STUDY PROTOCOL

Study Title: Rituximab - Immunotherapy for Schizophrenia spectrum disorder in adults. An open pilot study.

Protocol Number: RITS-PS-2019	**EudraCT Number:** 2018-004618-17
Protocol Date: 2019-01-28	**Protocol Version:** 1.2
Product: Rituximab	
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Signature Date

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1 ABBREVIATIONS AND DEFINITIONS OF TERMS

AAR = Any Adverse Reactions

ADEXI = Adult executive functioning inventory

ANCOVA = Analysis of covariance

ASD = Autism Spectrum Disorder

BDNF = brain-derived neurotrophic factor

BFCR = Bush-Francis Catatonia Rating Scale

BOCS = Brief Obsessive Compulsive Scale

BBQ = Brunnsviken Brief Quality of Life Inventory

CGI-I = Clinical Global Impression-Improvement scale

CGI-S = Clinical Global Impression-Severity scale

CHQ-8 = Client Satisfaction Questionnaire

CNS = Central Nervous System

CRIES-8 = Children's Impact of Events Scale

DMC = Data Monitoring Committee

DSM-5 = The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

GAF = Global Assessment of Functioning

HADS = Hospital Anxiety and Depression Scale

IVIG = intravenous immunoglobulin

M.I.N.I. = Mini International Neuropsychiatric Interview

MRI = Magnetic Resonance Imaging

MS = Multiple Sclerosis

NFL = neurofilament light chain

NGS = next generation sequencing

NIMH GOCS = National Institute of Mental Health Global Obsessive Compulsive Scale

NMDA = N-methyl-D-aspartate

NSE = euronspecific enolase

OCD = Obsessive-Compulsive Disorder

PANDAS = Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infection

PANS = Pediatric Acute-onset Neuropsychiatric Syndrome

PANSS-R = Positive and Negative Syndrome Scale

PGE = Patient's Global Evaluation of severity

PI = Principal investigator

PML = Progressive Multifocal Leukoencephalopathy

PNISSI = PsychoNeuroinflammatory Related Signs and Symptoms Inventory

PSP = Personal and Social Performance Scale

RAADS-R = The Ritvo Autism Asperger Diagnostic Scale-Revised

RDoC = Research Domain Criteria

SciLife Lab = Science for Life Laboratory

SLE = Systemic Lupus Erythematosus

SRIs = Serotonin Reuptake Inhibitors

SSD = Schizophrenia Spectrum Disorders

TB = Tuberculosis screen test

TNF = Tumor Necrosis Factor

UKU = UKU side effect rating scale

VGCC = Voltage-Gated Calcium Channel

WAIS = Wechsler Intelligence Scale

2 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

STUDY CENTER

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3 SYNOPSIS

TITLE	Rituximab - Immunotherapy for Schizophrenia spectrum disorder in adults. An open pilot study.
STUDY CENTER	Psychiatry Unit, Örebro University Hospital
AIM	To investigate whether rituximab added to treatment as usual (TAU) for 20 weeks in 12 treatment-resistant patients with schizophrenia spectrum disorder (SSD) will lead to clinically relevant overall improvement. Furthermore, the study aims to contribute to the understanding of underlying mechanisms by assessing inflammatory biomarkers in these patients.
OBJECTIVES	*Primary objective:* 1. To investigate whether severely ill, treatment resistant, psychiatric patients with schizophrenia spectrum disorder (SSD) are significantly improved after treatment with the immunomodulatory drug rituximab (anti-CD20 antibodies) compared to baseline. *Secondary objectives:* 2. To examine whether baseline levels of inflammatory markers predict treatment response. 3. To examine whether changes in inflammatory markers and B-cell depletion correlate with treatment response. 4. To examine metabolic mediators prior and after treatment and if they correlate with treatment response. 5. To examine whether there is a change in gut permeability after treatment. 6. To examine whether there is a change in cognition after treatment. 7. To investigate if there is a change in brain activity after treatment. 8. To investigate the patients' experiences of the novel treatment with a qualitative content analysis. 9. To assess safety and tolerability of rituximab during treatment for SSD

OUTCOME

Primary outcome

1. The primary outcome measure is change in symptoms measured as change in Positive and Negative Syndrome Scale (PANSS) score from baseline.

Secondary outcomes:

The secondary outcome measures are

1. Change in Personal and Social Performance Scale (PSP), measuring overall disability from baseline up to week 20
2. Change from baseline up to week 20 of illness severity (CGI-S) assessed by the clinician
3. Difference from baseline up to week 20 in inflammatory markers in blood (gene expression and proteins) in relationship to clinical response
4. Proportion of responders: Three different informants base their CGI-I evaluations on independent assessments: a) The treating clinician, b) The patient's self-assessment and c) A next of kin. If the mean value of these three is below 2.5 then the patient will be regarded as a responder (representing much or very much improved since baseline).
5. Frequency, seriousness and severity of side effects.

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Other outcomes:

The other outcome measures are:

1. B-cell depletion at week 5, and B-cell subpopulations at week 20 in relation to clinical response and baseline levels of B-cells
2. Change from baseline to week 20 in

cognitive functioning

3. Change from baseline to week 20 in diagnosis specific rating scales
4. Change in brain activity in fMRI.

Long-term outcomes:

<u>40 weeks follow – up</u>: Primary outcome for long term is identical to that used at 20 week:

1. Change in symptoms measured as change in PANSS score from baseline

The secondary outcome measures are:

1. Difference from baseline up to week 40 in inflammatory markers in blood (gene expression and proteins) in relationship to clinical response
2. Change in Personal and Social Performance Scale, measuring overall disability from baseline up to week 40
3. Change from baseline up to week 40 of illness severity (CGI-S) assessed by the clinician
4. Life quality measured with BBQ
5. Global improvement. Three different informants base their CGI-I evaluations on independent assessments: a) The treating clinician, b) The patient's self-assessment and c) A next of kin

Other outcome measures are:

1. B-cell subpopulations at week 40 in relation to clinical response
2. Change from baseline to week 40 in cognitive functioning
3. Change from baseline to week 40 in psychiatric rating scales

POPULATION

Twelve psychiatric patients (ages, 18-40).

INCLUSION CRITERIA

1) patient ages 18 to 40 years

2) a duration of illness exceeding 2 years

3) correspond to "Markedly ill", "Severely ill"

	or ”Among the most extremely ill patients” on the Clinical Global Impression – Severity scale (CGI-S) 4) Global Assessment of Functioning below 50 5) current Diagnostic and Statistical Manual for DSM-5 diagnoses of Schizophrenia spectrum disorder 6) treatment resistance, i.e. failing to remit despite adequate treatments 7) if female and with any risk for pregnancy, willing to use contraceptives 8) if antipsychotic treatment is prescribed the plasma concentrations of the drug must be tested and shown to be within therapeutic interval. 9) subjects should be judged by the investigator to be lucid and oriented to person, place, time, and situation when giving the informed consent. 10) immunoglobulin levels within the normal range
EXCLUSION CRITERIA	1) on-going immunomodulatory treatment 2) pregnancy or breast-feeding 3) weight below 40 kg 4) clinically relevant on-going infection 5) chronic infections 6) positive screening test for hepatitis B, C, HIV or tuberculosis 7) any change of psychotropic medication within the previous 4 weeks 8) “much” or “very much improved” already at baseline according to CGI-I i.e. scores of 1 or 2 by the clinician 9) severe heart failure (NYHA grade IV) or other severe heart disease or history of cardiac

arrhythmia or myocardial infarction

10) unable to make an informed decision to consent to the trial

11) in compulsory treatment

12) treatment with clozapine within the last 2 months

13) previous treatments with immunosuppressive agents

14) malignancy currently or within 2 years prior to inclusion

STUDY DESIGN

Open pilot study, in addition a qualitative study using content analysis approach.

Participants will be treated initially for 20 weeks and followed for 52 weeks.

STATISTICAL ANALYSIS

Determination of Sample Size

In this pilot study population of 12 participants is presumed to be large enough to determine any effect of rituximab.

Primary objective: Effect of rituximab on schizophrenia symptoms measured with PANSS

The primary outcome (as described in section 5.2) is difference between baseline and endpoint on PANSS scale. 40% reduction in the PANSS scale.

Analyses of laboratory data related to clinical response

Laboratory data will consist of: plasma protein concentrations and leukocyte mRNA expression values for a number of cytokines and related substances (inflammatory markers); total number of B-lymphocytes and number of cells from various B-cell subsets at week 5, 12 and 20, as measured through highly sensitive flow cytometry (HSFC); plasma proteins and lipids as measured through metabolomics analysis; and blood levels of markers for gut permeability.

In order to examine whether baseline characteristics can predict treatment response, baseline values of all these measurements will

be compared in “responders” and “non-responders” after completion of the study by means of t-tests or Mann-Whitney tests, as applicable. In order to examine whether changes in inflammatory markers and other measurements correlate with clinical response, the difference between baseline values and week-20 values (Δ-values) of all relevant measurements will be compared between “responders” and “non-responders” after completion of the study by means of t-tests or Mann-Whitney tests, as applicable. In addition, for each of these measurements, a generalized linear model (ANCOVA) will be built and a PCA analysis will be performed.

Analyses of other clinical data related to clinical response

For results on the scales (Personal and Social Performance Scale, CGI-S and diagnosis specific rating scales) the week 20 scores and the Δ-values (difference between scores at week 20 and baseline) will be compared by means of t-tests or Mann-Whitney tests, as applicable. They will also be included in an analysis of covariance (ANCOVA) model, controlling for baseline measures and other possible confounders, as demographic variables.

Analyses of long-term outcomes

The results at week 40 on these variables will be analysed with essentially the same methods as those at week 20, see above.

STUDY TERMINATION

LPLV – Last Patient Last Visit

4 BACKGROUND INFORMATION

4.1 Background

Schizophrenia and quality of life

Although psychiatric disorders are widespread, serious cases are concentrated among a relatively small proportion of cases with high psychiatric comorbidity (1-4). These individuals are usually unable to live independently, are repeatedly inpatients and have cognitive impairments. In addition to the suffering of having the disorder, the care of these patients involves high costs for society and constitutes a major burden for family and society as a whole. Quality of life is poor in patients with schizophrenia (5) and psychiatric patients as a whole have significantly lower quality of life than patients with physical disorders (6). Psychiatric disorders overall are the most disabling disorders among all disorders, according to the National Institute of Mental Health (2010). Life-expectancy is almost 19 years shorter for schizophrenic men compared to men in the general population and 16.3 years shorter for women with schizophrenia (7).

Treatment-resistance and schizo-obsessive disorder

A considerable proportion of the severely ill psychiatric patients does not improve despite adequate treatments. Among individuals with Schizophrenia spectrum disorder (SSD), treatment resistance occurs in approximately 30 % (8). Treatment resistance is associated with early onset (9), catatonia and comorbidity particularly with obsessive-compulsive disorder (10). This is sometimes referred to as schizo-obsessive disorder (11). This subgroup of schizophrenia, present in approximately 15 % of the patients, is characterized by severe OCD that tend to appear during the prodromal stages of psychosis. Schizo-obsessive patients have an earlier onset of psychosis compared to other patients with schizophrenia, and show more depressive symptoms, suicide attempts and motor abnormalities. They have increased rates of hospitalization, greater dysfunction, higher impairment in social behaviour, smaller social networks and poorer quality of life. They tend to be more socially hostile and more anxious, as evidenced by their greater number of panic attacks and phobias and they tend to be treatment resistant. In summary, obsessive compulsive symptoms seems to have a deleterious effect on schizophrenia outcome. However schizo-obsessive disorder has not yet been included in the DSM-5 diagnostic manual, for a review of schizo-obsessive disorder, see (12).

Psychiatric diagnostics and RDoC

Psychiatric diagnoses are solely based on reported symptoms and observed behaviours. Specific diagnostic biological markers have not been identified. Each diagnosis may have several different causes with complex interrelationships and symptom overlap.

A strong genetic component has been reported for most psychiatric diagnoses (13). Moreover, studies have shown a large overlap between different psychiatric disorders in terms of genetic risk factors; these are not disorder specific (14, 15). However, polygenic risk scores have been used in efforts to link certain genes to various clinical phenotypes such as treatment response, symptoms, imaging findings etc. In line with this, The National Institute of Mental Health (NIMH) launched Research Domain Criteria (RDoC) to support an agnostic, bottom-up reclassification, crossing traditional boundaries by setting aside conventional, categorical diagnostic entities (16). Recently motor abnormalities that cut-across many psychiatric, neuropsychiatric and neurological disorders have been proposed as a putative domain within the NIMH Research Domain Criteria framework (17). Motor abnormalities are common in neurodevelopmental disorders (i.e. schizophrenia, obsessive compulsive disorder, autism spectrum disorders and PANS) (18).

According to the researchers at NIMH it is obsolete to perform research based on categorical psychiatric diagnoses alone, without taking biological markers into account. RDoC intends to shift the focus of research (and eventually clinical practice) away from existing diagnostic categories, towards ”new ways of classifying psychopathology based on dimensions of observable behaviour and neurobiological measures”. To us this is the key issue. We question the value of the present psychiatric diagnostic entities in the severely mentally ill, treatment resistant, low function psychiatric patients with extensive comorbidity. These patients tend to present autistic, obsessive-compulsive and psychotic symptoms, and we can therefore expect extensive comorbidity in the patients in our study. The extent of the comorbidity needs to be explored as this may affect the outcome (example of comorbidity is shown in **figure 1**).

The immune system

The immune system is a host defense system comprising many biological structures and processes that protects against disease (19). The immune system must detect a wide variety of agents (pathogens) from e.g. viruses and distinguish them from the organism's own healthy tissue in order to function properly. In humans, the immune system is classified into subsystems: the innate immune system versus the adaptive immune system. The innate immunity is the first line of defence. It is more primitive, and dispatch foreign pathogenes without much precision. The innate immunity initiates the inflammatory response, in which white blood cells gather at the site of infection and release the inflammatory response with proteins that induce heat and swelling. The adaptive immune system consists mainly of cells called T lymphocytes and B lymphocytes, which can recognize a specific pathogen, resulting in a targeted attack against it. In a small percent of the population, the adaptive immunity instead cause autoimmune diseases such as multiple sclerosis or arthritis by attacking cells in the individual’s own tissues. For long researchers thought that the immune system simply worked by distinguishing an organism’s own constituents from non-self ones. But more recently this has shown not to be true. Eventually more complex theories have emerged.

The immune system regulates the body’s tissues in order to maintain equilibrium at all types of insults, regardless if they come from an external or internal source. However, pathogens can rapidly evolve and adapt, and thereby hide and avoid being recognized by the immune system. Nevertheless, multiple defence mechanisms have also evolved to recognize and neutralize pathogens. The immune system is also highly active in the healthy brain and essential to its functioning.

Immune cells, native to the CNS, are called *microglia* and exist in the healthy brain. Immune cells from elsewhere in the body (so-called peripheral immune cells) are not usually found in the healthy brain. The blood-brain barrier (BBB) protects the brain and keeps them out. Thus, the BBB protects the brain by separating the peripheral immune system from the neuroimmune system, however it is not a permanent wall. Pathogens can penetrate the BBB. The BBB is made from tightly packed endothelial cells (walls of blood vessels) that form a blockage. It prevents substances and immune cells to enter the parenchyma of the brain. Cells called astrocytes and a structure called the basement membrane reinforce the barrier. However, in the case of a brain disease the BBB becomes activated and allows immune cells to cross over.

How the immune system exerts its influence in the CNS is not fully understood. After all, apart from microglia, no immune cells are present within the parenchyma of healthy individuals. Proteins called cytokines, generated from peripheral immune cells, can affect the behaviour of other cells and they can influence the brain. Presumably, they enter the brain through areas that lack the regular BBB and could directly impact the brain through the vagus nerve (which runs from the brain to the abdomen). Moreover, immune cells within the meninges—the membranes that surround the brain—are thought to be another source of cytokines. Cerebrospinal fluid from the meninges enters the brain parenchyma through spaces surrounding the blood vessels (gap junctions) and thus can carry cytokines from the peripheral immune cells deep into the brain to influence neuron behaviour. Reversely the meninges contain lymphatic vessels that remove toxins and other waste from the brain parenchyma and thereby convey information about brain infections to the immune system. By these mechanisms, the immune cells in the meninges communicate with the brain. It is however still unclear how immune cells enter the meninges and produce their cytokines (19).

Inflammation as aetiology for severe psychiatric disorder

There is a strong genetic association, established by GWAS studies, between the human leukocyte antigen (HLA) locus and schizophrenia. Maternal immune activation, induced clinically through prenatal exposure to one of several infectious diseases, has shown to be a risk factor in the development of schizophrenia. HLA proteins are mediators of the T-lymphocyte responses, and genetic variability is well-established as a risk factor for autoimmune diseases as well as susceptibility to infectious diseases. Taken together, the findings strongly suggest that schizophrenia risk in a subgroup of patients, is caused by an infectious disease or an autoimmune process.

Immunological processes can cause mental illness and sequelae resulting in severe brain damage (20). Immune reaction causes inflammation, which in turn may result in elevated levels of anti-neuronal antibodies and cytokines in the blood and CSF, and signs of microglia activation (suggestive of inflammation) in the brain. Microglia cells, one of the main indicators of neuroinflammation, release cytokines, prostaglandins and glutamate in the brain. An activation of microglia has been observed in SSD according to brain imaging and post mortem studies (21).

NMDA receptor antibody encephalitis is an acute inflammation of the brain, first categorized in 2007 (22). It is caused by an immune system that attacks the NMDA receptor of the brain, mediated by autoantibodies that may diffuse across a disrupted blood-brain barrier and somehow reach the NMDA receptors of neuronal cell membranes.

The affected patients develop psychiatric symptoms such as agitation, psychosis, catatonia and paranoia. Grimacing and finger movements similar to those in PANS and chronic schizophrenia are also present. Consequently, patients with anti-NMDA receptor encephalitis run a risk of being misdiagnosed as schizophrenia. Subsequently, other types of antibodies have been linked to

psychiatric symptoms, and it is presently unknown to what extent patients diagnosed as schizophrenia may be afflicted by any of these putative autoimmune mechanisms.

Thus autoimmunity are evidently involved in the pathophysiology of schizophrenia but also in depression (23-25) and elevated levels of pro-inflammatory cytokines have been identified in various psychiatric disorders (26-28) (29) (30). In a highly influential GWAS study of schizophrenia (31), the genetic loci linked to schizophrenia were strongly enriched by B-lymphocyte lineages involved in acquired immunity (CD19 and CD20 lines). Out of multiple cell lines in the body, this B-cell enrichment was equalled only by cell lines derived from cortical brain regions, providing strong genetic support for immune dysregulation as a pathogenetic mechanism in schizophrenia, specifically implicating B-lymphocytes.

Several anti-neuronal antibodies are associated with personality change, psychotic and depressive symptoms (32). A childhood onset psychiatric disorder, Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infection, PANDAS (33) goes with OCD, but psychotic symptoms are likewise common in many of these patients (34). PANDAS is assumed to be an autoimmune reaction resulting from a streptococcal infection (35). The infection generates antineural antibodies assumed to cross-react with neuronal structures of the brain causing behavioral and motor abnormalities. This hypothesis resembles the known mechanism behind Sydenham's chorea; a childhood disorder characterized by involuntary movements and neuropsychiatric symptoms, such as obsessive-compulsive behavior (33). The patients are often prescribed off-label treatment with immunomodulatory drugs and antibiotics.

The hypothesis of PANDAS has been shown using rat models, both through active injection of antibodies into neuronal tissue of rats, as well as through passive transport (36). In the rat models, the antibodies caused compulsive behavior (manifested as increased induced-grooming) and motor abnormality (manifested as impaired food manipulation and beam walking), in line with the clinical picture of PANDAS. In addition, the administered antibodies cross-reacted with specific proteins responsible for important signaling cascades in the brain, such as dopamine and serotonin signaling cascades. IgG deposits were seen in striatum, frontal cortex and thalamus, areas proposed to have a central role in the symptomatology of PANDAS, and concomitant alteration of dopamine and glutamate levels in cortex and basal ganglia (37) in line with the pathophysiology of Sydenham's chorea and related neuropsychiatric disorders. Furthermore, the blood-brain barrier integrity seemed important in determining the distribution of antibodies in the neuronal tissue of the rats, as well as determining the neuropsychiatric outcomes (36).

Accordingly, immunological factors are hypothesized as being important determinants for the pathophysiology of several psychiatric disorders. Based on present knowledge on NMDA-receptor encephalitis and PANS, these mechanisms are more likely to be involved in the more severe, comorbid and treatment-resistant cases. The term *Immunopsychiatry* was recently coined (24) which covers this new field of research.

Cytokines in Schizophrenia spectrum disorder

Recent findings provide a strong evidence of a concomitant process of inflammatory activity in schizophrenia (38-40). The association between proinflammatory cytokines and schizophrenia is well accepted in the literature. Cytokine serum levels have been correlated with exacerbation/remission of the symptoms and with antipsychotic treatments (41, 42).

In a systematic review the existence of cytokines abnormalities in schizophrenia is confirmed (43). Immune imbalances such as increased levels of some cytokines (either at protein level or at mRNA expression), cytokine mRNAs, as well as cytokine gene polymorphisms have been reported with a large support in schizophrenia. Increased levels of the cytokines IL-6 in schizophrenia seem to be involved in its pathophysiology of schizophrenia. TNF-α contributes to the progress of the inflammatory response in schizophrenia. Moreover, the soluble receptors (sTNFR1 and sTNFR2) had increased levels in plasma or serum, and it has been considered an inflammatory marker in severe mental disorders. IL-10 Anti-inflammatory cytokine, whose levels were found altered in chronic patients with schizophrenia, may be due to an increased compensatory stimulus of the type 2 cytokine pattern. Also, IL-10 may suffer down-regulation by the use of antipsychotic drugs. Increased concentrations of IFN-γ were reported in several studies, correlating with the first episode of schizophrenia. Furthermore, high levels of IFN-γ were correlated with acute exacerbations and in subsequent antipsychotic treatment. IL-1β is a cytokine involved in neurodegenerative and neuroprotective processes, and in the modulation of synaptic plasticity. Reports have shown an increased concentration of IL-1β in schizophrenia. IL-8 is a chemoattractant cytokine. The role of IL-8 in schizophrenia is associated with treatment resistance and negative symptoms. Also, IL-8 levels are increased in schizophrenia. IL-2 and its soluble receptor (sIL-2R) levels were associated with schizophrenia and psychotic stages. These abnormal levels are also linked with antipsychotic treatments. IL-1 receptor agonist (IL-1Ra) is produced in response to diverse stimuli of inflammation such as IL-1β and IL-6. This cytokines is expressed in many cells, including neurons. Evidence shows that IL-1Ra levels are increased in schizophrenia, as well as the consequent immune activation and inflammatory processes. In studies of cytokine gene polymorphisms it is shown that IL-6 gene polymorphism is associated with the pathology of schizophrenia, which levels are increased. IL-6 gene polymorphism, specifically IL-6 -174G/C, was also linked to schizophrenia.

TNF-α is a pleiotropic cytokine (neuroprotective and neurodegenerative effects, and immune and inflammatory responses), which levels were significantly higher in schizophrenia. TNF-α gene is one of the most studied and best described polymorphism, correlating to schizophrenia susceptibility.

Cytokine mRNAs levels are correlated with schizophrenia: cognitive deficits and anatomic abnormalities such as decrease brain volume and abnormal cortical folding. IL-6, TNFR1/TNFR2 and IL-1β mRNAs were the mostly repeatedly investigated, and their levels are associated with the pathophysiology of schizophrenia.

In our own research group we have found similar associations. In a mixed sample of 40 severely ill psychiatric patients with obsessive-compulsive disorder, schizophrenia spectrum disorder, autism spectrum disorder and/or non-suicidal self-injury disorder and 40 healthy subjects, gene expressions of inflammatory-related genes were analysed using real-time qPCR. From plasma, protein levels were measured using electrochemiluminescence ELISA technology.

Cytokine levels for the IL-1 family showed significant higher levels for IL1Ra ($p<0.0001$) and IL-18 ($p=0.0005$) in patients compares to controls, but not for the IL-1β levels. Patients also had higher levels of the cytokines TNF ($p = 0.0001$) and IL-6 ($p <0.0001$), but not IL-8.

We found increase in patients' gene expression compared to controls in *CASP1* ($p = 0.0005$), *NLRP3* ($p = 0.0426$), *PYCARD* ($p = 0.0484$), *IL1B* ($p = 0.0014$) and *IL-1RN* ($p = 0.0013$). However, *IL18* levels did not differ. For more general markers of inflammation, *TNF* ($p = 0.0308$) was significantly increased compared to controls, but not *IL6* and *IL10*. Together our data indicate that individuals with severe psychiatric disorders have increased inflammatory activity (44).

The lack of reproducible biomarkers

A problematic dilemma concerning these concepts is the lack of reproducible biomarkers related to the hypothesised mechanisms. As already mentioned, in the case of PANS/PANDAS, treatment results, as well as clinical findings and animal studies, coherently support an autoimmune mechanism. In spite of this, the purported biomarkers have not hitherto been validated (64). This failure, however, does not prove that the hypothesis is wrong, just that the methods attempted to validate the hypothesis have yet been insufficient. It also compellingly suggests that patients may well have immunological processes that affect brain circuits, thereby producing psychiatric symptoms, while we are left unable to substantiate these processes with biomarkers. If the immunology is present in PANS/PANDAS, it may well be present in such adult OCD cases where biomarkers are difficult to identify.

It behoves ethical thinking to take responsibility for treatment research that potentially may result in novel methods for reducing suffering. This is the main incentive for our study, and we believe that the aggregate support for immunological mechanisms supersedes the lack of reproducible biomarkers. In this situation, a trial of a potent immune modulator, effective in autoimmune disorders, has the potential to move us forward towards an effective treatment for severe suffering.

Effects of Rituximab on the brain

Rituximab is a monoclonal antibody that binds to a membrane protein, CD20, which is located on the surface of pre-B cells and mature B lymphocytes. Binding of the antibody to the B cell leads to B cell cytolysis (cell death of specific B cells). The B cell fills multiple functions in the immune system, including as antigen presentator (activation of T cells and other cells), production of immunomodulating cytokines, and production of antibodies. In rituximab treatment, the antigen-presenting and immunomodulating function of the B cell is thought to be largely the property that gives rise to the treatment effect, i.e. not the elimination of the antibodies. CD20 is not expressed on hematopoietic stem cells, pro B cells (precursor to pre-B cells and B lymphocytes), plasma cells (challenged B lymphocytes produced antibodies) or in normal tissue, which means: 1) the effect of rituximab is reversible and upon completion of treatment, the patient eventually recovers its B cells and thus regains its function in the immune system, 2) already engineered plasma cells will continue to produce antibodies, and 3) other tissues in the body that do not express CD20 are not affected. A number of findings support that there is a B and T cell involvement in the psychiatric diseases we intend to investigate. In addition, communication between B and T cells clarifies how an anti-B cell therapy affects more immune system processes than just the production of antibodies. The hypothesis that the role of the immune system in mental illness not only is mediated by antibodies but also by interaction between B and T cells and cytokines was presented as an important information by several prominent researchers at the Swedish Medical Association's Translational Research Symposium for Immunopsychiatry, which was launched in April 2018. A possible effect of rituximab treatment may be that lack of activation via B cells contributes to the normalization of T-cell-driven inflammation in the brain, which in turn leads to a normalization of microglia activity and thus a decrease in symptoms. In summary, we consider that we have presented a scientifically based hypothesis that CD-20 antibodies may affect severe psychiatric disease.

Rituximab is a standard drug for the treatment of rheumatoid arthritis and is widely used for difficult-to-treat MS. The effect of rituximab on neuroinflammation has clearly been shown by its effect in the treatment of MS, which is a disorder of the brain. Rituximab is a first-hand treatment in primary B cell type lymphoma. It was previously assumed (based on early pharmacokinetic studies) that CNS lymphoma would be hard to access with rituximab. However, a recent study showed that addition of rituximab to a combined treatment was as effective as the addition of radiotherapy. CSF concentrations of rituximab, significantly lower than plasma levels, also appeared to be sufficient to contribute to this effect (45). In addition, one controlled randomized study showed a beneficial effect of rituximab on cerebral symptoms in patients with chronic fatigue symptoms (46). This study unraveled the connection between inflammation and chronic fatigue. For long chronic fatigue was gathered to solely have a psychological origin, thus the rituximab study had a dramatic impact on the understanding of the pathophysiological mechanism behind chronic fatigue.

In the case of rheumatoid arthritis (which in most cases is an extracerebral disorder), there are several case reports indicating that a significantly lower dose (< 10 %) than the currently recommended appears to be effective in a majority of cases, suggestive of a generous margin in dosage recommendations (47). In a pilot study on healthy volunteers, it was shown that < 1% of the recommended dose resulted in a clinically relevant depletion of B cells (48). The action of rituximab is thought to primarily be associated to its blocking effect on B cells in the periphery which subsequently leads to decreased B cell entry into the CNS (from the bloodstream), i.e. not due to a central blockage of B cells in the CNS.

The blood-brain barrier (BBB) can be damaged by a variety of factors, e.g. skeletal trauma. Inflammatory activity itself is probably another important factor, since it increases BBB permeability.

In this context, it should be emphasized that clinical psychiatry (at least in Sweden), lacks routines regarding the determination of BBB injury. As a result, even severely ill patients with difficult-to-treat psychiatric symptoms are normally not diagnosed with respect to barrier injury.

Immunomodulatory treatments

Intriguingly, treatments recommended for treatment-resistant psychosis are clozapine and minocycline. Both these drugs have immunomodulatory effects (49, 50) (51). A recent case-report presented a complete remission from a treatment-resistant schizophrenia after a bone marrow transplantation (52) and, conversely, another case-report described onset of treatment resistant psychosis after receiving bone-marrow from a schizophrenic donor (53). These studies strongly support the hypothesis of the immune system’s impact on psychiatric symptoms. However, to date very few immunomodulatory drug trials for severe psychiatric disorders have been published and rituximab has not been studied in any of them (54-56).

Why we want to study rituximab rather than other immunomodulatory drugs

In a case study rituximab was described as successful for treating PANS (57). Rituximab is included in the treatment guidelines of anti-NMDA receptor encephalitis (58) and is generally well-tolerated (59). Moreover, rituximab targets B-lymphocytes of CD19-CD20 lineage, exactly the cells that were found to enrich schizophrenia genome in the above-mentioned GWAS study. While rituximab selectively targets B-cells, studies have suggested that the treatment effect is largely antibody-independent and that other B-cell functions such as antigen presentation and cytokine production contributes to amelioration of autoimmune diseases after B-cell depletion therapies (60). B cells with increased production of inflammatory cytokines such as lymphotoxin, TNF and IL-6 are elevated in patients suffering from autoimmune disorders. Additionally, lower levels of cytokines associated with regulatory B cells, such as IL-10, is seen and B-cell depletion by rituximab has shown to normalize these immune perturbations (61, 62). In the present study we want to test if rituximab can ameliorate symptoms in treatment resistant markedly ill psychiatric patients with schizophrenia spectrum disorder (SSD).

Although the experience with immunomodulatory drugs such as corticosteroids, intravenous immunoglobulin (IVIG) and methotrexate is longer, as compared to rituximab, they have drawbacks: Corticosteroids strongly increase the risk for mania in psychiatric patients, which may further impair psychiatric health; IVIG is a blood-derived product, which makes its availability limited and furthermore, according to experience with patients with PANS, monthly treatments are often required to keep the patient symptom free. This makes IVIG costly and complicated. Trials with methotrexate on psychiatric populations have not yet been published, but we anticipate that psychiatric patients are reluctant towards a weekly intake of chemotherapy. Mycophenolate mofetil and azathioprine are other similar options used in autoimmune encephalitis and other autoimmune disorders, but not as far as we are aware, for psychiatric disorders. A fourth option is adding the antibiotic minocycline, which has anti-inflammatory properties, but minocycline must be dosed twice daily, which may affect compliance. Finally, since rituximab is regarded as sufficiently safe in large populations suffering from autoimmune disorders, thus we expect it to be likewise safe in psychiatric patients. Interestingly, a prevailing opinion on the mechanism of IVIG (hitherto the best immunomodulator in PANS treatment) is its modulation of B-cell function (63) which is also the main mechanism of rituximab). Accordingly, we expect the presumed benefits of rituximab to overrun the possible harm caused by this drug in markedly ill psychiatric patients with SSD.

Our experience of the treatment and the patient group

Already in 2013 we started our research on children and young adults assessed for Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Bacteria (PANDAS) and Pediatric Acute Neuropsychiatric Syndrome (PANS) (EPN in Stockholm 2014/551-31/2 and No. 2015 / 964-31). We interviewed 53 patients and their families in their homes all around Sweden. We interviewed each of them between 3 and 5 hours and tested their cognitive abilities. These children and young adults have obsessive-compulsive symptoms, but many also had psychotic symptoms and some were diagnosed with schizophrenia (34). We were alarmed by the extreme complexity of the symptoms in these patients, the poor treatment response to psychiatric medication and the extremely low quality of life that affected the whole family. Many patients were desperate and had spent large savings in search for relief. We also drew blood to investigate the diagnostic properties of the commercially marked panel for diagnosing PANDAS and PANS (the Cunningham panel) in these patients. When we compared the results with those of patients suffering from other psychiatric disorders and with healthy controls we could show that the Cunningham panel was unreliable. Healthy children had elevated levels to a high extent and the panel could not differentiate any of the groups (64). This led Wieslab, the company that marketed the Cunningham panel in Europe, to removed this analysis from their platform in December of 2017.

We also studied inflammatory markers in these patients in collaboration with researchers at KI (Thomas Poiret) and currently writing up findings supporting the idea of molecular mimicry as aetiology for PANDAS. More recently we have investigated gene expression of inflammatory markers in a pooled sample of 40 severely ill psychiatric patients (children and adults) with diagnoses such as SSD, OCD, non-suicidal self-injury disorder and ASD, and compared the markers with those of healthy age and sex matched controls from the same area (EPN in Uppsala, No 2016/091). Our preliminary data (as described above) shows large differences in gene expressions between the patients and the controls (manuscript in preparation) as well as elevated protein levels of cytokines and support the notion of elevated level of inflammation in psychiatric patients. Our results strongly support that our group of comorbid, severely ill psychiatric patients have an up-regulated immune system with inflammatory activity that do not correspond to detectable inflammation in the peripheral body. Hence, neuroinflammatory activity is a likely explanation. Also, we have followed the clinical picture in a large number of patients with PANDAS and PANS who were treated with IVIG or other immunomodulatory agents. Two of these PANDAS patients received rituximab because of comorbid autoimmune disorders (neuromyelitis optica and Mb Chron, respectively). Notably, psychiatric symptoms are not included in the symptomatology of these disorders. In both cases we noted remarkable improvements in the psychiatric symptoms, and in one patient all psychotic symptoms remitted (65).

Figure 1. A schematic illustration of diagnostic overlaps in severe psychiatric disorder

Overall, our research group consists of clinical researchers (psychiatrists) with extensive experience of patients with severe psychiatric illness, including children. The PI has worked decades as a senior consultant and currently assess treatment resistant psychiatric patients with SSD, OCD and ASD at the Psychiatric clinic in Örebro on weekly basis. Also Mats Humble, psychiatrist, PhD and collaborator in this study, has extensive clinical experience with severe treatment resistant psychiatric patients. Three doctoral students are involved in various projects related to inflammation in psychiatric patients. One of the PhD students, a nurse, is accustomed to perform blood tests in the homes of the patients, thus they don't have to go to a clinic. We closely collaborate with preclinical senior researchers at Therapeutic immune design, CMM, Karolinska institutet and the research group at iRiSC, Örebro University. Our collaborators have extensive experience in working with the research methods needed for our analyses and have long experience of the in vitro systems to be used on whole blood and its components. In the present study we will also collaborate with the Department of Clinical and Experimental Medicine at Linköping University and the Department of Radiology at Örebro University Hospital to conduct an imaging study on inflammation in CNS. We will collaborate with Nutrition-Gut-Brain Interactions Research Centre (NGBI) at Örebro University for research on the effect of rituximab treatment on gut microbiom. For research on metabolic markers we will work together with Man-Technology-Environment research centre (MTM) at Örebro University. For bioinformatics we will collaborate with Faculty of Health, Science and Technology at Karlstad University. Finally, we will collaborate with clinical researchers within the fields of occupational therapy, paediatrics, child and adolescent psychiatry and rheumatology. For health economy analysis we will collaborate with researcher at University health care research centre (UFC) in Örebro.

Clinical significance

In this trial we will study rituximab in markedly ill psychiatric patients diagnosed with schizophrenia spectrum disorder and with considerable impairment. Regularly they are treated with high dosages of psychiatric drugs nevertheless they suffer from symptoms as well as significant side effects.

The inflammatory hypothesis of psychiatric disorder predicts that rituximab treatment should improve the condition, and that the improvement should be associated with change in biological markers. Large gains by reduced suffering and lowered healthcare costs may be achieved with this treatment. If participants are improved this may entirely transform the outcome for large groups of psychiatric patients.

This study will be an interdisciplinary collaboration between clinicians and researchers within the fields of immunology, psychiatry, rheumatology, gastroenterology, psychology and laboratory disciplines, which may open up new work paradigms for exploring new treatments for severe psychiatric illnesses.

Trial design

Open pilot intervention study, in addition a qualitative study using content analysis approach.

5 STUDY OBJECTIVES AND ENDPOINTS

5.1 Objectives

Primary Objective

To investigate whether markedly ill, treatment resistant psychiatric patients, assumed to have an inflammatory aetiology for their symptoms and diagnosed with schizophrenia spectrum disorder (SSD), are improved after treatment with the immunomodulatory drug rituximab (anti-CD20 antibodies).

Secondary Objectives

- To examine whether baseline levels of inflammatory markers predict treatment response.
- To examine whether changes in inflammatory markers or B-cell depletion correlate with treatment response.
- To examine metabolic mediators prior and after treatment and if they correlate with treatment response.
- To examine whether there is a change in markers for gut permeability after treatment.
- To examine whether there is a change in cognition after treatment.
- To investigate the patients' experiences of the novel treatment with a qualitative content analysis.

To assess safety and tolerability of rituximab during treatment for SSD.

5.2 Outcomes

Primary outcome

The primary outcome measure is change in symptoms measured as change in PANSS score.

Secondary outcome

- Global improvement at week 20, according to a balanced assessment (mean value of three independent assessors: 1) The treating clinician, 2) The patient's self-assessment and 3) A next-of-kin) using the Clinical Global Impression-Improvement (CGI-I) scale (range 1-7; 1 representing "Very much improved").
- Change in Personal and Social Performance Scale (PCP) measuring overall disability
- Change from baseline up to week 20 of illness severity (CGI-S) assessed by the clinician
- Difference from baseline up to week 20 in inflammatory markers in blood (gene expression and proteins) in relationship to clinical response (CGI-I)
- Proportion of responders to treatment, i.e. rated as much or very much improved since baseline according to CGI-I assessed by three different informants: 1) The treating clinician, 2) The patient's self-assessment and 3) A next of kin. If the mean value of these three is below 2.5 then the patient will be regarded as a responder (representing much or very much improved since baseline).

- Safety and tolerability of rituximab during treatment for SSD measured with the questionnaire Any Adverse Reactions (AAR).

Other outcome measures

- B-cell depletion at week 5, and B-cell subpopulations at week 20 in relation to clinical response and baseline levels of B-cells
- Changes in cognitive functioning at 20 weeks:
    - Neuropsychological functioning assessed with e.g. visuospatial and executive tests
- Change from baseline on rating scales that measure psychiatric symptoms specific for each diagnosis (shown below) *during the past 2 weeks*
    - BOCS for measuring obsessive-compulsive symptoms
    - RAADS-R for measuring autistic symptoms
    - PANSS for measuring psychotic symptoms
    - BFCR to measure catatonic symptoms
    - HADS for measuring depressive and anxiety symptoms

Long-term outcomes

40 weeks follow –up:

Outcome measures for long term are identical to that used at 20 week.

Change in symptoms measured as change in PANSS score.

The secondary outcome measures are:

- Global improvement at week 20, according to a balanced assessment (mean value of three independent assessors: 1) The treating clinician, 2) The patient's self-assessment and 3) A next-of-kin) using the Clinical Global Impression-Improvement (CGI-I) scale (range 1-7; 1 representing "Very much improved").
- Difference from baseline up to week 40 in inflammatory markers in blood (gene expression and proteins) in relationship to clinical response
- Change in PSP, clinician administered version, measuring overall disability from baseline up to week 40
- Change from baseline up to week 40 of illness severity (CGI-S) assessed by the clinician
- Proportion of responders to treatment, i.e. rated as "Much improved" or "Very much improved" since baseline according to CGI-I
- Life quality measured with BBQ

Other outcome measures are:

- B-cell subpopulations at week 40 in relation to clinical response
- Changes in cognitive functioning at 40 weeks:

  - Neuropsychological functioning assessed with e.g. visuospatial and executive tests
- Change from baseline on rating scales that measure psychiatric symptoms specific for each diagnosis (shown below) *during the past 2 weeks*
  - BOCS for measuring obsessive-compulsive symptoms
  - RAADS-R for measuring autistic symptoms
  - PANSS for measuring psychotic symptoms
  - BFCR to measure catatonic symptoms
  - HADS for measuring depressive and anxiety symptoms

Follow-up, 1year after treatment start. Outcome is PANSS and CGI-I in relation to treatment and evaluated by the treating clinician, the patient's self-assessment and a next of kin.

Qualitative content analysis

At endpoint 1, after 20 weeks, a qualitative content analysis will be performed.

6 SELECTION AND WITHDRAWAL OF VOLUNTEERS

6.1 Inclusion Criteria

To be eligible for inclusion into this study, each patient must fulfil all of the following criteria:

1) Age: 18 to 40 years

2) Duration of psychiatric illness: exceeding 2 years

3) Rated "Markedly ill", "Severely ill" or "Among the most extremely ill patients" on the Clinical Global Impression – Severity scale (CGI-S)

4) Global Assessment of Functioning below 50

5) Current diagnosis according to Diagnostic and Statistical Manual for Mental Disorders, 5th edition (DSM-5) of SSD (schizophrenia spectrum disorder)

6) Treatment resistance, i.e. failing to remit despite adequate treatments

7) If female and with any risk for pregnancy: willing to use contraceptives.

8) If antipsychotic treatment is prescribed the plasma concentrations of the drug must be tested and shown to be within the therapeutic interval.

9) Subjects should be judged by the investigator to be lucid and oriented to person, place, time, and situation when giving the informed consent.

10) Immunoglobulin levels within the normal range

Treatment resistance is defined as poor general functioning (GAF<50) and minor or no persistent improvement from previous treatment attempts, according to the following specifications: At least 2 trials with different evidence-based drug treatments for schizophrenia and at least one from the second generation of anti-psychotic drugs. Both drugs should have been tested for a minimum of 4 months with adequate dosages. Clozapine must have been tested or considered not feasible

Fertile women will only be included in the study if they are using/agree to use adequate contraception during treatment with the study drug and for the following 12 months after the last treatment. Adequate contraception is defined as at least 1 of the following: 1) abstinence, 2) oral contraceptive (combined or progesterone alone), 3) implants of levonorgestrel, 4) estrogenic vaginal ring, 5) percutaneous contraceptive patches, 6) injectable progestogen, 7) intrauterine device (IUD) or 8) male partner sterilization. Non-fertile women are defined as any female that is surgically sterile (documented hysterectomy and/or bilateral oophorectomy or tubal ligation).

Participants with severe SSD may not be able to respond to all questions or fill out rating scales. However, we will then obtain independent information whether the patient is improved or not according to CGI-I and PANSS even though h/she may not be able to fill out rating scales. Thus our primary outcome measure can be used at all times regardless of the state of the patient.

6.2 Exclusion Criteria

Patients are to be excluded from the trial if ***any*** of the following criteria is fulfilled:

1) on-going immunomodulatory treatment

2) pregnancy or breast-feeding

3) weight below 40 kg

4) clinically relevant on-going infection

5) chronic infections

6) positive screening test for hepatitis B, C HIV or tuberculosis

7) any change of psychotropic medication within the previous 4 weeks

8) “much” or “very much improved” already at baseline according to CGI-I i.e. scores of 1 or 2 by the clinician

9) severe heart failure (NYHA grade IV) or other severe heart disease or history of cardiac arrhythmia or myocardial infarction

10) unable to make an informed decision to consent to the trial

11) in compulsory treatment

12) treatment with clozapine within the last 2 months

13) previous treatments with immunosuppressive agents

14) malignancy currently or within 2 years prior to inclusion

6.3 Withdrawal of patients

The patients can at any time choose to stop participating in the study without giving any reason for this. If possible, the reason for the patient leaving the study should be recorded. Data collected up to the subject’s leaving the study will be used in the analysis of the study.

The investigator can remove a patient from the study due to safety reasons or in case the patient is not able to comply with the protocol.

7 STUDY PLAN AND DESIGN

7.1 Study outline

After a screening visit, patients who meet the inclusion criteria will be asked to participate in this study. Plasma drug levels of the on-going medication will be measured before inclusion. Patients will be allocated to treatment with rituximab. If the patient is treated with anti-hypertensive drugs these shall be withdrawn 12 hours prior to rituximab treatment. Patients will receive the premedication 1-2 h prior to rituximab in their home or at the clinic. A nurse, experienced with rituximab treatment, will accompany the patient during the hours of rituximab infusion. Blood pressure will be measured before and after treatment and at every change of the speed of the infusion. The temperature will be measured before and after treatment. Mabthera will be administered according to the guidelines for safety of the patients, see supplementary file "*Rutiner för behandling med Mabthera inom ramen för studierna RITS-PO och RITS-PS*". Patients will rest for an hour after the termination of the infusion and will not be left alone during this time.

All patients will be treated with the study drug rituximab on one single occasion and followed for a total of 1 year. They will have a total of 13 visits (screening prior to inclusion can be divided into more than one visit if necessary) with a clinician or research nurse who is experienced with rituximab and with psychiatric patients. An extensive psychiatric interview will be performed with each patient and his/her next of kin at baseline. We will also review medical records in order to document previous symptomatology and treatment trials. A short video recording is optional. All participants will be examined for psychiatric, neurological and motor symptoms, cognitive functioning, and a range of biomarkers. However only blood sampling and the interview are prerequisites for participation, all other examinations and tests are optional. Prior and post treatment the optional lumbar puncture and fMRI will be performed for further diagnostic evaluation and assessment of blood-brain barrier function. Faeces samples will also be collected prior to treatment and endpoint 1 of this study (optional) as well as gut biopsy prior to treatment (optional). Patients are requested to remain on their standard psychiatric treatment until endpoint 1, and treatment should be stable for the last 1 month prior to baseline. Patients are allowed adaption of their on-going psychiatric treatments after week 20 but also earlier if the treating physician deems this necessary.

Biological and psychiatric assessments will be performed at pre-decided time points **(see table 1**). Patients will be followed by additional visits to a nurse for in-between visits.

Side effects will be monitored at each visit. Patients will continue their regular visits with the psychiatric staff.

At endpoint 1 (20 weeks) a separate interview lasting approximately 45 minutes will be held for a qualitative analysis.

7.1.1 Data collection, management, and analysis

Demographic background data and treatment history will be collected from interview and medical records at baseline.

Assessments will be made with established and well-validated rating scales and questionnaires in addition to one research interview that was developed by the principal investigator (PNISSI) (see list of all rating scales and questionnaires below). Sampling of biological materials will be done by a standard operating procedure (SOP) used for sampling for other current cohorts. We will also perform laboratory tests in a highly structured manner with well-validated methods in collaboration with researchers at Örebro University and Karolinska Institutet. Samples will be frozen and stored in Region Örebro County biobank (458) at -80 °C until analysis. All data collection forms will be stored at a safe place within Örebro hospital, Region Örebro County.

7.1.2. Rating scales and tests

If more than one clinician will assess the patients rating calibration training for obtaining agreement on scorings will be performed.

Due to the large data collection in combination with the severity of the disorder in these patients missing data will be unavoidable. We will try to avoid missing data by providing clarification if a patient cannot understand the meaning of a question. For each symptom rating scale the response rate is set at a minimum of 80 % of the questions. Isolated missing scores will be replaced with the individual mean.

Demographics

- Background data collection form
- Pans Data Collection Form provides information about inherited physical and psychiatric disorders within the family.

Diagnostic interviews

• Mini International Neuropsychiatric Interview (M.I.N.I. version 7) is a structured clinical interview for psychiatric disorders. In addition the childhood onset diagnoses from the M.I.N.I. kid version will be amended to the adult version of M.I.N.I. (66, 67)

• PsychoNeuroinflammatory Related Signs and Symptoms Inventory (PNISSI) in order to identify and measure symptoms related to neuro-inflammation and diagnose PANDAS and PANS (68)

Assessment of severity, improvement and quality of life

• Clinical Global Impression-Improvement scale (CGI-I) measures change of symptoms on a 7 point Likert scale. CGI-I will also be administered to the next-of-kin (69)

• Patient's Global Evaluation (PGE) provides a self-administered global measure of improvement on a 7 point Likert scale (70)

• Clinical Global Impression-Severity scale (CGI-S) provides a global measure of illness severity on a 7 point Likert scale (69)

• Global Assessment of Functioning (GAF) measures general disability and symptom burden (71)

• Personal and Social Performance Scale (PSP) (72). The PSP is a 100-point single-item rating scale, subdivided into 10 equal intervals. The ratings are based mainly on the assessment of patient's functioning in four main areas: 1) socially useful activities; 2) personal and social relationships; 3) self-care; and 4) disturbing and aggressive behaviours.

• Level 1 Cross-cutting symptom measure of global symptom severity is a patient or informant rated measure included in the DSM-5, which assesses mental health domains that are important across psychiatric diagnoses. It includes 13 domains. We will use it with the clinician’s assistance for adults (73)

• Brunnsviken Brief Quality of Life Inventory (BBQ) is a 12-item measurement of life satisfaction (74, 75)

• EuroQol Group EQ-5D™ (EQ-5D-5L) is an 5-item quality of life inventory (76)

Obsessive-Compulsive disorder

• Brief Obsessive Compulsive Scale (BOCS) maps self-reported symptoms and measures severity of obsessive-compulsive symptoms by a clinician (77)

• National Institute of Mental Health Global Obsessive Compulsive Scale (NIMH GOCS) is a global assessment of OCD severity (78)

Autism spectrum disorder

• Clinician-rated Severity of Autism Spectrum and Social Communication Disorder is a DSM-5 based measure for assessing severity of autism spectrum disorder (79)

• RAADS-R is a self-report to assess autistic symptoms. If the patient is unable to fill it out this can be done by a next-of-kin (80)

Schizophrenia spectrum disorder

• Clinician-rated Dimensions of Psychosis Symptom Severity is a DSM-5 based measure for assessing severity of a psychosis (79)

• Positive and Negative Syndrome Scale (PANSS) is a measure for psychotic symptoms in schizophrenia (81).

Catatonia

• Bush-Francis Catatonia Rating Scale (BFCR) is a 23 item measure for catatonic symptoms (82)

Anxiety and depression

• Hospital Anxiety and Depression Scale (HADS) is a combined brief self-report measure for anxiety and depressive symptoms (83)

Trauma

• The Children's Impact of Events Scale (CRIES-8) is an 8-item self-report to measure post-traumatic symptoms. In the present study it will be used in adults (84)

Personality syndrome

• The Personality Inventory for DSM-5 Brief Form is a brief self-report (79, 85)

Early onset psychiatric symptoms

• Five to fifteen-Brief (FTF-Brief) consists of 24 items representing 18 of the original 22 subdomains in the original Five to fifteen questionnaire for assessing childhood neurodevelopmental symptoms (86). The FTF-Brief has been validated by us to be used in retrospect in adults assessed in childhood for neurodevelopmental disorders, and reassessed in adulthood (87).

Cognitive tests

• Parts of Wechsler Intelligence Scale Revised (WAIS-III-R) and/or WISC-III; WAIS-III; D KEFS; Wechsler Memory Scale, and from PNISSI.

• Block design, digit span, letter number sequencing and digit symbol coding, visuospatial test

• Adult executive functioning inventory (ADEXI) is a 14 item self-report of working memory and impulsivity (88)

Motor skill test

• Nine hole peg test (89)

• Gross motor functioning (from PNISSI) (90)

Side-effects

- Safety and tolerability of rituximab measured in an interview (AAR).
- UKU side effect rating scale is a clinician-rated scale with 48 items (91).

In addition to UKU, adverse events will be checked with the following questions at each visit:

1. Have you noticed any new symptoms or problems since your last visit? If yes, which?
2. Do you gather these symptoms can be related to your rituximab treatment?
3. Have you noticed if any of your previous side effects have attenuated or increased? If yes, which ones?

Treatment satisfaction

- Client satisfaction questionnaire (CSQ-8) is an 8-item measurement that is designed to measure client satisfaction with services (92)

Other rating scales

- Bristol stool scale to evaluate the consistency of faeces and therefore obtaining a measure of transit through the gastrointestinal tract (93)

Physical examination

- General condition; blood pressure; heart rate; signs of joint inflammation; dermatological examination; height and weight
- Basic neurological examination

Video recording

Each patient will be video recorded at base line, week 12, 20 and 40 in order to enable blinded assessment by an independent rater, and for the patient's own evaluation.

7.1.3 Biochemical and other measures

There is still insufficient knowledge concerning inflammatory/immunological mechanisms of psychiatric disorders. Therefore, in order to increase our understanding and to extend the interpretational potential of clinical findings from our exploratory study, we will assess relevant immunological parameters, autoimmune biomarkers, genetic SNPs, etcetera. Additionally, we will apply routine safety biochemistry, recommended for rituximab in clinical use.

Also all subjects will be asked if they have received standard vaccinations and if they have had chicken pox, i.e. immunity to Varicella zoster (positive antibodies if unknown). If not then vaccinations must precede the treatment with rituximab.

Tests in blood and cerebrospinal fluid (CSF)

Blood and CSF will be drawn at baseline (before intervention), and at week 20 (after intervention). Analyses will include:

Blood only

At baseline, week 20 and week 40:

- Routine clinical tests for basic haematology, 5-partsdiff including neutrophil and lymphocyte count to be able to make the neutrophil/lymphocyte ratio and platelet counts, glucose, infection, liver function, renal function, electrolyte balance, thyroid status, 25-OH-vitamin D, PTH, vitamin B12, folat
- Expression levels of selected pro-inflammatory genes (e.g. IL-1β, caspase, IL-6, IL-8), analysed using real-time PCR and DNA microarray technology

At baseline and week 20:

- Biomarkers for gut permeability (e.g. I-FABP, GLP-2)

At baseline, week 5, 12, 20, and40:

- B-cell populations monitoring, by flow cytometric phenotyping (e.g. CD19, CD24, CD27, CD38, CD5), to quantify the impact of rituximab treatment *(if not normalized in week 40 a repeated test will be made in week 52)*

Routine clinical tests for 5-partsdiff including neutrophil and lymphocyte count to be able to make the neutrophil/lymphocyte ratio and platelet counts.*Only at prescreening/baseline:*

- Serology for hepatitis B, C and HIV
- QuantiFERON test (interferon-γ release assay) to check for response to *Mycobacterium tuberculosis*
- Drug concentration of psychotropic medication
- Assessment of autoimmune reactions against cerebral antigens (whole blood sample, stored in – 80° C) (application of novel findings from our group)
- T cell activation against selected, recombinantly produced, human, cerebral peptides, using the method FluoroSpot, where different cytokines will act as markers for T-cell activation (PBMC sample, stored in – 80° C) (application of novel findings from our group)
- Genetic analysis for identification of predisposing factors and possible outcome predictors (e.g. polygenic risk scores and single nucleotide polymorphisms (SNPs), related to relevant psychiatric disorders) by DNA microarray technology

Blood and CSF

At baseline, week 12 (blood only), 20 (blood and CSF) and week 40 (blood only):

- Inflammatory markers (e.g. IL-6, IL-1Ra, sTNFR1) and chemokines (e.g. CCL2, CXCL13), and other immune markers (e.g. sCD14, IL-10, complement C3 and C4), measured by means of electrochemiluminescence immunoassay (Mesoscale instrument), hsCRP, Neuronal damage assessment by measuring neuron specific enolase (NSE), S100, neurofilament light chain (NFL), and neurotrophic substances (brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF))
- Metabolomics, to explore molecular target mechanisms

Only at baseline:

- Titres of CNS-relevant autoantibodies (e.g. anti-NMDA receptor antibodies, voltage-gated calcium channel (VGCC) antibodies, transglutaminase antibodies, anti-nuclear antibodies (ANA))

CSF only

At baseline and at week 20:

- Routine tests for CSF (e.g. cell count, albumin and immunoglobulin quotients, oligoclonal bands)
- (If sufficient number of CSF cells are available): Expression levels of pro-inflammatory genes (e.g. IL-1β, caspase, IL-6, IL-8), analysed using real-time PCR and DNA microarray technology

Microbiology

Only at baseline:

- Routine test for presence of group A streptococci infection

Faeces

At baseline and at week 20:

Faecal samples will be collected at baseline (before intervention), and week 20 (after intervention). Patients will collect two spot samples of faeces in plastic containers at home at each time point. Analyses will include:

- F-calprotectin
- Metabolome
- Microbiome; Faecal sample for quantitative and qualitative analyses of faecal microbial composition by 16S rRNA-based next generation sequencing (NGS).

Urine

Only at baseline and week 20:

Urine samples will be collected at baseline (before interventions), and week 20 (after intervention) in parallel with collection of blood. Urine will be used for:

- Screening for pregnancy prior to the rituximab treatment (week 0)
- Illicit drug screen (baseline and week 20)

At baseline and week 20:

- 30 ml of urine, early morning sample, for assessment inflammatory markers, similar to those in blood

Brain

At baseline and week 20:

• fMRI for assessment of altered activity and extra cellular water, which may be a sign of inflammation

7.1.4 Procedures

7.1.4.1 *Metabolomics analyses*

The metabolomics analyses will utilize mass spectrometry (MS) coupled with chromatographic separation, using two analytical approaches with broad analytical coverage. For the global profiling of polar metabolites, GC coupled to high resolution quadrupole-time-to-flight mass spectrometry (GC-QTOFMS) will be used. The analytical protocol is similar as previously developed for GC×GC-TOFMS (94). For the analyses of molecular lipids, ultra-high performance liquid chromatography combined with quadrupole-time-of-flight mass spectrometry (UHPLC-QTOFMS) will be utilized (95).

Orešič and Hyötyläinen, who worked together over the past eight years, have an extensive knowledge of these platforms for the analyses of clinical samples. Taken together, these two platforms allow detection of over 2000 metabolites, covering all main metabolic pathways such as lipid metabolism, central carbon metabolism, amino acid metabolism, urea cycle, ketogenesis as well metabolism, as detection of several metabolites related to nucleotide metabolism as well as gut microbial metabolism. The methodology combined targeted, quantitative approach with semi-quantitative profiling.

The lipidomics platform proposed for this study was recently assessed as part of the US National Institute of Standards (NIST) lipidomics ring study, which comprised 31 laboratories worldwide (96). Blinded NIST standard reference plasma sample (NIST SRM 1950) has been used in the study and we obtained excellent results (mostly within 10 % of consensus quantitative values), which highlights the accuracy and robustness of our lipidomics protocol.

The Department of Natural Science and Technology at Örebro University has a state-of-the-art facility for metabolomics, with several MS instruments (GC-HRMS, 2 x GC-MS, 2 x UHPLC-QqQMS, GC-QqQMS, GC-QTOFMS, UHPLC-QTOFMS, UPLC2-MS, ICP-MS), several other analytical instruments (CE, UHPLC) and three more instruments are being purchased in 2017 (GC-MS, UHPLC-IM-QTOFMS, Orbitrap MS). This infrastructure can be utilised for the further characterization of the unknown metabolic markers. We are also currently setting up a database management system, including both the infrastructure (database, software) and the personnel for the task.

7.1.4.2 *Immune status analyses*

Analysis of inflammatory markers and immune status will be performed using a number of well-established methods. Quantification of cytokines and other soluble markers of inflammation in plasma will mainly be performed using electrochemiluminscent ELISA technology, which enables sensitive detection coupled with a broad detection range. Furthermore, this technology allows multiplexing, which reduces the amount of material needed. To monitor the effect of rituximab on immune status, B-cells will be quantified and phenotyped using flow cytometry. Transitional, naïve, memory and regulatory B cells will be identified using a combination of markers (CD19, CD24, CD27, CD38, CD5). Gene expression analysis of inflammatory genes will be performed using a combination of DNA microarray technology and real-time qPCR.

These methods are available through collaboration with researchers at iRiSC. iRiSC is an interdisciplinary research environment that includes 10 professors and a number of junior scientist, all holding expertise in inflammatory regulation. The infrastructure includes data analysis, instruments and personnel.

7.1.4.3 *Venipuncture blood collection*

Blood samples will be obtained for screening and routine clinical testing, analysis of pro-inflammatory cytokines, genetic analyses and screening for biomarkers. A registered nurse or trained phlebotomist will utilize a sterile technique to draw blood by venipuncture. Up to 150 ml of blood may be obtained three times during pre-study screening, visit 7, 8 and 12 (weeks -4-0; 12, 20 and 40), and 5 ml of blood will be obtained at visit 4 and 5 (week 2 and 5). Maximum blood volume is 770 ml. From the blood samples we will obtain biomarkers of inflammatory signaling and permeability of the gut using ELISA and Mesoscale methods.

7.1.4.5 *Lumbar puncture CSF collection*

To evaluate CSF levels of inflammatory cytokines, a lumbar puncture (LP) will be performed as described. LPs will be done in the L3/L4 or L4/L5 interspace following administration of local anesthetic. A volume of up to 20 ml of CSF will be collected in silicone-coated tubes for research. Tubes will be gently mixed to avoid gradient effects. CSF samples will be centrifuged at 2000g, 8°C, for 10 minutes to remove cells (for gene expression analyses) and other insoluble material, aliquoted into 1 ml tubes to eliminate the need for repeated freezing and thawing, and stored at -80°C. From the CSF samples, we will obtain biomarkers of inflammatory signaling using ELISA and Mesoscale methods. Mesoscale allows for complex multiplexing of up to 17 analytes in one sample, and will be the method of choice. The lumbar puncture is optional.

7.1.4.6 *Stool*

The *ex vivo* Ussing chamber model, well-established in the ORU lab will be used for examining gut permeability (97). Colonic specimens obtained by distal colonoscopy in an unprepared bowel, will be mounted in Ussing chambers and the effect of mucosal application of intestinal metabolites on intestinal transfer and permeability will be assessed in conjunction with histochemical analyses of the human biopsies. In all patients, *in vivo* segmental whole-gut permeability will be assessed by measuring 24-hour urinary recovery of five specific sugar probes.

<u>*Gut microbiome*</u>. **A**ltered gut microbiome in first episode psychosis patients has been reported. In a study by Schwartz (2018) (98), numbers of *Lactobacillus* group bacteria were elevated in first episode psychosis patients and significantly correlated with symptom severity along different symptom domains.

Stool samples are collected in the privacy of the patient's own home. A kit with three containers will be provided to collect faeces specimen that are kept in a special isolated bag. The patient is requested to place the bag in the freezer of her/his home. The bag shall be kept there until a transport is organized to the Örebro University hospital biobank by a research assistant. To provide a stool sample is optional.

7.1.4.7 *Brain imaging*

Patients will undergo an fMRI-based assessment lasting approximately 2 hours (optional). Prior to scanning patients will be asked to once again complete a MRI checklist. Patients will also be asked if they have taken benzodiazepines and/or hypnotics within 24 hours of initiating MRI; those who have will be asked to come back for a later session. During scanning, patients will undergo the following protocol:

1. Diffusion weighted imaging: 90 gradient orientations distributed in two shells of b=1000 and b=2000, plus 2 b0 images; 2 mm resolution isotropic—20-minute scan time. We will estimate neural inflammation using a measure of regional extra-cellular free water derived from the diffusion weighted imaging data. We will apply to these data a free-water estimation algorithm that calculates the degree of cellularly constrained versus extra-cellular water. This method has been used reliably to determine levels of regional neuro-inflammation in psychiatric conditions.
2. Brain-blood perfusion imaging: arterial spin labeling scan, 40 labeled and 40 unlabeled scans, 30 axial 5-mm thick axial slices, 4x4 mm in-plane resolution—6-minute scan time.
3. Resting functional MRI (fMRI) scanning: blood-oxygen level dependent scan, SMS factor = 3, GRAPA = OFF, 32 4-mm thick axial slices with 3x3 mm in-plane resolution—12-minute scan time.
4. High-resolution anatomical MRI scans:
    a. Whole-head, T1-weighted MRI scan, 1-mm isotropic voxels—6-minute scan time.
    b. Whole-head, T2-weighted MRI scan, 1-mm isotropic voxels—6-minute scan time.

We will also use various fMRI paradigms for assessing social anhedonia, e.g. by studying diminished motivation for social affiliation and lack of reward from social incentives (99). Reward processing abnormalities have been implicated in pathophysiology of negative symptoms in schizophrenia, such as anhedonia and avolition. One method to study reward is to use monetary incentive delay task and affective incentive delay task (100). This paradigm has previously been used to investigate neural correlates of reward processing in substance abuse, pathological gambling, depression, schizophrenia, binge eating, and ADHD (101). The results in the patients will be compared to those of healthy controls.

7.1.4.8 *Qualitative content analysis*

The 12 participants from the study will be interviewed about their experiences about participating in the study, using a semi structured interview guide. The questions will be focusing on the participants' experiences about the treatment, the approach from the staff their feelings about their psychiatric diagnosis. Our hypothesis is that the patients may have other thought about their psychiatric condition as it now can be viewed as an inflammatory disorder instead of purely a mental condition. Also we are interested in if the participants have reflected on any change in the approach of the staff since they now are regarded as "somatically ill patients" not simply "psychiatric patients". The interviews will be transcribed verbatim and analyzed with qualitative content analysis.

Table 1 Study plan

Week		
-4 – 0	Screening prior to treatment with rituximab Decision on inclusion / exclusion	• Evaluation of suitability • Vaccination check up (+varicella) • Information to not use attenuated vaccines, what risks are involved and signs of immunosuppression • Consent form • Information card about drug study participation • Demographics questionnaire including FTF-Brief • Treatment history • PNISSI and fine motor examination • Physical examination • Check anti-hypertensive medication* • Test for group A streptococcus • Blood sample • Routine safety: Test of hepatitis, HIV, TB and routine tests • Therapeutic drug monitoring • Urine: illicit drug screen and pregnancy test, inflammatory markers • Assessments (CGI-S and MINI, psychiatric rating scales, GAF, EQ-5D, Level 1 Cross-cutting symptom measure of global symptom severity) • Functioning: PSP and BBQ • Side-effects, AAR • UKU • Client satisfaction questionnaire (CSQ) • Cognitive tests Video recording I • Lumbar puncture (optional) • Faeces biopsy and sample (optional) • fMRI (optional)
0	**Baseline** *Rituximab dose 1000 mg*	• Assessments (PANSS, CGI-I**, PGE, CGI-S) • Blood sample (inflammatory markers)
2	Nurse check-up	• Side-effects, AAR
5, (+/- 1 week)		• Side-effects, AAR • Patient evaluated improvement (PGE) • Blood sample (B-cell population)
8	Nurse check-up	• Side-effects, AAR • Patient evaluated improvement (PGE)
12		• Assessments (PANSS, CGI-I**, PGE, CGI-S) • Side-effects, AAR • Patient evaluated improvement (PGE) • Blood sample (B-cell population) • Video recording II
16 (+/- 1 week)	Nurse check-up	• Side-effects, AAR • Patient evaluated improvement (PGE)
20-23	**20 w**	Interview + Assessments (PANSS, CGI-I*, PGE, CGI-S, and MINI, psychiatric rating scales, GAF, EQ-5D, Level 1 Cross-cutting symptom measure of global symptom severity) • PSP • Side-effects, AAR

		• UKU • Client satisfaction questionnaire (CSQ) • Physical examination • Cognitive and motor tests (from PNISSI and Nine-Hole Peg Test) • Video recording III • Blood sample • Lumbar puncture (optional) • Faeces sample (optional) • Urine sample • fMRI (optional)
20-24	Clinician	• Extended interview for content analysis
24 (+/- 1 week)	Nurse check-up	• Patient evaluated improvement (PGE) • Side-effects, AAR
32 (+/- 1 week)	Nurse check-up	• Patient evaluated improvement (PGE) • Side-effects, AAR
40-43	**40 w**	• Interview + Assessments (PANSS; CGI-I*, PGE, CGI-S, and MINI, psychiatric rating scales, GAF, EQ-5D) • PSP and BBQ • Client satisfaction questionnaire (CSQ) • Side-effects, AAR, UKU • Blood sample • Cognitive tests • Video recording IV
52 (+/- 1 week)	1 year follow-up	• Assessments (PANSS, CGI-I, PGE, CGI-S, GAF) • Side-effects, AAR

*if the patient is treated with antihypertensive medication these should be withdrawn 12 h prior to the rituximab treatment
**evaluated by clinician and next-of-kin

Table 2

Week			**-4 - 0**	**0**	1	2	5	8	12	16	**20**	24	32	**40**	**52**
Visit			**1**	**2**	3	4 (nurse)	5	6 (nurse)	7	8 (nurse)	**9**	10 (nurse/other)	11 (nurse)	**12**	**13**
Activity/ ***Assessment number***	**CRF Y/N**	**Time to complete**	**Pre-Study Screening**	**Baseline I:0**							**20w**			**40w**	
ENROLMENT Eligibility screen, Consent form, Demographics questionnaire, Treatment history, PNISSI, Physical examination, FTF-Brief. Routine blood tests, hepatitis, HIV, TB. Vaccination check up (+varicella). Therapeutic drug monitoring, U-illicit drug screening,		60 min	x												
Pregnancy test (females)			x												
INTERVENTION Rituximab, Dose 1: 4.5h				X:1											
ASSESSMENTS MINI, Psychiatric rating scales		45 min	x								x			x	x
Level 1 Cross-cutting symptom measure of global symptom severity			x								x				
GAF, PSP		5 min	x						x		x			x	x
CSQ, BBQ, EQ-5D		10 min	x								x			x	
PANSS		20 min		x					x		x			x	x
CGI-S (clinician)		5 min	x	x					x		x			x	x

CGI-I (clinician and next of kin)		10 min		x					x		x			x	x
PGE (patient evaluated improvement)		5 min		x			x	x	x	x	x	x	x	x	x
UKU		10 min	x								x			x	
Side effect open check up questions and AAR		10 min	x			x	x	x	x	x	x	x	x	x	x
Blood sample		15 min	x	x			x		x		x			x	
Lumbar puncture		30 min	x								x				
Faeces sample		5 min	x								x				
Urine test (morning sample)		5 min	x								x				
fMRI		120 min	x								x				
Gut biopsy		30 min	x												
Cognitive and motor test		30 min	x								x				
Video recording		10 min	x						x		x			x	
Extended interview for content analysis		45 min										x			
Nurse in-between visits/ progress notes		10 min				x		x		x		x	x		

BBQ = Brunnsviken Brief Quality of Life Inventory; CGI-S = Clinical Global Impression-Severity scale; CGI-I = Clinical Global Impression-Improvement scale; CSQ = Client Satisfaction Questionnaire; GAF = Global Assessment of Function; MINI = Mini International Neuropsychiatric Interview; MRI = Magnetic Resonance Imaging; PGE = Patient's Global Evaluation of improvement; UKU = Udvalg Kliniske Undersøgelser, side effect rating scale; Personal and Social Performance (PSP) Scale; EuroQol Group EQ-5D; Any Adverse Reactions (AAR).

7.2 Study Termination

Patients may withdraw from the study at any time, of their own choice or by investigator decision, due to safety reasons or in case the patient is not able to comply with the protocol. Data collected up to the end of follow-up will be used in the final analysis of the study. If a patient wants to discontinue the study participation, data collected until that time point will be analysed in the study.

Early termination of the study may occur due to decision of regulatory authority or ethics committee, safety issues or sponsor decision. The study is completed when the last patient has done their last study visit.

8 STUDY MEDICATION

8.1 Study medication

Rituximab/MabThera®
MabThera 1000 mg concentrate for solution for infusion,100 ml.
Active substance: Rituximab
ATC-code: L01XC02
Rituximab is a sterile, clear, colourless, preservative-free liquid concentrate designed for intravenous administration. The product is supplied at a concentration of 10 mg/mL in 500-mg (50-mL) single-use vials. Rituximab is formulated for intravenous administration in sodium chloride 9 mg/mL, sodium citrate dihydrate 7.35 mg/mL, polysorbate-80 0.7 mg/mL, and sterile water for infusion. The pH is adjusted to 6.5.

8.2 Administration of study drugs

A MabThera dose of 1000 mg will be given as intra-venous infusion in 1000 ml physiological saline (0.9 %, any brand provided by the pharmacy. Before administration of MabThera all patients will be given pre-medication to prevent or reduce possible side effects from MabThera.

Premedication is delivered 1-2 hours prior to rituximab and consists of T. Paracetamol 1000 mg, T. Loratadine 10 mg and T. Betapred (Betamethasone) 0.5 mg x 12 p.o (dissolved in a glass of water) in order to reduce the incidence of infusion-related reactions.

Patients remain on their stabilized treatments with psychotropic drugs during the first phase (20 weeks) of the study.

MabThera will be administered on one single occasion.

The MabThera treatment is administrated at the Rheumatology clinic at Örebro University Hospital. Study staff at the department of Rheumatology will prepare the saline bag for each patient infusion, adding MabThera. All bags will be labelled with a study specific label (**Error! Reference source not found.**). Each bag for infusion will be prepared just in time for each visit.

8.3 Storage and preparation of study medication

All study drug, MabThera and saline for dilution, will be available at the department of Rheumatology at Örebro University hospital.

9 ADVERSE EVENT, AE AND SERIOUS ADVERSE EVENT, SAE

9.1 Adverse Events, AE

An Adverse Event (AE) is any untoward medical occurrence in a patient/healthy volunteer administered a pharmaceutical product and which does not necessarily have to have a causal relationship with that treatment or usage. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical (investigational) product, whether or not related to the product.

Patients will be asked if they have experienced any AE by means of the following questions, administered at each visit:

- Have you noticed any new symptoms or problems since your last visit? If yes, which?
- Do you gather these symptoms can be related to a rituximab treatment?
- Have you noticed if any of your previous side effects have attenuated or increased? If yes, which ones?

We will also ask questions on specific adverse events that have been documented to be associated with rituximab treatment at each visit using the AAR. In addition, specific AEs, relevant for psychiatry, will be checked with the UKU Side Effect Rating Scale at **endpoint 1 and 2**.

The sponsor, as principal investigator or any investigator delegated to work in the study will record any AEs and assess the seriousness and severity (mild, moderate, severe) and whether the AE is related to the study medication. All investigators delegated to work in the study will report AEs to the principal investigator who is the sponsor in this study.

All AEs that are observed from the time of the administration of study medication until the patient leaves the study will be registered, analysed and summarized at the end of the study. Only serious adverse events will be recorded in the medical record.

9.2 Serious Adverse Event, SAE

Each adverse event is to be classified by the investigator as serious or non-serious. An adverse event that meets one or more of the following criteria/outcomes is classified as serious:

- Results in death
- Is life-threatening, i.e., the patient was, in the opinion of the Investigator, at immediate risk of death from the event as it occurred (It does not include an event that, had it occurred in a more severe form, might have caused death.)
- Results in persistent or significant disability/incapacity
- Requires in-patient hospitalization or prolongs hospitalization
- Is a congenital anomaly/birth defect
- Is another medically significant event that, based upon appropriate medical judgment, may jeopardize the volunteer and may require medical or surgical intervention to prevent one of the outcomes listed above (e.g., allergic bronchospasm requiring intensive treatment in an emergency room or home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse)

SAE that occurs during the study will be documented and reported to sponsor within 24 hours after the investigator is aware of the SAE. SAEs will be documented, analysed and summarized at the end of the trial. SAE will be followed until the participation in the trial is ended or they are resolved.

9.3 Suspected Unexpected Serious Adverse Reaction, SUSAR

The sponsor reports all SUSARs that arise during the trial to The Medicinal Product Agency and Uppsala's Ethics committee. Reports will be done by sending the CIOMS form to the MPA since sponsor does not have the resources to report electronically to the EudraVigilance database.

A SUSAR that results in death or is life-threatening must be reported promptly and no later than 7 days after it occurred and was known by sponsor. Relevant follow-up information should be sent within another 8 days. Any other SUSARs should be reported as soon as possible but not later than 15 days after being brought to sponsors attention.

10 STATISTICS

10.1 Determination of Clinical response

There is no power calculation due to the nature of this study, a pilot study. If at least 2 patients improve significantly we aim to continue with a controlled study.

Primary outcome

Reduction in PANSS score (30 items) is the primary outcome. 40% reduction in PANSS is regarded as a treatment response.

Secondary outcomes

CGI-I score 1 or 2 which corresponds to much or very much improved at week 20 is regarded as response. Assuming a SD of 1 in our sample we will be able to detect differences between the means before and after treatment corresponding to 0.64 points on the CGI-I scale.

Changes in PSP and clinician rated CGI-S score between enrollment and week 20.

PSP gives a score between 0-100 for disability.

CGI-S is a clinician rated measure of overall clinical severity that is rated on a scale between 1 and 7. This measure also has a floor effect that affects healthy participants, for a person with no clinical complaints or problems (which is most people in a population) will get a score of 1. This floor effect is not relevant to our study population. The score 7, which indicates the highest level of severity is phrased as "Among the most extremely ill patients". This may be true to the patient group in the present study. However, since patients currently in need of compulsory care are excluded from the study, a ceiling effect of the measure is unlikely.

Repeated measures of the PSP and CGI-S will be analysed using a repeated measures ANOVA.

10.2 Statistical methods and analytical plan

10.2.1 Primary objective: Improvement reflected in change in PANSS score

To investigate whether markedly ill, treatment-resistant, psychiatric patients according to our inclusion/exclusion criteria are significantly improved after treatment with the immunomodulatory drug rituximab (as addition to the patients' standard treatment).

We will analyse the clinical data on a modified intention-to-treat (ITT) definition basis, i.e. including treatment dropouts who received at least one dose of rituximab as well as completers. In addition, a completer-only analysis will be performed. *The primary outcome* is change in PANSS score, assessed by a clinician.

Secondary outcomes: CGI-I score (range 1-7). All CGI-I ratings are performed by three independent raters (the patient, his or her next of kin and a clinician). The mean value of these three values will be used as the patients' scores. If one of the values is missing, the mean will be calculated using only two values.

One secondary outcome is binary: the proportion of responders to treatment at week 20 (and similarly at week 40). Treatment response is defined as a mean value below 2.5 on the CGI-I (calculated as above), which corresponds to the scale step descriptors "much improved" or "very much improved". Non-response is defined as a mean CGI-I value of 2.5 or higher.

The continuous secondary outcome variables at the 20-week and 40-week end points (CGI-S, PSP, and diagnosis specific rating scales) will be included in analysis of covariance (ANCOVA) models, controlling for baseline measures and demographic variables.

Side-effects will be closely monitored and types, frequency, severity and seriousness will be reported.

10.2.2 Secondary objectives: analyses of laboratory data related to clinical response

These objectives are explorative, and our hypotheses are that clinical improvement will in various ways correlate with or depend on measures of inflammation.

1. To examine whether baseline levels of inflammatory markers predict treatment response.
2. To examine whether changes in inflammatory markers between baseline and week 20, and B-cell depletion at weeks 5, 12 and 20 correlate with treatment response.
3. To examine metabolic mediators prior to and after treatment and if they correlate with treatment response.
4. To examine whether there is a change in markers for gut permeability related to treatment.
5. To examine whether there is a change in CNS activity related to reward or signs of CNS inflammation.

Laboratory data will consist of: plasma protein concentrations and leukocyte mRNA expression values for a number of cytokines and related substances (inflammatory markers); total number of B-lymphocytes and number of cells from various B-cell subsets at week 5, 12 and 20, as measured through highly sensitive flow cytometry (HSFC); plasma proteins and lipids as measured through metabolomics analysis; and blood levels of markers for gut permeability. The study of these variables in psychiatric patients is still regarded as exploratory.

All these laboratory values (except metabolomics data) will be tested for normality (Shapiro-Wilk) and homogeneity of variances (Levene's test) and, if deemed necessary, log transformation will be used to achieve normality. If normality cannot be achieved through log transformation, non-parametric test will be used for analyses.

In order to examine whether baseline characteristics can predict response from rituximab, baseline values of all these measurements (except HSFC) will be compared between the two groups "responders" and "non-responders" after completion of the study by means of t-tests or Mann-Whitney tests, as applicable. Due to the explorative nature of this study, data will be examined both corrected and un-corrected for multiple comparisons. In addition, for each of these measurements, a generalized linear model (ANCOVA) will be built, with continuous measures of clinical improvement as dependent variable and possible confounders (e.g. age, sex and BMI) as covariates.

In order to examine whether changes in inflammatory markers and other measurements correlate with clinical response, the difference between baseline values and week-20 values (Δ-values) of all relevant measurements will be compared between the two groups "responders" and "non-responders" after completion of the study by means of t-tests or Mann-Whitney tests, as applicable. Due to the explorative nature of this study, data will be examined both corrected and un-corrected for multiple comparisons. In addition, each of these Δ -values will be introduced into a generalized linear model (ANCOVA), with continuous measures of clinical improvement as dependent variable and possible confounders (e.g. age, sex and BMI) as covariates. In addition, an exploratory principal component analysis (PCA) will be performed, in order to identify patterns of changes among the inflammatory markers that are related to clinical response.

The HSFC B-cell counts at week 5 and 12 will be used as validation of rituximab immunological impact. The clinical response will be compared with statistics as above between patients with complete ($<0.0001\times10^9$/L) and incomplete ($>0.0001\times10^9$/L) depletion of B-cells, respectively. Also B-cell subpopulations at week 20 will be correlated to clinical response.

Statistical analysis of the metabolomic data (baseline and Δ -values) will be performed similarly, however, correction for multiple comparisons by means of the Benjamini–Hochberg procedure and other methods for biostatistics and bioinformatics will be applied.

Δ -values of markers for gut permeability will be compared between pre and post treatment, in order to document whether rituximab has an effect on gut permeability.

For statistical analysis of genomic and microbiome data, appropriate methods for biostatistics and bioinformatics will be applied.

10.2.3 Analyses of other clinical data related to clinical response

1. Change in PSP, measuring overall disability from baseline up to week 20.
2. Change from baseline up to week 20 of illness severity (CGI-S) assessed by the clinician.
3. Change from baseline on rating scales that measure psychiatric symptoms according to psychiatric rating scales.
4. To examine whether there is a change in cognition after treatment.

5. To investigate the patients' experiences of the novel treatment with a qualitative content analysis.

For results on the scales (PSP, CGI-S and psychiatric rating scales) the week 20 scores and the Δ-values (difference between scores at week 20 and baseline) will be tested by means of t-tests or Mann-Whitney tests, as applicable. They will also be included in an analysis of covariance (ANCOVA) model, controlling for baseline measures and other possible confounders, as demographic variables. The cognitive tests will be analysed with established methods, and possible changes will be compared before and after treatment. The qualitative content analysis does not involve quantitative statistics.

10.2.4 Analyses of long-term outcomes (week 40)

1. Improvement according to PANSS and Clinical Global Impression-Improvement (CGI-I) at week 40
2. Differences between baseline and week 40 concerning CGI-S, PSP, BBQ, diagnosis specific scales and cognitive function
3. Inflammatory markers and B-cell populations at week 40

The results at week 40 on these variables will be analysed with essentially the same methods as those at week 20, see above.

11 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Monitoring

The study will be monitored at least once before start, during the study and after the study is completed. The monitoring will be performed to assure that the rights and wellbeing of the patients are protected; the study is conducted according to the protocol, that all essential documents are available, that data are collected, documented and reported according to ICH-GCP (Good Clinical Practice) and applicable ethical and regulatory rules and directives.

The aim of the monitoring is also to secure that collected data are correct, complete, legible and verifiable from source documents if applicable.

Monitoring will be done by monitors from the Clinical Trials Unit (Avdelningen för kliniska prövningar) at Örebro University Hospital, who are in no other way involved in the study.

11.2 Data monitoring

The PI and the research group associated with Örebro University will constitute a data monitoring committee (DMC). If any patient develops severe side effect, such as progressive multifocal leukoencephalopathy (PML), the study shall be stopped. The PI will have access to the results and make the final decision to terminate the trial.

12 ETHICS

12.1 Ethical Conduct of the Trial

The trial will be conducted in compliance with the protocol, in accordance with the latest adopted version of the Helsinki declaration, ICH-GCP and the applicable regulatory requirements.

12.2 Informed Consent

Patients who may be suitable to participate in the study will be informed about the study during one of their regular visits at the clinic and through personnel at inpatient clinics. We may also inform the patient interest associations for psychiatric disorders about the study; and patients living in psychiatric housing located in Region Örebro County, and enable patients from other parts of the country to participate, if we cannot find suitable patients within our catchment area. Patients will be given written information about the study, time to read it and the opportunity to ask the investigator any questions they may have. All patients will be asked to sign the written consent. Individuals who do not want to participate are not included in the study. The patients are informed that their participation is voluntary and that they may choose to withdraw from the study at any time, without repercussions. Only patients who are able to make an informed decision the study will be included.

If the patient agrees to participate in the study they will sign the informed consent form together with the investigator, who will then sign the consent form as well. A copy of the signed informed consent will be given to the patient.

12.3 Risk/benefit assessment

Patients will share personal information, which could be perceived as intrusive. However, all collected data will be coded and data will only be presented at a group level. Blood sampling and lumbar puncture can cause pain, but only for a brief period. The fMRI and gut examination can be experienced as difficult but it is not painful or dangerous. Handling urine and faeces samples may cause discomfort in the patient. In this study, only blood samples collected at baseline and week 20, in addition to responses to questionnaires at baseline and week 20 for assessment of outcome, are mandatory for participation.

The patients' expectations of the study and its intervention may be high, which can generate disappointment among those who do not experience a positive effect from the study drug.

Side effects of rituximab treatment may occur, some of those can be serious, but rituximab is usually well tolerated. Among patients with rheumatoid arthritis, even mild side effects are reported to be rare. According to the Summary of Products Characteristics (SmPC), between 1/100 and 1/1000 patients report depression and/or increased nervousness as a side effect from rituximab. The prevalence for these side effects is unknown among psychiatric patients. However, all side effects will be carefully documented in the present study. If the patient becomes depressed or severely anxious discontinuation of the study drug should be considered. Rituximab is a standard treatment for multiple sclerosis and rheumatoid arthritis in Sweden. Approximately 5,000 patients with rheumatoid arthritis is treated with MabThera (48/100 000) and as MabThera is prescribed off label for MS another 5 to15,000 patients receive this treatment yearly for this indication (227/100,000 are diagnosed with MS, Danish data). In Örebro, rituximab is administered to the rheumatoid arthritis patients on a day-ward at Örebro University hospital, run by specially trained nurses.

The side effects of rituximab include infusion reactions, severe skin and mouth reactions, hepatitis B virus reactivation and in extremely rare cases (1/25000 in patients with rheumatoid arthritis (102), progressive multifocal leukoencephalopathy (PML), a serious brain infection caused by the JC virus. We will collaborate with a senior rheumatologist who is experienced with rituximab. Since the treatment may activate latent infections, all patients will be screened for hepatitis and HIV prior to the study. The patients will be pre-medicated to reduce the risk of infusion-related side effects, and they are carefully monitored during the course of the study.

The reason to not use a standard drug for inflammation, such as cortisone, is because it may induce mania in susceptible individuals and is therefor not suitable for psychiatric patients.

We will film the patients on three different occasions. This is because we want to be able to show the patient if there is a treatment effect or not. It may also enable a completely external assessment of treatment effects. Since rituximab has not been tested in trials of psychiatric disorder an effect of the drug can be questioned. In such case we possibly could show video clips of treatment effects, if the patient allows it (but this is not included in this study). The film clips may also support a request for further treatment with the drug.

Early onset of severe psychiatric disability is associated with a particularly poor long-term prognosis. This is often the case for schizophrenia. Delayed treatment may cause persistent brain damage if associated with inflammation in the brain (which is presumed). We believe that earlier treatments may have better effects on the symptoms than treatment that are first provided after many years with the disease. Also, rituximab is frequently used for treating children and adolescents with MS (103). Thus it would be unjust to exclude them from participation in a trial with a medication that may prove helpful.

The patients will remain on their standard psychiatric treatment throughout the course of the study and are allowed to adapt their medication dose accordingly. Therefore, we do not put the patient's mental health at risk. The side effects of rituximab do not coincide with those that can be expected from psychiatric medication (apart from possibly clozapine), which implies low risk for add-on side effects.

To conclude, chronic psychiatric disorders that do not respond to treatments may cause severe suffering in the patient and his/her family members. It is also associated with poor quality of life and increased suicide risks. Thus the possible benefits from treatment with rituximab must be balanced against the risk of taking the drug. Moreover, even if the treatment proves ineffective for treating psychiatric symptoms, the trial would nevertheless be valuable; we will publish the results and other research groups can take part of our findings.

12.4 Considerations regarding group size

The study population is small, which may conceal a treatment effect of rituximab. For this reason, a large study population would be beneficial. However, rituximab has not been tested in a trial with psychiatric patients before; we do not know how psychiatric patients will respond to the drug. Therefore, inclusion of a large group of patients, at this point of time, cannot be defended on ethical grounds. Rather, we will be careful.

We will only include markedly ill psychiatric patients, which may endorse a selection of patients who is currently in a particularly poor phase of their disorder. Markedly ill patients with schizophrenia are rarely placebo responders. However, also chronic psychiatric disorders go with symptom fluctuations, and some symptoms may remit spontaneously. Improvement is most likely to happen in the most affected group.

13 DATA HANDLING AND RECORD KEEPING

13.1 Case Report Form, CRF

A study database with all patients included in the study will be generated. The patients' identity will be coded, names and social security numbers will not be entered in the CRF. The code list, identifying the patients, will be kept locked up, only accessible to study personnel.

13.2 Source data

Source data in the study will be defined in the source data list. All source data will be made available as requested for monitoring, audits and inspections for confirmation that all collected data is in keeping with the source data.

13.3 Data management

Data management and data handling will be completed in accordance with applicable regulations. Only study personnel will have access to and handle study data.

13.4 End of trial and archiving

The study will end when the last patient has completed the last visit. Data collected during the study will be archived for at least 10 years after the study has been completed.

Study results will be summarized and submitted to the Regulatory Authority and the Ethics Committee within 12 months after completion of the trial.

14 INSURANCE

The volunteers are covered by The Swedish Patient's insurance and The Swedish Pharmaceutical Insurance during the study.

15 PROTOCOL AMENDMENTS

Amendments, protocol additions and essential changes of the protocol may only be implemented after approval of a written application to The Ethics Committee and/or The Medicinal Products Agency.

16 PUBLICATION

An annual safety report will be submitted to the Medicinal Products Agency and the Ethics Committee.

Study results will be summarized and submitted in a report to the Medicinal Products Agency and the Ethics Committee within 12 months after completion of the trial.

The results will be submitted for publishing in suitable scientific journals and may be presented at meetings and conferences.

The results will only be presented on statistic group levels, so that no individual patient can be identified.

17 REFERENCES

1. Kessler RC, Avenevoli S, Costello J, Green JG, Gruber MJ, McLaughlin KA, et al. Severity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication Adolescent Supplement. Archives of general psychiatry. 2012;69(4):381-9.
2. Brent DA, Kolko DJ, Birmaher B, Baugher M, Bridge J, Roth C, et al. Predictors of treatment efficacy in a clinical trial of three psychosocial treatments for adolescent depression. Journal of the American Academy of Child and Adolescent Psychiatry. 1998;37(9):906-14.
3. Bejerot S, Ekselius L, von Knorring L. Comorbidity between obsessive-compulsive disorder (OCD) and personality disorders. Acta psychiatrica Scandinavica. 1998;97(6):398-402.
4. Rydén E, S. B. Autism spectrum disorder in an adult psychiatric population. A naturalistic cross sectional controlled study. Clinical Neuropsychiatry 2008;5:13-21.
5. Swain SP, Behura SS. A comparative study of quality of life and disability among schizophrenia and obsessive-compulsive disorder patients in remission. Industrial psychiatry journal. 2016;25(2):210-5.
6. Suliman S, Stein DJ, Myer L, Williams DR, Seedat S. Disability and treatment of psychiatric and physical disorders in South Africa. J Nerv Ment Dis. 2010;198(1):8-15.
7. Laursen TM. Life expectancy among persons with schizophrenia or bipolar affective disorder. Schizophr Res. 2011;131(1-3):101-4.
8. Meltzer HY. Treatment-resistant schizophrenia--the role of clozapine. Curr Med Res Opin. 1997;14(1):1-20.
9. Demjaha A, Lappin JM, Stahl D, Patel MX, MacCabe JH, Howes OD, et al. Antipsychotic treatment resistance in first-episode psychosis: prevalence, subtypes and predictors. Psychological medicine. 2017;47(11):1981-9.
10. Team POTSP. Cognitive-behavior therapy, sertraline, and their combination for children and adolescents with obsessive-compulsive disorder: the Pediatric OCD Treatment Study (POTS) randomized controlled trial. Jama. 2004;292(16):1969-76.
11. Poyurovsky M, Zohar J, Glick I, Koran LM, Weizman R, Tandon R, et al. Obsessive-compulsive symptoms in schizophrenia: implications for future psychiatric classifications. Comprehensive psychiatry. 2012;53(5):480-3.
12. Scotti-Muzzi E, Saide OL. Schizo-obsessive spectrum disorders: an update. CNS Spectr. 2017;22(3):258-72.
13. Consortium C-DGotPG. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet (London, England). 2013;381(9875):1371-9.
14. Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet (London, England). 2009;373(9659):234-9.
15. Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, Perlis RH, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nature genetics. 2013;45(9):984-94.
16. Insel TR. The NIMH Research Domain Criteria (RDoC) Project: precision medicine for psychiatry. The American journal of psychiatry. 2014;171(4):395-7.
17. Bernard JA, Mittal VA. Updating the research domain criteria: the utility of a motor dimension. Psychological medicine. 2015;45(13):2685-9.

18. Peralta V, Cuesta MJ. Motor Abnormalities: From Neurodevelopmental to Neurodegenerative Through "Functional" (Neuro)Psychiatric Disorders. Schizophrenia bulletin. 2017;43(5):956-71.
19. Kipnis J. The Seventh sence. Sci Am. 2018:28-35.
20. Barry H, Byrne S, Barrett E, Murphy KC, Cotter DR. Anti-N-methyl-d-aspartate receptor encephalitis: review of clinical presentation, diagnosis and treatment. BJPsych bulletin. 2015;39(1):19-23.
21. Trepanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP. Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. Mol Psychiatry. 2016;21(8):1009-26.
22. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. The Lancet Neurology. 2011;10(1):63-74.
23. Benros ME, Eaton WW, Mortensen PB. The epidemiologic evidence linking autoimmune diseases and psychosis. Biological psychiatry. 2014;75(4):300-6.
24. Khandaker GM, Dantzer R, Jones PB. Immunopsychiatry: important facts. Psychological medicine. 2017;47(13):2229-37.
25. Benros ME, Waltoft BL, Nordentoft M, Ostergaard SD, Eaton WW, Krogh J, et al. Autoimmune diseases and severe infections as risk factors for mood disorders: a nationwide study. JAMA psychiatry. 2013;70(8):812-20.
26. Mitchell RH, Goldstein BI. Inflammation in children and adolescents with neuropsychiatric disorders: a systematic review. Journal of the American Academy of Child and Adolescent Psychiatry. 2014;53(3):274-96.
27. Upthegrove R, Manzanares-Teson N, Barnes NM. Cytokine function in medication-naive first episode psychosis: a systematic review and meta-analysis. Schizophrenia research. 2014;155(1-3):101-8.
28. Yeon SM, Lee JH, Kang D, Bae H, Lee KY, Jin S, et al. A cytokine study of pediatric Tourette's disorder without obsessive compulsive disorder. Psychiatry research. 2017;247:90-6.
29. Bjorklund G, Saad K, Chirumbolo S, Kern JK, Geier DA, Geier MR, et al. Immune dysfunction and neuroinflammation in autism spectrum disorder. Acta neurobiologiae experimentalis. 2016;76(4):257-68.
30. Simsek S, Yuksel T, Cim A, Kaya S. Serum Cytokine Profiles of Children with Obsessive-Compulsive Disorder Shows the Evidence of Autoimmunity. The international journal of neuropsychopharmacology. 2016;19(8).
31. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421-7.
32. Pollak TA, Beck K, Irani SR, Howes OD, David AS, McGuire PK. Autoantibodies to central nervous system neuronal surface antigens: psychiatric symptoms and psychopharmacological implications. Psychopharmacology. 2016;233(9):1605-21.
33. Swedo SE, Leonard HL, Garvey M, Mittleman B, Allen AJ, Perlmutter S, et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. The American journal of psychiatry. 1998;155(2):264-71.
34. Hesselmark E, Bejerot S. Clinical features of Pediatric Acute-onset Neuropsychiatric Syndrome: Findings from a case control study of confirmed and suspected cases in British Journal of Psychiatry Open 2018;in press.
35. Husby G, van de Rijn I, Zabriskie JB, Abdin ZH, Williams RC, Jr. Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. The Journal of experimental medicine. 1976;144(4):1094-110.

36. Yaddanapudi K, Hornig M, Serge R, De Miranda J, Baghban A, Villar G, et al. Passive transfer of streptococcus-induced antibodies reproduces behavioral disturbances in a mouse model of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. Molecular psychiatry. 2010;15(7):712-26.
37. Brimberg L, Benhar I, Mascaro-Blanco A, Alvarez K, Lotan D, Winter C, et al. Behavioral, pharmacological, and immunological abnormalities after streptococcal exposure: a novel rat model of Sydenham chorea and related neuropsychiatric disorders. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2012;37(9):2076-87.
38. Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. The lancet Psychiatry. 2015;2(3):258-70.
39. Khandaker GM, Dantzer R, Jones PB. Immunopsychiatry: important facts. Psychol Med. 2017:1-9.
40. Khandaker GM, Dantzer R. Is there a role for immune-to-brain communication in schizophrenia? Psychopharmacology (Berl). 2016;233(9):1559-73.
41. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry. 2011;70(7):663-71.
42. Upthegrove R, Manzanares-Teson N, Barnes NM. Cytokine function in medication-naive first episode psychosis: a systematic review and meta-analysis. Schizophr Res. 2014;155(1-3):101-8.
43. Rodrigues-Amorim D, Rivera-Baltanas T, Spuch C, Caruncho HJ, Gonzalez-Fernandez A, Olivares JM, et al. Cytokines dysregulation in schizophrenia: A systematic review of psychoneuroimmune relationship. Schizophr Res. 2017.
44. Hylén U, Bartoszek J, Humble M, Särndahl E, Bejerot S, Eklund D. Enhanced priming state of the NLRP3 inflammasome and increased levels of inflammatory cytokines in patients with severe mental illness. Inflammation and immunity in disorders of the brain and mind; 16-17 November 2018; Barcelona The Lancet; 2018.
45. Swinnen LJ, O'Neill A, Imus PH, Gujar S, Schiff D, Kleinberg LR, et al. Phase II study of rituximab given in conjunction with standard chemotherapy in primary central nervous system lymphoma (PCNSL): a trial of the ECOG-ACRIN cancer research group (E1F05). Oncotarget. 2018;9(1):766-73.
46. Fluge O, Bruland O, Risa K, Storstein A, Kristoffersen EK, Sapkota D, et al. Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. PloS one. 2011;6(10):e26358.
47. den Broeder AA, Verhoef LM, Fransen J, Thurlings R, van den Bemt BJF, Teerenstra S, et al. Ultra-low dose of rituximab in rheumatoid arthritis: study protocol for a randomised controlled trial. Trials. 2017;18(1):403.
48. Schoergenhofer C, Schwameis M, Firbas C, Bartko J, Derhaschnig U, Mader RM, et al. Single, very low rituximab doses in healthy volunteers - a pilot and a randomized trial: implications for dosing and biosimilarity testing. Scientific reports. 2018;8(1):124.
49. Leykin I, Mayer R, Shinitzky M. Short and long-term immunosuppressive effects of clozapine and haloperidol. Immunopharmacology. 1997;37(1):75-86.
50. O'Sullivan D, Green L, Stone S, Zareie P, Kharkrang M, Fong D, et al. Treatment with the antipsychotic agent, risperidone, reduces disease severity in experimental autoimmune encephalomyelitis. PloS one. 2014;9(8):e104430.
51. Zhang L, Zhao J. Profile of minocycline and its potential in the treatment of schizophrenia. Neuropsychiatric disease and treatment. 2014;10:1103-11.

52. Miyaoka T, Wake R, Hashioka S, Hayashida M, Oh-Nishi A, Azis IA, et al. Remission of Psychosis in Treatment-Resistant Schizophrenia following Bone Marrow Transplantation: A Case Report. Frontiers in psychiatry. 2017;8:174.
53. Sommer IE, van Bekkum DW, Klein H, Yolken R, de Witte L, Talamo G. Severe chronic psychosis after allogeneic SCT from a schizophrenic sibling. Bone marrow transplantation. 2015;50(1):153-4.
54. Miller BJ, Goldsmith DR. Towards an Immunophenotype of Schizophrenia: Progress, Potential Mechanisms, and Future Directions. Neuropsychopharmacology. 2017;42(1):299-317.
55. Leon J, Hommer R, Grant P, Farmer C, D'Souza P, Kessler R, et al. Longitudinal outcomes of children with pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections (PANDAS). European child & adolescent psychiatry. 2017.
56. Husain MI, Chaudhry IB, Husain N, Khoso AB, Rahman RR, Hamirani MM, et al. Minocycline as an adjunct for treatment-resistant depressive symptoms: A pilot randomised placebo-controlled trial. Journal of psychopharmacology (Oxford, England). 2017;31(9):1166-75.
57. Frankovich J, Thienemann M, Rana S, Chang K. Five youth with pediatric acute-onset neuropsychiatric syndrome of differing etiologies. Journal of child and adolescent psychopharmacology. 2015;25(1):31-7.
58. Wandinger KP, Saschenbrecker S, Stoecker W, Dalmau J. Anti-NMDA-receptor encephalitis: a severe, multistage, treatable disorder presenting with psychosis. Journal of neuroimmunology. 2011;231(1-2):86-91.
59. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. The New England journal of medicine. 2004;350(25):2572-81.
60. Hauser SL. The Charcot Lecture | beating MS: a story of B cells, with twists and turns. Multiple sclerosis (Houndmills, Basingstoke, England). 2015;21(1):8-21.
61. Barr TA, Shen P, Brown S, Lampropoulou V, Roch T, Lawrie S, et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. The Journal of experimental medicine. 2012;209(5):1001-10.
62. Bar-Or A, Fawaz L, Fan B, Darlington PJ, Rieger A, Ghorayeb C, et al. Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? Annals of neurology. 2010;67(4):452-61.
63. Seite JF, Hillion S, Harbonnier T, Pers JO. Review: intravenous immunoglobulin and B cells: when the product regulates the producer. Arthritis & rheumatology (Hoboken, NJ). 2015;67(3):595-603.
64. Hesselmark E, Bejerot S. Biomarkers for diagnosis of Pediatric Acute Neuropsychiatric Syndrome (PANS) - Sensitivity and specificity of the Cunningham Panel. J Neuroimmunol. 2017;312:31-7.
65. Bejerot S, Hesselmark E, Mobarrez F, Wallén H, Hietala MA, Nybom R, et al. Neuromyelitis Optica Spectrum Disorder with Increased Aquaporin-4 Microparticles Prior to Autoantibodies in Cerebrospinal Fluid: A Case Report of a PANDAS patient. Journal of Medical Case Reports in press. 2018.
66. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of clinical psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57.
67. Sheehan DV, Sheehan KH, Shytle RD, Janavs J, Bannon Y, Rogers JE, et al. Reliability and validity of the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID). The Journal of clinical psychiatry. 2010;71(3):313-26.

68. Bejerot S, Hesselmark E, Humble M. The PsychoNeuroInflammatory related Signs and Severity Inventory (PNISSI). Brain, Behavior and Immunity. 2016;57.
69. Guy W. ECDEU assessment manual for psychopharmacology - revised. Rockville, Md: U.S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs; 1976.
70. Zohar J, Judge R. Paroxetine versus clomipramine in the treatment of obsessive-compulsive disorder. OCD Paroxetine Study Investigators. The British journal of psychiatry : the journal of mental science. 1996;169(4):468-74.
71. Soderberg P, Tungstrom S, Armelius BA. Reliability of global assessment of functioning ratings made by clinical psychiatric staff. Psychiatric services (Washington, DC). 2005;56(4):434-8.
72. Morosini PL, Magliano L, Brambilla L, Ugolini S, Pioli R. Development, reliability and acceptability of a new version of the DSM-IV Social and Occupational Functioning Assessment Scale (SOFAS) to assess routine social functioning. Acta Psychiatr Scand. 2000;101(4):323-9.
73. Association AP. Level 1 Cross-cutting symptom measure of global symptom severity. Diagnostic and Statistical Manual of Mental Disorders , Fifth Edition: American Psychiatric Publishing; 2013. p. 738-41.
74. Frisch MB, Cornell J, Villanueva M, Retzlaff PJ. Clinical validation of the Quality of Life Inventory. A measure of life satisfaction for use in treatment planning and outcome assessment. Psychological Assessment. 1992;4(1):92-101.
75. Lindner P, Frykheden O, Forsstrom D, Andersson E, Ljotsson B, Hedman E, et al. The Brunnsviken Brief Quality of Life Scale (BBQ): Development and Psychometric Evaluation. Cognitive behaviour therapy. 2016;45(3):182-95.
76. Devlin NJ, Shah KK, Feng Y, Mulhern B, van Hout B. Valuing health-related quality of life: An EQ-5D-5L value set for England. Health economics. 2018;27(1):7-22.
77. Bejerot S, Edman G, Anckarsater H, Berglund G, Gillberg C, Hofvander B, et al. The Brief Obsessive-Compulsive Scale (BOCS): a self-report scale for OCD and obsessive-compulsive related disorders. Nordic journal of psychiatry. 2014;68(8):549-59.
78. Tek C, Ulug B, Rezaki BG, Tanriverdi N, Mercan S, Demir B, et al. Yale-Brown Obsessive Compulsive Scale and US National Institute of Mental Health Global Obsessive Compulsive Scale in Turkish: reliability and validity. Acta psychiatrica Scandinavica. 1995;91(6):410-3.
79. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Arlington VA: American Psychiatric Association; 2013.
80. Eriksson JM, Andersen LM, Bejerot S. RAADS-14 Screen: validity of a screening tool for autism spectrum disorder in an adult psychiatric population. Molecular autism. 2013;4(1):49.
81. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 1987;13(2):261-76.
82. Bush G, Fink M, Petrides G, Dowling F, Francis A. Catatonia. I. Rating scale and standardized examination. Acta psychiatrica Scandinavica. 1996;93(2):129-36.
83. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta psychiatrica Scandinavica. 1983;67(6):361-70.
84. Öberg D, Söderström B, Broberg A. CRIES-8. Användarmanual för den svenska versionen av the Childen's Impact of Events Scale. Stockholm: Stockholms läns landsting; 2013.

85. Quilty LC, Ayearst L, Chmielewski M, Pollock BG, Bagby RM. The psychometric properties of the personality inventory for DSM-5 in an APA DSM-5 field trial sample. Assessment. 2013;20(3):362-9.
86. Kadesjo B, Janols LO, Korkman M, Mickelsson K, Strand G, Trillingsgaard A, et al. The FTF (Five to Fifteen): the development of a parent questionnaire for the assessment of ADHD and comorbid conditions. Eur Child Adolesc Psychiatry. 2004;13 Suppl 3:3-13.
87. Lugnegård T, Bejerot S. Retrospective parental assessment of childhood neurodevelopmental problems: the use of the "Five To Fifteen" in adults, submitted. 2018.
88. Holst Y, Thorell LB. Adult executive functioning inventory (ADEXI): Validity, reliability, and relations to ADHD. International journal of methods in psychiatric research. 2017.
89. Feys P, Lamers I, Francis G, Benedict R, Phillips G, LaRocca N, et al. The Nine-Hole Peg Test as a manual dexterity performance measure for multiple sclerosis. Multiple sclerosis (Houndmills, Basingstoke, England). 2017;23(5):711-20.
90. Bejerot S, Hesselmark E, Wallén H, Mobarrez F, Landén M, Schwieler L, et al. 57. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections perceived as conversion disorder. A case study of a young woman. Brain, Behavior, and Immunity. 2013;32:e16-e7.
91. Lingjaerde O, Ahlfors UG, Bech P, Dencker SJ, Elgen K. The UKU side effect rating scale. A new comprehensive rating scale for psychotropic drugs and a cross-sectional study of side effects in neuroleptic-treated patients. Acta psychiatrica Scandinavica Supplementum. 1987;334:1-100.
92. Larsen DL, Attkisson CC, Hargreaves WA, Nguyen TD. Assessment of client/patient satisfaction: development of a general scale. Evaluation and program planning. 1979;2(3):197-207.
93. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scandinavian journal of gastroenterology. 1997;32(9):920-4.
94. Castillo S, Mattila I, Miettinen J, Oresic M, Hyotylainen T. Data analysis tool for comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. Analytical chemistry. 2011;83(8):3058-67.
95. Nygren H, Seppanen-Laakso T, Castillo S, Hyotylainen T, Oresic M. Liquid chromatography-mass spectrometry (LC-MS)-based lipidomics for studies of body fluids and tissues. Methods in molecular biology (Clifton, NJ). 2011;708:247-57.
96. Bowden JA, Heckert A, Ulmer CZ, Jones CM, Koelmel JP, Abdullah L, et al. Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-Metabolites in Frozen Human Plasma. Journal of lipid research. 2017;58(12):2275-88.
97. Ganda Mall JP, Casado-Bedmar M, Winberg ME, Brummer RJ, Schoultz I, Keita AV. A beta-Glucan-Based Dietary Fiber Reduces Mast Cell-Induced Hyperpermeability in Ileum From Patients With Crohn's Disease and Control Subjects. Inflamm Bowel Dis. 2017;24(1):166-78.
98. Schwarz E, Maukonen J, Hyytiainen T, Kieseppa T, Oresic M, Sabunciyan S, et al. Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. Schizophr Res. 2018;192:398-403.
99. Xie WZ, Yan C, Ying XY, Zhu SY, Shi HS, Wang Y, et al. Domain-specific hedonic deficits towards social affective but not monetary incentives in social anhedonia. Sci Rep. 2014;4:4056.
100. Oldham S, Murawski C, Fornito A, Youssef G, Yucel M, Lorenzetti V. The anticipation and outcome phases of reward and loss processing: A neuroimaging meta-analysis of the monetary incentive delay task. Hum Brain Mapp. 2018;39(8):3398-418.

101. Dowd EC, Barch DM. Pavlovian reward prediction and receipt in schizophrenia: relationship to anhedonia. PLoS One. 2012;7(5):e35622.
102. Clifford DB, Ances B, Costello C, Rosen-Schmidt S, Andersson M, Parks D, et al. Rituximab-associated progressive multifocal leukoencephalopathy in rheumatoid arthritis. Archives of neurology. 2011;68(9):1156-64.
103. Salzer J, Lycke J, Wickstrom R, Naver H, Piehl F, Svenningsson A. Rituximab in paediatric onset multiple sclerosis: a case series. Journal of neurology. 2016;263(2):322-6.

18 APPENDIX 1, DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

PREAMBLE

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2 Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

GENERAL PRINCIPLES

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

RISKS, BURDENS AND BENEFITS

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

VULNERABLE GROUPS AND INDIVIDUALS

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

RESEARCH ETHICS COMMITTEES

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

PRIVACY AND CONFIDENTIALITY

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

INFORMED CONSENT

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

USE OF PLACEBO

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

POST-TRIAL PROVISIONS

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

• The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or

• Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient’s decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.