Exploring Glial Marker Activation and Neuroinflammation in Schizophrenia: A multimodal Approach

By Vilde Brecke

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Supervisor:
Kristiina Kompus, associate professor
Department of Biological and Medical Psychology, University of Bergen, Norway

Co-supervisors:
Helene Hjelmervik, post doc researcher
Department of Biological and Medical Psychology, University of Bergen, Norway

Rune Andreas Kroken, associate professor
Department of Psychiatry, Haukeland University Hospital, Bergen, Norway

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Abstract

Background: Research suggests glial cells of different classes play a central role in schizophrenia pathology. The glial perspective may help to better understand and treat underlying mechanisms. This master thesis investigated hypothesized group differences in activation in glial markers N-acetyl aspartate acid, myo-inositol and choline, with further exploratory analysis to symptom type and severity, peripheral inflammation-markers, and to diffusion tensor imaging (DTI) measures. **Methods:** The glial markers were acquired from magnetic resonance spectroscopy (MRS) imaging and processed with LCModel in four voxel placements. Clinical symptoms were indexed by the Positive and Negative Symptoms Scale (PANSS). Luminex Screening Human-Magnetic assayed inflammation-associated markers CRP and cytokines. The DTI data were processed using FSL and Tract-Based Spatial Statistics. The analysis included seventy-seven schizophrenia patients and controls (total N = 154) matched on age 18-65 (M = 30.23, SD = 10.23), handedness and gender (23,38% female). **The results:** Significant higher overall choline in patients compared to controls and voxel placement interaction effects for NAA in anterior cingulate cortex was found. There were trend-level myo-inositol and group interaction effects on the FA values. The relationship was negative in patients, and positive in controls. The results from the regression models indicated that it is difficult to predict positive and negative symptoms by glial markers as well as predicting glial marker levels by inflammation markers, after adjusting for known moderating factors. **Discussion and conclusion:** The study had limitations and technical issues. The group differences suggest glial dysfunction, which can have implications for understanding and treating schizophrenia.

***Keywords**:* schizophrenia, inflammation, multimodal, glial activation, biomarkers

Sammendrag

Bakgrunn: Forskningsresultater foreslår ulike typer gliaceller som sentrale i schizofreni patologi. Fremvoksende litteratur indikerer at gliaperspektivet kan bidra til å bedre forståelse og behandling for lidelsen. Denne masteroppgaven undersøkte hypotesen om gruppeforskjeller i aktivering i assosierte gliamarkører N-acetylaspartate acid, myo-inositol og choline, med videre utforskende analyser til symptomtype og alvorlighetsgrad, samt til perifere inflammasjonsmarkører, og til diffusjonsvektet avbildning (DTI). **Metode:** Gliamarkørene ble målt med magnetisk resonans spektroskopi-vektet (MRS) avbildning og prosessert med LCModel i fire vokselplasseringer. Kliniske symptomer ble registrert av Positive and Negative Symptoms Scale (PANSS). Luminex Screening Human Magnetic Assay analyserte inflammasjonsmarkørene CRP og cytokinene. DTI data ble prosessert med FSL og Trakt-Basert Spatiell Statistikk (TBSS). Analysen inkluderte syttisyv schizofrenipasienter og kontroller (N = 154) matchet i alder (*M* = 30.23, *SD* = 10.23), håndbruk og kjønn (23.38% kvinner). **Resultater:** Det ble funnet signifikant høyere nivåer av choline i pasienter sammenlignet med kontrollpersoner, og vokselplasseringseffekter for NAA knyttet til anterior cingulate cortex. Det var trendnivåfunn for myo-inositol og gruppe-interaksjonseffekter på FA verdiene. Assosiasjonen var negativ i pasienter, og positive hos kontrollpersoner. Resultatene fra regresjonsmodellene indikerte vanskeligheter med å predikere positive og negative symptomer, samt for prediksjon av glialmarkørnivåer gjennom cytokiner og CRP, etter justering for kjente modererende faktorer. **Diskusjon og konklusjon:** Studiet hadde flere begrensninger og tekniske problemer. Gruppeforskjellene foreslår gliadysfunksjon, noe som kan ha implikasjoner for forståelsen og behandlingen av schizofreni.

Nøkkelord: schizofreni, inflammasjon, multimodal, glial aktivering, biomarkører

Preface and Acknowledgements

This master thesis came about from the joy of the subject biological psychology at UiB, as it introduced me to topic of neuroinflammation and mental health. The field was mind blowing to me, and next the search begun to see if any research was being conducted in Bergen on the topic, and whether I could take part in any way. Research on inflammation and psychosis by Bergen Psychosis Research Group happened to be advertised in a doctor's waiting room, and from there the principal investigators dr. meds Rune Andreas Kroken and Erik Johnsen was contacted. Luckily, I was invited to Sandviken Hospital for a meeting, and from there the current project investigating glial activation and neuroinflammation in the Bergen Psychosis Project 2 (BP2) dataset started.

My deepest thanks to Kristiina Kompus for throughout supervision and encouragement, together with Helene Hjelmervik and Rune Andreas Kroken as co-advisors. Your expertise, insight and helpfulness have been absolutely priceless.

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Schizophrenia and Glial-Inflammation Activation: A brief introduction

Schizophrenia is a severe mental illness compromised by several disease phenotypes mainly associated with an inability to distinguish mind from environment, overall causing heavy disability for those affected. The underlying pathologies and mechanisms remain unclear. Converging evidence from neuropathological, biochemical and genetic studies suggests that glial cells of different classes could be involved in neuroinflammation, and hence could be one of the disease mechanisms playing a central role in schizophrenia pathology. Summing up the evidence, it is evident that the "glial perspective" may help to better understand the disease and identify promoting mechanisms and potential medication targets (Bernstein, Steiner, Guest, and Bogerts, 2014). Several studies and meta-analysis on inflammation mediators in schizophrenia is concluding with increased levels of cytokines that may reflect glial cells microglia and astrocyte activation in the central nervous system (Rothermundt et al., 2007). Inflammation is in the past decades increasingly investigated in association to schizophrenia as inflammation is increasing suggested to be a moderating link between genes and environment. Identifying schizophrenia-related biomarkers could aid earlier diagnosis and guide targeted personalized therapies. This project investigate the relationship of glial markers in brain in schizophrenia, and to inflammatory-associated markers in peripheral blood, as well as explore the relationships of the markers with positive and negative symptoms.

Schizophrenia

Schizophrenia has identifying features of positive and negative symptoms, yet is a heterogeneous population in terms of manifestation, course and duration, with a range of indicated mediators and potential origins in manifold. According to World Health Organization, it ranks top 10 worldwide disability and counts 3% of global burden (World Health Organization, 2015). Remaining an unresolved problem as cognitive deficits makes living with schizophrenia highly disabling, and one of the gravest mental diseases among young adults, intense effort is made to relive the affected (Wieronska, Zorn, Doller, and Pilc, 2016).

Regarding the manifestation of schizophrenia, the positive symptoms at core entails lost contact with reality, i.e. delusions and hallucinations. The most common type of hallucination involves hearing voices, and also see and smell other things that are not here. Delusions could be exemplified in people think they are someone famous or have special powers, or that they are being surveilled or foresee terrible future. The negative symptoms expresses as impaired motivation, social withdrawal and reduction in spontaneous speech, together with poorer cognitive performance compared to controls (Joyce and Roiser, 2007). The communication may not make sense or be meaningless, or give answers that are unrelated to the questions asked. Overall, cognitive impairment is key as it concerns thought, perception and memory, all found significantly aberrant in patients. "Cognition" as a term is understood as the ability to process information (Latin = to know, recognize) (Mitterauer, 2011), which is clearly impaired as the typically most characteristic symptom in the illness is the failure of differentiating between inner and outer world. Cognitive function has underlying mechanisms in a complex relation set between networks, potentially driven by various neurotransmitters, glial cells and neuromodulators (Miller et al., 2010; Miller and Goldsmith, 2017).

The term "schizophrenia" itself refers to a "*splitting of mind*" in Bleuler's original terminology (Bleuler 1950 cited in Phillips et al., 2003). Since its first description, the core features of split between thoughts (cognition) from feelings (emotion) together with flattening affect (anhedonia) is core to the experience (Bentall, 2013). Poor social skills and misinterpretation of social cues as revolving around the person instead for neutral and unrelated circumstances is often reported. Before onset, usually in early adulthood or late childhood, individuals are often considered normal except reports of vague experiences of own self and thoughts (Henriksen and Nordgaard, 2014). Increasingly difficulties with forming and understanding clear ideas, distinguishing relevant and irrelevant information and time conception are distorted (Cameron, Robertson and Nordahl, 1992). These are examples of cognitive symptoms, as it reflects lack of attention and memory (Silver and Feldman, 2005), and overall could be referred to as a "thought disorder". In the behavioral aspect, people may make strange postures or unpredictable actions. Furthermore, the lack of differentiating objects and individuals in environment from oneself, as patients experience what is taking place in the brain as real; it could be referred to as a loss of conceptual boundaries (Mitterauer, 2011). Overall, schizophrenia as a disintegration of the experience of self is a conceptualization shared among many researchers (Kean, 2009; Postmes,

Sno, Goedhart, Van Der Stel and Heering, 2014). Living with distortions are highly debilitating to the patients. The illness affects the ability to engage with others in socially acceptable ways, and to take care of themselves. As cognitive and negative symptoms precede positive manifestation, studies are aiming to entwine the distinct underlying mechanisms of the clinical manifestations.

Due to the heterogeneity in the population and the likelihood of several causing factors, one can speak of schizophrenia as a syndrome. Current research sums the disease to be statistically heritable, yet non-Mendelian, with possible roots in neurodevelopment deficits and epigenetic dysregulations of the brain genome playing a fundamental role in the course and manifestations of the disease that will be further discussed. Emerging models propose a disruption of the main systems in glial-neuronal interactions (Mitterauer, 2011), and research on neuroinflammation is promising for identifying potential mediating processes at work in schizophrenia.

Diagnosis and treatment.

Among the tools for assessing severity and presence of schizophrenia-associated symptoms, the positive and negative symptoms scale (PANSS; Kay et al., 1991) measures, in addition to the former mentioned characteristic positive and negative symptoms, also more general psychopathic themes like somatic concern and depression, as well as items addresses agency in the patients. Most patients experience thoughts to appear automatically and not coming from oneself. Furthermore, as diagnosis is currently relying merely on interview, which is often critiqued to be prone to subjectivity, more objective assessments like the use of biomarkers, is warranted (Horváth and Mirnics, 2014). Moreover, there is a great heterogeneity in symptom manifestations; it could be difficult to distinguish from other mental illness, such as bipolar disorder (Lichtenstein et al., 2009).

As of currently, there seems to be no cure for schizophrenia (Piltman, 2018), yet many find relief in medication, however, some patients are treatment resistant (Kroken et al., 2014). Concomitant treatment, like cognitive behavioral therapy and social skills training together with antipsychotic medication is suggested among the most optimal interventions as of now. Most patient has a lifelong diagnosis with poor prognosis where most relapse after a few years (Johnsen, Kroken, Wentzel-Larsen and Jørgensen, 2010), with interindividual variations in remission and relapse.

Regarding medical treatment, theories of the pathophysiology underlying schizophrenia have centered on neurotransmitters and their receptors, and therapeutic drug development has largely targeted dopamine, serotonin and glutamate systems (Kroken et al., 2014). Antipsychotic drugs remains as the pillar in the treatment, yet has small effect sizes in the cognitive domain (Bruijnzeel, Suryadevara, and Tandon, 2014), with the primary effect in reducing the positive symptoms. With short-term effectiveness, the vast majority of the patients experience decline in improvement along with increase in side effects (Johnsen, Kroken, Wentzel-Larsen & Jørgensen, 2010). Certain drugs, especially clozapine and olanzapine, are reported to give adverse metabolic effects such as weight gain, hyperglycemia and hypertriglyceridemia (Johnsen et al., 2010, Kroken et al., 2014). It is suggested that the elevated mortality within the patient group could be due to side effects contributing to physical illnesses, like cardiovascular diseases, diabetes and cancers (Cullen et al., 2012). Furthermore, medication is found to have detrimental effects like brain volume loss as indicated by a longitudinal volumetric study, indicating heavy influence by medication dosage (Ho, Andreasen, Ziebell, Pierson and Magnotta, 2011). Hence, identifying alternatives is of great interest.

Brain abnormalities in schizophrenia.

Several brain abnormalities are associated with schizophrenia. The functional deficits in schizophrenia suggests abnormalities in the brain as it parallels with evidence of dysconnectivity revealed by fMRI and DTI, together with abnormal structures such as enlarged ventricles as indicated by sMRI (Elkis, Friedman, Wise and Meltzer, 1995). Another line of research that includes post-mortem and genetic studies has demonstrated myelin-related abnormalities in schizophrenia, which further suggests not only functional but also anatomical disconnection between brain regions (Hakak et al., 2001). The correlation between the typical onset in teenage years or early adulthood and maturation of glial cell-derived myelin fits with the idea of faulty brain trajectories that manifests when conduction velocity is comprised (Fields, 2008).

Characteristic symptoms of schizophrenia like auditory hallucinations is one example of a symptom that is running parallel to myelin and white matter condition. White matter is hence suggested to be a potential reliable biomarker of schizophrenia. Biomarkers Definitions Working Group (2001) defines biomarkers as a characteristic that objectively measured and for them to be

evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological response to an intervention. Genes, gene expression products (transcripts and proteins) and metabolites are the main biomarker families (Rodrigues-Amorim et al., 2017.). In schizophrenia, aberrant EEG signal (e.g. Kompus et al., 2015), and structural and functional abnormalities indicated by MRI as previously mentioned are among the previously suggested biomarkers.

It is implied that brain abnormalities are among the underlying reasons for auditory hallucinations. Hearing voices is not exclusive to schizophrenia yet is the most characteristic feature occurring in 70% of patients (Hugdahl, Løberg & Nygård, 2009) and remain the most researched symptom. Auditory hallucinations (AHs) are also reported in mood disorders, personality disorders, post-traumatic stress disorder as well as in the general (non-clinical) population (Water, Blom, Jardi, Hugdahl and Sommer, 2018). Interestingly, the content and form of the auditory verbal hallucinations appear similar, however the response varies considerably with emotional valence being found as a predictor of functioning (Daalman et al., 2011). A difference in mean age onset for auditory hallucinations (AH) between healthy (12.4 years, SD = 13.6) and psychotic patients (*M* = 21.4 years, *SD* = 11.7) has been found (Dalmaan et al., 2011). This might be indicative of a difference in etiology within schizophrenia patients, something that have been interpreted by some authors to imply sub-types in the syndrome (Geisler et al., 2015). Synaptic density peaks during childhood, followed by extensive decrease of neuronal connectivity (pruning) during adolescence. Hence, the younger onsetters could be understood as a manifestation of maximal synaptic density, while the schizophrenia-related AHs are associated with aberrant synaptic connectivity obvious after myelin maturation. Such interpretations could aid in the differentiation of subtypes when similar manifestations occur.

In studies with children with early onset, not only did the subjects cross threshold of behavior, but abnormal evidence apparent also on brain imaging level (Hoffman & McGlashan, 2001). One study found abnormalities on a brain level in particular in superior temporal gyrus (STG) and dorsolateral prefrontal cortex (DLPFC) (Thompson et al., 2001). Findings like these have been interpreted as compelling evidence for the condition to be a brain disorder, in which behavior is the last thing to change (Insel, 2015). Hence investigating the biological architecture is a goal among researchers and research groups (e.g. NORMENT).

The Glial Perspective

Glial cells, inflammation and schizophrenia.

Structural, molecular and functional changes in glial cells is of interest as studies are suggesting abnormalities in all three types of glial cells (Bernstein et al., 2014). Exploring the immune signature in schizophrenia could aid for a better explanation, diagnostics and treatment. In degenerative and inflammatory illnesses, huddles of glial cells are found around malfunctioning neurons, which may either work as protectors and aid in restoration, or be neurotoxic when over-activated without stopping (Block, Zecca and Hong, 2007). Brain imaging and genetic analysis have yielded a wide range of glial cell-associated white matter abnormality data in schizophrenia (Takahashi, Sakurai, Davis and Buxbaum, 2011). White matter consists of myelin, which is produced by oligodendrocytes, one out of three major types of glial cells. Overall, neuroscience research suggest schizophrenia as a dysconnectivity syndrome, and as white matter forms connections between brain regions, and impaired connectivity indicated by reduced fractional anisotropy is a typical finding (Kubicki, Westin, Pasternak and Shenton., 2005), the role of glial cells seems pivotal. Glial cells have functions far beyond merely myelin production, like microglia's ability to produce pro- and anti-inflammatory reactions. An increasingly large body av research have found microglia activation in schizophrenia patients differing from controls, contributing to the formulation of a presence of low grade inflammation, or the "mild encephalitis" hypothesis as proposed by Bechter (Bechter, 2013). Several factors are identified to moderate and mediate schizophrenia, yet the majority of people do not develop schizophrenia despite being exposed to risk factors. Hence, the illness is interpreted to be mitigated by interactions across genetics, epigenetic and environmental risk factors (Miller et al., 2012).

Immune functions.

Anomalous immune function is increasing hypothesized involved in the pathophysiology of schizophrenia and could be of paramount importance as the mechanisms could be the missing link between genetic disposition and environmental factors, and its biological manifestations (Watanabe, Someya and Nawa, 2010). Increasing focus has been given to inflammation mechanisms the past decades, both genetic and environmentally derived, in particular during embryo state (viral infections, lack of nutrition, drug use) and trauma during birth such as neonatal

infections as well as hypoxia and autoimmune diseases (Brown, 2011). The presence of inflammation markers is demonstrated in peripheral blood, in cerebrospinal fluid (CSF), as well as in white and grey matter in schizophrenia (Najjar and Pearlman, 2015).

Inflammation is the complex biological response of inflammatory cells to pathogens, damaged cells or irritants. When inflammation occurs stereotypically as a response, it is referred to as the innate immunity in contrast to adaptive immunity that is specific to each pathogen, as in the example of vaccination against specific diseases. Both the acute response to damaging stimuli and the chronic one gives progressive change in the types of cells present at the location of inflammation, which is characterized by both destruction and healing of tissue (Horváth & Mirnics, 2014). Sources to inflammation could be environmental such as virus and bacteria, nicotine, pollution and obesity (Prasad, Tyagi, & Aggarwal, 2016), yet it also indicated in psychological sources such as stress, both short-term and prolonged (Watanabe et al., 2010). It is a known fact that the immune system is activated during normal stress response to prepare the body for being most adequate for action, which is a positive and normal response, however, it could become pathological if the response is not "switched off". However, there are also instances where the immune system is activated targeting the body itself, as in autoimmune disorders like HIV. However, reviewers are pointing to the nuance that the inflammation in schizophrenia is modest in comparison to inflammation pathologies like the example of HIV and rheumatoid arthritis (Serhan & Savill, 2005), and hence is often referred to as "low-grade" inflammation.

The Glial Cell Classes.

Each of the three glial cell classes - oligodendrocytes (OC), astrocytes and microglia are all found to confer a unique contribution to the pathophysiology of schizophrenia. Glia, or neuroglia, are non-neuronal cells engaged in the inflammation system and functions in maintaining balance in the body (i.e. homeostasis), forming myelin and support and protect neurons (Kroken et al., 2012).

Oligodendrocytes.

In schizophrenia, several studies are concluding that white matter is abnormal, supported by findings of lacking more oligodendrocytes than in normal in several brain regions. Myelin is the main component in white matter and is produced by oligodendrocytes. Myelin is essential for

the quality of conduction along the axons, synchronizing brain signals and contributing to synapse plasticity (Fields, 2008). Few of the developmental processes continues into adulthood, yet myelination is an exception. The development is later completed in women, a phenomenon that also is parallel in the gender differences in the onset of schizophrenia-related symptoms (Häfner, 2003). The temporal correlation of completion of myelination and disease onset suggests the debut to reflect maturation of myelinated tracts and manifests in misconnected networks (Davis et al, 2003). Studies are indicating underlying differences in onset of schizophrenia: the earliest onset cases are found to have more severe white matter abnormalities in contrast to the more regular early adulthood onset (Douaud et al, 2007; Szeszko et al., 2008) while less white matter changes were reported in late-onset (Jones et al., 2005). These findings might be the cue to the great heterogeneity in the pool of schizophrenia as there might be important differences among clinical subgroups that may manifest as different white matter pathology (Chen et al., 2013).

Fractional anisotropy (FA) is one of the most common indices of white matter representing the degree of spatial coherence in the fiber tracts. The FA value is altered by changes in the microstructure caused by for instance demyelination and inflammation (Alexander, Lee, Lazar and Field, 2007). One of the strongest supports for schizophrenia as a dysconnectivity syndrome stems from the replication of reduced FA in frontal and temporal lobes and in the fiber bundles that connect those (Roalf et al., 2013). However, the reduction is not limited to those areas, but also reported in parietal and occipital regions, which has been overall interpreted as global white matter alteration in schizophrenia (Roalf et al., 2013).

Astrocytes.

Astrocytes are actively controlling neuronal activity and synaptic information transmission (Mitterauer, 2011). They play a crucial part in supplying neurons and OCs with content for energy metabolism as well as a range of functions such as regulating neurotransmitter release, modulating the immune response and expressing neuromodulators (Sofroniew and Vinters, 2010), and several of astrocyte genes are found altered in schizophrenia. Furthermore, astrocytes is also implicated in the known dopamine dysregulation, and medication is thought to influence a hypothesized disturbed astrocyte metabolism (Kondziella, Brenner, Eyjolfsson and Sonnewald, 2007).

Astrocytes and oligodendrocytes: partners in crime?

There are overlaps in myelin deficits and neurotransmitter alterations. The role of glial cells is of pivotal importance for neuronal migration and synaptic functions such as glutamatergic and N-methyl-D-aspartate (NMDA) regulation, and abnormalities in such is indicated caused by astrocyte dysfunction (Bernstein et al., 2014). Their dysfunction could have detrimental impact such as reducing neuronal size, reducing levels of synaptic proteins as well as abnormalities in neurotransmission and functional dysconnectivity. Alterations in dopaminergic transmission as well as the glutamatergic system has long been investigated and implied in the disease's pathology. The observations of behavior similar to positive symptoms in schizophrenia induced by psychoactive drugs such as cocaine and amphetamines affecting glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonistic dopaminergic activity on these D2 dopamine receptors was the origin to the neurotransmitter imbalance hypothesis of dopamine and glutamate (Snyder, 1973 cited in Kolb and Whishaw, 2001). Further neurochemical conceptualization stems from the effects of glutamate receptor antagonists like phencyclidine (PCP) and ketamine producing both positive and negative symptoms, giving rise to the glutamate hypothesis, which stands in complement to the dopamine hypothesis (Gilmour et al., 2012). Altogether neurotransmission and tissue ties in loop as it is found that when glutamate is in excess it is damaging to myelin, (which is one suggestion to the abnormalities observed and progressive worsening (*Walterfang et al., 2011*).

Microglia.

Microglia cells are the predominant macrophages in the brain, making up 10% of the brain (Wood, 2003), executing three different morphologies of resting, activated or phagocytic state (Doorduin et al., 2005). Activated microglia are core to neuroinflammation for their role in removal of damaged tissue and infectious agents. Beyond phagocytosis, activated microglia also stands for cytokine production and is involved in the kynurenic acid (KYNA) pathway, which is connecting to serotonin and glutamate neurotransmitter systems (Kroken et al., 2014), two neurotransmitters traditionally important in the conceptualization and treatment of schizophrenia.

It is argued that microglial activation can distort neurotransmission, and hence be a source of psychotic symptoms (Steiner et al., 2013). Subtracting for such suggestions, their relation to PANSS symptoms index is indicated as likely and an interesting field to explore. The mechanisms behind the symptom manifestation and glial activation is suggested to lie in how microglial-

derived cytokines IL-1beta and IL-2 both have the ability to modulate catecholamine levels in the brain (Labuzek et al., 2005). Hence, microglia is important in neuronal protection as a reactant to pathogens, however, also in pathology when not deactivated (Block et al., 2007). Overall, microglia is the primary source of pro- and anti-inflammatory cytokines, which makes microglia a key player in neuroinflammation with the ability to produce or mediate a wide range of cellular responses (Kraft and Harry, 2011).

Cytokines.

The cytokine hypothesis of schizophrenia is deriving from a range of studies indicating abnormalities in the cytokine network, which might contribute to the neurodevelopmental and neurodegenerative findings previously presented in this master thesis.

Cytokines are key signaling molecules acting as regulators of acute and chronic inflammation, exerting their effects in the periphery and brain. This makes them a main connection between the central nervous system and immune system (Kubistova, Horacek, and Novak, 2012). Their ability to cross talk between the brain and immune system have be interpreted to highlight a gene-environment interaction in schizophrenia (Maric and Svrakic, 2011). Cytokines are deriving from both immune and nonimmune cells, binding to specific receptors on a range of target cells. When proinflammatory cytokine family, like interleukin (IL)-1beta, IL-6, and tumor-necrosis factor alpha (TNF-alpha) are activated, they facilitate vascular permeability and promote release of mediators that are a part of the complement system of immune functions. Under normal conditions, inflammation is controlled by homeostatic mechanisms. However, dysfunction in the feedback mechanisms that identifies whether the triggering processes is removed which further allows for anti-inflammatory repair process, leads to persistent inflammation. Dysfunction as such is observed in illnesses like rheumatoid arthritis, multiple sclerosis and Crohn's disease (Serhan & Savill, 2005). The low-grade inflammatory response observed in schizophrenia, both in central nervous system and in peripheral blood is suggested to be a result of disrupted blood-brain barrier (BBB), which is an important protector against pathogens, are is found important in neurodevelopmental and neurodegenerative diseases like the former mentioned, and is also found indicated aberrant in schizophrenia (Stolp and Dziegielewska, 2009).

Of the anti-inflammatory mechanisms, IL-10 is the hallmark of high antibody activity, also referred to as type 2 immunity (Spellberg & Edwards, 2001). Type 1 is high the phagocytic activity, which is suppressed by IL-10 and other t-helper type 2 cells. In immunosuppression or

severe systemic stress, the immune system responds with a Type 2 reaction. In other brain disorders such as formerly mentioned MS as well as Alzheimer's disease, immune cells is thought to cross the blood brain barrier (BBB) into brain tissue and when unregulated/dysfunctional, is found damaging. Microglia cells produce mainly type 1 immune response in the form of secreting cytokines like IL-6, while astrocytes produce type 2 cytokine IL-10 (Bernstein et al., 2014).

The direction of expression of the cytokines are not completely consistent within the literature. Overall, IL-1beta, IL-6, IL-10 and TNF-alpha, are among the cytokines found abnormal, however, their direction varies. TNF-alpha was found elevated in two studies both for chronic medicated patients and for first-episode unmediated patients (Boyajyan, Zakharyan and Khoyetsyan, 2012; Drexhage et al., 2010). However, other studies have found a decrease and others again found no change (Davison et al., 2016).

Furthermore, studies are indicating links between different markers and symptoms. For instance, IL-6 is in particular found associated to sustained attention (Holden et al., 2011 cited in Meyer et al., 2011). IL-12 and TNF-alpha is found elevated throughout illness duration, which is proposed by Miller and colleagues (2011) to be indicative as trait markers. TNF-alpha is thought to contribute to schizophrenia in its activation of the hypothalamic-pituitary-adrenal (HPA) axis as well as neurotoxic release of glutamate (Himmerich, Berthold-Losleben and Pollmächer, 2009).

Overall, it is generally suggested that member of the cytokine network may contribute to the pathogenesis of schizophrenia. In a meta-analysis, 40% of patients were found to have some form of inflammation (Osimo, Cardinal, Jones and Khandaker, 2018). Furthermore, in a summary of 99 studies, it was found that in 50% of the included studies, IL-6, TNF-alpha, and IL-1beta, was found to differ between patients and controls (Rodrigues-Amorim et al., 2017). Importantly, the identified changes are small, in particular in comparison to higher-grade inflammation like autoimmune disorders, again underlining schizophrenia as a low-grade inflammation.

CRP.

As a regularly accepted and applied biomarker for acute phase inflammatory response, C-reactive protein (CRP) has been used for diagnosing, monitoring treatments and progression in post-surgical situations (Kroken et al., 2014). CRP is synthesized in the liver as a main reactor to tissue damaging processes (Fathian et al., 2019). Cytokines IL-1beta, IL-6, and TNF-alpha among others, are indicated as main stimulators for the production of CRP and other acute-phase proteins (Wigmore et al. 2011, cited in Zakharyan and Boyajyan, 2014). In past years, it is found an inverse

relationship between CRP and cognitive performance in schizophrenia patients (Johnsen et al., 2016), and is consistently found elevated even in some studies across illness duration irrespective of medication status (Fernandes et al., 2016). One study suggested that IL-6 and CRP impair anisotropy in certain fiber tracts that are repeatedly found aberrant in schizophrenia patients, like inferior longitudinal fasciculus, and that the variation of the impact from the immune mediators suggests differences in their effect across the fiber pathways (Prasad, Upton, Nimgaonkar & Keshavan, 2014).

In conclusion, inflammatory mechanisms makes a healthy reaction yet may be a detrimental contribution to pathology, as proinflammatory cytokines plays a part in both neurogenesis and synaptic transmission, as well as cell death. The latter activation is found to have adverse effects, which manifests as the characterizing factors of several neurological disorders, such as in multiple sclerosis where myelin in destroyed (Hemmer, Kerschensteiner & Korn, 2015).

Biomarkers of glial cell activation.

Recent studies expand the research on glial cell markers by investigating in the brain in vivo with the aid of proton magnetic resonance spectroscopy (h-MRS). Studies suggest that neuroinflammatory disorders are related to elevated glial markers such as myo-inositol (mI) and Creatine and Choline, while concentration of neuronal metabolites like Glutamate and N-Acetyl aspartate Acid (NAA) are reduced (Reid et al., 2010). However, it is important to take note that normative metabolite concentrations are dependent on important clinical variables, such as age and gender, and there are individual differences (Chang et al., 2013). For instance, normal brain aging is associated with increased neuroinflammation, which in turn may lead to higher levels of glial metabolites like mI and Creatine in certain regions. This highlights the importance of matching the subjects in studies with MRS on age, gender and voxel placements in the brain regions. Three metabolite markers, choline, NAA and mI, are of particular interest.

Being the strongest in concentration in glia cells compared to neurons, choline is regarded in the research field as a glial cell marker. Choline is associated with inflammation in the light of the presence of more choline in glial cells which activation in triggered by inflammation and hence more choline must mean increased glial cells, which produce inflammatory mechanisms (Bernstein et al., 2014). Choline in the brain is higher than in plasma, but is dependable on the plasma concentration as the latter in influenced from and to peripheral organs. When cholinergic

neurons are activated, certain kinds of nutrition can increase acetylcholine release (Ross et al., 2010). Choline could also be understood as a marker of cell density, and reflect changes in phospholipid membrane formation (Chang et al., 2013). In general, studies have found higher choline in patients compared to controls (e.g. Plitman et al., 2018).

When glia are activated the cell volumes are enlarged which tend to correlated with elevated myo-inositol (mI) (Kantarci et al., 2008). mI is involved in maintenance of cell osmolality and phospholipid metabolism (Moore et al., 2000) and is evaluated as a glial cell marker due to higher expression in glial cells than neurons (Mahli et al., 2002). mI is further associated with aging, in that it increases with time. In neuroinflammation mI is increased and this might be taking part in hypomyelination, similar to the processes in MS. Decreased mI was found correlated to depressive symptoms which have led researchers to interpret it as a biochemical marker for such (Chiapelli et al., 2015). Otherwise, a potential target as administration have shown to improve mood in healthy volunteers (Moore et al., 1999).

N-acetyl aspartate acid **(**NAA) is found decreased in demyelination diseases such as multiple sclerosis (MS) and is associated with axonal injury (DeGraaf, 2011). Findings of reduced NAA in schizophrenia in medial temporal regions together with reduced anisotropy index is interpreted as lowered connection and myelination in axonal bundles (Schneiderman et al, 2007), and is therefore indicated as a glial marker. NAA is reckoned as a marker for neuronal viability and integrity, however it can be misleading to conceptualize is as structural marker (Reid et al., 2010). Due to its ability to recover should rather be viewed as a surrogate marker of neuronal health and dysfunction, rather than loss (Dwyer et al., 2018). In another study, specifically ACC was found to have lower levels of NAA compared to healthy controls (Reid et al., 2010). Overall is it thought to be more stable in healthy subjects with very low turnover and in general is prone to regional and developmental variations that might correlate with mental function (DeGraaf, 2011).

Models on glia and inflammation in schizophrenia.

There are several popular models on glia and neuroinflammation activation in schizophrenia. One model presents that synapses with non-functional astrocyte receptors can lead to uncontrolled synaptic information flux as no neurotransmitter can communicate to the receptor (Mitterauer, 2011). This model argues that it may cause a generalization of information processing

that may lead to manifestation of thought disorder such as hallucinations and delusions. It points to how the brain is unable to process information into categories when the oligodendrocyte-axon system is faulty.

Another proposed model on the characteristics of inflammation occurrence in schizophrenia postulates how abnormal expression of the inflammatory genes produce peripheral inflammation caused by either stress or pathogens (Meyer et al., 2011). The outcome of microglia-activated astrocytes releasing cytokines such as IL-6 and IL-10 that further leads to KYNA production, which block signaling to the NMDA receptor. Meanwhile microglia release cytokines IL-1beta and TNF-alpha, which ultimately promote production of neurotoxic substances. Further, the model proposes that irregular hypothalamus-pituitary-adrenal (HPA) axis function subsidize the inflammation, as there is a malfunction in the feedback-system. Pro-inflammatory cytokines has the power of mediating the activation of the axis, and this signaling is found to impair affective, emotional and social functions (Dantzer et al., 2008). This is expressed as for instance as flattened affect.

Takahashi and colleagues (2011) propose in their model that alterations in oligodendrocytes and subsequently myelin induce hyper-dopaminergic states in frontal lobes (Takahashi et al., 2011). In normal states when an inhibitory neuron receives enough glutamate input, it release a sufficient amount of GABA to inhibit excess dopamine release at dopaminergic terminals. However, if the communication neuron has unmyelinated axons, the inhibitory neuron fail to release the correct amount of GABA and fails to inhibit the excessive dopamine release. Another model could explain why typical antipsychotic medication is effective on reducing the positive symptoms. Ren, Wang and Xiao (2013) reviewed the effects of both typical and atypical medication like, quetiapine and olanzapine, and found them to promote the maturation of oligodendrocyte, which subsequently can restore myelin. This contributes to the restoring of the hyperactivity of dopaminergic neurotransmission.

Identifying Biomarkers in Schizophrenia with Imaging Methods

Many techniques are available to investigate brain chemistry, but most are indirect measures and only shows a fraction of the potential contributing factors of the molecular, structural and functional components (Goff et al., 2016). To with accuracy be able to identify an individual based one biomarkers could be a critical agent in identifying the disease state, identifying factors

contributing to underlying progression and predicting and monitoring response to treatment (Goff et al., 2016).

Multi approach complementary techniques are increasingly being applied for research on schizophrenia for a fuller understanding of the underlying pathophysiology. Cell firing as in action potential can be studied to give information on how cells are connected, yet the chemical changes is also crucial for understanding cell communication. No technique measure the large number of chemical signaling, as there are discrepancies in time and length scales. For instance, magnetic resonance spectroscopy (MRS) can measure biochemical profiles in the brain, whereas diffusion tensor imaging (DTI) is more sensitive to white matter structural differences. Further methods includes more traditional Structural magnetic resonance imaging (sMRI) revealing brain anatomy while functional magnetic resonance imaging (fMRI) highlights connectivity.

Magnetic resonance spectroscopy.

Magnetic Resonance Spectroscopy (MRS) is a non-invasively technique assessing the chemical metabolism/environment within a certain region of interest. While MRI identifies the anatomical location/tissue structure, MRS can compare the chemical composition of normal brain tissue to abnormal (DeGraaf, 2008). MRS exploit the magnetic properties of the hydrogen proton. The surroundings of the hydrogen proton(s), i.e., the molecule in which the proton is bound, influence its magnetic properties. This allows differentiation and identification of signals from different molecules. There are several metabolites, or products of metabolism, to evaluate. The frequencies of these metabolites are measured in units called pars per million (PPM) and can be visualized in a graph as peaks of varying heights (DeGraaf, 2011).

In order for the signal to be detected, pulse sequences creates magnetization in the transverse plane. There are two techniques mostly applied; PRESS (point resolved spectroscopy sequence) and STEAM (stimulated echo acquisition mode). The PRESS is most preferred for longer echo times and at field strengths of 3 T or lower, whereas STEAM are preferred for higher field strengths. Many metabolites may be measured with short echo via PRESS; however, some compounds are hard to disentangle due to low biological concentration and significant overlap in spectral profiles with other signals that are stronger at the same frequency (Dwyer et al., 2018). GABA is one of such, which then required the application of MEGA (Mescher-Garwood) spectral editing implemented to the PRESS giving MEGA-PRESS. To highlight some of the metabolites

available via MRS, there are neuronal markers like NAA and Glu, glial markers mI and Choline, and cell energy marker Creatine as well as inhibitory y-aminobutyric acid (GABA) (DeGraaf, 2011).

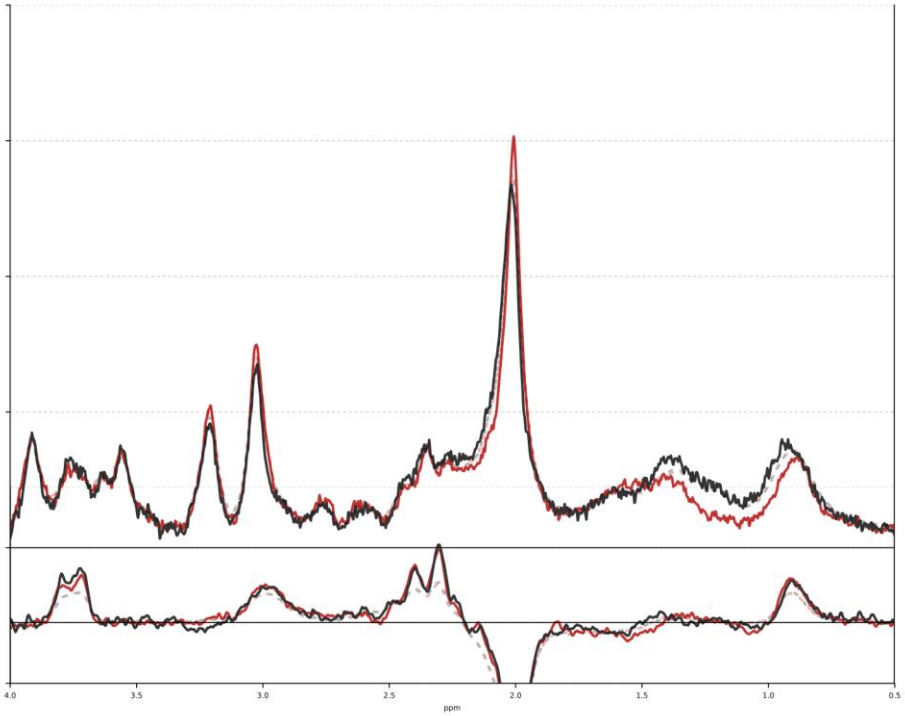

Figure 1*: Example of H-MRS spectra, taken from ACC in the current sample. The top line is the PRESS measures. The most pronounced peaks are from left to right: choline, creatine and N-acetyl aspartate acid (NAA) (red: patients, black: controls). The x-axis represents the unit parts per million (PPM) and the y-axis represents the various magnitude of the peaks. The lower line represents the MEGA-PRESS sequence.*

The x-axis values represents "chemical shift": the frequency of the received signal, as a value relative to what is called Larmor frequency and is expressed in parts per million (ppm). The different metabolites has different peaks, as seen in table 1 and in figure 1. NAA is the most pronounced peak. The y-axis indicated the intensity of the signal produced. The intensity is related to the concentration of the signal in question, yet the relation varies between the metabolites. For instance, the NAA peak at 2 ppm as seen in the figure is four times higher than the 3.55 ppm of mI, but it does not mean that it is four time as much of NAA than mI (Craven, 2018, unpublished).

Most of the signal from proton is in water (DeGraaf, 2011). The water signal is suppressed in the MRS sequences, which makes it easier to identify the weaker of the metabolite signals

(Gasparovic et al, 2006). Correction of cerebral spinal fluid (CSF) is required as well as correction distinguishing grey and white matter as the metabolite concentrations are found to vary with tissue type (Ernst et al., 1993 cited in Chang et al., 2013). Another important methodological factor is the use of reference marker as quantification typically relies on comparison to a reference signal (Dwyer et al., 2018). Cho or tCro is often used, however, they are both found to vary with age and stage of illness, which therefore might lead to drawing wrongful conclusions in the evaluation of spectral data/measures (Jansen et al., 2006 cited in Dwyer et al., 2018). Creatine is another typically used as internal reference as it has been considered a more stable metabolite. However, it has recently been found subject to gender effects, with particular variations with menstrual cycle (Hjelmervik et al., 2018). In this study, the authors point to how other studies use creatine as reference, yet the variations over the menstrual cycle is so prominent that it has important methodological implications for the use of creatine as reference, as uncritical use might bias results (Hjelmervik et al., 2018).

Table 1: *Typical resonances in ppm and concentration range in mmol/L for the glial marker metabolites and creatine (taken from DeGraaf, 2013)*

NAA:	2.0 PPM	7.5-17 mmol/L
Choline:	3.2 PPM	0.5-2.5 mmol/L
Myo-inositol	3.5 PPM	4-9 mmol/L
Creatine:	3.0 PPM	4,5-10,5 mmol/L

In conclusion, a force of researchers warrant further investigation of glial activation and neuroinflammation as altered neuro metabolite levels could be of clinical importance.

Assessing microstructure with diffusion tensor imaging.

Diffusion Tensor Imaging (DTI) can be applied as a method for characterizing changes or differences with neuropathology and treatment as it is highly sensitive to changes at the cellular and microstructural level (Alexander et al., 2007). DTI detects the directional movement of water molecules to image nerve fibers in the brain, and the preprocessed image provides a reconstruction of water movement along axons that should correspond to actual fibers (Kolb and Whishaw, 2001). The direction of the water movement is detected by a coil and interpreted by a computer, which provides images that easily detects abnormalities in neural pathways.

Two quantitative measures can be obtained from DTI: anisotropy indices and tract fiber orientations. Fractional anisotropy (FA) measure the amount of coherence of water diffusion, which reflects the amount of myelination in axonal bundles. Decreased anisotropy is interpreted as loss of integrity. In a study, Seok, Kim and colleagues (2007) found positive correlations between hallucinations and FA within the superior longitudinal fasciculus, which includes the arcuate fasciculus. Note there are regular changes in FA during life span. Studies in normal ageing have uniformly shown decreased FA in late adulthood, predominately in prefrontal, temporal, parietal lobes and in the corpus callosum (Sullivan et al., 2006 cited in Seok et al., 2007), which are also the areas of last myelination as well as most vulnerable to myelin breakdown as consequence of normal ageing (Bartzokis et al., 2004 cited in Seok et al, 2007).

Tract networks.

In a previous study, particularly two anatomically and functionally connected networks were implicated in schizophrenia (Nestor et al., 2004). The frontal-temporal network includes two cortical regions, inferior frontal and anterior temporal areas, and these are connected by the major fiber tract uncinate fasciculus (UF) (Ebbling and von Cammon, 1992 cited in Nestor et al., 2004). The other network is connected via the cingulate bundle (CB), and consist of the amygdala, nucleus accumbens, and medial dorsal thalamus. This dorsolateral prefrontal–cingulate network is believed to extract information about task regularities and contingencies so that rules can be acquired to guide thought and action (Miller, 2000, cited in Nestor et al., 2004). This network is believed demonstrated aberrant in how Nestor, Kubicki, and colleagues (2004) found significant lower scores compared to controls across neuropsychological test scores on intelligence, declarative-episodic memory, working memory and executive function. On the Wisconsin Card Sorting Test,

patients had a disproportionate number of perseverative errors relative to the controls. In the results, the left UF abnormalities, identified by DTI measures, correlated with deficits in declarative-episodic memory, but not in executive functioning. Whereas left CB abnormalities correlated with deficits in executive functioning, but not in declarative memory. The results from the study indicate a double dissociation between reduced DTI measures of the left UF and CB, and deficits in declarative memory and executive function, respectively. Taken together, such findings supports further a disconnection syndrome in schizophrenia.

Overall, the different imaging modalities results give further understanding of the pathology in question. The combination of imaging with blood markers can give a more robust support to the potential presence of neuroinflammation. Abnormal tract geometry, neuroinflammation and demyelination as suggested pathologies might co-occur, being part of the same pathology, or might occur in succession. For instance, tract geometry might because of abnormal brain development predating schizophrenia especially for the early onsetters, followed by neuroinflammation (because of psychosis onset), and demyelination (a consequence of inflammation and/or disease progression).

Statistics and Neuroscience

The outcome of a study could lead to clinical trials that may affect many individuals, and it is therefore of importance that the methods used are as effective and correct as possible, with main fundament in model building.

Imaging data are likely to not meet the assumptions of parametric tests, especially regarding distribution and random sampling, as research with such data often are comparing patients to controls, and the patients are not randomly selected participants. Increasingly, permutations test are applied for biological data as it makes fewer assumptions about the data (Winkler, Ridgway, Webster, Smith & Nichols, 2014). Permutations tests calculate all possible permutation of the data, and under the null hypothesis, use the outcome to estimate the distribution of the test statistic (Baume, 2015). This contributes to identifying critical areas, as there is a simulated null distribution. Randomization test within permutation tests refers to group membership being randomly assigned in the data, as in a null hypothesis where there are not difference between the groups, and when repeated multiple times, it simulate the null distribution. When there are not room for randomization, there must be week assumptions. With enough

replications run, typically 10000 iterations, the estimation of the distribution is argued to be very exact. To perform this, Permutation Analysis of Linear Models (PALM) is experimental software offering a range of methods, both parametric (e.g. MANCOVA) and non-parametric combination (NPC), and results in making fewer assumptions about the data like, offering statistics that are robust to heteroscedasticity (Winkler, Webster, Brooks, Tracey, Smith & Nichols, 2016). Classical methods based on means is not to be understood as invalid or without practical value. However, it could contribute to superficial understanding of the group comparison; some statecians argue (Wilcox and Rosselet, 2017). In sum, classical analysis methods are still used and valid, however, permutation methods, which only makes weak assumptions about the data, are increasingly applied and encouraged, especially for biological data (Winkler et al., 2014).

The Current Project: Aims and Hypothesis

Based on the studies reviewed in the introduction, it is clear that glial cell activation is associated to schizophrenia, and mostly differing from controls. The current project will focus on the glial activation related markers: choline, mI and NAA. To remind the reader, the three metabolites are considered glial markers due to choline and mI being higher expressed in glial cells than neurons (Bustillo et al., 2002; Mahli et al., 2002), and findings of reduced NAA in patients in medial temporal regions together with reduced DTI anisotropy index interpreted as lowered connection and myelination in axonal bundles (Tang et al., 2007). The current master project's research design is mixed, both within and between subjects, with the aim of exploring glial activation in schizophrenia.

The main investigation was hypothesized as 1) elevated levels of the glial activation markers mI and choline and decrease NAA in MRS spectra in patients compared to matched controls, with the glial markers acquired in 4 different locations across both hemispheres.

The further analysis was exploratory for 2) within patients for associations between the glial activation markers to immune mediators in peripheral blood. We suggested running analysis with the pro-inflammatory markers IL-1beta, IL-6 and TNF-alpha, together with the anti-inflammatory cytokine IL10 and CRP, fitted to prediction models in order to rule out a 0 hypothesis of no correlation between peripheral pro/anti-inflammatory cytokines and CRP to the glial markers.

Furthermore, the 3) symptom severity load indexed by two of the total scores from the Positive and Negative Symptoms Scale (PANSS) will be explored in relation to glial marker activation and the peripheral inflammation markers, as the literature are indicating impact on glial dysfunction on the symptoms, e.g. positive symptoms due to disconnection.

The final analysis was 4) to examine whether the MRS-measured glial markers predict regional differences in fractional anisotropy (FA) values in diffusion-weighted images, and test for differences in this relationship in patients and controls. If there are any significant group*marker interaction clusters, FA values will be extracted from these clusters and tested. Other studies have shown widespread lower FA in several brain regions in schizophrenia patients, indicating that white matter is in worse shape in patients. This could have to do with neuroinflammation, or demyelination. The tractograpy data indicate how strong the white matter tracts are between regions by the diffusion-coefficient fractional anisotropy (FA) in each voxel. The brain will be segmented into cortical areas and give estimated white-tracts between all of these to check for co-variance between identified tracts and the glial markers, i.e. the glial markers will be used as regressors to see which points in white substance have significant covariance between the glial markers and the diffusion-coefficient.

Methods

About the data

The data used in the current master thesis was collected and granted by the Bergen Psychosis Project 2 (BP2), under the principal leadership of dr. med. Erik Johnsen. Over 200 patients were included in the BP2.

Participants

After quality control, MR spectra were available for seventy-six (77) schizophrenia patients (SZ), and 77 healthy controls (HC) matched on age (mean age = 30.23, SD = 10.23), gender (18 female, 58 male) and handedness. Several structural and functional asymmetry differences exists between left- and right-handers (Sommer, Aleman, Ramsey, Bouma and Kahn, 2001). When not possible to match ambidextrous handedness, right-handed match was applied in line with other research done with the BP2 datasets (e.g. Hjelmervik et al., in prep). Age and gender are also crucial to match as they influence several biological variations, in particular white matter integrity and maturation (Alexander et al., 2007). The age was matched within +/- 3 years, except nine patients who were beyond this range (4-7 years). Patients and controls were also matched in terms of procedure protocols for scanning, as a scanner upgrade happened during the project duration. A subset of patients were MR-scanned repeatedly during follow-up; only the 1st session measures was included for the current study.

Table 2: *Medication exponation status. Measures for total 66 patients, missing nine.*

Never	Not past year	Not past 3 month	Ongoing	Started past 1-7 days
26	2	2	29	7

All patients were on medication at study start, with various exposure history (see table 2), and allocated to three different medications: amisulprid, aripiprazole and olanzapine. All patients were diagnosed with SZ according to the ICD-10 diagnostic manual (World Health Organization, 1992,

Norwegian translation; http://ehelse.no/standarder-kodeverk-og-referansekatalog/helsefaglige-kodeverk/kodeverket-icd-10og-icd-11). The study was approved by the Regional Committee for Medical Research Ethics at the University of Bergen (REK no 2010-3387), and conducted according to the Declaration of Helsinki. All participants received oral and written information about the study before signing a written consent form.

Exclusion criteria

Before data collection, subjects were inhibited to participate on several grounds: inability to understand spoken Norwegian, psychosis due to neurological conditions or other diagnosed disorders such as bipolar, and pregnant or breastfeeding women, and being unable to use oral antipsychotics.

Assessments

At baseline, general demographics data was collected, together with history of mental and physical illnesses, smoking, and education. Symptom severity was assessed in interviews by trained and certified personal and translated into scores on the Positive and Negative Syndrome Scale (PANSS).

PANSS (Kay, 1991).

The PANSS consist of 30 items including 7 on positive symptoms, 7 on negative and 16 on general psychopathology. A total score is achieved by summing across the topics giving numbers ranging from 7 - 49 on the positive and negative, and ranging 16 - 112 on the general psychopathology. The scoring on the PANSS range from 1 being absent and 7 being extreme. As data is gathered, the interviewers considers firstly whether the item is present at all by its definition provided by a manual. The highest rating is chosen even if the patient meets criteria for lower point too whether or not all elements of the description are observed; a standard within research.

Image acquisition and processing.

MRSI acquisition.

Imaging data were collected with 3T GE-Signa MRI scanner at the Haukeland University Hospital. First, an anatomical T1-weighted image was acquired of each subject using a 3D SPGR sequence. This was used for voxel placement according to anatomical landmarks. There were two MRS data collection protocols, differing in voxel placements. Protocol 1 measured from left Superior temporal gyrus (STG), right STG and anterior cingulate cortex (ACC), and was applied for thirty-nine of the patients and their matched controls. Protocol 2 recorded from left STG and left inferior frontal gyrus (IFG), performed on the remaining number of participants. Hence, all participants have obtained measures from left STG.

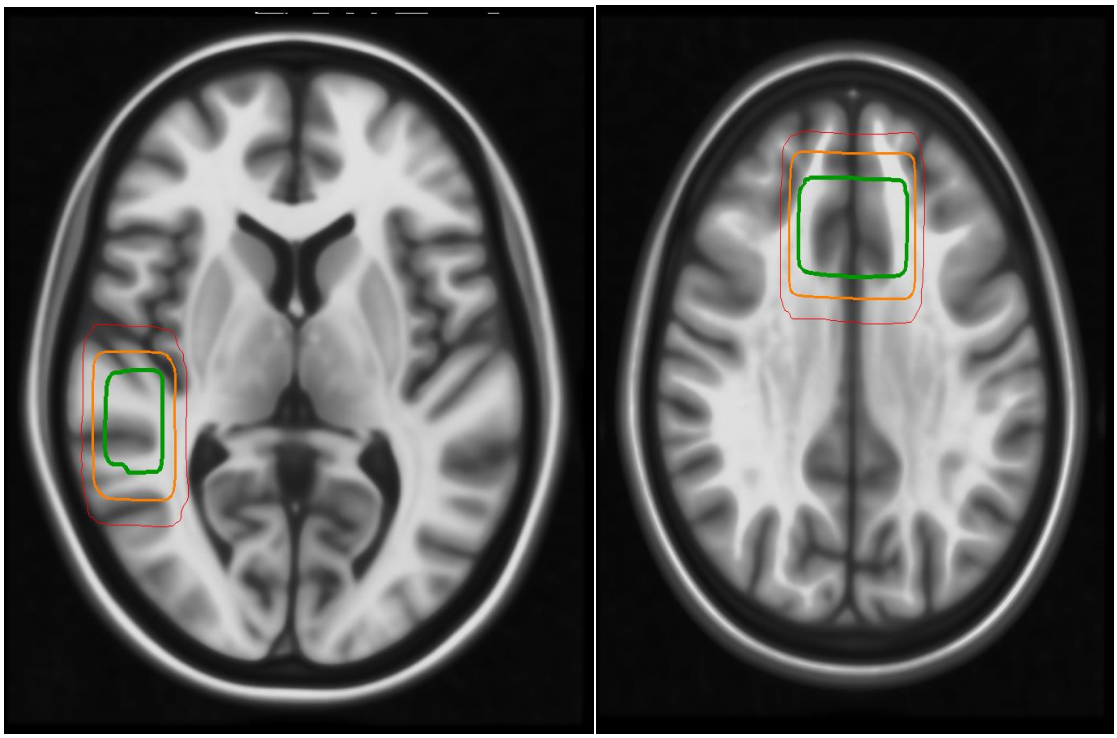

Figure 2*: Images of voxel placements in protocol 1: R STG (to the left) and ACC (right) in transverse view. The green box demonstrates the placement in one representative participant mapped onto a standard template. The orange and the red indicate respectively 95% and 60% confidence regions for the placements across the patient group.*

For protocol 1, the following parameters were used: Repetition time (TR)/echo time (TE)/flip angle (FA)/inversion time (TI) 7.74/2.9/500 and field of view (FOV) 260, which gives isotropic voxels of 1 × 1 × 1 mm^3). For protocol 1, the following parameters were used: TR/TE/TI 6.8/2.95/450 and FOV 256, giving isotropic voxels of 1 × 1 × 1 mm^3. Thereafter, MRS data acquisition was performed where ^{1}H-MRS-spectra of protocol 1 were obtained from the left and the right STG (voxel size 24 × 40 × 30 mm^3) and ACC (voxel size 40 × 40 ×25 mm^3) by using a single-voxel point-resolved spectroscopy (PRESS) sequence (TE/TR = 35 /1500ms, 128 repetitions), followed by a Mescher-Garwood PRESS (MEGA PRESS) sequence (TE/TR = 68/1500 ms, 128 repetitions), however MEGA-PRESS measures, i.e. GABA, is not included in the current thesis.

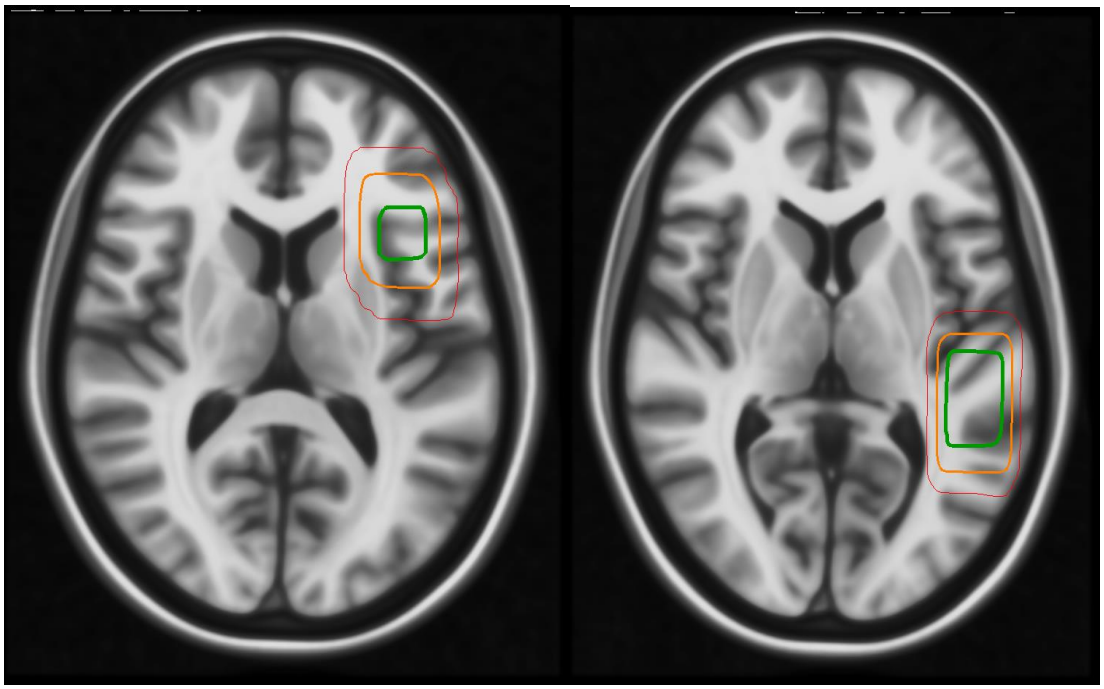

Figure 3*: Images of placements in Protocol 2: left IFG (to the left) and left STG (right) in transverse view. The green box demonstrates the placement in one representative subject mapped onto a standard template. The orange and the red indicate 95% and 60% confidence regions for the placements across the patient group.*

Measurement of protocol 2 were conducted from the left IFG (voxel size 24 × 38 × 28mm^3) and left STG (voxel size 24 × 30 ×31mm^3) obtained by using the PRESS and MEGA-PRESS sequences with identical parameters as above except that the MEGA-PRESS repetition number which was

set to 192. Voxel localization was performed according to anatomical landmarks using the T1-weighted structural image. Unsuppressed water reference spectra (eight repetitions) were acquired automatically after the acquisition of water-suppressed metabolite spectra in both protocols. A scanner up-grade was done between data collection of protocol 1 and 2, including a change of head-coil from 8 to 32 channels.

MRS data processing.

MRS data from the PRESS sequence were analyzed using the LCModel (Provencher, 2001) version 6.3-1J (Stephen Provencher, Inc., Oakville, ON, Canada), with the standard basis-set incorporating components from 15 metabolites (Alanine, Aspartate, Creatine, γ-aminobutyric acid, Glucose, Glutamine, Glutamate, Glycerophosphorylcholine, Phosphorylcholine, Lactate, myo-inositol (mI), N-acetyl aspartate acid (NAA), N-acetylaspartylglutamate, scyllo-inositol and Taurine). As a standard on the GE implementation of the PRESS sequence, unsuppressed water reference spectra were acquired automatically after the acquisition of water-suppressed metabolite spectra, and averaged before being processed and analyzed.

Diffusion Tensor imaging acquisition

White Matter (WM) diffusion was studied with Diffusion Tensor Imaging (DTI). In each 3D brain volume, the values indicated the strength of a certain type of diffusion-coefficient, Fractional anisotropy (FA), in each voxel.

DTI Data Processing

The DTI images were preprocessed with FMRIB Software Library's (FSL, version 4.1.2, fmrib.ox.ac.uk/fsl) (Smith et al., 2004). The DTI images were motion and eddy current corrected. Voxel wise statistical analysis of the DTI data was carried out using Tract-Based Spatial Statistics (TBSS) procedure (Smith et al, 2006), a part of FSL. For the next step, the FA data were aligned into 1 mm × 1 mm × 1 mm Montreal Neurological Institute (MNI) 152 Space using the FMRIB's Nonlinear Image Registration Tool (FNIRT). Then, the mean FA image (threshold of 0.25) was created and thinned to create a mean FA skeleton that represents the centers of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data were fed into voxel-wise cross-subject statistics. Subjects with poor image quality due to subject motion or other visible image artefacts (e.g. due to metal) were removed. Finally, the JHU

White-Matter Tractograpy Atlas was used to identify the tract content of the observed clusters with significant group and voxel placement interaction effects (Smith et al., 2006).

Laboratory.

The blood sample consisted of measures and analysis of cytokines Tumor-Necrosis Factor alpha (TNF-α), Interleukin-1beta (IL-1β), IL-6, IL-10 and C-reactive protein (CRP). The blood specimen were drawn from the participants in the fasting state between 8 and 9 PM by trained nurses and collected at serum gel tubes, centrifugation at 3300 rpm for 10 minutes, pipetted and stored at minus 40 centigrades, then moved without being thawed and stored at minus 80 centigrades until analysis. Cytokine measurements were done with the Luminex Screening Human Magnetic Assay from R&D Systems, Inc., 614 McKinley Place NE, Minneapolis, MN 55413

Statistical Analyses

Descriptive statistics on the datasets were run with IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Group differences in glial markers.

The glial markers myo-inositol (mI), N-acetyl aspartate (NAA) and choline (Cho) with group (patient versus control) and voxel placement were subjected to statistical analysis using linear mixed model regression models. This procedure enables to fit mixed effects models to data samples from normal distributions. The analysis was run with the different glial markers separately (see models in results), with the fixed factors group and voxel placement, and random factor subject.

Glial marker predict symptom severity.

Exploratory analysis examined the relationship between glial markers and symptom severity in patient group using mixed model analysis, with the variables PANSS positive and negative total, and voxel placement as fixed effects and again voxel placement as repeated effects.

Cytokines predict glial markers.

Hierarchical multiple regression was run separately for NAA, mI and Cho with predictors fitted into 2 models: (1) age, sex, medication group (how far in treatment), symptoms, (2) TNF-Alpha,

IL6, IL10, IL1b, and CRP to test if glial markers could be predicted by the theorized variables, with particular interest in inflammation markers' ability.

DTI data and glial markers.

After processing the DTI data creating FA values in TBSS, and matching the subjects with MRS data, 6 subjects was excluded due to missing data, leaving a total of 109 subjects to be analyzed. After the files were decompressed to suitable file type, tests were conducted to investigate if glia markers are related to regional changes in FA and whether this differ between patients and controls. Non-parametric permutation-based PALM package, was applied with 10000 permutations and threshold-free cluster enhancement (TFCE), with a significance level of $p < .01$ (family-wise error FWE corrected) adjusted for multiple comparisons by using the null distribution of the maximal voxel-wise test statistic (Winkler et al., 2014). A multiple regression model was set up containing the group effect (patient/control), the three metabolites (NAA, mI, Cho) and three group*metabolite interaction terms. Age, sex and scanner version were entered as covariates. Furthermore, for significant group*marker interaction clusters, FA values extracted from these clusters and tested only within patient group whether there is symptom severity effects.

Data management

Processing and analysis was performed on secure servers.

Results

Group differences in glial markers

Description for the means of the glial markers Choline, N-acetyl-aspartate acid (NAA), and Myo-inositol (mI), for all 154 patients and controls across voxels, are displayed in Table 3. The means were computed from all four regions measured; left Superior temporal gyrus (L STG), right STG, L inferior frontal gyrus (L IFG) and anterior cingulate cortex (ACC), resulting in one mean variable for each glial marker, and a four-level voxel placement variable. A linear regression model procedure was fitted to the data, with the glial markers as dependent variable, and group and voxel placement as fixed factors.

***Table 3**: Description of the glial marker values for patients and controls across regions*

	Glial marker	**Min**	**Max**	**Mean**	**Median**	**SD**
Patients	Cho	1,10	3,71	2,14	2,14	,49
	NAA	8,83	18,98	13,93	13,97	1,92
	mI	3,51	9,72	6,51	6,50	1,27
Controls	Cho	,91	3,16	2,04	2,05	,44
	NAA	8,74	19,32	13,85	13,95	1,69
	mI	1,49	11,48	6,34	6,40	1,45

Choline.

The fixed effects model estimated a significant main-effect of group on choline levels (F (1, 144,153) = 5.899, p = .016), with higher choline in patients (M = 2.14 SD = .49) compared to matched controls (M = 2.05, SD =.44). The median values seen in table 1 indicate close values to the means. Calculating the Cohen's d for the size of the differences yielded d = 0.18.

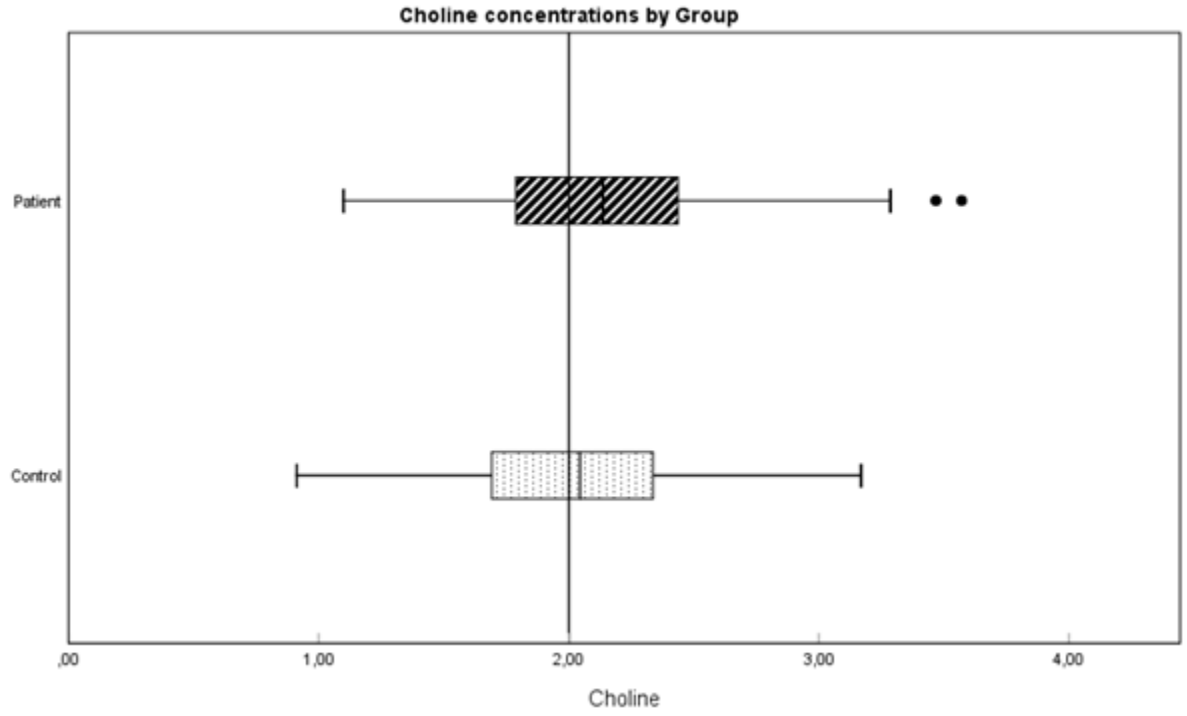

Figure 4*: Box plot demonstrating group differences in choline levels across regions. Top box's midline is demonstrating patients mean, which is visibly higher compared to the lower box's, representing control's mean. The lines are representing the range of variance, and in the patients' measures, there are also two outliers can be identified.*

No significant interaction-effects were found for the group x voxel placement analysis (F (3, 120. 611 = 1.410, p = .243). The main effect of voxel placement was however significant (F (3, 120, 611) = 173.089, p< .001) identified to anterior cingulate cortex.

NAA.

For NAA, there was no significant results for main-effect of group (F (1, 136,704) p = .484. For voxel placement, the fixed effects was significant F (3, 130,230) = .22.825, p = <.001.

There was no significant interaction effect for group x voxel, F (3, 130,230) =, p =.636.

mI.

There was no significant main-effect on mI by group, F (1, 141,717) = 1,300, p = .256, or main-effect for voxel placement, F (3, 130,263) = 88.217, p = .345. Finally, there was no significant interaction-effect for voxel placement and group, F (3, 130,263) = .155, p = .926.

Glial markers and Symptoms

The next set of analyses involved patients only. A linear multilevel mixed model was fitted to the data with PANSS (symptom severity index) measures and voxel placement as fixed effects, and glial marker as the dependent variable. The description of the severity index can be seen in table 4 below.

Table 4: *Description of PANSS severity scores for the patient population in the current sample. There is overall a higher positive total scores compared to negative total scores.*

N = 61	**Min**	**Max**	**Mean**	**Median**	**SD**
Positive Total	12	38	21,38	21	5,04
Negative Total	7	33	17,17	17,50	5,81

Choline.

There were non-significant main-effect of Cho for PANSS positive total index (F (17, 104) = 1.115, p = .350) and PANSS negative total index (F (18, 104) = .802, p = .694), with the only statistically significant effect being voxel placement main-effect (F (3, 104) = 41,421, p < .001). Finally, no significant interaction-effect for voxel placement on the total were found, pos*voxel, F (40, 12.659) = 1.704, p = .154), neg*voxel, F (43, 12.206) = 1.214, p = .373), indicating no significant difference in the relationship between choline and PANSS scores in the different regions.

NAA.

There were non-significant main-effect of NAA both for PANSS positive, F (17, 25.108) = .310, *p* =.992) and PANSS negative total scores, F (18, 26.111) = .398, *p* = .977) for the 59 subjects with data eligible for analysis. There were further non-significant main-effects of voxel placement for PANSS positive total scores, F (33, 5.386) = 1.041, *p* = .540) and for negative total scores (F (35, 4.729) = .1.294, *p* = .431), indicating that there were no significant difference in the relationship between the NAA and PANSS scores in the different regions.

mI.

Finally, the same lack of significance of mI on PANSS positive total (F (17, 256.903) = .302, *p* = .997) and negative total (F (18, 234,179) = .168, *p* = 1,000) was observed. No interaction-effect was observed for voxel placement and the total scores, pos * voxel (F (33, 38,051) = .870, *p* = .656) nor neg*voxel (35, 28.788) = .785, *p* = .754). Significance was observed for voxel placement main-effect, F (3, 34000) = .001, *p* < 001.

Cytokines and CRP predict glial markers, only patients.

Hierarchical multiple regression was run using the glial markers as dependent variables and with fitted Model 1 including the covariates given in table 6 below. We continued the hierarchical regression analysis by fitting model 2 using cytokines.

Table 5: *Cytokine and CRP description in patients on visit 1. The description of the values shows a wide variance for the 21 patients in the minimum to the maximum values indicated by the standard variation column to the right.*

N= 21	**Min**	**Max**	**Mean**	**SD**
IL-1B	,80	424,14	49,12	87,88
TNF-a	1,20	459,92	47,75	93,93
IL-6	1,55	566	53,34	140,32
IL-10	*,22*	*54,43*	*22,49*	*18,251*

CRP N = 59	*,0*	*29,70*	*1,72*	*3,57*

The glial measures applied derived from L STG due to most number of measures in sample, providing best power (N=75).

Table 6: *Regression models for glial marker level prediction. Model 1 consist of the known moderating factors in schizophrenia. Model 2 consists additionally of the cytokines and CRP.*

Model 1	**Model 2**
PANSS negative total, PANSS positive total, medication type, gender, age, and scanner version	*All variables in model 1, and in addition cytokines: IL-10, IL-6, IL-1beta, CRP, TNF-alpha*

Choline.

Model 1 gave us an R2 value of 512, and an adjusted R2 of .275. Model 2 obtained a R2 of .795 and adjusted R2 of .474. We compared model 1 with model 2, and found model 1 to explain 51.2% of the variance. The addition of the cytokines led to explaining additional 27, 9% of the variance, having the full model explaining 79,5% of the variance. Adding the cytokines (model 2) did not statistically significantly increase r2, F (5,7) = .1,909, p = .211. The full model was not statistically significant in predicting glial levels, R2 = .892, F (11,7) = 2,474, p = .119, adjusted R2 = .474.

Table 7: *Coefficients table: for cytokines regression analysis on choline. Only PANSS positive is significant to choline.*

	Model 1			**Model 2**		
Variable	**B**	**Beta**	**Sig.**	**B**	**Beta**	**Sig.**
(Constant)	.913		.079	1.482		.033
Gender	-,226	,346	.133	-,385	-,591	.023
Age	,009	,364	.187	,008	,340	.404

Fmri week	-,006	-,018	.950	,033	,102	.735
Allocated medication	-,055	-,139	.616	-,064	,142	.666
PANSS Positive	,048	,629	.018*	,034	,437	.251
PANSS Negative	-,011	-,195	.500	-,017	-,285	.529
IL-6				<0,001	-.004	.991
IL-10				-.015	-.798	.156
IL-1beta				.010	.828	.508
CRP				.032	.214	.418
TNF-alpha				-.007	-.437	.768

NAA.

Model 1 gave us an R2 value of .336 and an adjusted R2 of .005. Model 2 obtained a R2 of .588 and adjusted R2 of -.059. We compared model 1 with model 2, and found model 1 variables explaining 33.6% of the variance, and the addition of the cytokines led to additional 25.2%, explaining 58.8% of the variance. However, adding the cytokines (model 2) did not statistically significantly increase r2, F (5, 7) = .856, *p*= .553. The full model was not statistically significant in predicting glial levels, R2 = .588, F (11, 7) = .909, *p* = .575, adjusted R2 = -,059.

Table 8: *Coefficients table for cytokines regression on NAA. PANSS positive is significant.*

	Model 1			**Model 2**		
Variable	**B**	**Beta**	**Sig.**	**B**	**Beta**	**Sig.**
(Constant)	8,54		.043	14.46		.031

Gender	.192	.043	.866	-.481	-.109	.717
Age	.014	.086	.783	-,004	-,026	,963
Fmri week	-.351	-.163	.624	,224	-,344	,516
Allocated medication	.033	.012	.970	-,930	-,344	,516
PANSS Positive	.320	.616	.042*	,120	,231	,655
PANSS Negative	-.037	-.095	.788	,109	,277	,663

IL-6	-,010	-,702	,229
IL-10	,003	,023	,975
IL-1beta	,178	2,214	3230
CRP	,150	,149	,685
TNF-alpha	-,278	-2,437	,226

mI.

Model 1 gave us an R2 value of 548, and an adjusted R2 of -.050. Model 2 obtained a R2 of .823 and adjusted R2 of .172. We compared model 1 with model 2, and found model 1 variables explaining 30% of the variance, and the addition of the cytokines led to additional 37,8% explained, with the full model explaining a total of 67,8% of the variance. Adding the cytokines (model 2) did not statistically significantly increase r2, F (5, 7) = .1,642, p =.266. The full model was not statistically significant in predicting glial levels, R2 = .823, F (11, 7) = .1,339, p = .360, adjusted R2 = .172.

Table 9: *Coefficients table for cytokines regression on mI. None of the coefficients are significant.*

	Model 1			Model 2		
Variable	**B**	**Beta**	**Sig.**	**B**	**Beta**	**Sig.**
(Constant)	3.866		.097	4.452		.136
Gender	-.208	-.085	.749	-.731	-.298	.281
Age	.028	.299	.358	-.003	-.028	.955
Fmri week	-.696	-.581	.106	-.844	-.705	.095
Allocated medication	.600	.400	.241	1.168	.788	.124
PANSS Positive	.090	.313	.282	.156	.542	.256
PANSS Negative	-.008	-.039	.911	-.072	-.329	.560
IL-6				-.003	-.365	.465
IL-10				-.007	-.102	.876
IL-1beta				-.065	-1.451	.363
CRP				.215	.384	.259
TNF-alpha				.051	.810	.663

DTI and glial markers

Permutation analysis was run in PALM to test the relationship between the glial markers and group (patient/control) and FA levels. Main effects and group*glial marker interaction effects were included in the model. Glia markers were obtained from L STG, due to all participants undergoing measures from this voxel. Age, gender, and scanner version were added to the analysis as

covariates. The results yielded no main-effect for group or glial markers in L STG. However, at P<.01, scanner version gave significant main-effect results, an expected finding.

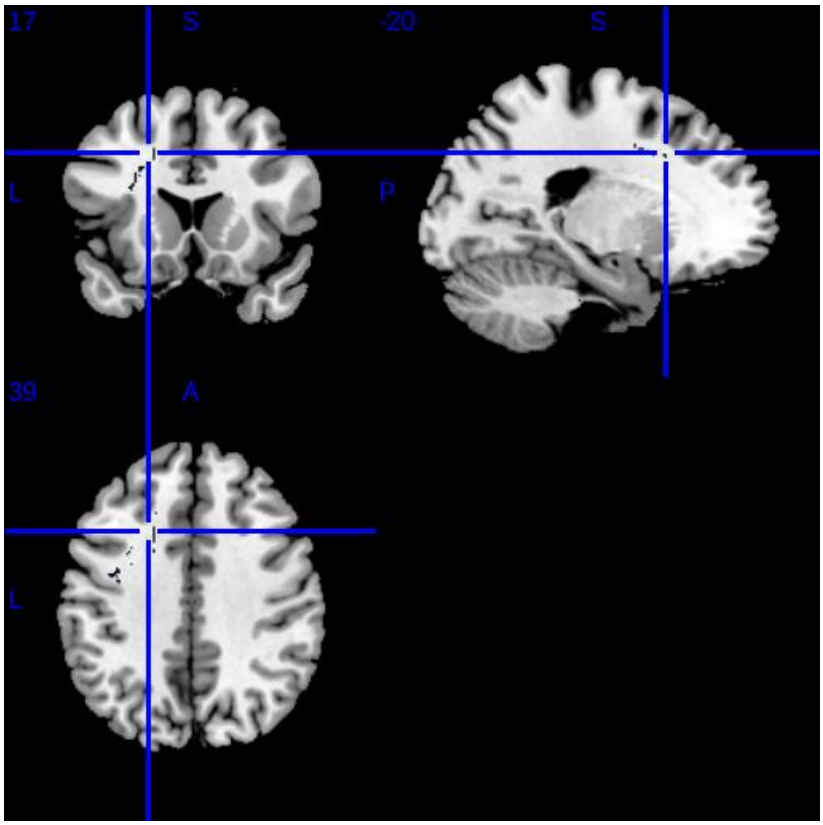

Figure 5*: The Trends-levels for mI in L STG x Group interaction effects in Superior Longitudinal Fasciculus (SLF). The black marks are indicating the placement of the trend-level effects, in coronal view (top left), sagittal (top right) and transverse (bottom left) view. The clusters were identified as SLF.*

Despite no statistically significant findings at p<.01, there were interesting trends in the analysis of the FA values for the mI in L STG x group interaction effect at $p<0.07$. Mainly the results were observed in the left hemisphere (see Figure X, Table N) fronto-temporally.

Table 10: *Cluster description for the trend-level mI x group interaction effects in FA analysis*

Analysis	Cluster Index	Cluster Voxels	Max/ p-value	MNI coordinates of peak voxel	Side	White matter tracts overlapping

				x	y	z		**with the clusters**
mI x Group interaction	1	2	1/0.07	-31	5	34	Left hemisphere	Superior longitudinal fasciculus L:4

As Table 10 shows, the cluster peak was at MNI coordinates -31, 5 ,34, and the closest white matter tract was left superior longitudinal fasciculus (4% overlap). Overall, the probabilities are low due to the tracts in question being large while the identified cluster are a minor part. The cluster consisting of 4% superior longitudinal fasciculus (SLF) in both identified voxels.

Further, mean FA values were extracted from the cluster to explore the direction of the interaction. Pearson correlation revealed significant negative correlation between FA and mI in L STG in patients, r = -.279, p = .045, and a non-significant positive correlation in controls, r = .192, p = .177.

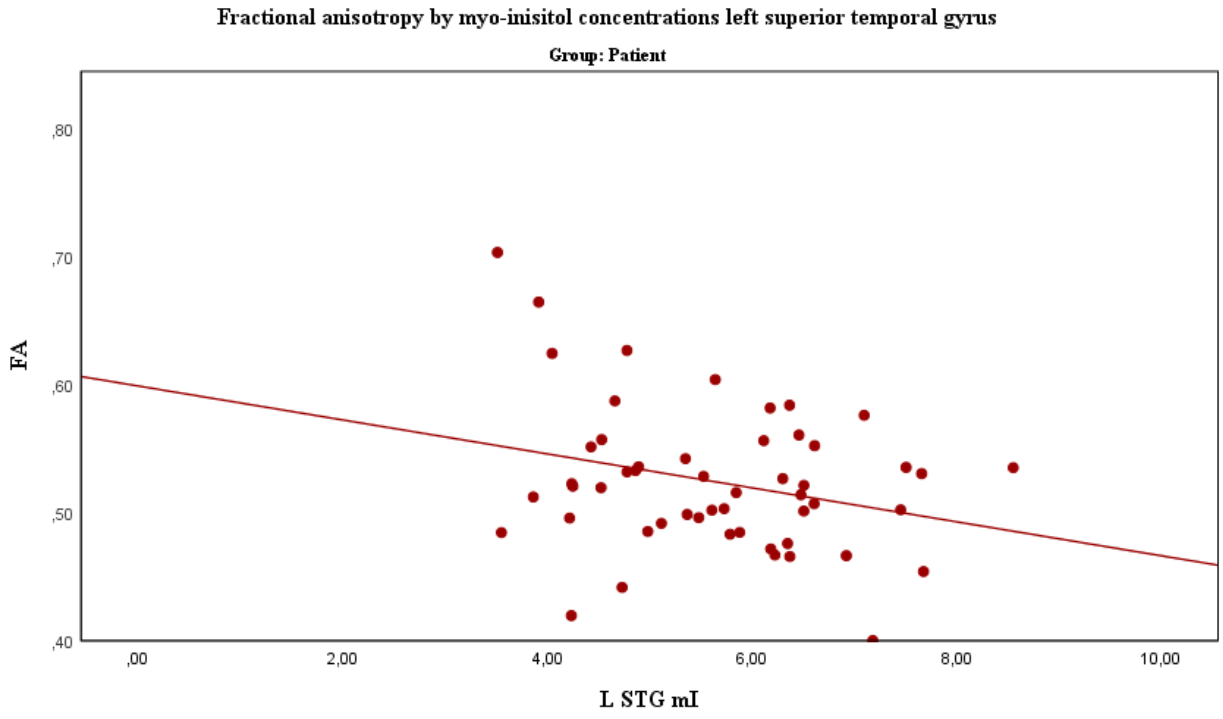

***Figure 6**: Interaction effects for mI in L STG and FA mean in patients. The direction of the association between mI and FA is negative, as indicated by the direction of the regression line.*

In Figure 6, the scatterplot indicated the negative correlation between mI and FA in patients of lower FA and higher mI. The x-axis demonstrate the mI in L STG levels for patients, ranging from 3.2 to 8.9 mmol/L, and the y-axis show the FA values ranging from .40 to .75. The main effect of mI increases as FA degree lowers, and the interaction of the effect is different between the groups, as indicated by the direction of the correlation. The interaction effect of mI from left superior temporal gyrus (L STG) is different between patients and controls. In patients FA decrease with increasing mI, while in controls, as seen in the figure 7 below, the FA increase with increase in mI.

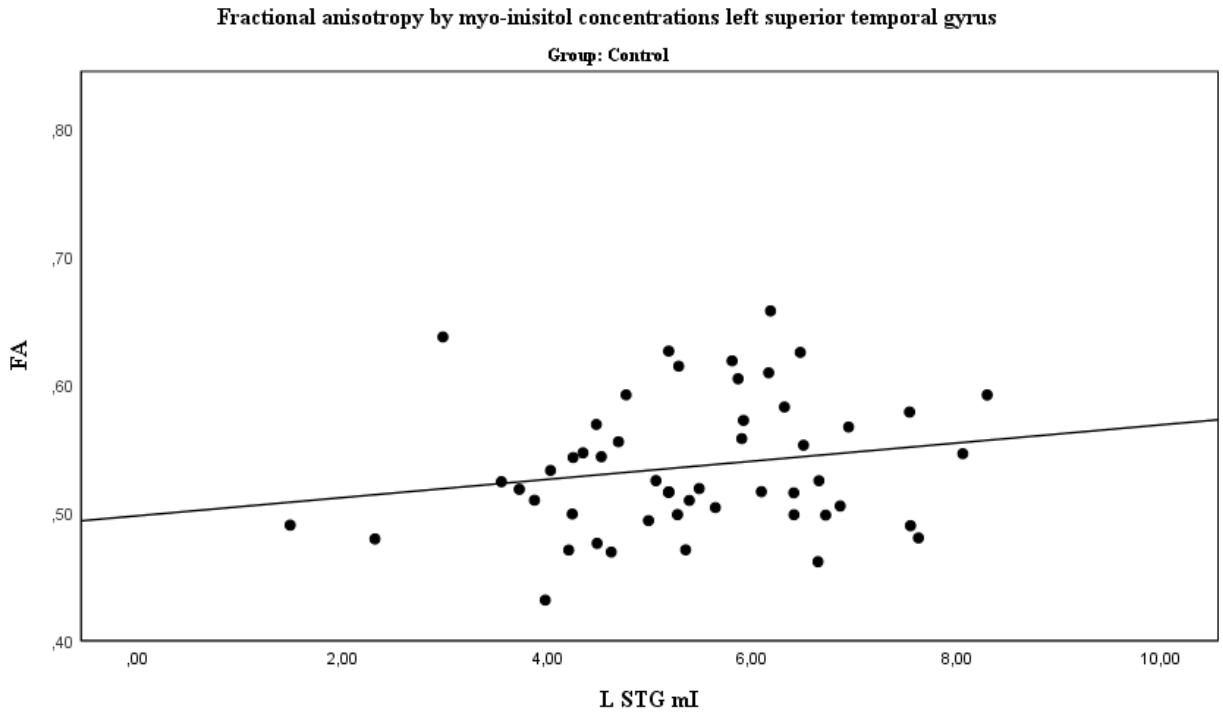

***Figure 7**: Interaction effects for mI in L STG and FA mean in controls. The effect was positive as indicated by the regression line, indicating increase in FA and increase in mI. OBS expand.*

In figure 7, the direction of FA increase with increase in mI is seen. The x-axis demonstrate the mI in L STG levels for patients, ranging from 1.6 to 8.6 mmol/L, and the y-axis show the FA values ranging from .40 to .68.

Table 11*: Description of Fractional Anisotropy (FA) values and myo-inositol (mI) levels in left superior temporal gyrus (L STG) in patients and controls.*

	FA (N = 52)	**L STG mI (N=75)**
Patients	.52 (SD =.06)	5,77 (SD =1,22)
Controls	.54 (SD = ,05)	1,37 (SD = 1,37)

The FA values were slightly lower in patients compared to controls, while mI was higher in patients than controls (See table 11).

Findings and Discussion

Summary of Findings

The current study investigated glial marker activation and neuroinflammation markers activation in schizophrenia by hypothesizing glial activation differences between participants and matched controls, and exploring the relationship between the markers and to symptoms. As hypothesized, there were group differences, with choline being statistically significant higher in patients across the brain compared to their healthy matches (low effect size). However, no significant group differences were found for N-acetyl aspartate acid (NAA) or myo-inositol (mI), yet all glial markers were higher in patients than controls on visual inspection of the means, yet considerably small effect sizes, $d = .005$ and $d = .12$, respectively for NAA and mI (very low effect sizes). Voxel placement main-effects were significant for NAA, and located to anterior cingulate cortex (ACC), which warrants future exploration of concentrations differing between brain regions. Further, the glial markers were tested to see if pro- and anti-inflammatory cytokines and CRP could predict them, with no statistically significant findings. In addition, dependency in glial cells to symptom severity and type indexed by PANSS total positive and negative scores was modelled and tested, with no significant findings. Overall, none of the models fitted the data in that they were not predicting the outcomes on the dependent variables. However, there might be several reasons for that which will be discussed further and are source for limitations in the study. The final analysis yielded trend-levels myo-inositol (mI) and group interaction-effects of fractional anisotropy (FA) values in the cluster identified. Inspecting the group effects revealed a negative correlation in patients between mI in left superior temporal gyrus (L STG) voxel placement and FA, meaning when mI increase the FA values goes down. In controls, the relationship was positive. Significant impact of the change of scanner version, i.e. image quality, were identified, indicating the importance to include such changes in analysis, like the current project have.

Group differences in glial activation

The key finding in the current project is the group differences in glial activation in schizophrenia patients compared to matched controls.

The significantly higher mean of choline concentrations across the brain in schizophrenia patients compared to matched controls, is finding in line with previous evidence of elevated choline levels in patients compared to controls (Bustillo et al., 2002). The finding of elevated choline in patients has previously been understood in the light of immune mechanisms at play, as there is more choline in glial cells compared to neurons (Gill et al., cited in Plitman et al., 2015). Overall, the findings could be understood to have implications for glial dysfunction. Another study also found higher Choline levels for first-episode psychosis (FEP) patients compared to controls (Plitman et al., 2018), which was also found at in a longitudinal study conducted by the same research fellowship (de la Fuente-Sandoval et al., 2011; de la Fuente-Sandoval, 2013). Bustillo and colleagues (2002) suggested high choline concentrations to reflect dysfunction in neuronal phospholipid membrane formation, slow glucose metabolism and/or larger than normal acetylcholine transmission.

There are no robust distinct metabolite levels established in schizophrenia, however effect sizes are interesting to evaluate for the understanding of the group differences found. In the current situation, the effect size (ES) for the group difference in choline was $d = .018$, which is considered small. However, as Cohen himself strongly emphasizes, it is not just the magnitude of the effect that is of interest, but the practical and clinical value too, and could be understood as "the extent of meaningful change in participants lives" (Cohen, 1979 cited in Durlak, 2009, p 917). Applied to the current situation, it is not possible to determine the practical or clinical benefits of the choline changes, as Durlak writes, "*it is worth a try in order to capture the full meaning of research findings*" (Durlak, 2009, p. 918). Hence, it is attempted to speculate in the implications of the results; exploring choline mechanisms, which could theoretically reach around 10% improvement in the patient population, calculated from the current ES. A number of significance regarding improvement in daily functioning.

Typical medication for bipolar disorder have tended to be lithium which is known to inhibit choline transportation which is thought to be the effective part of the effect treatment in rapid-cycling bipolar disorder (Stoll et al., 1996, cited in Moore et al., 2002). Such previous findings and

interpretation might also be speculated to translate to schizophrenia treatment. Bipolar and schizophrenia are known to have overlapping symptoms and emerging evidence points to shared genetics and phenotypes (Lichtenstein et al., 2009). In bipolar disorder, the adrenergic-cholinergic hypothesis postulates that the depression component is due to adrenergic underactivity and cholinergic overactivity, which results in increase of acetylcholine. Further, mania is associated to adrenergic overactivity and cholinergic underactivity (Moore et al., 2002). As the currently used dopamine receptor-blocking antipsychotic medication are found effectful for decreasing hallucinations and prevent major relapse, but not so much cognitive symptoms (Kroken et al., 2014), the finding of higher choline in patients than control is highly interesting in the light of exploring new treatment targets that might be promising to be effective on the cognitive and negative symptoms.

The finding of voxel placement main-effect from anterior cingulate cortex is also found in another recent study, where ACC was found to have lower levels of NAA compared to healthy controls (Reid et al., 2018). However, the direction is missing in the current study. Findings of reduced NAA in schizophrenia in medial temporal regions together with reduced DTI anisotropy index has as previously presented been interpreted as lowered connection and myelination in axonal bundles (Tang et al., 2007), and is therefore indicated as a glial marker. Similar to the current study, Plitman and colleagues (2018) found no differences between patients and controls in NAA concentrations, which was interpreted in the potential light of the patients being early in illness progression, subsequent to neuronal loss if NAA could be understood as an indicator for neuronal injury (Plitman et al., 2018). However, as earlier pointed out, due to NAAs ability to recover it should rather be viewed as a surrogate marker of neuronal health and dysfunction, rather than loss (Dwyer et al., 2018). Hence, such findings could be interpreted as a chronological development in metabolite level expression differences during illness progression. The changes in levels could speculated to occur in order as Choline is found being both early in the progression as indicated by the findings in FEP population (Plitman et al., 2018), and in the current thesis the sample selection is not limited to early stage participants. As Plitman argue, other study's findings of group differences in NAA is due to its occurrence later in the illness progression as NAA dysfunction could be due to imbalance in glutamate-GABA which result in excitotoxicity in combination with glial activity (Walterfang, Velakoulis, Whitford and Pantelis., 2011).

For mI, in neuroinflammation mI reported increased and is speculated to be due to hypomyelination, similar to the processes in MS, however, there were no significant group main-effects identified, yet group x voxel placement effects on FA, which will be discussed later on. Furthermore, several factors are identified to be considerably mediating the metabolite concentrations, such as illness progression. Several previous studies have included participants in different stages of the disorder, FEP persons together with chronic patients like in the current project, however, important variations might come forth regarding the metabolite profiles across illness progression, duration and severity.

A recent review suggested schizophrenia to be conceptualized as a syndrome consisting of "*several disease phenotypes with a range of distinct underlying pathologies*" (Kroken et al., 2019, page 2.). Immune mechanisms could be one of them, yet also related to energy metabolism or synaptic dysfunctions, and with choline being related to several processes that are glial-derived, it could seem unclear which specific mechanisms its levels are reflecting. Still, the findings could be speculated to have implications as a medication target worth exploring, which might be of practical significance.

Clinical Symptoms and Glial Markers

Contrary to expected the results from the analysis indicated little information from the three glial markers to the symptoms load index, both for positive and negative total scores. Furthermore, voxel placement yielded also little information about the PANSS values. Despite not statistically significant, the mixed model results could be speculated to that choline was better predicted by negative symptoms, than positive, findings that could arguably be in line with the suggestion of further explorations of choline target for negative symptoms, similar to the treatment in bipolar disorder as discussed in previous paragraphs. The results for myo-inositol indicated very poor (.997 and 1.000) prediction be the positive and negative symptoms respectively. For NAA, positive symptoms yielded very poor fit in the concentration prediction, with slightly better fit for negative symptoms.

The lack of findings of the glial markers predicting clinical symptoms could be due to several reasons. Unpublished studies on the same dataset as the current found significant associations when the symptoms were into specific analysis on metabolites GABA and Glutamate, and divided psychosis item on PANSS divided into high and low hallucinators (Hjelmervik et al.,

2019, in prep). Several authors warrants for further research with exact examination of the symptoms to their empirically and theoretically implied biological mechanisms, providing clearer indications for the interpretations of findings. This seems particularly important regarding exploration of glial marker activation as the current thesis are attempting to present in the introduction the wide range of glial cell functions, not merely inflammation. In addition, the different fit in the glial markers could be interpreted to indicate the importance of distinguishing the different classes' glial activation and their associations to different aspects of the schizophrenia syndrome. In this study, PANSS positive was found as a significant coefficient to choline that was significantly higher in patients. NAA also had significant coefficient findings for PANSS positive but no group differences, and finally no associations were identified to mI. However, previous studies found decreased mI to correlate to depressive symptoms (Chiapelli et al., 2015).

Mitterauer (2011) describes alternative dimensional descriptors for schizophrenia dividing into psychotic dimension, disorganized dimension and a negative deficit dimension. Perhaps two of the glial markers could be understood to represent some of these dimensions, with choline potentially being associated to negative symptoms and mI to the disorganized. The latter is further discussed under the findings from the DTI analysis. Mitterauser's (2011) model suggests that the lack of boundaries in information processing for patients are due to glial-neuronal interactions, as other illnesses associated to glial activation (i.e. inflammation) like previously mentioned inflammation-associated multiple sclerosis. Multiple sclerosis (MS) and rheumatoid arthritis are most indicated as more affected by inflammation processes than schizophrenia, distinguishing the illnesses in the current state of research as higher grade and lower grade inflammation, respectively. Interestingly, MS is not only indicated to have a strong inflammation component interpreted due to findings of elevated immune mediators, the same as the ones applied in the current thesis, and progressive demyelination, yet also share with schizophrenia cognitive impairments and psychotic symptoms, established in the diagnostic manual DSM (Chiaravalloti and DeLuca, 2008).

Cytokines Predicting Glia Markers

None of the glial markers was found predicted by the cytokines or CRP. Previous meta-analysis found that 40% of patients had some form of inflammation (Osimo et al., 2018) which gives source for the expected relation between the markers. Furthermore, in another summary of 99 studies, it was found that in 50% of the included studies investigating the cytokines IL-6, TNF-alpha, and IL-1beta, was found to differ between patients and controls (Rodrigues-Amorim et al., 2017). However, there are crucial technical issues concerning the analysis.

First, it was believed that the analysis would lack in power as only a number of 21 patients passed the quality test for the cytokines measures. The low number would have low statistical power, yet be able to give potential interesting insight into the relation between the markers despite not being able to give an estimation of more complex models with several parameters. It was considered that despite the technical issue with so few measures, the measures were believed to be representative as it was random missing data and not a matter of drop out. Drop outs are an issue in research, particularly for serious illnesses, and is typical in longitudinal studies, often with the results of that the most ill is not represented.

After analysis, great discrepancies in the measures for both cytokines and CRP, beyond what is considered possible range was observed. Frydecka et al (2018) did meta-analysis on both first episode (FES) and multiple episodes schizophrenia (MES) patients and identified the means in both cohorts. For instance, they identified IL-1beta to have a mean of 1.3 with 0.3 standard deviations in FES and for MES the mean was 1.5 with 0.5 standard deviations. In the current sample, the mean yielded 49.12 with 87.88 standard deviations.

It was identified that this was due to technical issues, as visual inspection of the dataset identified the variation to vary with the point of date measured. It was later confirmed as technical issues with the screening assay was found to skew proportions of the blood samples values to be a lot higher than what they typically range. If interpreting the current findings, the data is poorly fitting the models indicating that that it is difficult to predict glial markers by cytokines and that variance in the glial markers is not well explained. However, as it known that the blood samples are unreliable, interpretation of results with cytokines and CRP in the current master thesis will of course be faulty.

Usually, the interpretation of the regression outcome is to look at the increase of R and whether it is significant. Low Rs indicate poor model fit to the data, or that the covariates are not

contributing with much information that would increase the dependent variable. This would aid in the tradition for interpreting hierarchical regression analysis outcome when comparing the models. The results' low R's indicate poor model fit overall. The standard is to determine whether the model is a good fit for the data, and to finally inspect the coefficients unique contribution to the model.

However, the coefficients tables' could be worth mentioned for the other variables in the models. The output is understood as the significance levels of each coefficients indicate an effect when far enough from zero. For choline, significance was found from PANSS positive (.018), indicating that choline increase with .048 for each point on the PANSS positive. This finding is questionable, as the same associations has not been found in the mixed model analysis. The further coefficients were not significant, however, they suggests some tendencies. For instance, the choline levels decrease with the older scanner version and slightly increase with age. Furthermore, gender also showed tendencies, in line with previous studies have also found gender differences in choline, with particular variations in resonance in females depending on the menstrual cycle (Hjelmervik et al., 2018). For NAA, none of the coefficients was significant in model 1. However, it could be speculated if the results indicate that with increase in PANSS positive total scores, there is a rise in NAA, and decrease with PANSS negative total score increasing. In addition, the levels seems to increase with age and if being male. For mI, there were no significant coefficients effects.

Trend-level mI x Group Interaction Effects on FA

There was no replication of other studies' findings of significant group differences in mI; however, there was an interesting trend-level (0.07) in the group and mI interaction effect on FA. In previous findings of elevated mI, this was interpreted as hypomyelination, and decreased mI have been found correlated to depressive symptoms (Chiapelli et al., 2015). Furthermore, the negative correlation in patients between mI in L STG and FA value could be suggested to imply that in schizophrenia subjects, when mI increase, the FA values goes down. The decreased FA values in patients compared to controls are perhaps reflecting aberrant tissue environment. The previous finding by Chiapelli and colleagues (2015) and the current thesis' findings could be speculated to be of shared processes between mI and FA, which seems to be some sort of imbalance in patients compared to controls. The imbalance could be interpreted in how mI is thought of as a reflection of cell density, and FA as degree of coherence in tracts. However, if the data allowed to

be modelled, the plots would align in an obvious pattern. Furthermore, there was no obvious relationship between mI and FA on the visual inspection of the scatterplot of the data.

In line with previous studies, the FA was overall lower in patients compared to controls. The lower FA is found across illness progression in FEP and chronic patients compared to controls (Szeszko et al., 2008). Mitterauer (2011) propose that the incoherence hypothesis of schizophrenia is based on the consistent findings of decrease or loss of oligodendrocytes and the myelin sheath that in normal situations wrap axons. The axons conduct information, and when normal the brain is able to compose and construct meaning from a wealth of input. In cases of demyelination, the oligodendrocyte-axon system cracks and the brain is unable to generate the information received into categories. Mitterauer (2011) describes alternative dimensional descriptors for schizophrenia dividing into psychotic dimension, disorganised dimension and a negative deficit dimension. It could be speculated if the two of the glial markers could be understood to represent two of these dimensions, with choline potentially being associated to negative symptoms and mI to the disorganised dimension, as abnormalities in white matter may be responsible for symptoms of incoherence.

The trend for the interaction effect was identified in clusters mostly in superior longitudinal fasciculus (SLF). The superior longitudinal fasciculus is the fiber bundle that link posterior parietal cortical areas to different frontal cortical regions (Karlsgodt et al., 2008). Despite the values derived from the cluster analyses indicate the probabilities are quite low (4%), it could be due to the identified white matter tract is quite large, and the cluster is only taking up a small part of it. The involvement of superior longitudinal fasciculus (SLF) is recurrently implied to be a region importance in schizophrenia. Alterations in SLF are indicated as robust in the early progression of the disorder (Ruef et al., 2012 cited in Karlsgodt et al, 2008), as well as in bipolar disorders with manifesting psychosis (Lin et al., 2011 cited in Karlsgodt et al., 2008). Furthermore, the findings have been replicated in high-risk individuals (Karlsgodt et al., 2008). The current findings are suggestive to SLF as a region with alterations in schizophrenia, and to be associated with mI measured in L STG, which warrants exploration in future studies.

In conclusion, the current project suggest glial abnormalities in schizophrenia patients.

Limitations

There are limitations in the master thesis. The current sample is not characterized as low (n = 154), however, due to quality issues, the number of valid measures are in some voxels down to 37. Furthermore, low sample sizes are not accurate in detecting subtle metabolite changes, whereas large ones are critiqued to be prone to detecting differences too small to have a clinical significance. Due to scanner update during the data collection period, there are differences in protocols which results in different number of measures between the voxels. As a reminder, for instance in L STG there are 119 measures for NAA (all participants), while only 52 in R STG. The missing measures limits the exploration of the voxel placements and measured metabolites in power.

There are known covariates that could have benefitted the robustness of the findings if included. For instance, controlling for scanner version is supported by the significant main effect observed. Smoking is found to impact inflammatory markers (Fernandez et al., 2012) and obesity (Khandaker and Dantzer, 2016), hence, smoking status and BMI should be controlled for. Furthermore, so could the duration of illness and previous medication exposure, as these as repeatedly causing differences.

In addition to the faults in the blood sample analysis, there are other features of the analysis models that could be critically discussed. The regression models have a relative large amount of variables, which could make analysis prone to Type 1 errors in multiple comparisons. With several variables added to the model at once, it could be that some of the added variables to contribute, but is cancelled out by the lack of information in the other variables in the model. An alternative could have been to add one variable at a time in the model.

Traditionally, corrections for multiple comparisons like the Bonferroni has been the go-to solution. This method divides the alpha level .05 on the number of added variables. However, adding such a conservative correction on biological data might make the conclusions prone to Type 2 error, i.e. accepting the null hypothesis when there are actual differences. Imaging Statisticians Lindquist and Gelman, (2009) suggest that the whole issue of correction can be avoided if one multilevel model is builded from the start. In the current study, it is also attempted to build a model of glial activation markers and neuroinflammation markers in schizophrenia patients. Multilevel models are shifting estimates and intervals towards each other, and hence it is important to include covariates known to be implying in the topic like age for myelin.

One of the strengths of the current project is its attempt to build models informed by current practice and emerging findings, and therefore the individual variables' contributions to the models are not reported as many of these variables single correlations are already much researched and established, without an overall model context.

Theoretical limitations

Most studies on inflammation in schizophrenia use mean or median due to this lack of established cut-off scores which usually is referred to healthy controls (Monastero & Pentyala, 2017*)*. Hence, the levels are only considered abnormal if they are aberrant from the controls if the values are differing around two Standard Deviations. This is problematic. In the current study, there was only measures for patients, which, given the measures were without technical issues, would allow only for investigation of regression effects and not effect size.

It is found that there will be revealed differences among individuals when the level of the proteins reach a certain number. In a study cut-off level of CRP was set to 3.8 mg/L, which then yielded significant correlations to PANSS positive symptoms (Weiser et al., 2014 cited in Kroken et al., 2019). Further research on the current data set could have potential to reveal associations with symptom severity as other studies have found if the sample was grouped into subtypes based on CRP levels, i.e. low, medium, and high. Interestingly, following the previously mentioned study, other researchers are applying the same value as inclusion criteria for individuals recruited to test the effect of immune-modulating drugs. This might be a first step to a consensus on a cut-off score. Despite brain imaging techniques great ability to investigate microstructure and chemical changes, proteins like CRP and cytokines are far more cost-effective as most general practitioners offices have the ability to measure such.

Future Research

The choline elevations appear to be a promising area of investigation going forward. It would be interesting to explore to specific symptoms and the cytokines and CRP, as there is a new version of the blood analysis on the way. The trend-levels of mI and group interaction effects on FA is also interesting in the light of other studies findings on the blood brain barrier (BBB).Further exploration in the dataset was outside the scope of the current project. However, non-reported analysis in the dataset yielded replication of previous studies' findings of positive correlation between S100B with mI within patients (Rothermundt et al, 2007). Chiapelli and colleagues (2015,

p 356) interpreted their finding to support "the hypothesis that activation of astrocytes may play an important role in the pathogenesis of schizophrenia at least in a subgroup of patients". There is evidence of increased blood-brain barrier (BBB) permeability in patients (Najjar et al., 2017), and the effect of such is pro-inflammatory cells and molecules to enter the brain, which has already discussed in introduction and above about cytokines, can have adverse impact on tissue.

Conclusion

In sum, the current project cannot not contribute to the role of neuroinflammation in schizophrenia from the cytokine and CRP to glial cell markers or symptoms, however, it provides information of glial related-markers' activation to be aberrant compared to match controls. Overall, the implications the current study is to further explore the glial dysfunction in schizophrenia, and encourage for further investigation of the potential immune signature, identify potential new medication targets, and build towards revealing the order of biological changes in the illness.

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