = 0.025) (Fig 3). These subgroup findings were however not significant after the Bonferroni correction for multiple testing. In addition, there was a trend towards higher levels of IFN-γ in agitated psychosis patients when compared to non-agitated psychosis patients (20.90 ± 38.14 vs 3.49 ± 9.16, Mann-Whitney U = 105.0, p = 0.055), although not significant. No other differences in cytokine levels between the two groups reached the level of significance.

Associations between cytokines and possible confounding factors. Serum levels of TNF-α did not differ significantly between smokers and non-smokers (14.93 ± 20.85 vs 16.81 ± 20.85, Mann-Whitney U = 8890.0, p = 0.696). Similar findings were reproduced for all the other measured cytokines. Age was negatively correlated with IFN-γ (rho = -0.150, p = 0.007), while no other significant associations between age and cytokines were found. In addition, there were no significant correlations between BMI and any of the measured cytokines.

Stratification by gender showed that both female and male patients with agitation had higher levels of TNF-α than non-agitated patients of the respective gender (female: 20.84 ± 18.17 vs 14.08 ± 18.23, Mann-Whitney U = 1681.5, p = 0.030, male: 24.27 ± 19.06 vs 16.82 ± 18.95, Mann-Whitney U = 1492.0, p = 0.045). In addition, TNF-α levels did not differ significantly between females and males (15.13 ± 19.79 vs 17.75 ± 19.31, Mann-Whitney U = 11440.0, p = 0.143) (Fig 4). Levels of IL-6 (Mann-Whitney U = 10864.0, p = 0.017) and IL-10 (Mann-Whitney U = 10415.5, p = 0.003) were significantly higher in male subjects. No other gender differences in cytokine levels were detected.

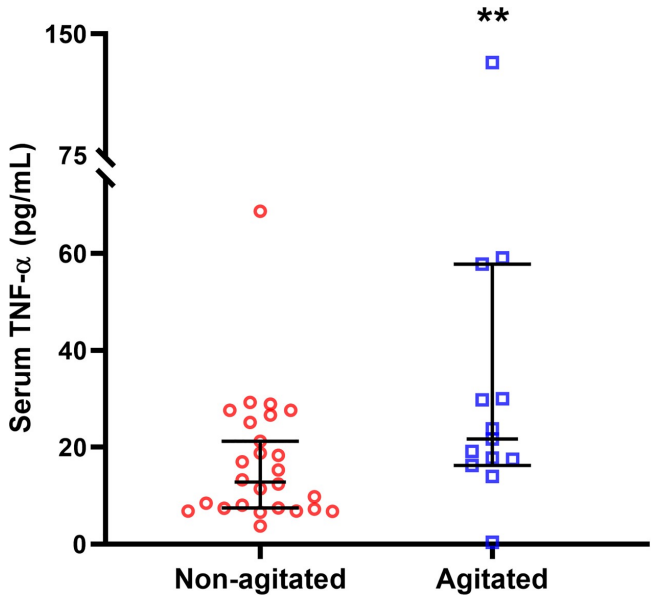

Fig 2. TNF-α levels in psychosis group. Agitated psychosis patients had significantly higher serum levels of TNF-α compared to non-agitated psychosis patients (21.71 ± 27.00 vs 12.84 ± 18.15 pg/ml). data are expressed as median ± 95% confidence interval. **p = 0.027.

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Discussion

Summary of results

The present study indicates that serum TNF-α is increased in acutely agitated psychiatric inpatients when agitation is measured by PANSS-EC. This finding was present in both the general acute psychiatric population, within the diagnostic subgroup of non-affective psychosis and when the subgroup of non-affective psychosis was excluded from the population. However, the finding only remained significant in the general population when correcting for multiple testing. Serum levels of TNF-α were not affected by gender, age, smoking status or BMI. In agitated patients with psychosis, there was a trend towards increased IFN-γ when compared to non-agitated patients with psychosis. Increased IFN-γ was related to younger age but not to other potential confounding factors.

Elevated TNF and agitation

Previous studies on patients with schizophrenia have shown an association between increased PANSS-EC and the inflammatory marker CRP [11, 12]. As CRP is produced by the liver when stimulated by cytokines such as TNF-α, these studies are in line with our finding of increased TNF-α levels in agitated patients with schizophrenia as well as in the general acute psychiatric population. Hostility is one of several symptoms seen in the syndrome of agitation. Hostility is also one of the items scored in the PANSS-EC. Therefore, the association of TNF-α and IFN-γ with hostility reported in healthy subjects are might also in line with our results [20].

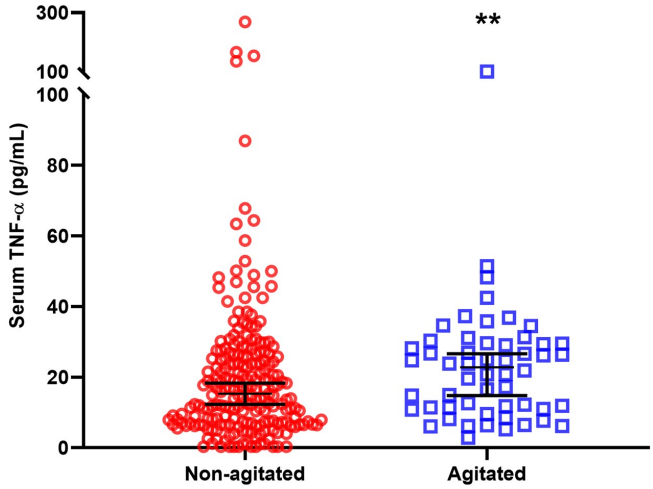

Fig 3. TNF-α levels in patients without psychosis. In a group of patients with other diagnosis than psychosis (psychosis group excluded), serum levels of TNF-α were significantly elevated in agitated patients compared to non-agitated patients (22.84 ± 18.23 vs 15.31 ± 19.22 pg/ml). Data are expressed as median ± 95% confidence interval. $^{**}p = 0.025$.

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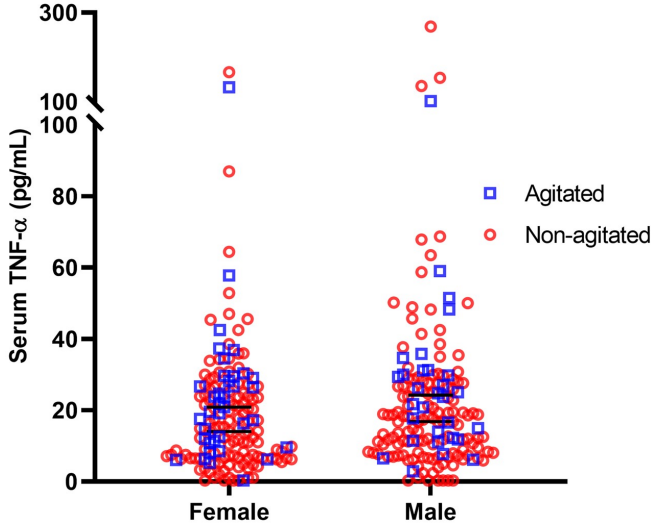

Fig 4. Serum TNF-α stratified by gender. No statistically significant differences were found in TNF-α levels between males and females (p = 0.143). TNF-α was significantly elevated in both male and female agitated patients when compared to non-agitated of the respective gender (female: 20.84 ± 18.17 vs 14.08 ± 18.23, p = 0.030, male: 24.27 ± 19.06 vs 16.82 ± 18.95, p = 0.045).

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Li et al. found that agitation measured by PANSS-EC was correlated with the cytokines IL-17, IL-23 and TGF-β in patients with schizophrenia [13]. This finding is not in line with our results, as we found agitation to be associated with TNF-α. However, Li et al did not measure TNF-α and IFN-γ, and we did not measure IL-17 or IL-23. It is possible that our findings would be more in line if measuring the same cytokines.

A few studies have also investigated the association between aggression and cytokines. As aggression and agitation are two overlapping clinical signs with several symptoms in common, these findings are also relevant to our study. Previously, levels of IFN-γ, IL-10 and TGF-β have been reported to be associated with aggression in schizophrenia patients [13, 14]. In line with this, we found a trend towards an association between IFN-γ and agitation in the sub-group non-affective psychoses. Interestingly, this was not seen in our total population, indicating that it may be a phenomenon specific for non-affective psychoses. The phenomenon should be further explored in a larger study and comparing with other diagnostic groups. For IL-10 and TGF-β we could not confirm the findings previously reported in a single study. It is also possible that patients with agitations have slightly different changes in the immune system than those seen in patients with aggression. However, only two studies is too limited to conclude and the phenomena should be further explored.

Some of these cytokines may be described to suppress or enhance each other, a phenomenon that could be part of the discussion. However, the patterns of interaction between different cytokines in immunology, as well as in neurophysiology, may have to be revised. Also, as stated decades ago the effect of cytokines on behavior may be context-dependent [21]. Thus, we do not comment further on in this article.

Potential confounders

The possible confounders age, gender, BMI and smoking, affected few cytokines in our study. This is in contrast to the established consideration of high BMI and obesity as a driver of chronic low-grade inflammatory state [22]. This knowledge is mainly yielded from animal studies, epidemiological studies and other populations without a psychiatric comorbidity [23]. However, in a study from an acute psychiatric population elevation of inflammatory markers, independently of BMI, has been reported [24].

Previous studies in acute psychiatry have shown that high levels of inflammatory markers might also be independent of smoking, age and gender [12, 25]. The same finding was reproduced in a population of depressed patients where the association between cytokines and anhedonia was independent of gender [26]. It is possible that the association between possible confounders and inflammatory markers is overridden by inflammation related to the acute psychiatric state in our population. Also, this study is on cytokines, not CRP and changes in TNF-α might be less related to BMI than CRP.

Medications is another potential confounder. The effect of clozapine and other neuroleptics as well as other psychoactive medications on the immune system is well known [27]. A slight effect of medications on levels of cytokines in psychiatric populations has been reported, as reviewed by Goldsmith et al [2]. Unfortunately, we did not record the use of medications in this study. The acute setting with blood samples withdrawn the first day after admission reduces the relevance of this issue, as most patients would be medicated later. Also, assessment of medications used immediately previous to admission is difficult in this clinical setting; patients are psychiatrically very ill and may not be able to actually report use of medications correctly. In addition, many patients are admitted due to exacerbation after discontinuation of medications. Other patients have not started medications when acutely admitted, maybe for the first time. Use of psychoactive substances is a similar issue.

Independently of the cause of the altered cytokines and the potential confounders, we suggest that the findings may be of clinical relevance. The purpose of our study was to reveal factors that may be a cause of agitation, and consequently a target for prevention and relief of agitation. No matter whether the potentially altered cytokines—that potentially cause agitation—are an internal trait of the psychiatric disorder or a consequence of known or unknown confounding factors (like medications) they may be a target for clinical improvement. Clinical relief of symptoms and signs is the main goal of clinical research. We fully agree that any confounding factor should be explored. However, at the same time the clinical relevance must be kept in mind. Thus, we suggest our findings are interesting also independently of confounding factors. In this context, it may even be argued that high CRP should not have led to exclusion.

Limitations and strengths

There are several limitations to the current study. The study population in the psychosis group is relatively small. However, the population is rather high when including all patients and when comparing the number with other studies in acute psychiatry.

We do not have a healthy control group that could extend the interpretation of the study. We did however aim to investigate characteristics of agitation and thus comparing with non-agitated patients may be more suitable than comparing with (non-agitated) healthy controls.

No data on medication was available and we did not control for substance abuse in our analyses. Theoretically, medications as well as substances affecting agitation also might affect the immune system. Substance abuse is also a possible confounder that we did not adjust for in our analyses. However, we did not find any significant differences in substance abuse between the agitated and non-agitated group. The cross-sectional design is another limitation, making it impossible to draw any conclusion on the causal connection between inflammation and agitation.

All blood samples were drawn during the first 24 hours of admittance to a closed inpatient ward, when the symptoms were most prominent and patients acutely ill. We used a validated and well-known tool for assessing the syndrome of agitation, which simplifies the comparability with other studies in the field. Furthermore, the psychiatric department recruiting the study participants is the only inpatients service in the catchment area, reducing the effect of socioeconomic differences.

Conclusions

The present study indicates that there is an association between the syndrome of agitation and TNF-α in an acute psychiatric population. A similar trend was reproduced to the psychosis subgroup. The findings need to be replicated, but may lead to therapeutic interventions in the future.

Supporting information

S1 File. Full dataset.
(SAV)

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Writing – original draft: Jeanette Brun Larsen.

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Paper II

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BRIEF RESEARCH REPORT
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Cytokines in Relation to Motor Activity in an Acute Psychiatric Population

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Background: Deviations in motor activity are important clinical features of several psychiatric disorders in an acute state. Immune activity is associated with several psychiatric disorders and may affect motor activity. We aimed to examine the association between immune activity measured as serum levels of cytokines and deviations in motor activity, in an acute psychiatric setting.

Methods: Data on motor activity and immune markers were available on 277 patients admitted to an acute psychiatric inpatient department. The degree of increased or decreased motor activity was clinically assessed at admission. Serum concentrations of the following immune markers were measured: interleukin (IL) -1β, IL-4, IL-6, IL-10, tumor necrosis factor (TNF) -α, interferon (IFN) -γ, and transforming growth factor (TGF) -β.

Results: Scores of increased motor activity were negatively correlated with IFN-γ (rho = –0.128, p = 0.033) in an acute psychiatric population. There was also a trend towards an association between motor activity and TGF-β (rho = 0.118, p = 0.050). In a multiple-linear-regression model correcting for age, gender, and body-mass index (BMI, kg/m^2), the association did not remain significant. No significant correlations between motor retardation and circulating cytokines were found.

Conclusions: After adjustment for potential confounders our study did not reveal any significant association between cytokines and motor activity. However, there is an indication of increased Th17 and decreased Th1 responses in relation to increased motor activity in line with the few previous reports in the field. The phenomenon however needs further exploration.

Keywords: cytokines, psychomotor retardation, agitation, depression, psychosis, acute psychiatric care

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INTRODUCTION

Altered motor activity is gaining increased interest within psychiatric research and may be a prominent finding in an acute psychiatric setting (1, 2). Traditionally, increased or abnormal activity is seen in ADHD, tic disorders, affective disorders, anxiety, and schizophrenia (3). Motor symptoms may also characterize different subtypes of unipolar depression and predict treatment response (2, 4).

Evidence supports a role of immune activity in the etiology and pathogenesis of psychiatric disorders (5). Several studies have demonstrated altered systemic levels of cytokines in patients with schizophrenia, bipolar disorder, and unipolar depression compared to healthy controls (6). These cytokine alterations may also be more prominent in an acute psychiatric setting (6).

Immune activity often is classified into different profiles based on effector mechanisms and characterized by a set of cytokines promoting those effector mechanisms. Th1 profile is characterized by cytokines such as interferon (IFN) -γ and tumor necrosis factor (TNF) -α and mediates potent responses to viruses. Th2 profile is characterized by interleukin (IL) -4 and IL-10. Th2 mediates certain B-cell responses (e.g., immunoglobulin-E production) and opposes Th1. Th17 is characterized by cytokines such as IL-17 and TGF-β and mediates other effector mechanism in the immune system (7).

Cytokines may be the factor mediating altered motor activity in certain psychiatric conditions (8). One mechanism by which cytokines may influence motor activity, is through alterations in neural activity and dopamine metabolism in the basal ganglia (9). It is also shown that treatment with cytokines such as IFN-α induces psychomotor retardation and depressive symptoms in patients with hepatitis (10). Finally, an association between motor activity, psychomotor retardation, and cytokines in outpatients with major depression has been described (11). Agitation is an important clinical syndrome consisting of several symptoms and signs, including increased motor activity. In patients with Alzheimer's disease, a previous study demonstrated that increased IL-1β was associated with agitation (12). However, few previous studies have investigated the association between increased motor activity only and immune markers.

The aim of this study, was to assess the association between circulation levels of cytokines, motor retardation, and increased motor activity in a sample of patients with a variety of severe mental disorders admitted to an acute psychiatric department. Because changes in motor activity are more common symptoms in certain diagnostic groups, unipolar depression and non-affective psychosis were chosen as subgroups.

MATERIALS AND METHODS

Setting and Participants

This cross-sectional study was conducted in the acute psychiatric inpatient wards of St. Olav's University Hospital, Trondheim, Norway. All acutely admitted inpatients between September 2011 and March 2012 were asked to participate. At the time of inclusion, the psychiatric department served a catchment area of 228.000 inhabitants (≥18 years old) and represented the only psychiatric inpatient acute unit in the area. Of the total 654 admitted patients in the inclusion period, 382 (58.4%) patients were included in the study. The study was approved by the regional committee for ethics (REC Central number 2011/137) and registered at ClinicalTrials.gov (NCT01415323). All patients gave their written informed consent prior to inclusion. The inclusion process was conducted by specialists in clinical psychology or psychiatry in order to secure that all included patients had the mental capacity to give their consent. The study was conducted according to the Declaration of Helsinki.

Exclusion Criteria

The following exclusion criteria were applied: (1) chronic or ongoing infections, (2) comorbid autoimmune diseases, (3) C-reactive protein (CRP) levels above 35 mg/L, or (4) lack of patient consent. When patients had multiple admissions, we only included the first admission in our analyses.

Diagnostic Evaluation

Patients were diagnosed according to the International Classification of Diseases-10 (ICD-10) Criteria for Research (13). The diagnoses were set in a consensus meeting in the treatment staff, always including at least two senior psychiatrists of whom one had personally examined the patient. For subgroup analyses, we included patients with non-affective psychosis (ICD-10 F20–29) and unipolar depression (ICD-10 F32 and F33).

Assessments

Sociodemographic history, comorbid medical conditions, smoking status, substance abuse, and psychiatric symptoms were recorded after an interview by a staff member. In addition, participants were screened in a general medical examination and routine blood tests, including CRP and leukocyte count. Height and weight were measured for calculation of the body mass index (BMI, kg/m^2).

The degree of motor retardation and increased motor activity was assessed by an experienced clinician using the Symptomatic Organic Mental Disorder Assessment Scale (SOMAS). SOMAS is a 5-item scale developed to assess atypical depressive symptoms. Item B rates the degree of motor retardation, and item C rates the degree of increased motor activity when the patient was most dysthymic during the previous 24 h (14). Both items are modified from the Positive and Negative Syndrome Scale (PANSS), where item B was modified from PANSS item "motor retardation" (general psychopathology scale, item G7), and item C was assessed from PANSS item "hyperactivity" (positive scale, item P4).

For analyses, patients were subdivided into two groups: with or without increased motor activity according SOMAS item C. If the patients were scored as ≥2 on SOMAS item C, they were grouped as motor active. Similarly, patients were separated into the two groups with or without motor retardation according to SOMAS item B. A score on SOMAS item B ≥ 3 was set to group the patients as motor retarded. In order to simplify the interpretation of findings on SOMAS item B, it was reverse-coded. Therefore, a higher score on both SOMAS item B and C would be interpreted as more severe symptoms of motor retardation or increased motor activity. Subgroup analyses were performed on patients with diagnoses non-affective psychosis group and unipolar depression.

Serum Analyses of Immune Biomarkers

Blood samples were collected on 9 ml serum tubes with SiO2 without gel between 08.00 and 13.00 (median at 10:00) at the first working day after admission. Strict instructions regarding fasting were not given, though most patients would be fasting overnight. Samples were immediately cooled on ice, protected from daylight, and centrifuged within 30 min (15 min, 1,500 g, 4°C). Serum samples were stored at −80°C until further analysis in a registered Biobank (Biobank1, St. Olav's University Hospital, Trondheim, Norway). The following parameters were analyzed bymultianalyte profiling Milliplex MAP assays: IL-1β, IL-4, IL-6, IL-10, TNF-α, and IFN-γ (Millipore Corporation, Billerica, MA, US). TGF-β1 was measured by a Bio-Plex Pro TGF-β Assay (Biorad Hercules, CA, US). Intra- and interassay coefficients of variance were less than 10%. The range of detected values was IL-1β: 0.06-198.72 pg/ml; IL-4: 0.92-286.01 pg/ml; IL-6: 0.10-576.42 pg/ml; IL-10 0.10-1125-33 pg/ml; TNF-α: 0.70-268.89 pg/ml; IFN-y: 0.04-1529.70 pg/ml; and TGF-β: 13.07-415.61 ng/ml. The number of samples and percentage under the detection limit was as follows: IL-1β 236 (74.2%), IL-4: 242 (76.1%), IL-6: 177 (55.7%), IL-10:177 (55.7%), TNF-α: 11 (3.5%), IFN-y: 68 (21.4%), and TGF-β: 0.

Statistical Analyses

Statistical analyses were done using SPSS version 24.0 for Windows. The level of significance was set at $p \leq 0.05$, and all analyses were two-tailed. Significant findings were adjusted for multiple testing with the Bonferroni correction (α/k where k = the seven tested cytokines giving $\alpha/k = 0.007$). Data normality was assessed by using a Kolmogorov-Smirnov test. The distribution of all serum cytokines was skewed, and only TGF-β became normally distributed after logarithmic transformation. Descriptive statistics were calculated by using chi-square tests for categorical variables and student's independent samples t-tests or Mann-Whitney U test (depending on distribution) for continuous variables. The Spearman correlation coefficient was calculated for the relationship between cytokines and SOMAS. Additionally, we examined the difference in cytokine levels between the groups with and without increased motor activity by using student's independent samples t-test or Mann-Whitney U test if the data were not normally distributed. The same statistical methods were applied when comparing cytokine levels between the groups with and without motor retardation.

RESULTS

Sociodemographic, Clinical, and Inflammatory Characteristics of the Sample

Of the total 382 patients included in the study, 24 were excluded due to infection or autoimmune diseases. This left us with 358 patients for whom serum samples were available, and cytokines were analyzed in 318 patients. For 277 of these 318 patients we also had complete measures for altered motor activity (increased or reduced motor activity). The main ICD-10 diagnostic categories in the 277 patients were unipolar depression (22.7%), substance-use disorders (15.9%), schizophrenia (9.7%), bipolar disorder (12.7%), neurotic, stress-related, and somatoform disorders (10.1%), and personality disorders (8.3%), and other diagnoses (20.6%).

The demographic, clinical, and immune data for the total study population, the non-affective psychosis group, and unipolar depression group are given in **Table 1**.

Relation Between Serum Inflammatory Markers and Measures of Motor Activity

When all patients were analyzed together, scores of increased motor activity were significantly negatively correlated with IFN-γ (rho = −0.128, p = 0.033, **Table 2**). In addition, we found a trend

TABLE 1 | Demographic and clinical parameters.

	All patients N = 358	Non-affective psychosis N = 48	Unipolar depression N = 73	*p*-value[a]
Age (years), mean ± SD	38.9 ± 14.8	40.1 ± 11.8	40.1 ± 14.9	0.756[b]
Gender (female), N (%)	174 (49)	19 (40)	42 (58)	0.053[c]
Smoking, N (%)	175 (49)[d]	25 (66)	28 (25)	**0.045[c]**
BMI (kg/m^2), mean ± SD	25.5 ± 5.9[e]	27.6 ± 6.2	24.9 ± 5.4	**0.015[f]**
Higher education (above high school), N (%)	50 (14)	2 (4)	14 (19)	**0.017[c]**
Unemployment (incl. sick leave), N (%)	255 (71)	41 (85)	42 (58)	**0.002[c]**
Alcohol use upon admission, N (%)	99 (28)	9 (19)	15 (21)	0.808[c]
Substance abuse, N (%)	78 (22)	8 (17)	11 (15)	0.813[c]
Motor retardation score[g], mean ± SD	1.6 ± 0.7	1.5 ± 0.8	1.7 ± 0.9	0.205[f]
Motor activity score[g], mean ± SD	1.5 ± 0.9	1.6 ± 1.0	1.3 ± 0.5	0.084[f]
Number with blood samples, N (%)	318 (89)	40 (83)	68 (93)	0.088[c]

[a]*Comparison between non-affective psychosis and unipolar depression. Significance with a p-value < 0.05 is indicated in bold text.*
[b]*Independent students' samples t-test.*
[c]*Chi-square test.*
[d]*Missing 60 (10 within non-affective psychosis group and 11 within unipolar depression group).*
[e]*Missing 89 (15 within non-affective psychosis group and 11 within unipolar depression) observed (data not shown).*
[f]*Mann-Whitney U test.*
[g]*Motor retardation and motor activity were assessed by items form a Symptomatic Organic Mental Disorder Assessment Scale (SOMAS). Both items were scored on a scale from 1–5. BMI, body mass index; SD, standard deviation.*

TABLE 2 | Correlation coefficients (rho) between serum cytokines, motor retardation, and motor activity[a].

	IL-1β	IL-6	TNF-α	IFN-γ	IL-10	IL-4	TGF-β
All patients							
-Motor retardation	0.112	0.066	–0.027	0.090	0.061	–0.043	–0.042
-Motor activity	0.001	–0.010	–0.034	–0.128*	–0.002	0.057	0.118
Non-affective psychosis							
-Motor retardation	0.154	0.165	0.002	0.191	0.194	–0.181	–0.153
-Motor activity	0.052	0.251	0.029	0.000	0.085	0.080	–0.054
Unipolar depression							
-Motor retardation	–0.089	–0.003	–0.047	–0.007	–0.056	0.059	0.173
-Motor activity	–0.046	–0.133	–0.181	–0.173	–0.239	–0.002	0.107

*$p \leq 0.05$.
[a]*Motor retardation and motor activity were scored on a scale from 1–5.*
IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

towards a positive correlation between TGF-β and increased motor activity (rho = 0.118, p = 0.050). No significant correlations where found between cytokines, motor retardation, and increased motor activity analyzing the two subgroups non-affective psychosis and unipolar depression (**Table 2**). In a multiple-linear-regression model correcting for age, gender, and BMI, the associations between increased motor activity and IFN-γ (beta = −0.112, t = −1.726, p = 0.086) and TGF-β (beta = 0.123, t = 1.904, p = 0.058) did not remain significant. No findings remained significant after correcting for multiple testing with the Bonferroni correction.

Comparisons of Cytokine Levels Based on the Presence or Absence of Increased Motor Activity and Motor Retardation

Mean log-transformed values of TGF-β were significantly higher in patients with increased motor activity compared to those with no increase in motor activity (11.31 ± 0.34 vs. 11.20 ± 0.45, t = −2.11, df = 236.41, ES = 0.016, p = 0.036) (**Figure 1**). The increased-motor-activity group had significantly lower levels of IFN-γ compared to the group without increased motor activity (2.27 ± 11.52 vs 6.00 ± 20.04, U = 7213, d = 0.13, p = 0.032) (**Figure 2**). These findings were however not significant after the Bonferroni correction for multiple testing. No other differences in cytokine levels between groups reached the level of significance. When comparing cytokine levels between the two groups with and without motor retardation, no statistical differences were detected (data not shown).

DISCUSSION

After correcting for multiple testing and confounders, we did not find any significant association at the 0.05-level between motor activity and cytokines. However, a trend towards an association between increased motor activity and lower serum levels of IFN-γ and higher levels of TGF-β in patients admitted to an acute psychiatric ward was seen. No statistically significant association

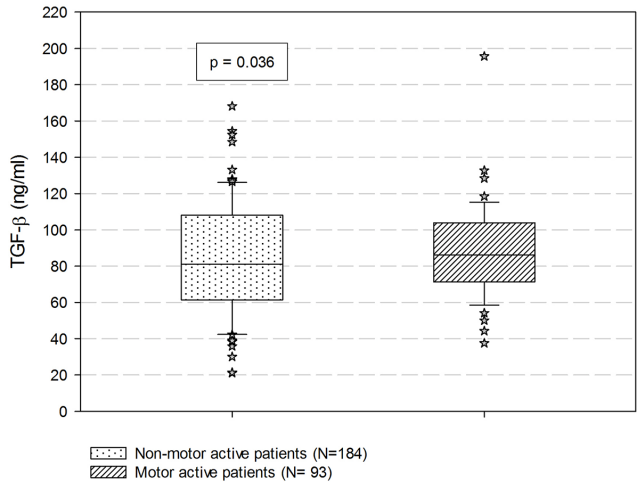

FIGURE 1 | Comparisons of serum TGF-β based on the prevalence of increased motor activity. P-value is estimated by student's independent samples t-test with log transformed values. Data are expressed as median with percentiles. TGF-β, transforming growth factor β.

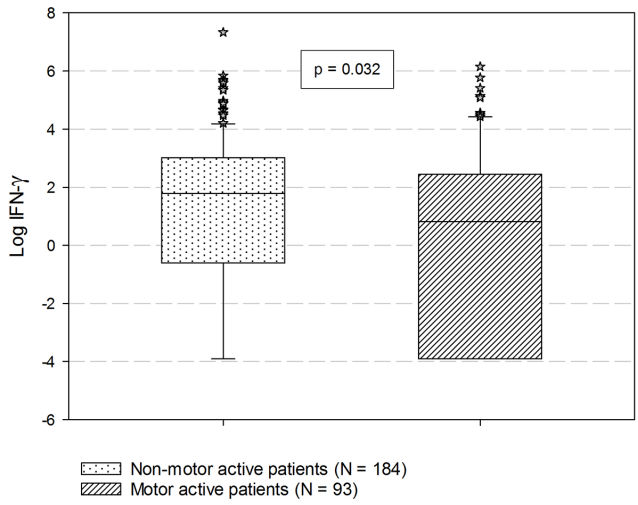

FIGURE 2 | Comparisons of serum IFN-γ based on the prevalence of increased motor activity. P-value is estimated by Mann-Whitney U test. Data are expressed as median with percentiles. IFN-γ, Interferon-γ.

between motor retardation and cytokines was seen. To our knowledge, this is the first report on the relation between immune markers and motor activity in an acute inpatient psychiatric population.

Aggression is a psychiatric sign associated with increased motor activity (15). In a recent study on inpatients with schizophrenia, aggressive behavior was associated with increased levels of Th17 cytokines TGF-β, IL-17, and IL-23 (16). In the present study, we examine motor activity only, but our finding of a trend towards increased TGF-β may be in line with the report on aggressive behavior. However, our findings did not remain significant after corrections for multiple testing and confounders. One might therefore also interpret this previous finding as not being in line with our study.

The present study did not show any significant difference in cytokine levels in relation to motor retardation. This may be somewhat surprising as other studies have indicated a relation between motor retardation and Th1 cytokines. Increased Th1 response has been demonstrated in association with low number of steps per day (17) as well as reduced psychomotor speed (11) in outpatients with major depression. Also, treatment with IFN-α increases the risk of depression and reduced psychomotor speed in patients with hepatitis C (18) and is associated with higher degree of motor retardation in depression (19). However, none of these studies were conducted in an acute psychiatric population, making our results not directly comparable. It is possible that other inflammatory factors are stronger in the acute population masking an association between Th1 and retardation.

There are several limitations and strengths to the current study. We did unfortunately not calculate power for this part of the study. The degree of motor retardation and increased motor activity was assessed by items from SOMAS, which is validated for this purpose. However, the items are published in a previous study (14, 20), and is also shown to correspond with findings in actigraphy (2). For future studies actigraphy should be included. Finally, even though alterations in cytokines in relation to motor activity were influenced by age, gender, and BMI, cytokines still may be clinically important.

Several of the serum cytokines had a high percentage of samples below the detection limit. Although this was not the case for IFN-γ and TGF-β, it may have affected the analyses of other pro-inflammatory cytokines, increasing the risk for type-II error. Further, the results for subgroup analyses are limited by a relatively low number of participants in the subgroups non-affective psychosis and unipolar depression and by the lack of a healthy control group. However, it is interesting as it suggests that alterations in immune activity are more related to symptoms than diagnostic group.

The study population was also relatively heterogeneous with the possibility of confounding factors. Thus, all findings need replication and should be interpreted with care.

We were however able to include severely ill patients in acute states with a variety of psychiatric diagnoses. The blood samples were drawn during the first 24 h of the admission, the period in which the symptoms were most prominent. In addition, the clinic recruiting the study participants is the only acute psychiatry inpatient service in the catchment area, reducing the effect of socioeconomics. All patients in the area needing acute psychiatric services were admitted to this unit. Also, our total sample size is relatively large, compared to other studies in the field.

CONCLUSIONS

Our study comparing levels of immune markers in an acute setting did not reveal any significant associations between altered motor activity and cytokine levels. However, a trend towards a Th17 profile among patients with increased motor activity was seen. The finding should be further explored because it may have implications for predicting and treating deviations in motor activity.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Committees for Medical and Health Research Ethics (REC) Central Regional, Trondheim, Norway. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JL did all statistical analyses, took a leading role in selection of analyses and interpretation of the results, and wrote the original draft for the manuscript. AS did all the laboratory analyses on cytokines. AS took an equal part in planning and discussing the choice of analyses, interpretation of results and writing of the manuscript. VI supervised on statistics and took an equal part in choice and discussion of the statistical analyses as well as writing of the manuscript. AV initiated the study and headed the inclusion of patients in the clinic. AV took an equal part in interpretation of the results and writing of the manuscript. SR collaborated in initiation and performance of the study in the clinic. SR together with JL took a leading role in choice of laboratory analyses as well as interpretation of the data and in writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2019.00920/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material paper 2

The Symptomatic Organic Mental Disorder Rating Scale (SOMAS).

The ratings of all items are based on the investigator's personal examination of the patient, and/or information in the case records and/or information from the hospital ward staff.
The scorings are 1 - 10 on the following 5 items:

A: Degree of observable change in symptoms during the previous 24 hours

1: The symptoms have been completely stable throughout 24 hours.
3: Minor changes in symptoms during the past 24 hours (e.g., increased symptoms in the morning as in a depressive episode).
5: Some change of symptoms (e.g., breakthrough of depressive symptoms in hypomania).
8: Frequent alternation of symptoms, dominating more than half of the day.
10: Rapid fluctuation of symptoms from one half hour to the next.

B: Degree of motor retardation, rated during the period or periods of the previous 24 hours in which the patient was most depressed.*

1: The patient has been almost completely immobile and virtually unresponsive to external stimuli.
3: Movements are extremely slow, resulting in a minimum of activity and speech. The patient is mostly sitting idly or lying down.
5: The patient has slow movements, and speech may be characterized by poor productivity, including long response latency, extended pauses, or slow pace.

8: Slight diminution in rate of movements and speech.

10: No motor retardation.

C: Degree of increased motor activity, rated during the period or periods the previous 24 hours when the patient was most depressed.*

1: No increased motor activity.

3: The patient is slightly agitated with hypervigilance or has a tendency towards mild overarousal. The speech is slightly pressured.

5: The patient is clearly agitated and overaroused with affected speech and motor activity.

8: Marked excitement dominates the period and restricts attention and vital functions such as eating and sleeping.

10: The excitement is so extreme that interpersonal interaction is virtually impossible. The patient has acceleration of speech and motor activity resulting in incoherence and exhaustion.

D: Degree of patient's insight into his or her condition/symptoms

1: Mature and thoroughly considered attempt at explaining the condition. This explanation may or may not be psychotic.

3: The patient has been thinking of various possible explanations and has come up with a well-founded opinion about some of them.

5: The patient wonders about different causes of the condition, but is unsure.

8: The patient has one or several ideas about the cause, without any considered argumentation.

10: Patient is totally bewildered to what has happened or to what causes the condition.

X: Not possible to score; e.g. due to incapability to communicate verbally.

E: Degree of the patient's concern in finding an explanation for his or her condition/symptoms

1: Considerable engagement in finding an explanation of the condition.

3: Moderate engagement in finding an explanation of the condition.

5: Some engagement in finding an explanation of the condition.

8: Minimal engagement in finding an explanation of the condition.

10: The patient does not wonder at all what may have caused the condition.

X: Not possible to score; e.g. due to incapability to communicate verbally.

* Items B and C are modifications of two PANSS items (Kay SR, Fiszbein A, Opler LA. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schiz Bul 1987;13:261-76.)

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Paper III

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The association between cytokines and psychomotor speed in a spectrum of psychotic disorders: A longitudinal study

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ABSTRACT

Background: In schizophrenia, impaired psychomotor speed is a common symptom predicting worse functional outcome. Inflammation causes changes in white matter integrity, which may lead to reduced psychomotor speed. Therefore, we wanted to investigate if peripheral inflammation assessed with cytokines affected performance on psychomotor speed in patients with a spectrum of psychotic disorders.

Methods: The current study is a prospective cohort study, including participants from a pragmatic, randomised controlled trial comparing three atypical antipsychotics in patients with a spectrum of psychotic disorders. For the purposes of this sub-study, we analysed drug treatment groups collectively. Psychomotor speed was assessed at baseline, and at weeks 6, 12, 26 and 52 of follow-up, using the neuropsychological tests trail making test (TMT) A and B, and symbol coding. Serum concentration of the following cytokines were measured: interleukin (IL)-β, IL-2, IL-4, IL-6, IL-10, IL12 p70, IL-17a, interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Blood samples were collected at baseline and after 1, 3, 6, 12, 26, 39 and 52 weeks. We analysed the effect of cytokines levels on psychomotor speed over time in linear mixed effects models.

Results: In our linear mixed effects models controlling for possible confounders, IFN-γ had a significant negative effect on TMT-A and symbol coding performance. None of the other tests for psychomotor speed were significantly associated with cytokines. Overall psychomotor speed performance increased significantly across the study period while cytokine levels remained stable.

Conclusion: Our study indicates a negative association between IFN-γ and psychomotor speed, which might be of importance when understanding the mechanisms behind psychomotor deviations in psychotic disorders.

1. Introduction

Deviations in psychomotor speed have been demonstrated in patients with various mental disorders, including schizophrenia and major depression (Bruder et al., 2014; Dickinson et al., 2007; Rybakowski and Borkowska, 2002). In patients with schizophrenia, psychomotor speed

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; MRI, magnetic resonance imaging; MS, multiple sclerosis; PANSS, Positive and Negative Syndrome Scale; RCT, randomised controlled trial; SCID-I, Structured Clinical Interview for DSM-IV Axis I Disorders; TMT, Trail Making Test; TNF, tumor necrosis factor; BMI, body mass index.

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impairment is associated with worse functional and social outcome (Nuechterlein et al., 2011; Sanchez et al., 2009). Further, subjects at clinical high risk for psychosis show impairment in several cognitive domains, including psychomotor speed (Anda et al., 2019; Zheng et al., 2018). Interestingly, psychomotor speed may be a strong cognitive predictor for whether a subject in a potential prodromal state actually do develop psychosis (Pukrop et al., 2007).

In general, psychomotor speed is associated with reduced white matter integrity in magnetic resonance imaging (MRI) studies of healthy volunteers and patients with schizophrenia (Karbasforoushan et al., 2015; Wang et al., 2020). This relation is prominent in several areas of the brain, including the basal ganglia (Tsapanou et al., 2019). Inflammation is one pathway that might alter both white matter integrity and connectivity of the basal ganglia (Felger et al., 2016; Najjar and Pearlman, 2015), subsequently affecting psychomotor speed (Felger et al., 2016; Walker et al., 2017). Theoretically, this is possible knowing that peripheral cytokines can cross the blood-brain barrier both ways (Banks, 2015), bind to glial cells (Prieto and Cotman, 2017), and induce damage to oligodendrocytes and microglial cells (Martino et al., 2000). Additionally, inflammation and cytokines might influence dopamine levels in the basal ganglia (Petrulli et al., 2017), influencing performance on tests for psychomotor speed (Capuron et al., 2012).

The possible link between psychomotor speed and inflammation is of particular interest when studying patients with psychosis. There is increasing evidence for a linkage between psychosis and inflammation (Kroken et al., 2018; Pape et al., 2019). Notably, patients with schizophrenia have higher levels of inflammatory markers including cytokines, compared to healthy controls (Goldsmith et al., 2016b; Miller et al., 2011). Changes in cytokine levels across the course of illness is seen across a range of mental health disorders (including schizophrenia), with a common pattern where cytokine levels fluctuate between acute and chronic states (Goldsmith et al., 2016b; Momtazmanesh et al., 2019). There is a need for studies on inflammation in relation to signs and transdiagnostic symptoms (Khandaker et al., 2017). Psychomotor speed impairment may be one such transdiagnostic sign, evident in several mental disorders.

A few previous studies on psychotic disorders identified an association between reduced psychomotor speed and cytokines such as interleukin (IL) -6 and tumor necrosis factor (TNF) -α (Frydecka et al., 2015; Goldsmith et al., 2020). However, the results are conflicting as other studies found no significant association (Hori et al., 2017; Martínez-Cengotitabengoa et al., 2012). Moreover, previous studies are limited by cross sectional designs, which preclude conclusions about causality. Nor can cross sectional studies take into account that both cytokines and psychomotor speed might change over time. Therefore, we wanted to explore the association between cytokines and psychomotor speed in a longitudinal study including patients with spectrum of psychotic disorders. The aim was to investigate whether cytokine levels affected psychomotor speed over time.

2. Methods

2.1. Setting and participants

The current study is a prospective cohort study with participants from the BeStInTro study, a pragmatic, randomised controlled trial (RCT) comparing three different atypical antipsychotics in the treatment of schizophrenia spectrum disorders. The primary outcome results from this study are recently published in Lancet Psychiatry (Johnsen et al., 2020). The follow-up period was 52 weeks with visits at baseline as well as after 1, 3, 6, 12, 26, 39 and 52 weeks. Blood samples were collected at all these visits. Participants underwent neuropsychological testing at baseline, and at week 6, 12, 26 and 52. For the analyses of the present study, the patients were not subdivided into treatment groups.

Three treatment centers in Norway and one in Austria participated in collecting the data between Oct 20, 2011, to Dec 30, 2016. Inclusion criteria were age ≥18 years and a psychosis spectrum disorder (ICD-10 diagnosis F20-29) with active psychotic symptoms. Active psychosis was defined as a score ≥4 on at least one of the following Positive and Negative Syndrome Scale (PANSS) items: Delusions (P1), Hallucinatory behavior (P3), Grandiosity (P5), Suspiciousness/persecution (P6) or Unusual thought content (G9) (Kay et al., 1987). Exclusion criteria were inability to understand the site language, pregnancy, breastfeeding, limbic encephalitis, hypersensitivity to any of three antipsychotic drugs tested in the main study, or somatic disorders known as precautions for the drugs tested (prolactin-dependent tumors, pheochromocytoma, risk of torsade de points or narrow-angle glaucoma). The study protocol and population are previously described in detail elsewhere (Johnsen et al., 2020).

All patients gave written informed consent prior to inclusion. The study was approved by the Regional Committees for Medical and Health Research Ethics and the Norwegian Medicines Agency in Norway, and the Ethikkommission der Medizinische Universität Innsbruck and the Austrian Federal Office for Safety in Health Care in Austria. This work was carried out in accordance with the Declaration of Helsinki. The study is registered at ClinicalTrials.gov (NCT01446328).

2.2. Clinical assessments

Diagnoses were set according to the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and converted to ICD-10 diagnoses in line with Norwegian clinical guidelines. Sociodemographic data were recorded at baseline. We assessed alcohol and drug abuse with the two scales the Clinical Alcohol Use Scale and Clinical Drug Use Scale (Drake et al., 1990). At baseline, we recorded information on demographic variables such as ethnicity, smoking status, gender and age. In addition, we interviewed participants with PANSS at each visit. Height at baseline, and weight were also measured throughout the entire study period in order to calculate body mass index (BMI, kg/m^2). Participants underwent testing with Trail Making Tests A (TMT-A) and B (TMT-B) (Bowie and Harvey, 2006; Crowe, 1998), at baseline and at weeks 6, 12, 26 and 52. Symbol coding was conducted at baseline and weeks 6, 26 and 52 (Keefe et al., 2008). All raw scores from the cognitive tests were converted to t-scores according to available norms and manuals.

2.3. Immune biomarkers

Fasting-state blood samples were collected between 8 and 10 a.m. Before centrifugation at 3300 rpm for 10 min, the blood was left to clot at room temperature for 20–120 min. The aliquoted serum was frozen at -40 °C and stored at -80 °C. Cytokine analyses were conducted with a Multiplex immunoassay (High Sensitivity 9-Plex Human ProcartaPlex™ Panel (ThermoFisher Scientific, Waltham, MA, USA)). The following cytokines were analysed: IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 p70, IL-17a, interferon (IFN)–γ and TNF-α. The panel was performed in accordance with the manufacturers' instructions, but we substituted the universal assay buffer with 1X phosphate buffer saline (PBS) with 0.10% tween in order to improve fluorescence intensities. Using the manufacturers' recommendations for sample dilutions and standard curve concentrations, all samples and standards were assayed in duplicates. A Luminex 200 (R&D Systems, Inc., Minneapolis, MN, USA) was used for measuring the fluorescence intensities, and two lots of reagents with similar upper and lower detection limits (ULOQ/LLOQ) were used for all samples (21 plates).

The detection limit was defined as the lowest detected value within the standard curve. Samples below the detection limits were set to half of the detection limits. Summarizing all visits, the number of samples and percentage under the detection limit was as follows: IL-1β: 229 (30.1%), IL-2: 41 (5.4%), IL-4: 162 (21.3%), IL-6: 239 (31.4%), IL-10: 74 (9.7%), IL-12 p70: 171 (26.3%), IL-17a: 40 (6.1%), IFN-γ: 158 (20.8%) and TNF-α: 146 (19.2%). Mean inter- and intra-assay coefficients of variation (CV) are given in Table A1.

2.4. Statistical analyses

Statistical analyses were performed using R version 3.6.2 for Windows (www.R-project.org). The level of significance was set at $p \leq 0.05$ and all analyses were two-tailed. We evaluated data normality with Shapiro-Wilk normality test and evaluation of the QQ-plots. TMT-A, TMT-B and symbol coding t-scores were close to normally distributed. All cytokine values were heavily right skewed, and thus log transformed cytokine values were used in the statistical analyses. We assessed associations between demographic variables, cytokines, and tests for psychomotor speed with Pearson's correlation for continuous variables, and with Mann Whitney *U* test or *t*-test (depending on the normal distribution) for dichotomous variables.

We used linear mixed effects models to see if log transformed cytokines, TMT-A, TMT-B, or symbol coding changed over time alone without adjusting for the effect of each other. A random intercept for each patient was included to account for dependencies in the data due to repeated measures from the same participants. Secondly, we calculated the Pearson correlation coefficient for the relationship between log transformed cytokines, and TMT-A, TMT-B, or symbol coding at baseline and at end point. Finally, we used linear mixed effects models to assess the relationship between log transformed cytokines and tests for psychomotor speed. We made one model for each psychomotor test, including the test score as dependent variable, and cytokines and visit as independent variables. Again, a random intercept for each patient was included in order to account for dependencies in the data. The following possible confounders were included in the model as independent variables: age, gender, BMI, ethnicity, smoking, study site, antipsychotic drug and PANSS positive subscale score.

Due to the possible confounding effect of including patients with various psychotic disorders, we conducted sensitivity analyses where patients with F22 Delusional disorder and F23 Acute and transient psychosis were excluded. After excluding these patients, we conducted the same linear mixed effect models investigating the association between log transformed cytokines and tests for psychomotor speed correcting for possible confounders.

3. Results

The total sample consisted of 144 patients. At baseline, serum cytokine measurements were available for 140 patients, TMT-A scores for 101 patients, TMT-B scores for 89 patients and symbol coding scores for 92 patients. At end point, there were cytokine measurements from 58 patients, TMT-A scores for 47 patients, TMT-B scores for 40 patients and symbol coding scores for 41 patients. A total of 79 patients had measurements of cytokines and all three psychomotor tests at baseline, and 39 patients had both at end point. Tables 1 and 2 show demographic and clinical parameters at baseline.

3.1. Change in psychomotor speed and cytokines over time

Overall, the mean level of t-score for the tests TMT-A, TMT-B and symbol coding increased during the study period in linear mixed effects models, being most pronounced for TMT-A (Fig. 1). From baseline to end point, there were significant changes in t-scores for TMT-A (mean change = 6.3, p-value < 0.001), TMT-B (mean change = 4.9, p-value < 0.001) and symbol coding (mean change = 4.8, p-value < 0.001). Further, a linear mixed effects model found a statistically significant improvement on performance for the TMT-A t-score from baseline to 6 weeks (mean change = 2.7, p-value = 0.011), and from 12 to 26 weeks (mean change = 3.5, p-value = 0.009). Between the other two visits, there were no significant change in TMT-A t-score (Table 3). TMT-B t-score improved significantly from baseline to 6 weeks only (mean change = 3.7, p-value = 0.001) (Table 3). Symbol coding t-scores only had a significant improvement from baseline to 26 weeks (mean change = 3.3, p-value = 0.021) (Table 3). None of the cytokines changed significantly from baseline to end point in a linear mixed effects model (Fig. 2 and Table A2).

Table 1
Demographic and clinical parameters at baseline (N = 144).

	N	%	
Gender (female)	51	35.4	
Higher education (above high school)[a]	25	19.0	
Employed[b]	36	26.5	
Smokers[c]	84	66.1	
Alcohol misuse or dependence[a]	13	9.6	
Drug misuse or dependence[b]	27	19.9	
Diagnosis			
F20 Schizophrenia	84	58.0	
F21 Schizotypal disorder	2	1.4	
F22 Delusional disorders	21	14.6	
F23 Acute and transient psychosis	18	12.5	
F25 Schizoaffective	10	6.9	
F28–F29 Other and unspecified psychosis	9	6.3	
	Mean	**SD**	**Range**
Age	31.7	12.7	18–65
BMI[d]	25.5	6.0	16.4–54.8
PANSS total score	77.6	15.3	44.0–142.0
PANSS positive score	21.1	4.9	12.0–38.0
TMT-A (sec)	35.5	22.5	12–181
TMT-B (sec)	91.9	53.8	32–335
Symbol coding (t-score)	35.8	12.9	12–76

Abbreviations: BMI; body mass index, PANSS; Positive and Negative Syndrome Scale, TMT; Trail Making Test.
[a] Missing 9.
[b] Missing 8.
[c] Missing 17.
[d] Missing 22.

Table 2
Cytokine levels at baseline.

	Mean	SD	Range
IFN-γ (pg/ml)	5.3	9.4	0.04–70.87
IL-1β (pg/ml)	1.2	2.8	0.01–22.74
IL-10 (pg/ml)	1.6	2.5	0.02–22.10
IL-12 p70 (pg/ml)	3.2	8.2	0.12–80.82
IL-17a (pg/ml)	33.3	46.6	0.02–279.43
IL-2 (pg/ml)	25.5	31.9	0.06–217.47
IL-4 (pg/ml)	21.2	34.9	0.10–227.41
IL-6 (pg/ml)	2.0	3.1	0.07–15.19
TNF-α (pg/ml)	46.1	76.1	0.11–445.08

Abbreviations: IFN; interferon, IL; interleukin, TNF; tumor necrosis factor.

3.2. Relationship between psychomotor speed and cytokines

At baseline and end point, no Pearson's correlation coefficients reached the level of significance for the relationship between cytokines and any of the test TMT-A, TMT-B, or symbol coding (Table A3). In linear mixed effects models including measurements from all visits and correcting for possible confounders, only IFN-γ had a significant negative effect upon TMT-A (model estimate = -2.923, p = 0.011) and symbol coding (model estimate = -2.564, p = 0.038). None of the other cytokines had a significant effect upon TMT-A or symbol coding (Table 4). For TMT-B, we found no significant associations with the tested cytokines in a linear mixed effects model (Table 4).

In linear mixed effects models excluding patients with F22 and F23 ICD-10 diagnoses, IFN-γ was still significantly associated with TMT-A (model estimate = -3.059, p = 0.042), but not symbol coding (model estimate = -1.882, p = 0.212). In addition, IL-4 was associated with TMT-A (model estimate = 2.506, p = 0.030) and TMT-B (model estimate = 2.417, p = 0.046). None of the other tested cytokines were significantly related to TMT-A or TMT-B (Table A4). In these linear mixed effects models, no cytokines had a significant effect upon symbol coding

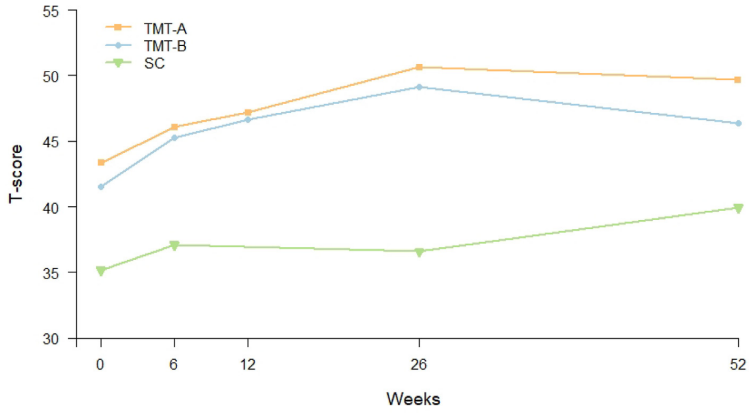

Fig. 1. Estimated mean t-score of psychomotor speed over time. Data estimated in linear mixed effect models including three different tests for psychomotor speed. Abbreviations: TMT; trail making test, SC; symbol coding.

Table 3
Change in tests for psychomotor speed (t-score).

	TMT-A			TMT-B			SC		
	Estimates	SE	P	Estimates	SE	p	Estimates	SE	p
Baseline (0 weeks)	43.3	1.1	0.000	41.5	1.1	0.000	35.1	1.2	0.000
Change 0–6 weeks	2.7	1.1	**0.011**	3.7	1.1	**0.001**	1.9	1.1	0.084
Change 6–12 weeks	1.1	1.1	0.337	1.4	1.2	0.224	–	–	–
Change 6–26 weeks	–	–	–	–	–	–	-0.5	1.3	0.704
Change 12–26 weeks	3.5	1.3	**0.009**	2.5	1.3	0.064	–	–	–
Change 26–52 weeks	-1.0	1.4	0.499	2.8	1.5	0.055	3.3	1.4	**0.021**

Estimates from the linear mixed effects models. No measurements of SC at week 12.
Abbreviations: SC; symbol coding, TMT; Trail Making Test.

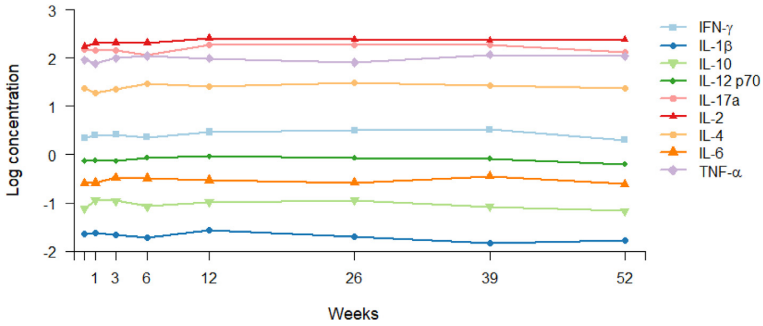

Fig. 2. Estimated mean levels of cytokines over time.
Data estimated in linear mixed effect models including log transformed cytokine values. Abbreviations: IFN; interferon, IL; interleukin, TNF; tumor necrosis factor.

(Table A4). As in the main analyses described above, these models were also conducted including several possible confounders for correction.

4. Discussion

Our findings indicate that higher levels of IFN-γ had a significant negative effect upon psychomotor speed (assessed by TMT-A and symbol coding) in patients with a spectrum of psychotic disorders. No other cytokines were significantly associated with psychomotor speed. In addition, we found that although patients improved significantly on tests for psychomotor speed during the study period of 52 weeks, the tested cytokines remained stable without any significant changes.

To our knowledge, this was the first study to include IFN-γ when investigating the association between inflammatory markers and psychomotor speed in patients with severe mental disorders. Therefore, it is difficult to find studies that are directly comparable with the finding regarding IFN-γ and psychomotor speed in our study. However, previous studies have found an association between performance on tests for

Table 4
Estimated effect of log-transformed cytokines on t-scores from test of psychomotor speed.

	TMT-A			TMT-B			Symbol coding		
	Estimates	SE	*p*	Estimates	SE	*p*	Estimates	SE	*p*
IFN-γ	**-2.923**	**1.14**	**0.011**	-1.131	1.07	0.294	**-2.564**	**1.22**	**0.038**
IL-1β	0.161	0.55	0.768	-0.199	0.55	0.716	0.444	0.60	0.463
IL-10	0.835	0.58	0.149	0.550	0.56	0.330	-0.039	0.59	0.948
IL-12 p70	0.959	1.27	0.451	-0.867	1.26	0.493	1.044	1.31	0.427
IL-17a	-0.264	0.96	0.782	0.849	0.94	0.370	-1.602	1.15	0.166
IL-2	0.951	1.30	0.465	-0.607	1.27	0.635	0.248	1.346	0.854
IL-4	0.648	0.77	0.399	1.297	0.73	0.079	0.668	0.92	0.468
IL-6	-0.327	0.61	0.594	0.220	0.61	0.719	-0.576	0.68	0.399
TNF-α	-0.007	0.83	0.993	-0.217	0.83	0.793	1.516	0.92	0.103

Estimates from linear mixed effect models. The models included age, gender, BMI, ethnicity, smoking, study site, antipsychotic drug and PANSS positive score as independent variables.
Abbreviations: IFN; interferon, IL; interleukin, TMT; trail making test, TNF; transforming growth factor.

psychomotor speed (including TMT-A, TMT-B and symbol coding) and other pro-inflammatory cytokines in schizophrenia patients (Frydecka et al., 2015; Goldsmith et al., 2020). Additionally, Bulzacka et al. found that schizophrenia patients with increased levels of high-sensitivity C-reactive protein (hs-CRP, increased level defined as > 3 mg/ml) performed significantly worse on TMT-A and TMT-B when compared to patients with normal hs-CRP levels (Bulzacka et al., 2016). The same association between worse performance on TMT-A and hs-CRP have been proven in patients with both bipolar disorder and major depressive disorder (Dickerson et al., 2013; Krogh et al., 2014). CRP is known to induce the production of IFN-γ (Van Vré et al., 2008). Therefore, findings of CRP and IFN-γ in relation to psychomotor speed, might be an expression of the same immunological mechanisms. For future research, CRP should be included.

Of the three tests for psychomotor speed, only TMT-A and symbol coding were significantly associated with a cytokine. Although all three tests included in this study measure psychomotor speed to some extent, they differentiate in whether they assess other cognitive domains as well. More specifically, TMT-A tests psychomotor speed and visuospatial abilities, whereas TMT-B also draws on higher cognitive abilities including working memory (Bowie and Harvey, 2006; Crowe, 1998). The symbol coding test measures psychomotor speed and attention (Keefe et al., 2008). In other words, TMT-A could be classified as the purest measure of psychomotor speed of the three. Keeping the differences of the tests in mind, our finding might indicate that IFN-γ is related to psychomotor speed and attention. In the future, including more tests that measure psychomotor speed more solely would be helpful to differentiate this association further (Hubel et al., 2013; Skogan et al., 2018).

After sensitivity analyses excluding patients with delusional disorder and acute transient psychosis, IFN-γ was still associated with TMT-A, but not symbol coding. In these analyses we lost some statistical power, and this might explain the loss of a significant association between IFN-γ and symbol coding. In addition, we found an association between IL-4 and the two tests TMT-A and TMT-B. Although false positive findings are more probable when performing additional statistical tests, the spectrum of schizophrenia patients might represent a different entity. Regarding blood levels of cytokines, a meta-analysis concluded that certain cytokines are trait markers (Miller et al., 2011), and that not all cytokines normalize during treatment (Tourjman et al., 2013).

In general, IL-4 represents a different part of the immune system than IFN-γ (Zhu et al., 2010) Whereas IFN-γ is important for type 1 T helper (th1) cells (Li et al., 2014), IL-4 is classified as part of the th2 response (Zhu et al., 2010). In our study, the association between IL-4 and IFN-γ is inverse, which makes sense considering the underlying biological function of these cytokines. More specifically, IFN-γ had a negative effect on psychomotor speed, whereas IL-4 had a positive effect. To our knowledge, no previous studies investigating the association between cytokines and psychomotor speed in psychosis included IL-4 or other th2 cytokines. However, a few previous studies found increased levels of th2 cytokines in schizophrenia (Borovcanin et al., 2012; Guo et al., 2015), although the results are conflicting (Momtazmanesh et al., 2019).

The majority of tested cytokines were not significantly associated with psychomotor speed in our study. This finding was somewhat surprising as it is contrary to a few previous studies which found an association between several cytokines and psychomotor speed in schizophrenia patients (Frydecka et al., 2015; Goldsmith et al., 2020). Still, our findings are in line with one study which also failed to find any significant correlation between plasma levels of the two cytokines IL-6 and TNF-α, and psychomotor speed in patients with schizophrenia (Hori et al., 2017). Similarly, no association between cytokines (IL-6, IL-10 and TNF-α) and psychomotor speed was found in a group of patients with bipolar disorder (Mora et al., 2019). In a longitudinal study by Krogh et al. including patients with major depressive disorder, only hs-CRP predicted an improvement on TMT-A, but not the cytokine IL-6 (Krogh et al., 2014). However, as mentioned above, none of these studies included IFN-γ or IL-4 in their analyses, making it difficult to meaningfully compare our results. Finally, differences between our and other studies regarding selection and exclusion criteria (i.e. diagnoses, somatic comorbidity, and substance abuse) might also explain different findings.

Interferons are potent cytokines with a major antiviral function (Pestka et al., 2004). IFN-α is commonly used as treatment for chronic hepatitis C together with an antiviral drug (AASLD-IDSA., 2021). When given IFN-α, patients do develop decreased psychomotor speed and depressive symptoms compared to controls (Majer et al., 2008). Another study has demonstrated that patients developing depression after IFN-α treatment have more reduced psychomotor speed when compared to somatically healthy patients with major depression (Capuron et al., 2009). Although IFN-γ and IFN-α are two different classes of interferons (class I and II), they have several similarities and these studies on IFN-α and psychomotor speed are therefore of relevance (Pestka et al., 2004).

On a neurobiological level, reduced psychomotor speed is associated with reduced white matter integrity in several parts of the brain, such as corpus callosum and basal ganglia (Karbasforoushan et al., 2015; Tsapanou et al., 2019; Wang et al., 2020). Such reduced white matter integrity may be induced by inflammation and peripheral cytokines (Benedetti et al., 2016; Najjar and Pearlman, 2015). In a study including patients with bipolar disorder, IFN-γ was associated with reduced white matter integrity in several networks of the brain (Benedetti et al., 2016). Further, IFN-γ is damaging to brain myelin through several mechanisms (Popko and Baerwald, 1999). Most importantly, through inducing apoptosis in oligodendrocytes (Buntinx et al., 2004; Horiuchi et al., 2006). In addition, activation of macrophages and microglia are of significance (Popko and Baerwald, 1999). These effects of IFN-γ on myelin are of importance in the pathology of the demyelinating disease multiple sclerosis (MS) (Kebir et al., 2009). Interestingly, reduction in psychomotor speed (measured by symbol coding and TMT-A) are the most common cognitive impairments in patients with MS (Grzegorski and

Losy, 2017; Storm Van's Gravesande et al., 2019; Van Schependom et al., 2015).

When considering the pathophysiological mechanisms behind altered psychomotor activity, the basal ganglia need to be mentioned. In patients with schizophrenia, Yang et al. found an association between psychomotor slowing and basal ganglia activity (Yang et al., 2004). Following administration of IFN-α, hepatitis C patients do develop psychomotor slowing on neuropsychological tests, which is associated with reduced connectivity in the basal ganglia (Felger et al., 2016). An inflammatory stimulus (i.e. IFN-α or endotoxin) can influence the basal ganglia not only through reduced connectivity, but also alter dopamine metabolism and glutamate levels (Capuron et al., 2012; Eisenberger et al., 2010; Haroon et al., 2015). In fact, this relation between basal ganglia and inflammation might also involve reduced white matter integrity (Benedetti et al., 2016). In other words, it is possible that several pathophysiological mechanisms are important when understanding the link between psychomotor speed and inflammation.

In the current study, cytokines remained stable throughout the entire observational period of 52 weeks. Several studies have shown that some cytokines change from acute states of schizophrenia to more chronic phases (Çakici et al., 2021; Goldsmith et al., 2016a; Momtazmanesh et al., 2019). Still, certain cytokines remain unchanged during the course of schizophrenia, indicating that these cytokines are trait markers (Capuzzi et al., 2017; Tourjman et al., 2013). Importantly, patients from our study were in both acute and more chronic phases of schizophrenia. We did execute linear mixed effects models within the group of drug naïve patients, but here no cytokines affected the performance on psychomotor speed tests significantly (data not shown). The lack of an association between IFN-γ and TMT-A in the drug naïve group, could be due to low statistical power. Also, we did not distinguish between treatment groups, or responder's vs non-responders in our analyses. These are factors known to be of relevance when considering changes in cytokines over time in schizophrenia patients (Momtazmanesh et al., 2019; Mondelli et al., 2015; Tourjman et al., 2013).

In contrast to the cytokines remaining stable, patients improved significantly on tests for psychomotor speed during the study period. This in line with other studies demonstrating that schizophrenia patients treated with antipsychotics improve on overall cognitive performance (Fathian et al., 2019; Gold et al., 1999), and psychomotor speed over time (Hughes et al., 2003; Townsend et al., 2002). In this particular study population, we have found the same improvement in overall cognitive performance (Anda et al., 2021).

There are several limitations that need to be considered in this study. Only IFN-γ was significantly associated with psychomotor speed in the main analyses, and it is largely unclear why none of the other cytokines were associated with psychomotor speed. Considering a few previous studies on the topic, we expected to find an association between psychomotor speed and several inflammatory cytokines. However, as all tests for psychomotor speed changed significantly during the study period, it should have been possible to detect a relation with cytokines if present. The study was not powered for testing associations between cytokines and psychomotor speed and the lack of associations for other cytokines than IFN-γ may be a type II error. Even though correction for treatment groups did not have any effect on the main findings, we do not know from this study if antipsychotics in general influence the association between psychomotor speed and cytokines.

However, the study also has several strengths. Importantly, we used a hypothesis driven approach. An extensive panel of cytokines representing different immune responses were included. Regarding psychomotor speed, we included three validated and well-established neuropsychological tests. Although none of these tests are the gold standard for psychomotor speed, they are all considered sound measures of psychomotor speed. When measuring neuropsychological tests repeatedly, improved scores can be a result of practice effects. However, the tests included in this study is proven relatively stable to such effects (Rodriguez-Toscano et al., 2020). As already mentioned, TMT-A is the purest measure of psychomotor speed of the three, supporting that IFN-γ is associated with psychomotor speed specifically. Further, a longitudinal study design including a relatively large study sample, are also important strengths. Although being a RCT, the study was designed with few exclusion criteria, resembling a real-life sample of patients with psychotic disorders. Finally, we corrected for several potential confounders such as age, gender, BMI and severity of psychotic symptoms.

In conclusions, our findings suggest an association between IFN-γ and psychomotor speed over time. Future studies should elaborate this association further, including both a wide range of inflammatory markers and several tests for psychomotor speed.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2021.100392.

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Appendices

Table A1: Coefficients of variation for serum cytokines

	Intra-assay CVs	Inter-assay CVs
IFN-γ	10.6	9.3
IL-1β	10.5	13.6
IL-10	10.0	4.7
IL-12 p70	9.7	5.9
IL-17a	10.6	3.7
IL-2	10.0	12.9
IL-4	10.1	5.2
IL-6	10.7	11.7
TNF-α	9.6	6.0

Abbreviations: CV; coefficients of variation, IFN; interferon, IL; interleukin, TNF; transforming growth factor

Table A2: Change in log-transformed cytokines from baseline to end point

	Estimates	SE	p-value
IFN-γ	0.042	0.09	0.649
IL-1β	-0.133	0.16	0.398
IL-10	-0.052	0.14	0.701
IL-12 p70	-0.070	0.07	0.289
IL-17a	-0.058	-0.11	0.597
IL-2	0.145	0.09	0.098
IL-4	-0.001	0.09	0.988
IL-6	-0.024	0.14	0.866
TNF-α	0.075	0.10	0.448

Data estimated in linear mixed effect models

Abbreviations: IFN; interferon, IL; interleukin, TNF; transforming growth factor

Table A3: Correlation coefficients (r) between tests for psychomotor speed and cytokines

	TMT-A baseline (n = 98)		TMT-A end point (n =42)		TMT-B baseline (n = 86)		TMT-B end point (n = 40)		SC baseline (n = 90)		SC end point (n = 41)	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
IFN-γ	-0.139	0.174	-0.188	0.234	0.029	0.793	-0.208	0.197	-0.152	0.153	-0.134	0.403
IL-1β[a]	-0.016	0.889	-0.053	0.745	0.106	0.378	-0.177	0.288	0.031	0.794	0.135	0.413
IL-10	0.008	0.940	0.185	0.242	-0.004	0.972	0.121	0.456	-0.090	0.370	0.186	0.244
IL-12 p70[a]	-0.096	0.388	-0.228	0.157	0.081	0.498	-0.253	0.125	-0.074	0.530	-0.078	0.636
IL-17a	-0.045	0.684	-0.171	0.291	0.110	0.353	-0.256	0.121	0.001	0.990	-0.217	0.185
IL-2	-0.021	0.837	-0.077	0.627	0.110	0.312	-0.184	0.256	-0.015	0.890	-0.122	0.449
IL-4	-0.006	0.837	-0.038	0.809	0.139	0.202	-0.103	0.527	0.057	0.596	-0.003	0.983
IL-6	-0.059	0.956	-0.078	0.625	0.129	0.237	-0.163	0.317	-0.037	0.726	-0.010	0.951
TNF-α	-0.070	0.495	-0.120	0.449	0.050	0.650	-0.243	0.131	-0.060	0.574	-0.081	0.613

[a]24 missing at baseline, 5 missing at end point

[b]23 missing at baseline, 5 missing at end point

Abbreviations: IFN; interferon, IL; interleukin, SC; symbol coding, TMT; trail making test, TNF; tumor necrosis factor

Table A4: Estimated effect of log-transformed cytokines on t-scores from test of psychomotor speed in patients with schizophrenia spectrum disorders

	TMT-A			TMT-B			Symbol coding		
	Model estimate	SE	*p*	Model estimate	SE	*p*	Model estimate	SE	*p*
IFN-γ	**-3.059**	**1.48**	**0.042**	-1.303	1.536	0.399	-1.882	1.493	0.212
IL-1β	-0.077	0.68	0.9104	-0.255	0.724	0.726	0.535	0.758	0.483
IL-10	0.638	0.68	0.349	0.521	0.697	0.456	-0.400	0.694	0.566
IL-12 p70	-0.255	1.47	0.863	-1.495	1.588	0.349	0.446	1.467	0.762
IL-17a	-0.451	0.95	0.636	0.782	0.997	0.435	-1.610	1.165	0.172
IL-2	1.172	1.29	0.364	-0.094	1.357	0.945	1.113	1.323	0.403
IL-4	**2.506**	**1.14**	**0.030**	**2.417**	**1.193**	**0.046**	-0.056	1.208	0.963
IL-6	-0.132	0.65	0.838	0.350	0.699	0.617	-0.350	0.725	0.631
TNF-α	-0.594	0.91	0.513	0.385	-0.846	0.385	1.874	1.00	0.065

Data estimated in linear mixed effect models. The models included age, gender, BMI, ethnicity, smoking, study site, antipsychotic drug and PANSS positive score as independent variables. Patients with ICD-10 diagnoses F20, F21, F25 and F28-29 were included. N = 58 at baseline, N = 27 at end-point.
Abbreviations: IFN; interferon, IL; interleukin, TMT; trail making test, TNF; transforming growth factor

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