From the Department of Clinical Neuroscience
Karolinska Institutet, Stockholm, Sweden

PET STUDIES ON THE IMMUNE CELL MARKER TSPO IN FIRST EPISODE PSYCHOSIS PATIENTS

Karin Collste

Stockholm 2017

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Published by Karolinska Institutet.
Printed by E-Print AB 2017.

ISBN 978-91-7676-695-8

Institutionen för Klinisk Neurovetenskap

PET studies on the immune cell marker TSPO in first episode psychosis patients

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Leksellsalen, Eugeniahemmet, Karolinska Universitetssjukhuset, Solna

Fredagen den 19:e maj, 2017, kl 09.00

av

Karin Collste
Leg läkare, specialist i psykiatri

Huvudhandledare:
Docent Simon Cervenka
Karolinska Institutet
Institutionen för klinisk neurovetenskap

Bihandledare:
Professor Lars Farde
Karolinska Institutet
Institutionen för klinisk neurovetenskap

Docent Lena Flyckt
Karolinska Institutet
Institutionen för klinisk neurovetenskap

MSc PhD Anton Forsberg
Karolinska Institutet
Institutionen för klinisk neurovetenskap

Fakultetsopponent:
Professor Jarmo Hietala
University of Turku
Department of Psychiatry

Betygsnämnd:
Docent Håkan Karlsson
Karolinska Institutet
Institutionen för neurovetenskap

Docent Janet Cunningham
Uppsala Universitet
Institutionen för neurovetenskap
Divisionen för psykiatri

Professor Mark Lubberink
Uppsala Universitet
Institutionen för kirurgiska vetenskaper
Divisionen för radiologi

To my family

"There are more things in heaven and earth, Horatio, than are dreamt of in your philosophy."

– Hamlet, Act I, Scene V, by William Shakespeare, 1564-1616.

ABSTRACT

Several lines of evidence are indicative of a role for immune activation in the pathophysiology of schizophrenia. Nevertheless, studies using positron emission tomography (PET) and radioligands for the translocator protein (TSPO), a marker for glial activation, have yielded inconsistent results. In the present thesis the primary aim was to investigate immune activation in brain in early schizophrenia by examining brain TSPO availability in a cohort of first-episode psychosis (FEP) patients, never before exposed to antipsychotics.

In our first study we assessed the reproducibility of the second generation radioligand [^{11}C]PBR28 by performing repeat measurements in 12 healthy control subjects. We found a medium test-retest reproducibility, but high reliability in [^{11}C]PBR28 binding. A numerically lower variability was detected for subjects examined in the morning of separate days, as opposed to morning and afternoon of the same day, where higher afternoon TSPO levels were observed using secondary methods of quantification. The results suggest that diurnal variation may be a potential confounder in clinical studies.

In our second study we examined 32 healthy individuals, using [^{11}C]PBR28, of which 26 had repeat PET measurements. We found a strong association between TSPO availability in brain and blood cells, both at baseline and when analyzing change between two PET examinations. There was also a significant correlation between change in peripheral leukocyte numbers and change in brain TSPO. The results suggest interplay between central and peripheral TSPO at physiological conditions, and that measurement of radioligand binding in blood cells may be a way to control for peripheral immune function in PET studies of TSPO in brain.

In our third study we examined 16 antipsychotic-naïve FEP patients and 16 control subjects with PET and [^{11}C]PBR28. A significant decrease in TSPO availability in brain was detected in patients as compared to controls. The results indicate that the lack of increase in TSPO availability in earlier studies of schizophrenia was not caused by antipsychotic medication. The observed decrease suggests reduced numbers or altered function of immune cells in brain in early schizophrenia.

Finally, we examined the same cohort of FEP patients and control subjects with respect to the relationship between TSPO availability in brain and peripheral blood cells, as well as chemokine levels. The ratio between binding in brain and blood cells was significantly lower in patients as compared to control subjects. Moreover, we observed a correlation between TSPO binding in brain and levels of the chemokine YKL-40 in cerebrospinal fluid, in different directions among patients and controls respectively. These preliminary results suggest a dysregulation of brain immune cells in early schizophrenia.

Future studies combining TSPO PET with pro- and anti-inflammatory immune markers are needed to clarify the role of the immune system at different stages of the disease.

LIST OF PUBLICATIONS

I. **Karin Collste**, Anton Forsberg, Andrea Varrone, Nahid Amini, Shahin Aeinehband, Igor Yakushev, Christer Halldin, Lars Farde, Simon Cervenka. Test-retest reproducibility of [^{11}C]PBR28 binding to TSPO in healthy control subjects. *Eur. J. Nucl. Med. Mol. Imaging*. 2016, 43(1), 173–183.

II. Naoki Kanegawa, **Karin Collste**, Anton Forsberg, Martin Schain, Ryosuke Arakawa, Aurelija Jucaite, Mats Lekander, Caroline Olgart Höglund, Eva Kosek, Jon Lampa, Christer Halldin, Lars Farde, Andrea Varrone, Simon Cervenka. In vivo evidence of a functional association between immune cells in blood and brain in healthy human subjects. *Brain, Behavior and Immunity,* 2016, 54; 149-157.

III. **Karin Collste**, Pontus Plavén-Sigray, Helena Fatouros-Bergman, Pauliina Victorsson, Martin Schain, Anton Forsberg, Nahid Amini, Shahin Aeinehband, Sophie Erhardt, Christer Halldin, Lena Flyckt, Lars Farde, Simon Cervenka. Lower levels of the glial cell marker TSPO in drug-naïve first episode psychosis patients as measured using PET and [^{11}C]PBR28. *Mol Psychiatry*, online publication, Feb 14, 2017.

IV. **Karin Collste**, Funda Orhan, Ryosuke Arakawa, Sophie Erhardt, Lilly Schwieler, Lena Flyckt, Lars Farde, Göran Engberg, Simon Cervenka. Comparison of central and peripheral immune cell activity as measured using TSPO PET and chemokine levels in drug-naïve first-episode psychosis patients. *Manuscript*.

CONTENTS

LIST OF ABBREVIATIONS

ABSS	Arterial blood sampling system
ANCOVA	Analysis of covariance
AUC	Area under the curve
BBB	Blood brain barrier
CGI	Clinical global impression
CNS	Central nervous system
CNV	Copy number variation
COV	Coefficient of variance
CSF	Cerebrospinal fluid
Da	Dalton
DOI	Duration of illness
DUP	Duration of untreated psychosis
DVR	Distribution volume ratio
FEP	First episode psychosis
GM	Gray matter
GWAS	Genome-wide association studies
HAB	High affinity binder
HCT	Hematocrit
HRRT	High resolution research tomograph
ICC	Intraclass correlation coefficient
IFN	Interferon
iv	Intravenously
KYNA	Kynurenic acid
LAB	Low affinity binder
LP	Lumbar puncture
MAB	Mixed affinity binder
MHC	Major histocompability complex
MRI	Magnetic resonance imaging
MSS	Mean sum of squares
NMDA	*N*-methyl-$_{D}$-aspartic acid
PANSS	Positive and negative syndrome scale
PCP	Phencyclidine
PET	Positron emission tomography

ROI	Region of interest
SBR	Signal-to-background noise ratio
SD	Standard deviation
SUV	Standardized uptake value
TAC	Time activity curve
TNF	Tumor necrosis factor
TSPO	Translocator protein
V_T	Volume of distribution
WAPI	Wavelet-aided parametric imaging
2TCM	Two tissue compartment model

1 INTRODUCTION

1.1 RATIONALE FOR THIS THESIS

Schizophrenia is a life-long, often disabling disease, affecting young people on the threshold of adult life. Apart from the obvious suffering due to the severity of symptoms, the schizophrenic patient has an increased risk of somatic morbidity, in particular cardiovascular disease, an increased risk of concomitant substance abuse and, moreover, an increased risk for suicide, which leads to a life expectancy of 10-25 years less than non-schizophrenic individuals (*1*, *2*). In addition, the disease leads to increased yearly costs for society estimated at around half a million Swedish kronor per individual (*3*). Existing treatment is insufficient and only a minority of patients reach full remission. Thus, the discovery of novel treatment possibilities is crucial in order to improve patients' lives and reduce costs. However, the pathophysiology of the disease is not fully understood. Involvement of the immune system has been suggested in the development of schizophrenia, indicated by several lines of research (*4*). Microglia cells of the central nervous system (CNS) play a crucial role in the immune response of the healthy brain, and activation of microglia has been suggested as a possible pathophysiological mechanism in schizophrenia (*5*, *6*).

With position emission tomography (PET) it is possible to image the chemistry in the brain *in vivo*. For the past decade, PET studies have been conducted imaging the translocator protein (TSPO), seen as a marker for activated microglia, however with inconclusive results.

The general aim for this thesis was to evaluate the reproducibility of a recently developed PET radioligand for the TSPO receptor, [^{11}C]PBR28, in healthy subjects, and thereafter investigate TSPO as a biomarker in early schizophrenia.

1.2 SCHIZOPHRENIA

Even though patients suffering from both depression and mania have been described quite frequently in history, the first clear description of schizophrenia is found in a case-report from London as late as 1810 (Haslam 1810). During the 19th century a series of case reports of psychotic illnesses were published and at the same time the idea emerged that psychiatric illness could be treated.

1.2.1 Definition

At the end of the 19th century the German psychiatrist Emil Kraepelin brought together the three concepts hebephrenia, catatonia and paranoia into the new diagnosis dementia praecox (Kraepelin 1893). Later the Swiss psychiatrist Eugen Bleuler coined the term schizophrenia for this condition (Bleuler 1911), and described the four characteristic symptoms of the illness: autism, ambivalence, disturbances of association and affective disturbances.

According to the American Psychiatric Association's fourth version of the Diagnostic and Statistical Manual of Mental Disorders the following criteria for schizophrenia must be met:

Characteristic symptoms: Two (or more) of either:

1. Delusions
2. Hallucinations
3. Disorganized speech
4. Grossly disorganized or catatonic behavior
5. Negative symptoms (for example lack of motivation or poverty of speech)

Each of these symptoms should be present for a significant portion of time during a 1-month period (or less if successfully treated). In addition, social and/or occupational dysfunction must be present, and the duration of the disturbance must be at least six months (or less if successfully treated). Furthermore, schizoaffective disorder, mood disorder and substance abuse or a general medical condition must be excluded. In May 2013 the latest version of the diagnostic and statistical manual, DSM-5, was launched. This version contains minor changes to the definition of schizophrenia, however, the abovementioned main characteristics of the disease remain intact (*7*).

1.2.2 Cognitive deficits

Kraepelin's observation of dementia as part of the disease later named schizophrenia captured both the negative symptoms and cognitive deficits most often apparent in schizophrenia. Indeed, cognitive impairment has since been established to constitute part of the disability in schizophrenia (*8*). A recent meta-analysis of cognition in neuroleptic-naïve, early schizophrenia confirms earlier findings, and establishes the impairment irrespective of medication with neuroleptics (*9*).

1.2.3 Epidemiology

1.2.3.1 Prevalence and incidence

Schizophrenia affects young people on the verge of adulthood, with a similar prevalence worldwide. A meta-analysis of international studies estimated the point prevalence to 0.46 % (*10*), comparable to the prevalence 2005 in Stockholm of 0.35 % (department of Public Health, Karolinska Institutet). However, incidences differ. A review of 100 studies from 33 countries reveal a rather large range of incidences between the different studies (the majority range from 7.7 till 43.0 per 100 000) with a median of 15.2 per 100 000 individuals and year (*11*). This can be compared to the yearly incidence in Stockholms Län of 20-25/100 000 inhabitants (department of Public Health Epidemiology, Karolinska Institutet).

1.2.4 Risk factors

The cause of schizophrenia is to a great extent poorly understood, but several factors have been associated with an increased risk for developing the disease. The largest single risk factor is, however, heredity.

1.2.4.1 Heritability

In one meta-analysis of twin studies in schizophrenia, heritability has been estimated at 81 % (95 % confidence interval 73-90 %) (*12*). However, in the general population heritability estimates tend to be lower, and an estimate calculated from the Swedish Multi-generation Register was 64 % (*13*).

1.2.4.2 Pregnancy and birth

Having an older father is associated with an almost three times increased risk for schizophrenia (*14*, *15*), and complications during pregnancy and birth also contribute to an increased risk of later developing the disease (*16*). Moreover, infections, such as Influenza virus and Toxoplasma Gondii, during pregnancy have been associated with later onset of schizophrenia (*17–19*).

1.2.4.3 Socioeconomic status and urbanicity

The risk of developing schizophrenia increases for individuals growing up in an environment of low socioeconomic status (*20*). It has also been demonstrated that the incidence rate is higher in urban than in rural settings (*11*), however, a later review from the same group investigating the prevalence of the disease showed no difference due to environment (*10*).

1.2.4.4 Migration

Both first- and second generation migrants have an increased risk of developing schizophrenia (*21*), to a greater degree for refugee migrants as compared to non-refugee migrants, according to a recent Swedish epidemiologic study (*22*). It has also been demonstrated that the risk increases depending on the country of origin, suggesting that psychosocial factors of the assimilation process may be of importance (*23*).

1.2.4.5 Gender differences

An increased risk for males of developing the disease has been shown (*11*). This is congruent with the observation that males often present more severe symptomatology and show poorer long-term outcome than females, however, this increased risk ratio was not accompanied with an increase in prevalence between the sexes (*10*).

1.2.4.6 Cannabis

Cannabis use during adolescence has been reported associated with a subsequent two-fold risk increase for schizophrenia, even when adjusting for confounders, such as use of other drugs, certain pre-morbid personality traits predisposing for schizophrenia, and the use of cannabis as "self medication" of incipient psychosis (*24*). However, later studies have suggested that mechanisms of genetic-environmental interplay underlie the association between cannabis and psychosis (*25*, *26*).

1.2.5 Treatment

The methods for calming patients that were available up to the19th century were often barbaric, such as imprisonment in cages, or special "coffins for lunatics" (*27*), and not until the 1930s different types of therapies, such as insulin shock, cardiazoline injections and lobotomy were invented (*3*). Of the novel therapeutics developed at that time electro convulsive treatment (ECT) is the only one still in use. The breakthrough in schizophrenia therapy arrived with the invention of the neuroleptics in the 1950s. A few of the first introduced drugs are still in use, but numerous antipsychotics have since been developed. A common feature to all is blocking of the dopamine D_2 receptor, even though more recent neuroleptics have a broader receptor profile (*28*) and targets for example serotonergic and histaminergic pathways as well. Besides neuroleptics, different types of non-pharmacological treatment, such as psychological treatment, psychoeducation and family intervention is often a necessary addition (*29–31*). Even so, with existing treatment only a minority of patients reach full remission of psychotic symptoms (*32*). Moreover, treatment of the cognitive impairment often seen in schizophrenia is complicated (*33*, *34*), and both symptom severity and impaired cognition contribute to decreased quality of life (*35*).

1.2.6 Pathophysiology

Disease-specific symptoms of schizophrenia include delusions and hallucinations, implying defects in interpreting the meaning and source of an experience (*36*). Our understanding of the pathophysiology behind schizophrenia has increased over the years, since advances in neuroimaging and genetic studies have been made. Accordingly, several hypotheses have emerged.

1.2.6.1 Neurodevelopmental model

According to this model, early interference of normal neuronal development, such as synaptic pruning, apoptosis and axonal outgrowth, leads to later development of schizophrenia (*37–39*). This disturbance is thought to arise either during early neurodevelopment, or adolescence (*40*). The cause is suggested to be both genetic and environmental factors, such as obstetric complications and prenatal infections, resulting in brain pathologies leading to dysconnectivity, enlarged ventricles, and gray and white matter alterations (*41*). Indeed, longitudinal studies of individuals with attenuated psychotic symptoms, later developing into manifest psychosis, have made it possible to detect the earliest findings, such as reduced cortical gray matter volumes and enlarged ventricles (*42–49*).

1.2.6.2 Excitation-inhibition imbalance model

The psychotropic drug phencyclidine (PCP) and ketamine induce psychotic symptoms, including paranoia, depersonalization/derealization and perceptual abnormalities, mimicking those in schizophrenia (*50*). Both PCP and ketamine are antagonists of *N*-methyl-$_D$-aspartic acid (NMDA) receptors, a receptor in the glutamatergic neurotransmission. A hypofunction of the NMDA receptor has been implicated in the pathophysiology of schizophrenia (*51*, *52*),

and leads to an imbalance of the inhibitory/excitatory interplay of neurons (*53*). Elevated levels of the NMDA-receptor antagonist kynurenic acid (KYNA) have been demonstrated in both CSF and post-mortem brain of schizophrenia (*54–57*), (see section 1.3.5). Moreover, the inhibitory neurotransmitter GABA, also implicated in schizophrenia (*58–61*), was just recently found to be decreased in CSF of FEP patients, the decrease unrelated to medication status, and GABA levels were negatively correlated to symptom severity (*62*). In addition, recent genome-wide association studies (GWAS), as well as copy number variation (CNV) studies similarly implicate genes involved in glutamatergic neurotransmission (*63*).

1.2.6.3 Dopamine hypothesis

The dopamine hypothesis has dominated the field since the 1950's. Its origin was empiric findings that amphetamine induces psychotic symptoms in healthy individuals and worsens symptoms in individuals with schizophrenia (*64*). Moreover, antipsychotic drugs were found to affect the dopamine system (Carlsson 1963), by targeting the dopamine D_2-receptor (*65*). Modern imaging studies using Positron Emission Tomography (PET) have led to further insights into the dopaminergic system in schizophrenia. Hence, dopamine synthesis and release seems to be elevated in the striatum in schizophrenia, together with reduced dopamine release in prefrontal cortex (*66–68*). The striatal dopamine up-regulation has also been demonstrated in the prodromal phase of the disease (*69*).

It has been hypothesized that NMDA hypofunction leads to changes in the neuronal interactions of the glutamate, GABA and dopaminergic systems (*36*).

1.2.6.4 Immune hypothesis

Immunological factors as part of the pathophysiology in schizophrenia have been discussed since 1845, when Esquirol described psychotic cases as an "epidemic"(*70*). In more recent years an immune hypothesis of schizophrenia has developed. Support for this hypothesis come from epidemiologic (*71*), genetic (*39*, *63*) and clinical studies (*72*, *73*), see below.

1.3 INVOLVEMENT OF THE IMMUNE SYSTEM IN SCHIZOPHRENIA

The human immune system consists of an innate immune response, mediated by for example neutrophils and macrophages and thought of as the first line of defense, and an adaptive response, involving immunological memory with T lymphocytes that recognizes antigens and causes destruction of infected cells and B lymphocytes that secrete antibodies (*74*). Links between schizophrenia and the immune system were assumed more than a century ago (*75*). Over the years an increasing amount of evidence point in the direction of the immune system as part of the pathophysiology behind the disease.

1.3.1 Genetic studies

In recent years, the possibility to implement large genetic studies has increased. A recent GWAS in schizophrenia, including more than 36 000 cases and 113 000 controls, detected 108 genetic loci associated with schizophrenia, associations enriched among genes expressed

in tissues with important immune functions (*63*). The strongest genetic relationship in schizophrenia implicates variations in a locus coding for the major histocompability complex (MHC). The MHC locus contains genes coding for many antigen-presenting molecules, including complement component 4A (C4A), known to regulate synapse pruning in mice. Increased expression of C4A was indicated in schizophrenia, suggesting a cause for the reduced number of synapses found in the schizophrenic brain (*39*).

1.3.2 Epidemiological studies

Epidemiologic studies corroborate associations between schizophrenia and infection by several common viruses during the pre- or perinatal period (*71*). Moreover, there is significant support that severe prenatal infection and immune activation during pregnancy increases the risk for developing schizophrenia. This has been demonstrated in animal studies as well as in human epidemiological studies (*76*). Similarly, infection during childhood is associated with increased risk for later developing the illness (*77*).

1.3.3 Pharmacological studies

Anti-inflammatory agents have been proposed as add-on therapy in schizophrenia, and an increasing amount of reports have been published. Several studies using a Cox-II inhibitor as add-on to neuroleptics have demonstrated positive results in reducing psychotic symptoms (*78*). In addition, one meta-analysis of five placebo-controlled treatment studies reported a significant effect of non-steroidal anti-inflammatory drugs (NSAID) as augmentation therapy in reducing psychotic symptoms in schizophrenia (*79*); however, a more recent meta-analysis found aspirin to augment the effect of neuroleptics on psychotic symptoms, but not the Cox-II inhibitor celecoxib (*80*). A way of sorting the contradictory results of these types of studies could be to investigate subgroups in schizophrenia, which would benefit from this add-on therapy.

1.3.4 Immune markers

1.3.4.1 Cytokines

Cytokines are molecules involved in cell signaling, and contribute to immune cell migration towards sites of trauma, inflammation and infection. Elevations of several cytokines have been demonstrated in plasma and CSF of both medicated, chronic, as well as in early, neuroleptic-naive stages of schizophrenia (*72*, *73*). These include interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-12, IL-1β, interferon (IFN)-γ. According to one meta-analysis, the most replicated plasma finding in acutely relapsed chronic patients was IL-6 and in first-episode psychosis (FEP) patients TNF-α. However, there was no difference in plasma levels of IL-6 in stable, medicated patients as compared to controls (*72*). Another publication including studies of neuroleptic-naïve FEP patients reported elevated serum levels of the cytokines IL-1β, sIL-2r, IL-6 and TNF-α (*73*). Studies of cytokine levels in the cerebrospinal fluid (CSF) are fewer and lacking in power (*72*). Nevertheless, IL-6 have been found elevated

in CSF of chronic patients with schizophrenia (*81–83*), and levels of IL-1β elevated in medicated FEP patients (*84*) later diagnosed with schizophrenia.

1.3.4.2 Chemokines

Immune cell migration to infected or injured tissue is mediated by specific chemokines, chemoattractant proteins, secreted by immune cells. In addition to cell migration, functions include adhesion of leukocytes to vessel walls, and various tissue responses, such as proliferation, differentiation and angiogenesis (*85*). Moreover, chemokines have been hypothesized to play a role in the central nervous system (CNS), by having effects on neurotransmission, neuromodulation and even blood-brain barrier (BBB) permeability (*86*). Impact of chemokines on neurodegenerative diseases, such as multiple sclerosis (MS), stroke, Alzheimer's (AD) and Parkinson's (PD) diseases, have been demonstrated (*86*). Elevations of chemokine levels have also been proposed in psychiatric disorders, such as schizophrenia (*87*). Indeed, elevated levels of the chemokine, monocyte chemoattractant protein (MCP)-1 have been associated with the disease (*88*), and the chemokine chitinase-3-like protein 1 (YKL-40) has been genetically linked to schizophrenia (*89–91*).

1.3.5 Kynurenic acid (KYNA)

KYNA, released by astrocytes of the CNS, is a metabolite in the degradation of tryptophan, an antagonist to the NMDA-receptor, and has been found elevated in schizophrenia. In vitro findings suggest that KYNA may be of importance for both dopaminergic and glutamatergic neurotransmission (*92*). Moreover, elevated cortical KYNA levels in rats have been associated with impairment of cognitive domains, such as spatial working memory, learning and sensory gating (*93*), In addition, it has been proposed that the cytokine IL-6 induces increased production of KYNA in schizophrenia (*83*), suggesting a link between the immune system and symptoms of schizophrenia.

1.3.6 Immune cells of the CNS

1.3.6.1 Microglia

Microglia cells are considered the resident macrophages of the brain and constitute 5-20 % of brain glial cells and around 10 % of the total cell population of the brain. At physiological conditions, microglia, unlike monocytes that constantly are being renewed from bone marrow, are maintained via self-renewal in the brain (*94*). In the healthy brain microglia appear in a ramified form, with small cellular bodies and long, slim processes. Microglia in this form are highly mobile, surveying the surroundings of the brain. In the damaged brain, either from injury, infection or neuronal dysfunction, microglia become activated, thus changing into a reactive, amoeboid form. In this form microglia can proliferate, phagocytose cellular debris and, moreover, secrete immune markers, such as cytokines, chemokines and neurotrophic factors, as well as present antigens (*95*, *96*), (figure 1).

Activated microglia can be classified in two groups, depending on the mode of activation. The pro-inflammatory phenotype M1 produces for example TNF-α, IL-1β and IL-6, in

defense against pathogens and tumor cells, however, in addition causing cell damage and increased inflammatory response. The anti-inflammatory M2-type microglia secretes cytokines, such as IL-10 and arginase-1, down-regulating inflammation and promoting repair and tissue remodeling (*97*, *98*).

1.3.6.2 Astrocytes

Astrocytes have been reported to constitute around 20 % of the human glia cell population (*99*). Morphologically, there are four classes of astrocytes in the human brain: interlaminar, protoplasmic, fibrous and varicose projection astrocytes. The functions of astroglia is not fully understood, however, participation in intra-neuronal communication and coordination, metabolic support and even a role in cognitive function specific to humans have been proposed (*100*). Recent findings reveal two forms of astrocytes, the neurotoxic A1 and neuroprotective A2. It is proposed that activated microglia induce the A1 form by secreting the cytokines interleukin (IL)-1α, tumor necrosis factor (TNF) and subcomponent (C1) q after CNS injury, contributing to neuronal cell death. The A2-form on the other hand is induced by ischemia and promotes neuronal survival and tissue repair (*101*).

Increases in microglia and, in some studies, of astrocytes, have previously been reported post-mortem in the brain of schizophrenia patients (*5*, *6*, *102*, *103*). However, later meta-analyses of post-mortem microglia and astrocyte markers are inconclusive (*104*, *105*). The varying results might be due to methodological variations, such as differences in identifying and counting the cells and use of different immunohistochemical markers. Moreover, the most commonly used marker for microglia does not differentiate between the different phenotypes.

1.3.7 Immunological interplay between the CNS and the periphery

Previously the central nervous system (CNS) was seen as an immunologically privileged part of the body, due to the blood brain barrier (BBB), preventing immune cell entry from the periphery. However, findings during the last decade have demonstrated that there is indeed an interaction between peripheral immune cells and the CNS, with entrance to the CNS via the meninges during inflammation (*106*). In addition, a lymphatic system was recently discovered in the dural sinuses, able to carry both fluid and immune cells from the CSF, and connected to the deep cervical lymph nodes (*107*).

1.4 POSITRON EMISSION TOMOGRAPHY

The soft tissue of the brain is encompassed by the bony structure of the scull, preventing damage, but also complicating investigation of the brain. Using ionizing radiation is one possible measure to overcome this obstacle and study the brain in vivo. Positron emission tomography (PET) is an imaging technique using radiolabeled compounds, or radioligands, in nanomolar concentration to target specific receptors of the brain. The PET compound used determines what can be measured in each PET examination. The ligands are often labeled with a short-lived radionuclide, for example ^{11}C, which has a half-life (T1/2) of 20.3 minutes.

Ideal radioligands should have a high affinity together with a high selectivity for the target, and if useful as a CNS-ligand must be lipophilic in order to pass the BBB (*98*).

The radioligand is injected in the subject intravenously and spreads throughout the blood volume. Reaching the vessels of the brain, passage over the blood brain barrier (BBB) is necessary to enter the brain, and a small portion of the injected radioligand thus reaches the brain. In the brain tissue the radioligand binds to the target receptor. The radionuclide decays, whereby a positron is emitted. This positron travels a short distance (about 1 mm for ^{11}C), until it reaches an electron, its antiparticle. This results in the annihilation of the positron-electron pair, with the emission of a pair of photons (γ-particles) in opposite directions at approximately 180˚ as a consequence (*108*). These photons are eventually detected by the PET system. Two photons detected within a short time frame at an 180˚ angle are registered as a coincidence, and a large number of such coincidences are registered during a PET examination (figure 1). All coincidences at a given time point constitutes a 2D image of the annihilations. Placing all the 2D images from different time points of the examination in order creates a 3D representation of the distribution of the radioligand.

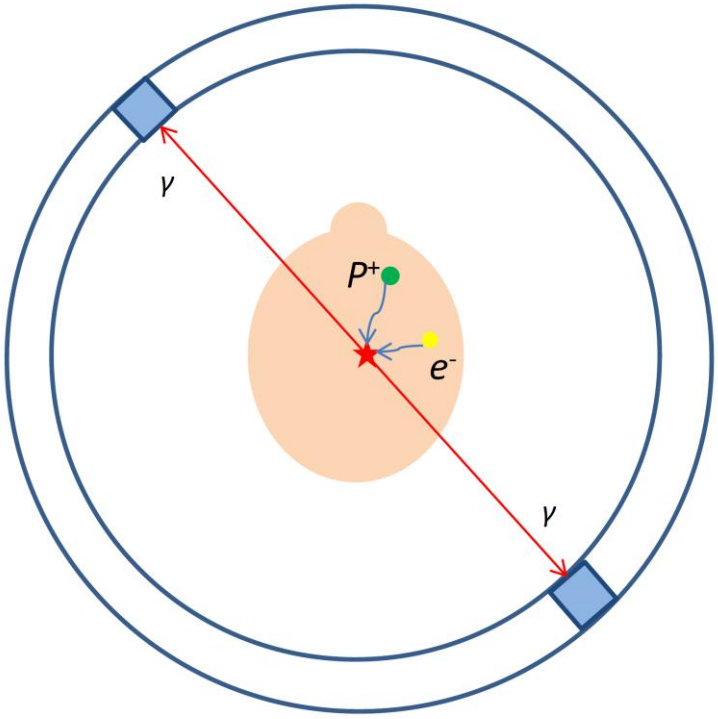


Figure 1. Schematic figure illustrating collision of a positron (p+), emitted at decay of the radionuclide, and an electron (e-), within the brain tissue of the subject. At the site of annihilation (red star) the two coincidence photons (γ-particles) generated, move away at 180^{o} angle, and are subsequently detected by the PET system.

Spatial resolution of an imaging system is described as the ability to discriminate between two closely located, separate objects that are being imaged. The spatial resolution of the high resolution research tomography (HRRT) system used in this thesis is approximately 1.5 mm in the centre, and 2.4 mm at 10 cm distance from it.

1.5 PET RADIOLIGANDS FOR THE IMMUNE SYSTEM

Several possible neuroinflammatory targets have been proposed, imaging different aspects of the immune system of the brain. Of these the receptor of the translocator protein (TSPO) is the most studied (*98*).

1.5.1 Imaging the TSPO receptor

The TSPO receptor is an 18-kDa protein located in the outer mitochondrial membrane of many cell types of the body, including microglia and astrocytes of the CNS. Previously an important role in cholesterol synthesis was prevailing; however, recent findings have challenged this concept. Nevertheless, a role in neurosteroid synthesis and cellular energy consumption have been suggested (*109*). Expression of TSPO in the healthy human brain increases in response to neuroinflammation. In microglia the up-regulation of TSPO is relatively fast after injury, in contrast, astrocytes show a more delayed, but persistent increase in TSPO expression (*110*, *111*).

In blood, expression of TSPO have been demonstrated in monocytes and polymorphonuclear neutrophils in particular, and to a lesser extent in lymphocytes, platelets and erythrocytes (*112*).

1.5.1.1 [^{11}C]PK11195

[^{11}C]PK11195 is the most used of the radioligands imaging translocator protein, and up until some years ago the only PET tracer available for imaging TSPO. However, [^{11}C]PK11195 has some limitations, including poor signal-to-background noise ratio (SBR) and a high level of non-specific binding (*113*).

1.5.1.2 Second generation TSPO radioligands

In recent years, a large number of second-generation PET radiotracers for TSPO have been developed. They all share the feature of improved SBR; however, an additional trait is a difference in binding affinity to TSPO among human subjects. It is now established that approximately 10 % of the population exhibits low binding capacity to TSPO. This is explained by a polymorphism in the gene coding for TSPO (*114*), resulting in high affinity binders (HABs), with a single high affinity binding site for TSPO (major allele), low affinity binders (LABs), with a single low affinity binding site (minor allele), and mixed affinity binders (MABs), with equal numbers of the high and low affinity binding sites. The distribution of HABs, MABs and LABs in the population is approximately 49 %, 42 % and 9 % respectively (*115*). However, the frequency of the minor allele differs, thus, among Caucasians the minor allele is present in around 30 % of the population, the major in 70 %, whereas the minor allele is less prevalent in African Americans (25%), Han Chinese (2%) and Japanese (4%) populations (*114*).

These differences in binding capacity to TSPO, using second generation radiotracers, mean that the binding genotype must be considered in human PET studies, in order to interpret data correctly.

1.5.1.3 [^{11}C]PBR28

One of the second generation TSPO radioligands developed is [^{11}C]PBR28. It has an aryloxyanilide structure (figure 2) and belongs to a group of proteins that exhibits high specific binding to TSPO. [^{11}C]PBR28 has a 10-fold higher affinity to TSPO than [^{11}C]PK11195 (*116*, *117*).

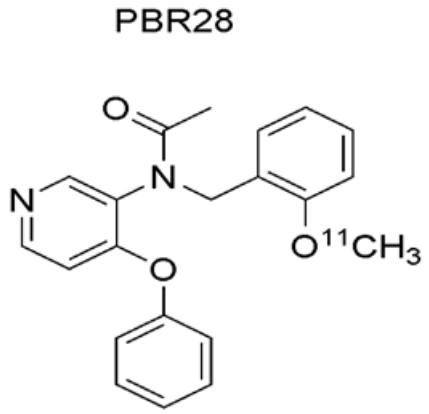


Figure 2. Schematic illustration of [^{11}C]PBR28.

1.5.2 Quantification of TSPO

TSPO is present throughout the brain. Thus, when quantifying TSPO binding, no region of the brain is devoid of binding, and may be used as a reference region. Instead, the use of a compartmental model is required, using radioactivity in tissue together with radioactivity in arterial plasma, correcting for the metabolism of the tracer (*116*, *118*).

1.5.2.1 The two-tissue compartment model (2TCM)

The two tissue compartments are defined as the radioactivity concentration of non-displaceable radioligand in the brain (CND) and the radioactivity concentration of radioligand specifically bound to receptors (CS). In this model, four rate constants (K1, k2, k3 and k4) are used to interpret the time curves for the radioligand in different regions of the brain. This model has been shown to be suitable for the quantification of [^{11}C]PBR28 binding (*117*), (figure 3, page 18).

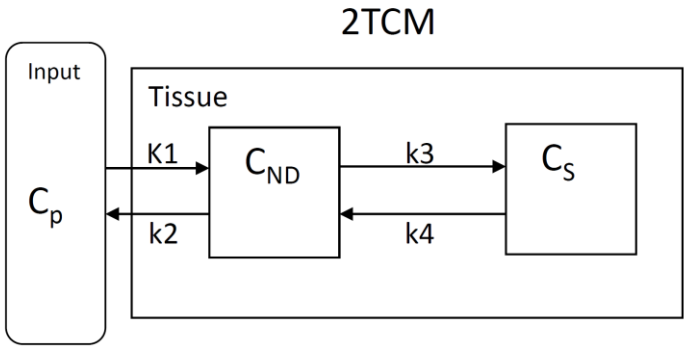


Figure 3. 2TCM model: One compartment for binding in plasma (C_P), and two exchangeable tissue compartments; one for the non-displaceable component (C_{ND}) and one for the specific binding (C_S). K1 and k2 are rate constants for transport from plasma to tissue and back, k3 and k4 are rate constants for transport from the non-displaceable compartment to the specific and back.

1.5.2.2 Logan graphical analysis

The linear graphical analysis, or Logan plot (*119*) can be used to estimate TSPO binding also where very low specific binding is expected. The Logan plot does not assume any particular model structure. The slope of the plot when the linear phase has been reached corresponds to the plasma volume, plus the total volume of distribution (V_T) of the radioligand. The slope can then be expressed as follows:

$$\left[\frac{K_1}{k_2}\left(1+\frac{k_3}{k_4}+V_p\right)\right] \qquad (1)$$

Where $K1$ to $k4$ are rate constants, and V_p is the regional plasma volume.

1.5.2.3 Parametric imaging

Parametric mapping approaches can be used to filter out noise from the signal we aim to detect. With these algorithms outcome measures for each voxel are calculated. One example of parametric mapping is the wavelet-aided parametric imaging (WAPI) method. Here, signal patterns are identified, with the use of a "wavelet filter", thus differentiating signal components (with lower frequency) to noise (higher frequency), and facilitating subsequent deletion of the noise (*120*).

1.6 PET STUDIES OF TSPO IN SCHIZOPHRENIA

Compared to other neurodegenerative diseases there are few PET studies of the immune system in schizophrenia. The two first were published less than a decade ago and used the older radioligand [^{11}C]PK11195, which had previously shown elevated binding to TSPO in for example Alzheimer's, Parkinson's and Huntington's diseases (*121*). These two first studies demonstrated an increase in TSPO binding in schizophrenic patients as compared to

controls (*122*, *123*). However, the studies were small, all patients were on antipsychotic medication, which might influence the result (*124*), and moreover, the outcome measure used has shown low accuracy and reliability (*125*). Further studies of TSPO in schizophrenia have consequently used second generation TSPO radioligands. Takano et al used [^{11}C]DAA1106 and examined chronic patients, with mean duration of illness (DOI) of 18.8 (SD 2.2) years. No difference in binding between patients and controls was detected (*126*), however, authors did not control for TSPO genotype. In another study, using the radioligand [^{18}F]-FEPPA, patients had on-going psychotic symptoms, and shorter DOI (177.3 (SD 105.7) months), however, no evidence of a difference in TSPO binding as compared to controls was demonstrated (*127*). Patients in both of these studies were on medication with neuroleptics.

One study from 2015, using [^{11}C]PBR28, presented an increased binding both in long-term medicated patients and in un-medicated Ultra High Risk Individuals (*128*). These results were derived from the relative measure distribution volume ratio (DVR), as opposed to the volume of distribution (V_T). However, this method has been criticized (*129*); thus, the whole brain (WB) values used in the normalization were ~20 % lower among patients than controls, seemingly driven by significant decreases in white matter of patients. In addition, WB was used as covariate in the statistical analysis, and not as denominator in a ratio. When the authors estimated TSPO binding using the 2TCM no significant difference was detected in either group of patients, as compared to controls.

Since this study was presented an array of similar PET studies in schizophrenia have been published, using [^{11}C]PK11195 and second generation TSPO ligands. The first used [^{11}C]DPA-713 in medicated, recent-onset schizophrenia, with a DOI of 2.2 (SD 1.4) years. Numerical decreases in TSPO binding was seen in patients as compared to controls, however, no significant differences (*130*). In a subsequent publication, investigating [^{11}C]PK11195 binding in recent-onset schizophrenia (DOI 1.3 (SD 1.1) years), no difference was detected between patients and controls (*131*).

Two additional studies have been published; one using [^{11}C]PK11195, with a total of 16 patients with schizophrenia (DOI 9 (SD 7) years), whereof a subgroup were neuroleptic-naïve (n=6) (*132*), and one using [^{18}F]FEPPA, with 19 FEP patients (DOI 33.6 months), where 14 were neuroleptic-naïve (*133*). None of these studies presented a difference in TSPO binding between patients and controls.

Of all the TSPO studies in schizophrenia to date, only minor subgroups of patients have been naïve to neuroleptics, thus the impact of neuroleptics on TSPO availability have not yet been possible to rule out.

2 AIMS

The overall objective of the thesis was to investigate the immune system in first-episode psychosis. The specific aims were to investigate:

1. The reproducibility of the TSPO ligand [^{11}C]PBR28 in healthy control subjects.
2. The association between TSPO binding in brain and blood cells.
3. TSPO availability in brain of neuroleptic-naïve first-episode psychosis patients.
4. The relationship between TSPO binding in brain and peripheral blood cells, together with levels of chemokines, in first-episode psychosis patients.

3 MATERIALS AND METHODS

3.1 ETHICAL CONSIDERATIONS

The local Ethical review board and the Radiation Safety Committee approved all studies. The test-retest study in paper I was moreover approved by the Medicinal Products Agency in Sweden. The subjects provided verbal and written informed consent before participation.

The PET measurement procedure is standardized and has been applied in more than 2000 examinations in human subjects over the last 25 years. The procedure is generally well tolerated. During a PET measurement, each subject is exposed to radioactive energy equivalent to the background radioactivity which a person living in Stockholm for one year encounters derived from the atmosphere and surrounding buildings.

In conjunction with the PET examination some subjects may experience discomfort by lying still with the head fixated during 90 minutes, and the administering of both venous and arterial catheters may also be perceived as uncomfortable. Lumbar punction may in rare cases give rise to post-puncture headache. Necessary measures to minimize the risk for adverse events were taken, such as the use of an experienced anesthesiologist for administering the arterial catheter, test of blood flow and continuous monitoring for as long as the catheter was in place and the use of insertion techniques that minimizes the risk for post-puncture headache.

The first episode psychotic patients in papers III and IV were included directly after admittance for their first acute psychotic episode. At our centre, experience regarding this vulnerable patient group has been acquired by examining more than 100 young, neuroleptic-naïve first-episode patients with schizophrenia in previous PET-projects. The time to plan, prepare and conduct the MR and PET-examinations has been performed within a week and does not represent a delay of initiation of antipsychotic drug treatment with regard to current clinical guidelines.

When performing research, a potential risk of adverse events must be weighed against the benefit of advancement in knowledge about a severely disabling disease.

3.2 SUBJECTS

In paper I and II healthy individuals were included, whilst in paper III and IV both first-episode psychotic patients and healthy control subjects participated.

3.2.1 Karolinska Schizophrenia Project (KaSP)

The clinical platform for the recruitment of patients in paper III-IV consisted of the Karolinska Schizophrenia Project (KaSP), a broad collaboration within the Centre for Psychiatric Research in Stockholm, with the overall purpose to study the immune system in schizophrenia. The clinical partners at the time of the studies were Norra Stockholms Psykiatri, Prima Barn- och Vuxenpsykiatri and Psykiatri Nordväst.

3.2.2 Patients

3.2.2.1 First-episode psychosis (FEP)

The definition of a first-episode psychosis patient differs in the literature (*134*). We chose to define an FEP patient as an individual having their first contact with a psychiatric clinic, due to onset of psychotic symptoms, and meeting the criteria for a schizophrenia spectrum disorder, according to the DSM-IV.

3.2.2.2 Paper III-IV

In papers III-IV 16 FEP patients, mean age 28.5 (SD 8.4) years, were recruited from outpatient clinics and hospital wards of three psychiatric clinics in the northwestern area of Stockholm (see section 3.2.1). All patients were experiencing their first episode of psychotic symptoms. Their mean duration of illness was 7.9 (9.6) months and total PANSS score 77.4 (18.3). The patients had not yet been exposed to antipsychotic medication. To enable participation, medication with anxiolytics and sedatives was permitted, thus five patients received benzodiazepines (diazepam (5 mg per day) n=1 and oxazepam (10–30 mg per day) n=4) on the day of the PET measurement. One patient was prescribed paroxetin 20 mg per day. Two of the patients were nicotine smokers. A further exclusion criterium was current use or history of abuse of illegal drugs, including cannabis. All patients included participated in one PET measurement each.

3.2.3 Healthy subjects

Control subjects were recruited by advertisement. They were deemed healthy according to their medical history, physical examination, routine laboratory test and magnetic resonance imaging (MRI) of the brain. Moreover, a negative illegal drug-screening test, including cannabis, was ascertained. None of the control subjects were nicotine users. A further exclusion criterium was relatives with a psychotic illness. None of the healthy subjects were on any medication at the time of the study.

3.2.3.1 Paper I

Twelve healthy subjects, six males and six females, mean age 23.9 (SD 3.1) years, were included in paper I. In addition to the abovementioned examinations for healthy subjects, a Mini International Neuropsychiatric Interview (MINI) for psychiatric diagnoses was performed to ascertain their previous or current psychiatric health. All subjects in this paper performed two PET measurements each. Six subjects had the two examinations in the morning and afternoon of the same day, six on two separate days, 2-5 days apart.

3.2.3.2 Paper II

In paper II two cohorts of healthy subjects were included. Cohort one consisted of 13 subjects, seven male and six female, mean age 24.1 (SD 3.0) years, cohort two of nine males and 10 females, mean age 42.2 (SD 13.4). Subjects in cohort one performed two PET measurements, either on the same day (n=6), or on separate days (n=7). In cohort two 13

subjects performed two PET measurements on separate days, and six subjects participated in one measurement each.

3.2.3.3 Paper III and IV

In paper III and IV 16 healthy control subjects were included to enable comparison with the FEP patients, mean age of the control subjects were 26.4 years (SD 8.4). In addition to the examinations mentioned in section 3.2.3, a Mini International Neuropsychiatric Interview (MINI) for psychiatric diagnoses was performed to ascertain their previous or current psychiatric health.

3.3 MAGNETIC RESONANCE IMAGING (MRI)

An MRI was performed in a 3 Tesla system for each subject prior to the PET examination. A T2-weighted sequence was used to rule out pathology and T1-weighted images for definition of anatomical brain regions and for coregistration with PET images.

3.4 PET EXAMINATIONS

The PET measurements were performed at the Karolinska university hospital in Solna, and the High Resolution Research Tomograph (HRRT, Siemens Molecular Imaging, USA) were used for all examinations.

To fixate the head in the PET system, an individual plaster helmet was made for each subject, prior to the examination. Each subject achieved both a peripheral venous cannula for injection of the radioligand, and an arterial catheter in order to sample arterial blood throughout the PET examination.

A sterile phosphate buffer (pH 7.4) containing radioligand was injected intravenously as a bolus over approximately 10 seconds; hereafter the cannula was flushed with a 10 mL saline solution.

Brain radioactivity was measured for up to 91 minutes, except for one subject in paper I where the acquisition time was 60 minutes, two subjects in paper II (one in cohort 1, PET 2 60 min.; and one in cohort 2, PET 1 50 min) and in papers III-IV one patient (85 min) and two controls (77 min), due to technical issues.

3.5 ARTERIAL BLOOD SAMPLING

An automated arterial blood sampling system (ABSS) was used during the first five minutes of all PET examinations. In addition, arterial blood samples were drawn manually at subsequent time points throughout the examinations.

3.6 IMAGE PREPROCESSING

In all papers, MR images were realigned, segmented and co-registered to PET images using SPM5 in Matlab R2007b (Wellcome Trust Centre for Neuroimaging, London, UK; The Mathworks, Natick, MA, USA). Correction for head movement in PET examinations was

performed using a frame-by-frame realignment algorithm, where all frames were realigned to the first minute of acquisition. 3D PET summation images were obtained by integrating 4D PET images over time, and reoriented MR images were thereafter co-registered to the 3D PET summation images, thereby creating parameters of rotation and translation.

3.7 BRAIN REGIONS OF INTEREST

In papers I-II regions of interest (ROIs) were defined using the Automated Anatomical Labelling (AAL) System in SPM5. The primary ROI in paper I was gray matter (GM), in addition, regional binding in thalamus, putamen, hippocampus, lateral frontal cortex and lateral temporal cortex (all of these brain regions considered of interest in research on the role of immune activation in neuropsychiatric disorders) was investigated. In paper II all AAL ROI's were combined into one large whole brain (WB) ROI, since there was no hypothesis of a regional difference in the relationship between brain and blood cells binding.

In papers III-IV ROI's were defined using the FreeSurfer software (version 5.0.0) (*135*). The primary ROI of paper III was GM, in addition, white matter, frontal cortex, temporal cortex and hippocampus (considering their interest in research in schizophrenia, as well as for comparison to previous TSPO PET studies) were examined. In paper IV GM was selected as the primary ROI.

3.8 METABOLITE CORRECTED ARTERIAL PLASMA INPUT FUNCTION

Preprocessing of arterial blood was performed for all papers using Kaleidagraph 4.1 software (Synergy Software). In short, after dispersion correction, interpolation of manual arterial blood samples and integration with the continuous sampling from the ABSS was performed, thus creating a continuous blood time-activity curve. A plasma curve was estimated by multiplying the first 5 min of the blood curve by the plasma-to-blood ratio curve, and the remaining part of the plasma curve estimated by interpolating the discrete plasma measurements.

In papers I and II, correction for radioligand metabolism, using individual parent fraction data, was performed in PMOD v3.2 (PMOD Technologies Ltd, Zurich, Switzerland), with a three-exponential model, yielding an arterial plasma input function. In papers III and IV correction for radioligand metabolism was performed in Matlab R2007b, using individual parent fraction data, thus interpolating the discrete metabolite data and multiplying it with the plasma, resulting in the final input function.

3.9 QUANTIFICATION OF RADIOLIGAND BINDING

3.9.1 2TCM

For the main analyses in paper I and III [^{11}C]PBR28 binding was quantified using two-tissue compartmental modeling, with the metabolite-corrected arterial plasma as input function (see also section 1.5.2.1).

3.9.2 2TCM-1K

In paper III a modified 2TCM model was used in addition, in order to enable comparison with a previous TSPO PET study (*128*). In this model an additional vascular compartment is added. This compartment is hypothesized to account for vascular endothelial binding, and the uptake is thought to be irreversible.

3.9.3 DVR

In paper III a relative measure to estimate TSPO binding was used as well. The distribution volume ratio (DVR) is calculated as the ratio between regional V_T and V_T of a chosen reference region (equation 2). In this case gray matter (GM) was chosen as the region of interest (ROI) and whole brain (WB) as the reference region. V_T of both GM and WB was estimated from both the 2TCM and 2TCM-1K models. Also this approach was used for comparison with a previous publication (*128*).

$$DVR = \frac{V_{T.ROI}}{V_{T.REF}} \qquad (2)$$

3.9.4 Logan graphical analysis

In paper II linear graphical analysis, or Logan plot (*119*) was chosen to estimate [^{11}C]PBR28 binding, in order to quantify TSPO binding also in the LAB individual, where very low specific binding is expected (see also section 1.5.2.2).

3.9.5 Parametric imaging

In order to compare the reference ROI-based method of analysis with parametric imaging, the wavelet-aided parametric imaging method (WAPI) was applied in paper I (see also section 1.5.2.3).

3.9.6 Radioactivity in blood cells

The blood constitutes of blood cells and plasma, according to figure 4.

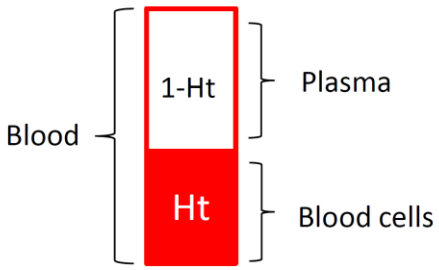


Figure 4. Schematic illustration of plasma and blood cells in whole blood. Figure courtesy Naoki Kanegawa.

In paper II and IV the radioactivity in blood cells was calculated from the radioactivity in blood (C_{Blood}) and in plasma (C_{Plasma}) and hematocrit value (HCT), according to equation 3:

$$C_{Blood.cells} = \frac{[C_{Blood} - C_{plasma} * (1 - HCT)]}{HCT} \qquad (3)$$

Generating a time activity curve (TAC) for radioactivity in blood cells. V_T of blood cells was then estimated as the ratio of area under the curve (AUC) for blood cells, to AUC of the whole scan time (AUC input).

$$V_{T.Blood.cells} = \frac{AUC_{Blood.cells}}{AUC_{Input}} \qquad (4)$$

3.9.7 Binding in brain normalized for peripheral binding

In paper II and IV normalized V_T was calculated as follows:

$$V_{T.Normalized} = \frac{V_{T.Brain}}{V_{T.Bloodcells}} \qquad (5)$$

3.10 CLINICAL EVALUATIONS

Patients in paper III were assessed for psychotic symptoms with the Positive And Negative Syndrome Scale (PANSS) (*136*), and their level of functioning with the Clinical Global Impression (CGI) scale (*137*, *138*).

3.11 COGNITIVE TESTING

Cognitive functioning, using the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB) (*139*, *140*), was assessed in all subjects in paper III.

3.12 CHEMOKINES IN BLOOD AND CSF IN PAPER IV

3.12.1 Lumbar puncture

In paper IV lumbar puncture (LP) and blood sampling was performed in both patients and control subject within two weeks of PET measurements, and cerebrospinal fluid (CSF) and plasma was obtained for analyses of chemokine levels in 11 patients and 13 controls.

3.12.2 Analysis of chemokines

Levels of the chemokines YKL-40 and MCP-1 in CSF and plasma were measured using an electrochemiluminescence assay. In short, the Meso Scale Discovery (Meso Scale, Gaithersburg, MD, USA) human chemokine panel 1 kit, measuring MCP-1 (cat no. N05047A-1) and the human YKL-40 kit (cat no. N451MCA-1) was used, following the protocol set by the manufacturer (www.mesoscale.com), all samples assayed in duplicates, the intra-assay coefficient of variation less than 25 %.

3.12.3 TSPO genotyping

All subjects were genotyped for the rs6971 polymorphism in the TSPO gene using a TaqMan based polymerase chain reaction assay (Applied Biosystems® QuantStudio™ 7).

3.13 STATISTICS

All statistical analyses were performed using IBM SPSS Statistics 22 or 23 (Armonk, NY), or R (version 3.2.4 'Very Secure Dishes').

3.13.1 Paper I

In paper I the absolute variability (V) was expressed as the difference in V_T between the first and second PET measurements (PET1 and PET2), relative to the mean of the two values as follows:

$$V = \frac{ABS(V_{T.PET1} - V_{T.PET2})}{\frac{1}{2}(V_{T.PET1} + V_{T.PET2})} * 100 \quad (6)$$

Absolute variability (V) was calculated for each subject separately, and from these separate values of V, the mean absolute variability of the whole sample was calculated.

The intraclass correlation coefficient (ICC) was calculated as follows, from McGraw and Wong:

$$ICC = \frac{MSS_{subj} - MSS_{error}}{MSS_{subj}(k-1)MSS_{error} + \frac{k}{n}(MSS_{meth} - MSS_{error})} \quad (7)$$

Where k and n represent the number of observations and subjects respectively, MSS_{subj}, MSS_{meth} and MSS_{error} denote the mean sum of squares for the subjects, methods, and residual error respectively.

The direction of change between two measurements was assessed using the following equation:

$$D = \frac{(V_{T.PET2} \quad V_{T.PET1})}{V_{T.PET1}} * 100 \quad (8)$$

Effect of gender and genotype on the reproducibility of [^{11}C]PBR28 was assessed using a Mann-Whitney U test. Differences in radiochemical data between subjects were tested using the paired t test. Related samples Wilcoxon signed ranks test was used to test differences in [^{11}C]PBR28 binding in the subgroup performing PET in the morning and afternoon of the same day, as well as group differences in radiochemical data between the two subgroups.

3.13.2 Paper II

Coefficient of variance was calculated as the ratio of the standard deviation to the mean. The change in brain and blood cell binding was estimated according to equation 9.

$$\Delta V_T = \frac{V_T^{PET1} - V_T^{PET2}}{\frac{1}{2}(V_T^{PET1} + V_T^{PET2})} * 100 \quad (9)$$

The change in blood cell parameters, as well as the ratio between V_T in brain and blood cells (normalized V_T) was calculated in a similar fashion. Student's *t*-test was used to calculate differences of mean V_T values between HABs and MABs. Partial correlation was performed to investigate the relationship between V_T values in brain and blood cells, with TSPO genotype as covariate; then separate analyses were performed using Pearson's correlations for each genotype group. Partial correlations were also used to investigate the relationships between V_T values and blood cell counts. On account of the small sample sizes, Spearman's correlation was used to assess the relationship between the changes in V_T values in relation to leukocyte cell counts, in each genotype subgroup. Pearson's correlations were performed for all subjects combined in the main analyses of the relationship between change in V_T between brain and blood cells, as well as a partial correlation, to avoid a confounding effect of genotype. Spearman's correlation was performed for the separate analysis in the groups examined during the same day, or on separate days with short interval, respectively.

3.13.3 Paper III

The difference between patients and controls in [^{11}C]PBR28 binding in GM was examined using a univariate analysis of covariance (ANCOVA). GM V_T derived from 2TCM was used as dependent variable, group (patients versus control subjects) as independent variable and gender and genotype as covariates. Additional univariate ANCOVAs was performed to examine group differences in binding in frontal cortex, temporal cortex, hippocampus and white matter, each having sex and genotype as covariates.

3.13.4 Paper IV

Logistic regression was performed to investigate differences in chemokine levels between patients and controls, with age as covariate, due to the small sample size. An ANCOVA; with gender and genotype as covariates, was used to compare V_T blood cells between the groups. Partial correlation was performed to examine the relationship between TSPO in brain and blood cells, with gender and genotype as covariates. Partial correlations were also performed to investigate the relationship between TSPO in brain and chemokine levels in CSF, and V_T in blood cells and chemokine levels in plasma, respectively, using age, gender and genotype as covariates.

4 RESULTS AND COMMENTS

4.1 PAPER I

[^{11}C]PBR28 is a second generation radioligand for the translocator protein (TSPO). The primary aim of paper I was to investigate the test-retest reproducibility of the ligand, by determining the regional volume of distribution (V_T) in two consecutive PET measurements in healthy subjects. 12 subjects were examined twice, six in the morning and afternoon of the same day, and six in the morning of two different days. V_T in gray matter (GM) estimated using a two-tissue compartmental model (2TCM) was the primary endpoint in the analysis of reproducibility.

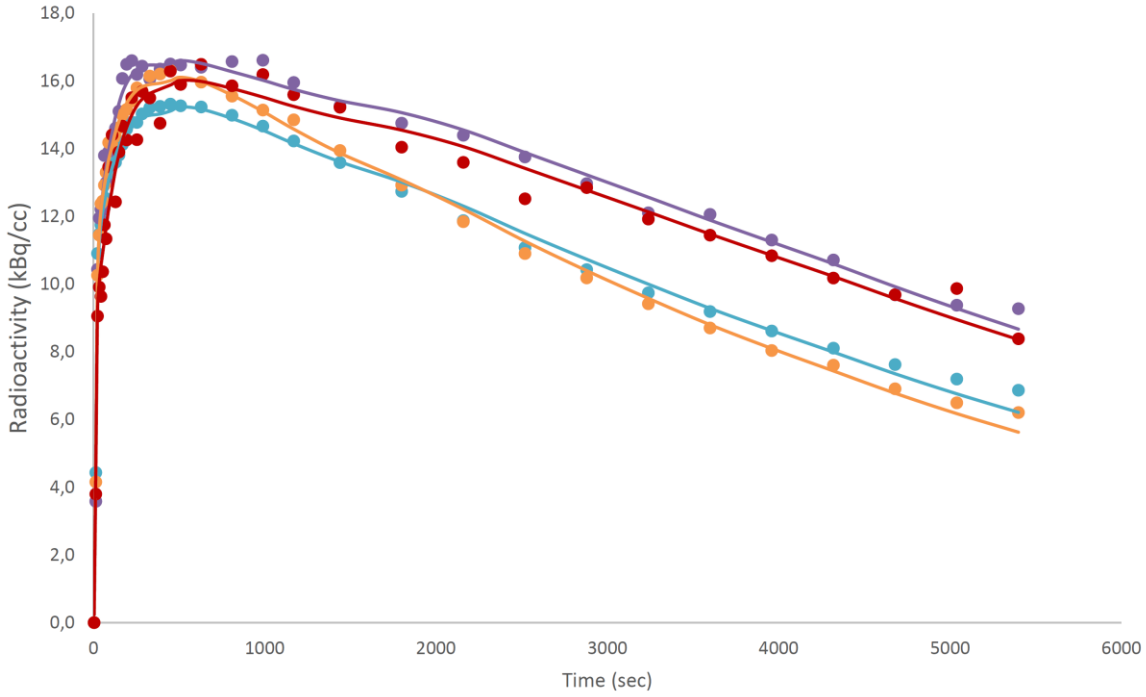


Figure 5. Representative figure with radioactivity data (dots) from subject 2 showing the 2TCM-model fit (line) to the time-activity-curve (TAC) for gray matter (light blue), thalamus (purple), lateral frontal cortex (yellow) and hippocampus (red).

The absolute variability in V_T in the GM was 18.3±12.7 %. Regional ICC values ranged from 0.90 to 0.94, except in the white matter (WM) region (0.32), where there was also a higher inter-individual variability (48.3±39.8 %). Calculations made separately for each genetic group of HABs and MABs, rendered values of V_T ICC in the GM of 0.89 and 0.91, respectively. When reducing the time of analysis to 63 min, similar values for the variability in V_T (16.9± 14.9 %) were obtained.

When analyzing the subjects who performed PET on the same as opposed to separate days, respectively, we found a numerically lower absolute variation in the separate-day group as compared to the same-day group (15.9±12.2 vs. 20.7±20.7), however, not significant

(p=0.59) (figure 6). In the same-day group, the difference in GM V_T between morning and afternoon examinations was significant for the 63-min (p=0.028), but not for the 91-min analysis.

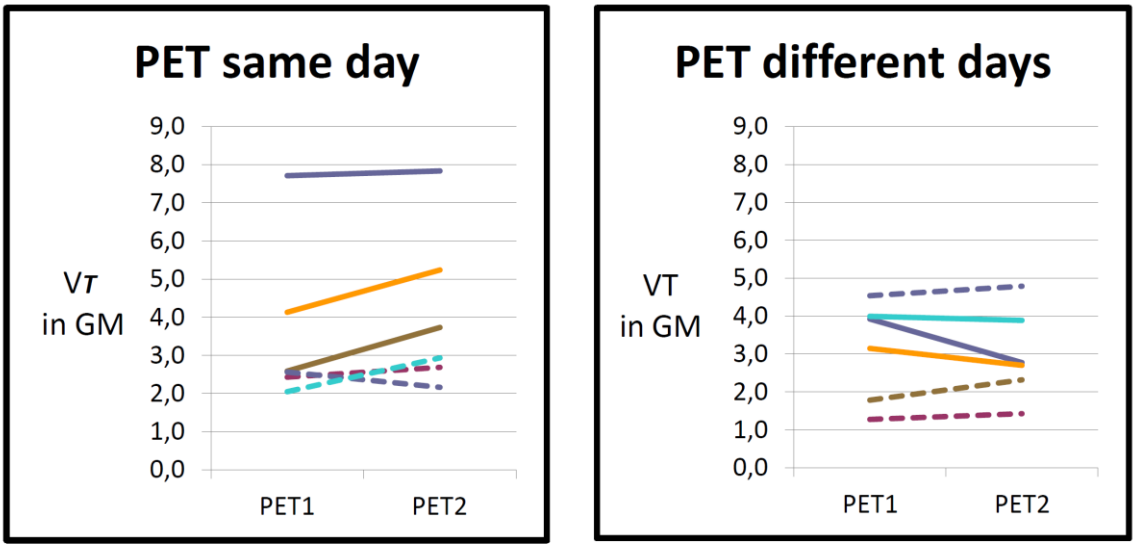


Figure 6 showing V_T values for repeat measurements of the two subgroups performing PET in the morning and afternoon on the same day, and those with PET in the morning of separate days. Straight lines represent HABs, dashed MABs. V_T values in gray matter (GM) estimated using 2TCM.

Average GM V_T for the HAB subjects was 4.3±1.8 for both measurements combined. The corresponding value in MAB subjects was 2.6±1.1. Genotype did not affect absolute variability (17.6 % in MAB subjects vs. 19.1 % in HAB subjects; p=1.00).

Using quantification with WAPI, V_T in the GM of all subjects ranged between 1.3 and 7.7, and correlation with the 2TCM-derived values was high (r=0.989, p<0.001, all GM values combined, n=24). The mean difference in absolute variability between 2TCM and WAPI was 0.6±9.0% (p=0.838). The difference between morning and afternoon examinations using WAPI-generated GM V_T was statistically significant for both 91-min and 63-min analyses (p= 0.028). When using WAPI, the absolute variability in V_T of the GM was 17.8±12.7% (figure 7, page 33).

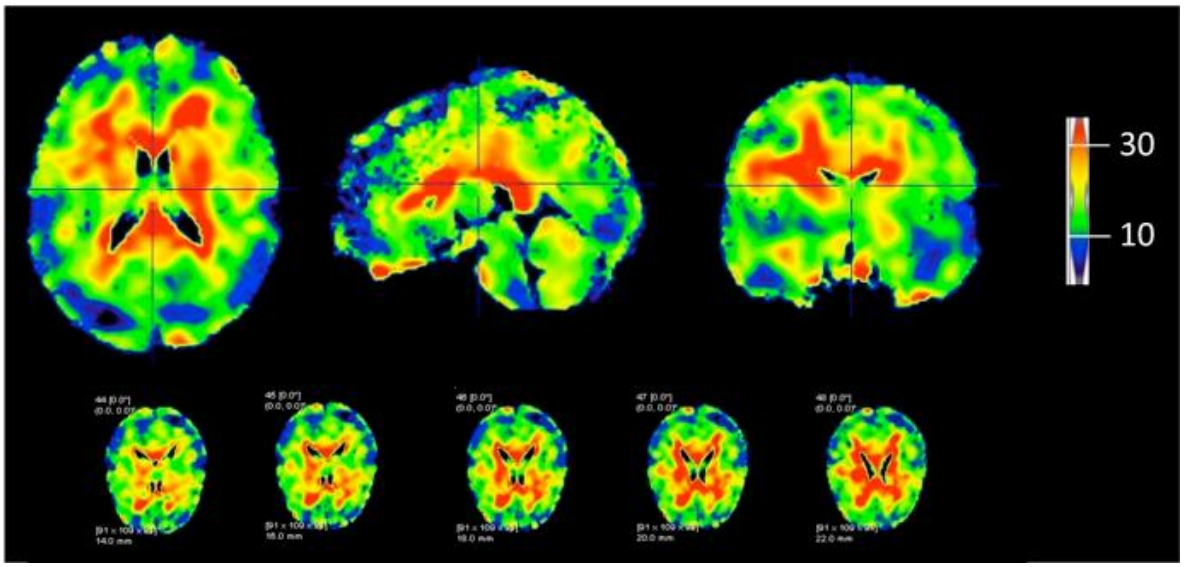


Figure 7.Woxel-wise absolute variability in all subjects, based on wavelet-aided parametric images (WAPI). Scale bar represent percent absolute variability.

In a test-retest reproducibility study of the gold standard TSPO ligand [^{11}C]PK11195 the mean absolute variability was calculated (from reported rate constants) to be 15.9 % for V_T in GM (*125*), thus similar to the findings reported on [^{11}C]PBR28 in paper I. However, ICC values for GM was considerably lower (0.47) for [^{11}C]PK11195, and in specific brain regions, mean difference in binding potential of [^{11}C]PK11195 was much higher than mean regional differences of [^{11}C]PBR28 V_T, indicating that [^{11}C]PBR28 is more reliable for quantification of TSPO levels than [^{11}C]PK11195.

The findings in paper I of high variability combined with high ICC values for [^{11}C]PBR28 can mathematically be explained by higher inter-subject variability. Nevertheless, the variability discovered in paper I is in line with previous findings of variability in studies of [^{11}C]PBR28 (*115*, *117*, *141*). Moreover, the lack of reference region for TSPO ligands means that analysis of blood data is advisable (*142*), a procedure that may introduce other sources of variation, when several steps of analysis are introduced. In addition, [^{11}C]PBR28 metabolize rapidly, thus very small amounts of parent compound remain towards the end of the examination. This means that small analytic errors could affect estimation of V_T at this stage. Thus, alternative methods of quantification without the need for blood data in the analysis, such as image-derived input function (*143*), would be an important goal for future methodological research.

Genotype was not found to affect the absolute variability, thus does not have to be taken into account in clinical within-subject studies.

Since the immune system has been reported to show circadian variation in plasma (*144*, *145*), we also investigated whether there were any diurnal changes in TSPO availability. We could not detect any significant difference in the main outcome measure between morning and

afternoon examinations, although, a trend-level increase in 91 min 2TCM GM V_T-values, and moreover, significant increases in 63 min 2TCM GM V_T and in WAPI-based values were demonstrated. In addition, plasma AUC was significantly decreased in the afternoon PET examination, as compared to the morning. These findings, although in a limited sample, suggest a diurnal component in TSPO availability.

4.2 PAPER II

The primary aim of paper II was to investigate the relationship between central and peripheral TSPO availability, and a secondary aim to attempt to reduce variability in TSPO binding in the brain by accounting for binding in blood cells. Healthy subjects were included (n=32), were a subgroup (n=26) had repeat PET measurements. V_T in GM was estimated using Logan graphical analysis, and TSPO binding in blood cells was calculated from blood and plasma radioactivity and hematocrit (HCT) value, according to equation 3, section 3.9.6.

TSPO in blood cells was lower in MABs than in HABs, at a trend-level significance (p=0.066), and both V_T brain and V_T blood cells of the one LAB individual was <1, corroborating lack of specific binding. A strong positive correlation was seen between V_T brain and V_T blood cells among both HABs and MABs together and in both PET 1 and PET 2 (n=31, r=0.85, p=2.1*10^{-9}).

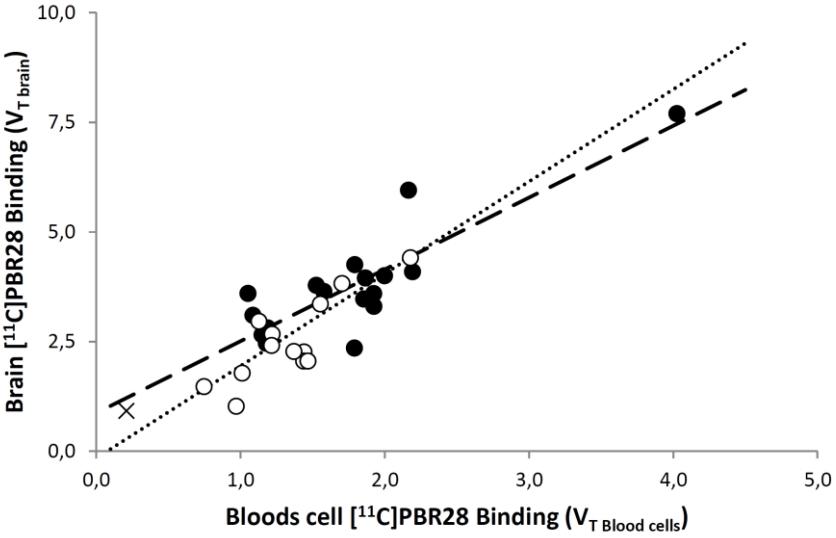


Figure 8. Scatter-plot of values for V_T brain in relation to V_T blood cells. Filled dots representing HABs, hollow MABs, and x the LAB individual. Dashed line representing the regression among HABs, and dotted line among MABs.

Changes in V_T brain and V_T blood cells were also positively correlated (n=25, r=0.60, p=0.002, when LAB individual excluded). The correlation persisted also when dividing subjects according to time elapsed between the PET measurements.

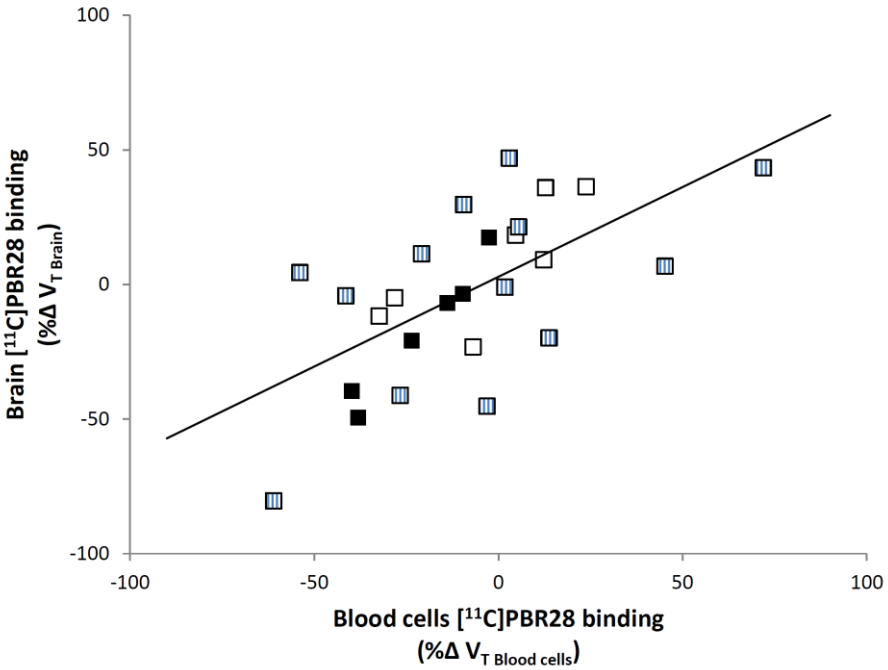


Figure 9. Scatter plot of % change (Δ) in V_T brain in relation to change in V_T blood cells in all individuals with repeat PET measurements. Filled boxes represent PET performed on same day, morning and afternoon, blank boxes PET on separate days with short interval, and blue boxes on separate days, long interval.

Change in leukocyte numbers and change in V_T brain between two successive PET examinations was significantly correlated (n=12, r=0.63, p=0.038), whereas change in leukocyte numbers correlated on a trend-level significance to change in V_T blood cells (r=0.60, p=0.052).

The variability between the two PET measurements for each individual was decreased when V_T brain was normalized to V_T in blood cells (using the ratio of V_T brain/V_T blood cells), as compared to V_T brain alone. Thus, coefficient of variance (COV%) was reduced from 40.4 to 23.4 in HABs and MABs combined, from 34.9 to 22.0 in HABs, and from 37.5 to 22.4 in MABs. So, in a comparison with paper I, for the subgroup of subjects investigated with two PET measurements, short interval (n=12), the absolute variability was decreased from 20.1 ± 14.7% (for V_T brain) to 12.4 ± 8.4% (for the ratio of V_T brain/ V_T blood cells).

The positive correlations between TSPO binding in brain and blood cells, the correlations between changes in V_T brain and blood cells between repeat measurements, as well as the correlations between changes in leukocyte numbers and V_T brain indicate an interaction between central and peripheral immune cells.

The brain was long considered an organ immunologically secluded from the rest of the body. However, an increasing amount of studies demonstrate an interaction between the immune system in the periphery and in the brain (*146*), In addition, TSPO availability in brain has

been shown to increase after peripheral immune activation (*147*, *148*), without disruption of the blood brain barrier (BBB). At the same time, interaction between the immune system of the periphery and the CNS can also come about via direct entry of immune cells or immune markers via the BBB (*149*), and moreover, a lymphatic system of the brain was recently discovered (*107*).

Our findings in paper II of interplay between TSPO availability in brain and peripheral blood cells in healthy individuals point in the same direction as the current literature, and need to be further examined in a clinical setting.

4.3 PAPER III

In paper III we aimed to investigate the TSPO availability among a group of neuroleptic-naïve, first-episode psychotic (FEP) patients, in comparison with healthy control subjects. In patients an assessment of psychotic symptoms was performed using PANSS and level of functioning using CGI. All subjects underwent assessment of cognitive functioning, with the MATRICS battery. Sixteen FEP patients and 16 control subjects were examined with PET using [^{11}C]PBR28. The main outcome measure was [^{11}C]PBR28 V_T in GM.

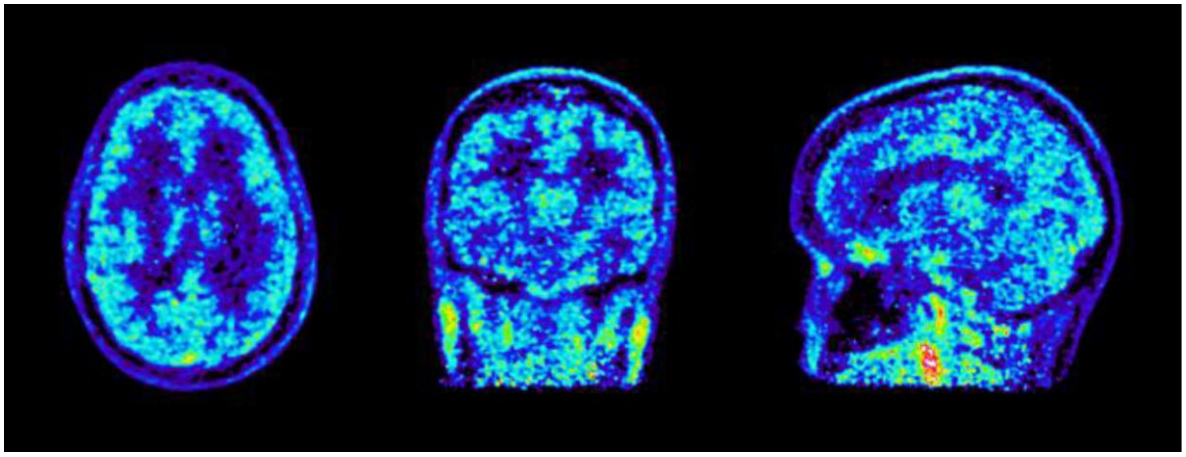


Figure 10. Summation PET image in a first-episode psychosis patient using [^{11}C]PBR28.

We found a significant effect of both TSPO genotype and gender on V_T, thus, in the main statistical analysis of group difference in V_T between patients and controls, used genotype and gender as covariates. A significant decrease in V_T was found between patients and controls in the ANCOVA (F=6.19, df=1.28, p=0.019), more prominent in the subgroup of HAB patients (effect size of 0.38 in HABs, as compared to 0.02 in MABs, partial eta squared) (figure 11, page 37). The finding remained when excluding the patients that were on medication with benzodiazepines at the time of the study (F=6.71, df=1.23, p=0.016). When analyzing different brain regions, a significant decrease in patients' V_T was found in hippocampus, frontal and temporal cortex, but not in white matter (figure 11). In addition to 2TCM, we also performed quantification with 2TCM-1K and DVR. The former rendered a significant decrease in patients (F=6.66, df=1.28, p=0.015), but the latter showed no difference between the groups, either using 2TCM or 2TCM-1K V_T.

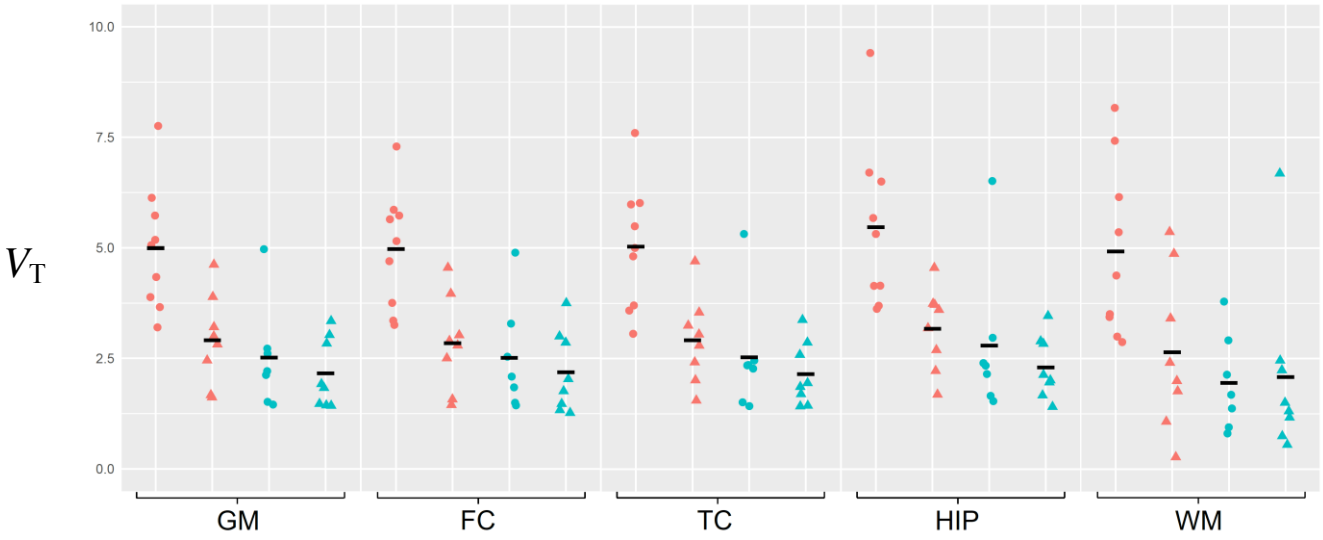


Figure 11. Jitterplot of regional V_T values in FEP patients and controls. Round markers representing controls and triangular markers patients, red markers representing HABs and blue markers MABs. GM=gray matter, FC=frontal cortex, TC=temporal cortex, HIP=hippocampus and WM=white matter.

No correlation was seen between V_T and either of the PANSS-scores, CGI or duration of illness (DOI). The patients performed worse than the controls in all of the tested domains in the MATRICS battery, however, no significant correlation was found between V_T and cognitive measures after Bonferroni-correction.

TSPO availability in schizophrenia has been studied only for the last decade. Initial findings of elevated TSPO binding (*122*, *123*) has in more recent years been succeeded by observations of no difference, or even trend-level decreases in patients (*150*). Our finding is novel in several respects; first, ours is a larger sample than the previous PET studies investigating TSPO in schizophrenia, including the ones examining neuroleptic-naïve psychotic patients (*132*, *133*) (n=6 and n=14 respectively). Second, the duration of illness (DOI) was considerably longer for the neuroleptic-naive patients in both of these studies, with DOI of 2 to 6 years (Holmes et al), or a mean of 33.5 months (Hafizi et al) respectively, as compared to ours, where it was less than eight months. Third, we used a radioligand with improved characteristics, such as increased signal-to-background-noise ratio and higher affinity for TSPO, in comparison with the older ligand [^{11}C]PK11195 (*151*), used by Holmes and colleagues.

We did not find support for the idea that schizophrenia is characterized by activation of microglia. Nevertheless, convincing evidence of an alteration of the immune system as part of the pathophysiology behind the disease have indeed been demonstrated (*75*). So how can we interpret decreased TSPO availability in FEP?

It may be that the immune system fails to adapt to various pathological processes emerging from the psychotic state, and thus may promote the development of the disease. A recent finding of a positive association between schizophrenia and the autoimmune disease Systemic Lupus Erytematosus (SLE) (*152*), as well as previous reports of elevated auto-antibodies in subgroups of patients with psychosis (*153*), points in this direction.

Microglia are highly dynamic cells, continuously surveying the surroundings already in their non-active state. When triggered by disturbances in the CNS homeostasis, they transform into the amoeboid, "active" form, secreting cytokines and chemokines and phagocytosing damaged or dying neurons (*154*). Their activation can be described as a continuum between two functional states of polarization; either the classical, pro-inflammatory M1, or the alternative, anti-inflammatory M2 (*155*). The phenotypes of microglia are regulated by pro- or anti-inflammatory cytokines, transcription factors, acute phase proteins and differences in metabolic states (*156*), determining whether proliferation, phagocytosis, neuromodulation or pruning of superfluous synapses shall be on the agenda (*157*). Altogether, alterations in TSPO may thus reflect differences in microglial activation, and future directions for research include characterization of the various microglia phenotypes in relation to TSPO.

TSPO is not only expressed in microglia, but also in astrocytes and in vascular endothelial cells of the brain, as well as in the periphery (*158*, *159*). Thus, lower levels of TSPO binding may not reflect a dysfunction of microglia, but of other CNS cells. Moreover, in a recent animal study, using an infection-mediated mouse model of neurodevelopmental disruption, with disease-relevant behavioral abnormalities, TSPO expression was found to be reduced not only in microglia, but also in astrocytes and vascular endothelial cells of the CNS (*160*). Interestingly, according to an ischemic stroke animal model activation of microglia seems to be preceding that of astrocytes (*161*).

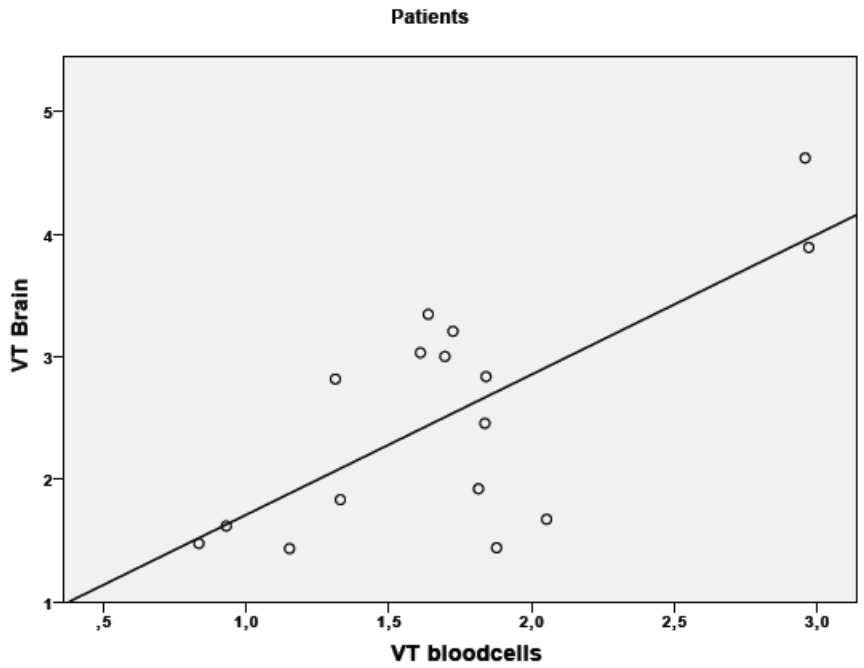
Besides immunological functions, TSPO has been proposed to be involved in the steroidogenesis of the cell, in oxidative processes and in the regulation of programmed cell deaths (*158*), and a recent animal study suggests an even more fundamental role for TSPO in the energy production of the cell (*162*). Thus, alterations in TSPO binding in FEP may reflect other pathophysiological processes of the disease.

4.4 PAPER IV

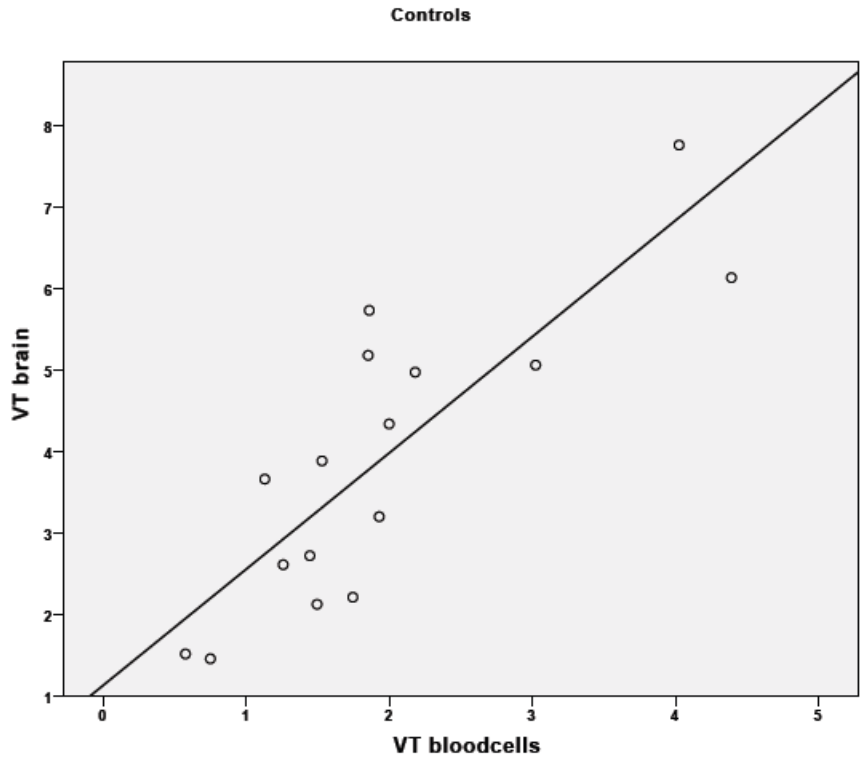
Our aim with paper IV was to investigate the availability of TSPO in peripheral blood cells of FEP and to relate this to TSPO binding in brain. Moreover, we aimed to examine levels of the chemokines YKL-40 and MCP-1 in both plasma and CSF in relation to TSPO.

We estimated TSPO binding in blood in the group of patients and control subjects participating in paper III. Of these, 11 patients and 13 control subjects contributed plasma and CSF samples for analyses of chemokines. TSPO binding in blood cells was calculated from blood and plasma radioactivity and hematocrit (HCT) value, as described previously, and chemokines analyzed using electrochemiluminescence assay.

No difference was seen in V_T blood cells of patients as compared to controls; nevertheless, a significant correlation was detected between V_T brain and V_T blood cells in both patients (r=0.63; p=0.017) and controls (r= 0.68; p=0.007) (figure 12).



A



B

Figure 12. Scatterplots of V_T brain in relation to V_T blood cells. A) Shows the relationship in FEP patients (n=16), where the correlation was significant, controlling for gender and TSPO genotype (r=0.63; p=0.017). B) Shows the corresponding relationship among control subjects (n=16) (r=0.68; p=0.007).

Moreover, the ratio of V_T brain/V_T blood cells showed significant reduction in patients as compared to control subjects (F=16.54, df=1.27, p<0.001) (figure 13).

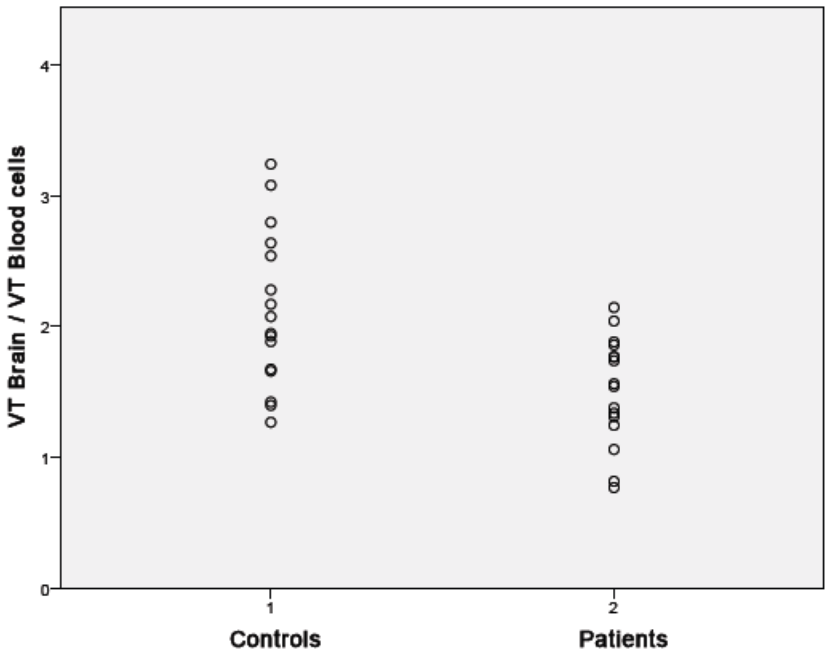


Figure 13. Showing the ratio between V_T brain and V_T blood cells in both patients and control subjects, with a significant difference between the two groups in an ANCOVA (F=16.54; df=1.27; p<0.001), using gender and genotype as covariates.

In addition, plasma YKL-40 levels were significantly increased in patients, and YKL-40-levels in CSF and V_T brain correlated significantly and positively among patients (r=0.78; p=0.021) (figure 14A), whereas in controls these variables were negatively correlated (r=-0.88; p=0.009) (figure 14B, page 41).

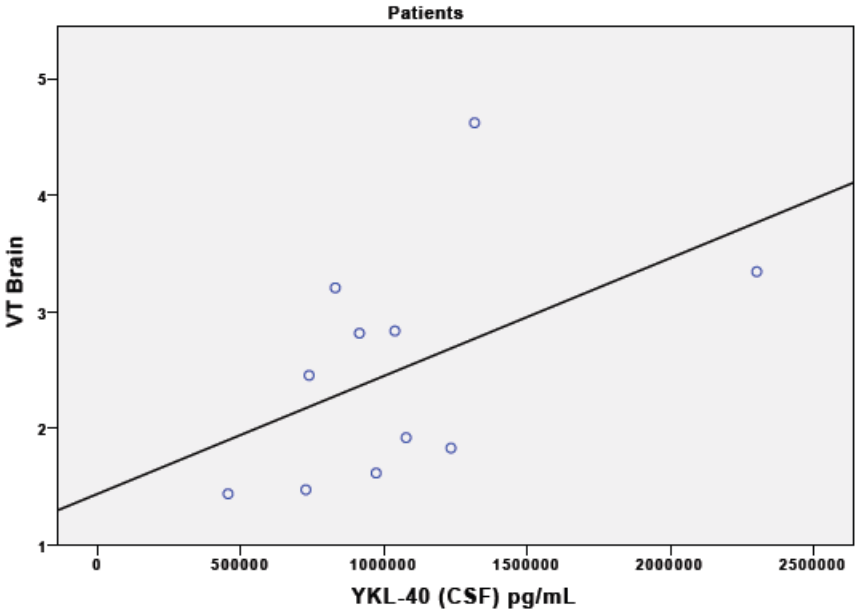


Figure 14 A

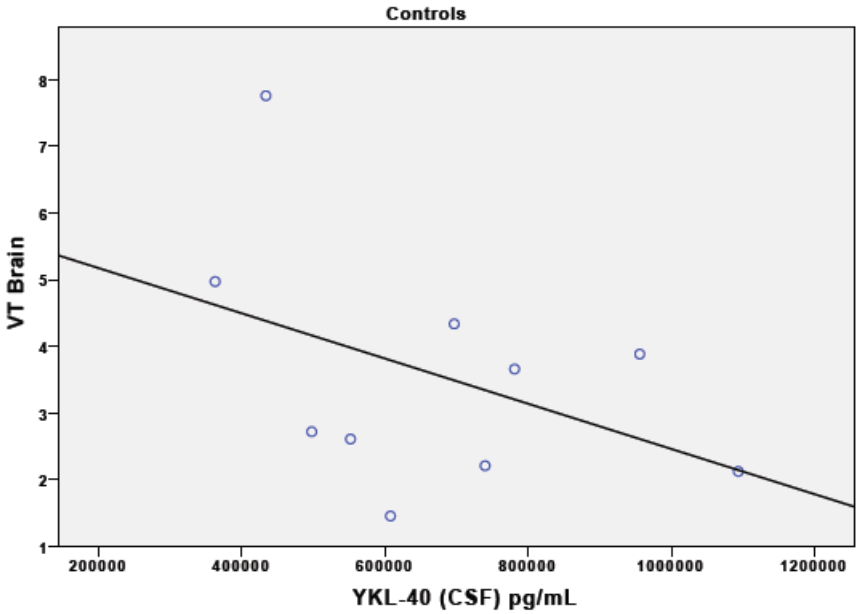


Figure 14 B

Figure 14. Scatterplot of the relationship between V_T brain and the levels of YKL-40 in CSF, with A) (previous page) showing this relationship in patients, with a significant positive correlation when controlling for age, gender and genotype (r=0.78; p=0.021), and B) in control subjects, where the corresponding correlation was negative (r=0.88; p=0.009).

The observation of a positive correlation between V_T in brain and blood cells supports maintenance of a peripheral-to-central immune function in FEP patients, even in the absence of a significant alteration in TSPO binding in blood cells. Nevertheless, the elevated chemokine levels in plasma suggest a pro-inflammatory state in early psychosis.

Chemokines have been but sparsely investigated in schizophrenia (*87*), however, an increasing amount of studies point to their relevance for the disease (*163*, *164*). YKL-40 is a chemokine secreted by both microglia and astrocytes of the CNS, and is thought of as a biological marker for non-infectious inflammatory diseases, such as Alzheimers', Parkinsons' and other neurodegenerative diseases (*165*). It has also been genetically linked to schizophrenia (*89–91*). Here we present unique data of an association between the chemokine YKL-40 in CSF and TSPO binding in brain, in different directions among patients and controls respectively, raising further questions about the role of YKL-40 in schizophrenia. One explanation to this pattern of disparate correlations is that the relationship between TSPO up-regulation in brain and elevations in central YKL-40 expression is put out of action in early schizophrenia.

5 SUMMARY OF FINDINGS

5.1 TEST-RETEST VARIABILITY OF [^{11}C]PBR28

Mean absolute variability in [^{11}C]PBR28 binding ranged from 13.8 % to 25.9 %, depending on the method of quantification used and region examined, and whether or not diurnal changes were controlled. Furthermore, ICC values for V_T in all GM regions ranged from 0.90-0.94, indicating a high reliability. A voxel-based quantification approach rendered similar reproducibility, and reducing the time to 63 min did not affect variability.

5.2 RELATIONSHIP BETWEEN TSPO BINDING IN BRAIN AND BLOOD CELLS

TSPO availability in brain correlated strongly and positively to binding in blood cells, at both baseline and when analyzing change between two PET examinations. There was also a significant correlation between change in leukocyte numbers and change in TSPO binding in brain, and moreover, a trend- level correlation to change in TSPO binding in blood cells. The inter-individual variability in brain V_T was reduced when controlling for V_T in blood cells.

5.3 ASSESSMENT OF TSPO BINDING IN NEUROLEPTIC-NAÏVE FEP PATIENTS

We demonstrated a significant reduction in [^{11}C]PBR28 binding in patients compared to healthy control subjects in GM as well as in secondary regions of interest. There was no correlation between GM V_T and clinical or cognitive measures, after correction for multiple comparisons.

5.4 COMPARISON OF CENTRAL AND PERIPHERAL IMMUNE CELL ACTIVITY IN NEUROLEPTIC-NAÏVE FEP PATIENTS

There was no difference in TSPO availability in blood cells between patients and controls, however, a significant positive correlation was found between TSPO in brain and blood cells, of similar magnitude in both groups. The ratio between V_T in brain and V_T in blood cells was significantly lower in patients than in controls. We found no correlation between TSPO availability in blood cells and plasma levels of chemokines. Nevertheless, a positive correlation between CSF levels of YKL-40 and TSPO in brain was found in patients, while this correlation was negative in controls.

6 FINAL REMARKS AND FUTURE PERSPECTIVES

Treating first-episode psychotic patients comprises several obstacles. The suffering individual is most often a youth on the threshold of adulthood, surrounded by a family in crisis. The available treatment options are insufficient and often leave a lot to be desired. Thus, finding paths for novel medical strategies is vital and urgent for treatment development in schizophrenia.

We started this project with the hypothesis that TSPO availability in early schizophrenia would be enhanced, due to microglia activation. However, in our clinical study in FEP patients we obtained evidence for a decrease in TSPO binding in brain. This finding has an essential impact on the field of TSPO PET in schizophrenia, since it rules out the effect of neuroleptics on TSPO availability. Furthermore, it raises further questions as to in which cell types of the CNS the decreased binding takes place, and if in microglia, which phenotype(s) it is that expresses lowered levels of TSPO. Is it mainly the neuroprotective capacity of microglia that is affected? One is also tempted to follow up patients included at onset of psychosis, to investigate whether TSPO availability differs later on, as the disease progresses. A further research direction would be to investigate individuals with respect to TSPO at an earlier stage, in order to determine the onset of the disturbance in receptor availability.

In our final paper we investigated peripheral and central levels of chemokines in the same set of patients as in paper III, and found elevations of plasma levels of YKL-40. This chemokine has previously been found associated to disease progression in several inflammatory and neurodegenerative diseases; however not fully investigated in schizophrenia. Hence, this finding is of importance, even though the sample size is small. Moreover, we found associations between YKL-40 levels in CSF and TSPO availability in brain, which further supports the notion of a disturbance in immune regulation in early psychosis. This preliminary study needs to be supplemented with a larger sample of CSF and plasma, in relation to TSPO. In addition, to enable differentiation between microglia and astrocytes, markers for the latter should be included.

In the past, attempts at treating schizophrenia with add-on anti-inflammatory medication have been made, with some effect on psychotic symptoms. It is not impossible that this may prove to be a feasible route in the future. However, before establishing antiphlogistics as clinical practice in schizophrenia, a more thorough knowledge is necessary of the role of CNS immune cells, within the different phases of the disease. In the quest for a more cohesive picture, imaging the TSPO receptor can be helpful in establishing protein expression. Furthermore, concomitant evaluation of the different cellular sources of expression is necessary, in order to determine the significance of the outcome.

7 ACKNOWLEDGEMENTS

It is a truth universally acknowledged, that a single PhD student in possession of a PET project, must be in want of benefactors! My deepest thanks go to:

All the wonderful patients and control subjects that participated! Without you the studies could not have been made

My main supervisor, associate professor *Simon Cervenka*, my guide through this PhD-project, for always being positive, helpful and supportive! For all the work you have put in my project during these years and for always being available for questions and discussions. I could not have wished for a better supervisor!

Co-supervisor professor *Lars Farde*, our mentor in the PET community, for invaluable scientific input throughout both KaSP and PET-studies

Co-supervisor associate professor *Lena Flyckt*, for recruiting me to research in the first place, for initiating KaSP and for your generous support all along

Co-supervisor Dr *Anton Forsberg*, for excellent hands-on support with Kaleidagraph and Pmod in the beginning, as well as helpful assistance throughout

Director of the PET-group professor *Christer Halldin* for superb research conditions!

Our excellent research nurses of the PET group *Nina Knave*, *Karin Olsson* and *Opokua Britton Cavaco* for taking such good care of the study subjects and for making work in the PET lab efficient and fun!

Our excellent research nurses of KaSP, former and present; *Minna Juntura*, *Henrik Gregermark*, *Martin Szabo*, *Joakim Eckerström* and *Marie Adolfsson* for invaluable help with inclusion and organization

Helena Fatouros-Bergman for outstanding work with cognitive evaluations and for great support and helpfulness in KaSP

Naoki Kanegawa for excellent research ideas!

Pontus Plavén-Sigray for being such a wiz on statistics and generally a really great guy ;-)

Funda Orhan for skillful analyses at FYFA and for being calm and supportive in hectic times!

Ryosuke Arakawa for invaluable work with blood binding analyses

Shahin Ahinehband for genotyping all subjects

Igor Yakushev for great work with WAPI

Andrea Varrone for excellent scientific input and generous support!

Martin Schain for invaluable math-support and great singing!

Per Stenkrona for your never-ending willingness to assist in practical matters

Granville Matheson for generous support and for your lovely voice!

Sophie Erhardt, *Göran Engberg* and *Lilly Schwieler* at FYFA, for excellent scientific input to both KaSP and manuscripts

Fredrik Piehl for invaluable scientific input to KaSP, as well as immense clinical skills

Katarina Varnäs for scientific input, *Zsolt Cselenyi* for creating the pipeline, *Johan Lundberg* for practical assistance, *Akihiro Takano* for scientific input, and *Jacqueline Borg* for scientific input and supportive talks

All the chemists of the PET-group for excellent work in the lab, with a special thanks to those who were specifically involved; *Zhisheng Jia*, *Obaidur Rahman* and *Mahabuba Jahan* for radioligand synthesis, *Nahid Amini*, *Ryuji Nakao* and *Stefan Martinsson* for metabolite analyses, *Sangram Nag* and *Arsalan Amir* for overall support and *Carsten Steiger* for QC. Thank you all!

Göran Rosenqvist for taking such good care of the equipment down in the PET-lab and for general helpfulness, *Urban Hansson* for excellent computer support, and *Julio Gabriel* and *Ann Axelsson*, former and present PET-technicians, for invaluable help during examinations

Karin Zahir for indispensable administration of the PET-group!

My employer *Prima psykiatri*, who granted me funds for the greater part of my research

Marianne Wennmalm for excellent mentorship

Pauliina Victorsson for being my comrade-in-arms as a PhD-student, in both KaSP and the PET-group, for being my clinical colleague and also very dear friend!

Magdalena Nord for great friendship and support!

Patrik Fazio for great support whilst sharing the struggle towards the end of our respective PhD-projects, *Patrik Mattson* for interesting psychiatric discussions, and room-mate *Mikael Tiger* for great discussions on both research, clinical and family matters

Members of the PET-group: *Jenny Häggkvist*, *Max Andersson*, *Emma Veldman*, *Ämma Tangen*, *Jonas Svensson*, *Miklòs Toth*, *Lenke Tari* and *Marie Svedberg* for great lunch-time company!

All my wonderful co-workers at Lotsa for taking excellent care of patients during my absence, and for creating a great work environment; *Inger Brown*, *Susanne Buchmayer*, *Gunnel Eriksson*, *Maria Larsen*, *Georg Dester*, *Alexandra Åkerström*, *Regina Barra-Somai* and *Anneli Enegren*. Manager of the psychosis section of Prima *Helena Kyrö* for making it

possible to combine research and clinical work and for helpful support. *Emelie Johansson* for help including patients and for the lovely picture!

David Aler for excellent proof reading and support!

All my friends for making PhD-life endurable: Members of the Lidingö *Knoll och Tott* mafia for lovely dinners and afterworks. Dear friends and colleagues *Sigrun Reibring*, *Caroline Ek*, *Louise Eggimann*, *Jessica Welin-Berger* and *Janet Post*. Thank you all for great friendship and lovely times!

My parents *Ann* and *Hans* and brother *Olle* for life-long love and support!

Finally, my wonderful family; my bonus sons *Erik* and *Johan* and my darling daughters *Emma*, *Karolina* and *Sofia* for filling my life with happiness and meaning!

Sven, my love, for endless support and encouragement, without you I am lost

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