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Master thesis in Medicine

Mikael Redsäter

Henrik Zetterberg
Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy, University of Gothenburg, Gothenburg and Mölndal, Sweden

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Blood-brain barrier dysfunction in patients with bipolar disorder

Mikael Redsäter[1], Ulf Andreasson[1], Erik Pålsson[1], Kaj Blennow[1], Mikael Landén[1,2,3], Carl-Johan Ekman[2], Henrik Zetterberg [1,4],

[1] Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy, University of Gothenburg, Gothenburg and Mölndal, Sweden.
[2] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden.
[3] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[4] UCL Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

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Abstract
Background: Bipolar disorder (BPD) is a common psychiatric disorder, characterized by recurrent episodes of mania/hypomania and depression. Similar to a majority of other psychiatric disorders, complete aetiology and/or pathogenesis are unknown in BPD. Modern research has proposed an association between an impaired blood-cerebrospinal fluid (CSF) or blood-brain barrier and subgroups of patients suffering from different psychiatric conditions, such as schizophrenia, depression and other affective disorders. The purpose of this study was to evaluate whether dysfunction of the blood-brain barrier is over represented in a cohort of bipolar patients, compared to an age- and gender-matched healthy control group, i.e. quantify and further validate past research findings and aim the study against a specific disorder where documentation is limited.

Methods: Subjects included 134 BPD patients and 86 healthy controls. Serum and CSF were collected from all subjects and analyzed for albumin concentration by immunonephelometry, where after CSF/serum albumin ratio was determined by dividing CSF with serum albumin concentration. CSF/serum albumin ratio was then compared between patients and controls using Mann-Whitney U-test.

Results: CSF/serum albumin ratio was significantly elevated in BPD patients as compared to controls, suggesting impairment of the blood-brain barrier in BPD. When excluding 35 patients and controls, on magnetic resonance imaging (MRI) of the brain displaying signs of somatic or neurological diseases known to impinge the blood-brain barrier, the significant difference in CSF/serum albumin ratio between BPD patients and controls remained. Moreover, after stratifying patients according to type of BPD, the group consisting of BPD I patients significantly excelled.

Conclusions: The findings statistically establish that a subgroup of individuals with BPD has elevated CSF/serum albumin ratio, indicating that an impaired blood-brain barrier may play a role in or reflect the disease process in BPD, although the complete mechanisms causing the impairment are still unknown and further studies are required.

Keywords

Bipolar disorder, cerebrospinal fluid, blood-brain barrier

1. Introduction

1.1. The past

For more than 100 years mankind has tried to find the aetiology and/or pathogenesis of psychiatric disorders as bipolar disease (BPD), depression and schizophrenia. Already in the late-nineteenth century several scientists, such as Emil Kraepelin, believed that some kind of bacteria was involved in the aetiology [1]. One of the grounds behind this theory was that many of the patients suffering from microbial diseases developed psychiatric disorders. Accordingly, the eager to find an explanation to why some people develop psychiatric disorders has driven scientists and researchers for a long time, during which several infectious agents have been suggested as possible underlying factors; Treponema pallidum, Borrelia burgdorferi, herpes simplex viruses, Epstein-Barr virus, cytomegalo virus, influenza, measles, rubella, mumps, polio, etc. [2].

The interest in finding the aetiology for psychiatric disorders has varied during the last 100 years. For example, the research in this field, in the western world, became less intense when Sigmund Freud presented his theories with psychological explanations to psychiatric illness in the 1930´s. However, the theories that imply that infections can cause, or at least somehow are linked to, psychiatric illness are still wide spread among neurological researchers.

1.2. The present

Modern research indicates that, even before birth and during childhood, infectious agents can increase the risk of psychotic syndromes and schizophrenia later in life [3-9]. Studies during the last decade postulate that the inflammation process itself may be involved in the pathomechanisms linked to e.g. depression. Increased CRP (C-reactive protein, an acute-phase protein that rises in response to inflammation) levels in blood from depressed patients, and after a depressive state, have been observed, which supports an involvement of

inflammation in the disease [10-14]. In 2001, Zorrilla et al. published a comprehensive meta-analysis, consisting of more than 180 studies of patients with depressive disorder, which shows that several interesting immunological changes related to the inflammation process can be observed in patients with depression [15]. Findings from different recent studies also propose a correlation between inflammatory reactions and other psychiatric disorders, both schizophrenia and affective ditto [16-21].

1.3. The blood-brain barrier

In parallel to the interesting research on psychiatric illnesses and their relationships to the immune system, the question rises whether the inflammation in the central nervous system (CNS) originates from within the CNS or if it is somehow transferred through e.g. an impaired blood-brain barrier. If the latter assumption is correct, some individuals may be predisposed to psychiatric disorders if their blood-brain barriers, for some reason(s), are dysfunctional or more vulnerable. As there are a number of established causes of dysfunction in the blood-brain barrier, such as; hypoxia, head trauma, hypertension and tumours [22], it opens for a variety of possible aetiological factors for psychiatric disorders. Also different kinds of dementia illnesses have been reported to depend, to some extent, on a damaged barrier [23-25]. The concept that an impaired blood-brain barrier may precipitate development of psychiatric disorders also creates a possible interpretation of the previously mentioned findings [3-9], which suggest a correlation between prenatal/childhood infections and psychiatric illness later in life:

An infection early in life may create a damage or vulnerability in the blood-brain barrier, which later in life can be an aetiological factor for psychiatric disorders.

In analogy with the blood-brain barrier impairment theory, studies have shown a significant association between an impaired blood-brain barrier and patients with both paranoid

psychosis [26] and schizophrenia [27, 28]. Within these three rather small studies, with a total of 64 patients included, it was found that as many as one third of the affected patients had an impaired blood-brain barrier.

Current research shows that schizophrenia shares phenotypic traits with BPD, including for example psychotic symptoms [29, 30]. Also epidemiological [31] and genetic studies [32, 33] suggest that these syndromes partially share genetic vulnerability traits, which caused us to hypothesize that bipolar patients may have similar defects in the blood-brain barrier.

Moreover, a study from 1995 with 90 suicide attempters, whereof 44 were diagnosed with either depression or dysthymia, revealed that 18% displayed signs of an impaired blood-brain barrier, which significantly separated them from the controls [34]. Also, consistent with the blood-brain barrier dysfunction theory, a recent (2009) study in Germany on 63 treatment-resistant affective and schizophrenic spectrum disorder patients revealed that 29% had blood-brain barrier dysfunctions. This study even showed that as many as 38% (9/24) of the affective spectrum disorder (including bipolar disorder) patients exhibited blood-brain barrier dysfunction [35].

1.4 Means to measure the blood-brain barrier dysfunction

The best-established biomarker for the integrity of the blood-brain barrier is the ratio of the albumin concentration in CSF to serum or plasma (CSF/serum albumin ratio) [36]. Albumin is synthesized in the liver and released to plasma, where it serves as a carrier protein and colloid oncotic pressure regulator. It enters the CSF across the blood-CSF barrier in plexus choroideus and the blood-brain interstitial fluid barrier along the capillaries. Comparing albumin levels in lumbar CSF and serum gives an estimate of the integrity of the blood-brain barrier as a whole, at least for this particular type of molecule. Around 150 times lower

concentration of albumin is expected in the CSF compared with serum, providing the blood-brain barrier function is intact [36].

1.5. Bipolar disorder

Bipolar disorder (BPD) is a common psychiatric disorder, affecting about 1-3 % of the population [37]. It is characterized by recurrent episodes of mania (or hypomania) and depression, interspaced with periods of euthymic mood [38]. Cognitive dysfunction is frequent, also in euthymic bipolar patients, including impairment in executive function and verbal memory [39]. The disease often, indirectly, affects families, friends and employers, and can be the actual background to homelessness, financial troubles, broken relationships, drug abuse, suicide and other tragic life stories. According to DSM IV, BPD is classified in three different subtypes; BPD I (presuming at least one episode of mania), BPD II (recurrent depressions and circles of hypomania) and BPD NOS ([not otherwise specified] patients with bipolar affective disease not fulfilling criteria for I or II). In this study we have further divided patients into BPD III (merely mania/hypomania induced by antidepressives) and BPD V (unipolar depressions and heredity for BPD), according to e.g. Akiskal et al. [40]. In addition, we have included three patients diagnosed, according to DSM-IV, with cyclothymia (circles of hypomania during two years and not fulfilling criteria for actual depression) or SDM ([schizoaffective disorder manic type] either depressive or manic episode, concurrent with schizophrenia).

1.6. Aim

The purpose of this study was to evaluate whether dysfunction of the blood-brain barrier is over represented in a cohort of bipolar patients, compared to a healthy control group, which thereby quantify and further validate past research findings that proposes a correlation

between a damaged blood-brain barrier and psychiatric illness. Additionally, subjects in this study consist of a diagnostically heterogeneous large patient group, which is not well documented with regards to blood-brain barrier function in previous studies. Also, the study aimed to control if a potential difference in blood-brain barrier function, between patients and controls, is still of significance when the subjects are followed up and further investigated to find supplementary medical conditions, or other confounding factors, which may be the actual cause of the blood-brain barrier dysfunction.

Subjects and methods

2.1. Patients

Patients were recruited from the St. Göran bipolar project, enrolling patients from the bipolar unit at the Northern Stockholm Psychiatric Clinic, Stockholm, Sweden. The BPD group consisted of 134 patients and their respective diagnoses where distributed as follows: Bipolar disorder type I; 64 patients (41 women, 23 men), Bipolar disorder type II; 53 patients (32 women, 21 men), bipolar disorder type III; 7 patients (2 women, 5 men), bipolar disorder type V; 1 patient (man), bipolar disorder NOS; 6 patients (3 women, 3 men), cyclothymia; 1 patient (woman), schizoaffective disorder manic type; 2 patients (1 woman, 1 man). Supplementary demographics and clinical characteristics of the study population are presented in table 1.

The key clinical assessment instrument used for diagnostics was the Affective Disorder Evaluation (ADE), developed for the NIMH-sponsored Systematic treatment Enhancement Program of Bipolar Disorder (STEP-BD) [41]. The ADE interviews were conducted by board-certified psychiatrists working at the tertiary care bipolar out-patient unit or by residents in psychiatry completing their psychiatric training at this unit [42]. Furthermore, instruments utilized to exclude not suitable patients with other psychiatric diagnoses, alcohol or drug

abuse were three psychiatric tools; Mini International Neuropsychiatry Interview (M.I.N.I) [43], the Alcohol Use Disorders Identification Test (AUDIT) [44] and the Drug Use Disorders Identification Test (DUDIT) [45], whereof the two latter are self-report questionnaires. Other exclusion criteria were inability to complete the standard clinical assessment or incapability of providing informed consent. The patient data was presented at a diagnostic case conference, where the final diagnostic decision was made by a consensus panel of experienced board-certified psychiatrists specialized in bipolar disorder. The general criteria for inclusion were patients at the age of minimum 18 years, who met the DSM-IV criteria for any bipolar disorder, i.e., type I, II, NOS (not otherwise specified), cyclothymia, or schizoaffective syndrome manic type.

Information was collected about number of depressive, manic, and mixed episodes, as well as age at onset, history of psychosis and current medications. The lifetime severity of bipolar disorder was rated using the Clinical Global Impression (CGI) rating scales. This 7-point scale reflects the clinician's rate of the severity: 1=normal or not at all ill, 2=borderline mentally ill, 3=mildly ill, 4=moderately ill, 5=markedly ill, 6=severely ill and 7=extremely ill. For ethical reasons, patients continued to take their prescribed medications at the time of CSF sampling. More detailed information about the work-up and diagnostic assessments have been described previously [42].

2.2. Controls

The subjects collected to get age- and sex-matched healthy, population-based, controls consisted of 86 individuals picked in a random selection, by Statistics Sweden (SCB), and contacted by mail. Persons interested of participating in the study contacted the study team, which conducted a preliminary telephone screening to exclude severe mental health and neurological issues as well as substance abuse. Eligible individuals were scheduled for a one-

day comprehensive assessment. Of the controls that received the invitation mail, 14% contacted the research team. This is on par with other studies of similar nature according to SCB. Of those controls that volunteered, 75 were excluded at the telephone interview mainly due to drug use (N=16), changed their mind (N=14), somatic ill-health (N=12), metal objects in body excluding MRI (N=10), heredity in first degree relative of bipolar disorder or schizophrenia (N=9), ongoing mental health diagnoses (N=6), pregnancy (N=5), and moved out of area (N=2). One subject had no documented reason for exclusion. Furthermore, one subject failed to show up for the assessments.

Control subjects underwent a psychiatric interview by experienced clinicians using MINI [43], which included the affective disorders section, and the same investigations the patients have undertaken, including self-rating scales, somatic tests, blood tests and lumbar puncture. Because the assessments of controls might reveal pathology, case conferences were held between examining clinicians, primary investigator and study coordinator in order to decide whether or not including such individuals in the study. It was thus decided to allow past minor depressive disorders, isolated episodes of panic disorder, eating disorders or obsessive compulsive disorder which remitted spontaneously or with short term psychotherapy.

Substance abuse was screened for at telephone interview by the nurse, in the psychiatric interview, by AUDIT [44] and DUDIT [45] as well as by determining serum carbohydrate-deficient transferrin (CDT). Over consumption of alcohol as revealed by CDT or responses in self-tests indicating large consumption (> 8 standard drinks per time more than 2 times per week, and/or amnesia and/or loss of control more than once per month) resulted in exclusion of these subjects from the study.

Other exclusion criteria were neurological conditions other than mild migraines, untreated endocrinological disorders, pregnancy, dementia, recurrent depressive disorder, suspected

severe personality disorders (based on interview and SCID-II personality assessment), a family history of schizophrenia or bipolar disorder in first-degree relatives.

2.3. Ethics

The study was approved by the Regional Ethics Committee in Stockholm and conducted in accordance with the latest Helsinki Protocol. All enrolled patients and controls consented orally and in writing to participate in the study after the nature of the procedure had been fully explained.

2.4. MR-imaging

During the study 114 of 134 patients and 85 of 86 controls underwent magnetic resonance imaging (MRI) of the brain. MRI scans were acquired at the MR Research Centre, Karolinska University Hospital, Stockholm. Sagittal and coronal T1 weighted, axial and coronal T2 weighted, and axial fluid attenuation inversion recovery (FLAIR) T2 weighted scans were acquired using a 1.5T scanner (General Electric Signa Excite) and subsequently examined for clinically significant anatomical abnormalities by a senior radiologist.

2.5. CSF sampling

CSF sampling (lumbar puncture) was performed when the participants were in a stable euthymic mood. Subjects fasted overnight before the CSF collection, which occurred under standard conditions at 9.00 a.m. The spinal needle was inserted into the L3/L4 or L4/L5 interspace, withdrawing a total volume of 12 ml of the CSF (gently inverted to avoid gradient effects) which were divided into 1.0-1.6 ml aliquots and subsequently stored at -80˚C pending analysis. Identical procedures were performed for both patients and controls.

2.6. Analyses of the blood-brain barrier function

To detect impairment of the blood-CSF barrier function in the subjects, the ratio between the albumin concentration in the cerebrospinal fluid (mg/L) and that in the serum (g/L) were determined. An increased CSF/serum ratio was interpreted as an impairment of the blood-brain barrier [46-49]. Albumin levels were measured in clinical routine by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). Intra- and inter-assay coefficients of variation were below 10% for all analyses.

All biochemical analyses and measurements were performed at the Clinical Neurochemistry Laboratory in Mölndal, Sweden, by experienced and board-certified laboratory technicians, blind with respect to clinical information, using methods accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC).

2.7. Statistics

The statistics software packages Prism 5 (Graph Pad, La Jolla, USA) and PASW Statistics 18 (IBM, New York, USA) were used for the statistical analysis. As the quantitative data were not normally distributed, non-parametric methods were used. When comparing data between two or more groups, we used the Mann-Whitney U-test, or the Kruskal-Wallis test with Dunn's post hoc test, respectively. For dichotomous variables Fisher's exact test was utilized and correlation analysis was performed using Spearman's rho coefficient. All p-values lower than 0.05 were regarded as significant.

3. Results

3.1. Demographics

We compared data from 134 CSF and serum samples collected from patients suffering from BPD with data from 86 healthy, age- and sex-matched individuals (Table 1). As expected, the patient group did not differ from the control group in respect to sex (p=0.58) or age (p=0.53). There was a significant difference in CSF/serum albumin ratio between men and women in the patient group (p<0.0001, Figure 1a) and we could, also in the control group, see a difference between men and women, although not significant (p=0.12, Figure 1b). A significant correlation between CSF/serum albumin ratio and age was notable in both patient and control groups (r_s= 0.31, p=0.0003, and r_s=0.33, p=0.0022, respectively, Figure 2a and 2b).

3.2. Patient-controls comparisons

CSF/serum albumin ratio was significantly elevated in BPD group as compared to control group (p=0.0093, Figure 3a). When stratifying the patient group according to BPD subtype (I, II and NOS, NOS including type III/V/NOS/Cycl/SDM) patients with increased CSF/serum albumin ratio were found in all diagnostic subgroups, although most patients with elevated albumin ratio were found in the BPD I subgroup (p=0.046, Figure 4a). From the scatter plots it was evident that a limited number of BPD patients had clearly elevated albumin ratios, and that these patients contributed the most to the significant difference in albumin ratio on a group level.

When excluding a total of 35 patients and controls displaying abnormal MRI findings (as Wilson´s disease, multiple sclerosis and meningioma) the p-value slightly changed, although a statistically significant difference between cases and controls remained (p=0.020, Figure 3b). Again, after excluding patients and controls with positive MRI findings, stratifying patients

according to BPD subtype (I, II and NOS), the statistical differences remained fairly intact and the subgroup of BPD I patients still excelled (p=0.062, Figure 4b).

CSF/serum albumin ratio did not correlate with any of the following parameters; experienced psychosis or not, CGI, or number of manic or depressive episodes (data not shown), although correlation were found with both age at onset and duration of illness (r_s= 0.16, p=0.059, and r_s= 0.20, p= 0.024, respectively, Figure 5a and b). There were no significant differences in CSF/serum albumin ratio between patients receiving no treatment, standard lithium treatment, antiepileptic drugs, or antidepressives (data not shown). However, there was a strongly significant difference between CSF/serum albumin ratio and treatment with antipsychotic drugs (p=0.0003, Figure 6).

In addition, we also compared the CSF/serum albumin ratio from both patient and control groups to the age-adjusted reference limits normally used in our laboratory (Table 2), noting that 20 (15%) of the BPD patients had albumin ratios above reference limits as well as 7 (8%) of the controls. When comparing the patients to controls, with respect to elevated CSF/serum albumin ratio according to mentioned reference values, no statistical significance was obvious (p=0.15)

Discussion

4.1. Findings and interpretations

When comparing CSF/serum albumin ratio in a population of patients suffering from BPD of different types with an age- and sex-matched control group consisting of healthy individuals, we found that the CSF/serum albumin ratio was significantly elevated in BPD patients. This finding indicates that an impairment of the blood-brain barrier is more common among BPD patients than in a healthy population. Based on similar past findings in studies made on patients with different psychiatric conditions such as paranoid psychosis, schizophrenia and

affective disorders [26-28, 34, 35], combined with the suggested genetic relation between most mentioned disorders and BPD [29-33], we expected to find a subgroup, also among the patients in our study group, with an increased CSF/serum albumin ratio.

Significant differences were found in CSF/serum albumin ratio when comparing men to women in the BPD group, but not in the control group. The differences in CSF/albumin ratio between men and women are interesting, raising e.g. the question whether separate reference values should be used in clinic, although it is not relevant in this patient-control sex-matched study. Also the observation that a significant correlation between CSF/serum albumin ratio and age was notable in both patient and control group is considered irrelevant in this study, since patients and controls are age-matched as well.

We have ruled out possible positive correlations between patients with blood-brain barrier impairment and; history of psychosis or not, CGI, and number of manic or depressive episodes, in order to ensure that these variables are no confounders. Regarding age at onset and duration of illness we found a correlation between CSF/serum albumin ratio and both these items, but the slope of the correlation was not very far from zero and thus considered clinically irrelevant. After excluding 20 patients and 15 controls, displaying signs of organic injuries from e.g. Wilson's disease or overt multiple sclerosis in the brain MRIs, we repeated Mann-Whitney tests, which revealed that even now our BPD patients were over represented in the category with impairment of the blood-CSF barrier.

As we, by screening tests, interviews, MRI and clinical diagnostics, have eliminated many known differential diagnoses [23-25] and other factors [22] that may cause the increased CSF/serum albumin ratio or the impairment of the blood-CSF barrier, it is possible to make the interpretation that impairment and/or elevated ratio may be caused by one of, or a combination of, the following factors:

- BPD in itself.
- Side effects from either BPD or medications for the bipolar disorder/other medical conditions.
- An unknown element (see; 4.3. *Analyses, albumin ratio and blood-CSF barrier, advantages and limitations,* for examples*)* before, or during, the onset of the BPD, indicating that the impairment/elevated ratio may have caused or acted as a triggering factor for BPD.

Obviously, we can not leave out the possibility that, despite thorough psychiatric and neurological evaluation, the diagnostic tools for ruling out other medical conditions or factors, affecting the blood-brain barrier, used in this study may be too limited.
Noteworthy is also the fact that, after stratifying patient groups according to type of bipolar disorder, we can observe that most patients with elevated albumin ratio were found in the BPD I subgroup, both before and after exclusion of patients and controls displaying abnormal MRI findings, which, with a cautious interpretation, may suggest a stronger correlation between the manic state of the bipolar disorder and an impaired blood-brain barrier.
Incidentally, we discovered that as many as 20 (15%) of the BPD patients had a CSF/serum albumin ratio above the age-adjusted upper reference limit used in our laboratory as compared to 7 (8%) of the controls, nevertheless no statistical significance between the groups, with respect to elevated CSF/serum albumin ratio, was to be found. We have decided not to make any interpretation of this finding, see both; *4.2. Patients and controls, advantages and limitations,* as well as; *4.3. Analyses, albumin ratio and blood-CSF barrier, advantages and limitations,* for comments regarding the decreased power in comparisons between patient data and historical reference values.

4.2. Patients and controls, advantages and limitations

All patients included in this study were recruited, in euthymic state, from a bipolar unit at a psychiatric clinic in Stockholm, Sweden. They were all diagnosed with some type of BPD by skilled psychiatrists and evaluated using a number of psychiatric tools, including ADE [41] for clinical assessment and MINI [43] for excluding patients with other psychiatric disorders (see methods). To further ensure the liability of the diagnostic evaluation the patient data was discussed at a diagnostic case conference, where the final diagnostic decision was made by a consensus panel of experienced board-certified psychiatrists specialized in bipolar disorder.

Since a number of patients during the time of the CSF and serum sampling were using different kinds of prescribed drugs (as well as combinations of drugs) for the bipolar disorder, and in some cases other medical conditions, we can not completely rule out the potential long-term effect they may have on the blood-brain barrier. However, our study showed no significant difference in CSF/serum albumin ratio between patients treated with lithium, antiepileptic or antidepressive drugs, and the authors are not aware of any research describing such effect/s.

On the other hand, we found that patients treated with antipsychotic drugs had higher CSF/serum albumin ratio as a group. This may suggest an association between blood-brain barrier dysfunction and severity of disease (in clinical practice, mostly patients with treatment-resistant BPD are considered for antipsychotic regimes) or that the drugs itself somehow affects the blood-brain barrier. In advantage of the latter hypothesis, haloperidol (antipsychotic drug) has been found to alter the BBB permeability in a rat model [50]. Additionally, no association was found between CSF/serum albumin ratio and any of the following items: experienced psychosis or not, number of manic episodes, number of depressive episodes, which all may be considered as factors reflecting the severity of disease.

More research is needed to examine the precise cause-and-effect relationships underlying the association between blood-brain barrier dysfunction and the use of antipsychotic drugs. For more possible effects from drugs on the blood-CSF barrier, see: *4.3. Analyses, albumin ratio and blood-CSF barrier, advantages and limitations.*

Several past studies of the blood-brain barrier in patients with psychiatric disorders have, as a comparison to the patient data, used historical reference values constructed either from more or less heterogeneous disease groups [47]or neurologically and psychiatrically healthy individuals[48]. In our opinion this decreases the power to detect small differences in CSF/serum albumin ratio, as compared to our approach of using data derived in parallel from age- and gender-matched healthy controls. All controls were evaluated in a telephone based interview to exclude mental illness and symptoms of neurological disease, followed by a one-day comprehensive psychiatric assessment including the same investigations and psychiatric test tools as the patients, in order to ensure mental and neurological health. Also case conferences were conducted with examining clinicians, primary investigators and study coordinator to decide whether or not to include specific controls in the study. Some individuals were allowed in the control group despite minor depressive disorders, isolated episodes of panic disorder, eating disorders or obsessive compulsive disorder which remitted spontaneously or with short term psychotherapy, which may have influenced the study, although the experienced case conference decided the risk was exiguous. For more advantages of this study compared to studies that used previously mentioned reference values [47], see: *4.3. Analyses, albumin ratio and blood-CSF barrier, advantages and limitations.*

Kornhuber et al. have proposed a correlation between an impaired blood-brain barrier and alcohol consumption [51]. To rule out possible effects from alcohol on the CSF/serum albumin ratio in this study both patients and controls have been screened for alcohol- and other substance abuse by AUDIT [44] and DUDIT [45], as well as by determining serum

carbohydrate-deficient transferrin (CDT). Also, there are no documented studies on effects on the blood-brain barrier after completed detoxification or on subjects with a past history of alcohol abuse, yet these variables should be considered.

4.3. Analyses, albumin ratio and blood-CSF barrier, advantages and limitations

All CSF sampling, as well as CSF and serum analyses, were conducted under standard conditions and standard procedures, and also conducted identical in terms of patients and controls. The ratio between the albumin concentration in the cerebrospinal fluid (mg/L) and that in the blood serum (g/L) were determined to evaluate the function of the blood-brain barrier, which by several authors is considered as the gold standard [35, 52, 53]. Increased CSF/serum albumin ratio in patients, compared to controls, was interpreted as an impairment of the blood-brain barrier in BPD [36]. This interpretation is accepted and used as standard procedure among neurologists and scientists in the neurological field [46-49].

A possible bias when comparing past established reference values of albumin ratios (derived with X ml CSF withdrawn) to new patient data (derived with Y ml CSF withdrawn) is that albumin ratio decreases when volume of CSF withdrawn from the subject increases (explained by the rostro-caudal concentration gradient [35]), which in this study has been eliminated by withdrawing the same volume of CSF from both patients and controls.

Also, by age-matching the patients and controls, along with application of CSF/serum ratios, this study ruled out possible biases caused by, probably all known, age-related differences in CSF (as well as serum) albumin levels. Another advantage with age-matched, as well as sex-matched, patient-control studies, instead of comparing CSF/serum albumin ratios to mentioned reference values [47], is the obvious differences in ratio correlating to sex and age in our study.

Reduced CSF flow rate induces, according to general assumptions and experience in clinical neurology, increased concentrations of albumin in the CSF, which may alter the CSF/serum

ratio. According to Bechter et al. (2010), a variety of different mechanisms, such as; "low CSF production rate, increased flow resistance in the subarachnoid space, or reduced outflow into venous blood due to blocked arachnoid villi" [35] are proposed to reduce CSF flow rate. This indicates that in absence of past MRI and CSF flow rate charts from all patients and controls it is not possible to completely rule out the possibility that any of these mechanisms impinge the result of this study, although recent MRI and clinical assessment should have eliminated most mechanisms. However, research regarding the influence of medical treatment on CSF flow rate is limited and as all subjects in this study continued to take their prescribed medications at the time of CSF sampling, this source of bias should be considered.

It is notable that there are a number of already established causes of dysfunction in the blood-brain barrier, such as; hypoxia, head trauma, hypertension and tumours [22]. Furthermore, different kinds of dementia illnesses, vascular dementia in particular, are characterized by barrier dysfunction [23-25]. All these causes are however also, most likely, ruled out (by MRI and thorough clinical evaluation) in the current study.

Another observandum is that this study merely provides the albumin concentrations and CSF/serum ratios during a euthymic period (trait marker). Changes in albumin concentrations, as well as changes in CSF flow and blood-brain barrier function that may affect the albumin concentrations, might be more apparent during manic and/or depressive episodes (state marker). Nevertheless, it may also be argued that measuring albumin concentrations during euthymia provides a more reliable picture of BPD albumin concentrations, as these might be affected by secondary effects that appears during mania/depression (like altered sleeping behavior, motor activity etc.) not specific to bipolar disorder.

4.4. Conclusions

In a well defined, diagnostically heterogeneous, cohort of patients suffering from bipolar disorder we discovered a subgroup of patients, displaying signs of impairment in the blood-brain barrier. When comparing CSF/serum albumin ratios from a healthy, population-based, age- and sex-matched control group to data from the patient cohort significant differences occurred, providing compelling evidence of an association between bipolar disorder and impairment of the blood-brain barrier. This evidence is also in consistency with similar research on several different psychiatric disorders. After stratifying patients according to different types of bipolar disorders we can also state that the strongest association to CSF/serum albumin ratio was found with the BPD I subgroup, significantly separating this group when comparing to the other types of BPD and controls. This finding makes it tempting to speculate whether that the blood-brain barrier dysfunction somehow is more related to the manic state of the bipolar disorder.

Unfortunately, the cross-sectional design of the study makes it impossible to address cause-and-effect relationships underlying the detected associations as well as to designate which of the bipolar disorder and the blood-bran barrier impairment that came first (in somewhat similar to the unanswered philosophical causality dilemma about the chicken or the egg [54]), which both requires research with a different approach.

On the other hand, evidence from this study should stimulate further research on longitudinal cohorts. Such studies could, for example, answer the question if patients with blood-brain barrier impairment are at increased risk of bipolar disorder or vice versa. Finally, among bipolar patients with blood-brain barrier damage we found a number of treatable somatic or neurological conditions (such as Wilson’s disease and multiple sclerosis), which underscores the importance of thorough somatic and neurological inquiry of patients clinically assessed with bipolar disorder.

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The authors declare no conflict of interest.

References

1. Baruk, H., *[Discovery of the "deep personality" and revision of the Kraepelin concept of autonomic mental disease]*. Ann Med Psychol (Paris), 1989. **147**(1): p. 47-56.
2. Yolken, R.H. and E.F. Torrey, *Are some cases of psychosis caused by microbial agents? A review of the evidence.* Mol Psychiatry, 2008. **13**(5): p. 470-9.
3. Meyer, U., et al., *Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge.* Neuropsychopharmacology, 2008. **33**(2): p. 441-56.
4. Buka, S.L., et al., *Maternal exposure to herpes simplex virus and risk of psychosis among adult offspring.* Biol Psychiatry, 2008. **63**(8): p. 809-15.
5. Dalman, C., et al., *Infections in the CNS during childhood and the risk of subsequent psychotic illness: a cohort study of more than one million Swedish subjects.* Am J Psychiatry, 2008. **165**(1): p. 59-65.
6. Brown, A.S., et al., *Serologic evidence of prenatal influenza in the etiology of schizophrenia.* Arch Gen Psychiatry, 2004. **61**(8): p. 774-80.
7. Gattaz, W.F., A.L. Abrahao, and R. Foccacia, *Childhood meningitis, brain maturation and the risk of psychosis.* Eur Arch Psychiatry Clin Neurosci, 2004. **254**(1): p. 23-6.
8. Koponen, H., et al., *Childhood central nervous system infections and risk for schizophrenia.* Eur Arch Psychiatry Clin Neurosci, 2004. **254**(1): p. 9-13.
9. Abrahao, A.L., R. Focaccia, and W.F. Gattaz, *Childhood meningitis increases the risk for adult schizophrenia.* World J Biol Psychiatry, 2005. **6 Suppl 2**: p. 44-8.
10. Lanquillon, S., et al., *Cytokine production and treatment response in major depressive disorder.* Neuropsychopharmacology, 2000. **22**(4): p. 370-9.
11. Danner, M., et al., *Association between depression and elevated C-reactive protein.* Psychosom Med, 2003. **65**(3): p. 347-56.
12. Ford, D.E. and T.P. Erlinger, *Depression and C-reactive protein in US adults: data from the Third National Health and Nutrition Examination Survey.* Arch Intern Med, 2004. **164**(9): p. 1010-4.
13. Cizza, G., et al., *Plasma CRP levels in premenopausal women with major depression: a 12-month controlled study.* Horm Metab Res, 2009. **41**(8): p. 641-8.
14. Kling, M.A., et al., *Sustained low-grade pro-inflammatory state in unmedicated, remitted women with major depressive disorder as evidenced by elevated serum levels of the acute phase proteins C-reactive protein and serum amyloid A.* Biol Psychiatry, 2007. **62**(4): p. 309-13.
15. Zorrilla, E.P., et al., *The relationship of depression and stressors to immunological assays: a meta-analytic review.* Brain Behav Immun, 2001. **15**(3): p. 199-226.
16. Hanson, D.R. and Gottesman, II, *Theories of schizophrenia: a genetic-inflammatory-vascular synthesis.* BMC Med Genet, 2005. **6**: p. 7.
17. Davis, K.L., et al., *White matter changes in schizophrenia: evidence for myelin-related dysfunction.* Arch Gen Psychiatry, 2003. **60**(5): p. 443-56.
18. Sperner-Unterweger, B., *Immunological aetiology of major psychiatric disorders: evidence and therapeutic implications.* Drugs, 2005. **65**(11): p. 1493-520.
19. Irwin, M.R. and A.H. Miller, *Depressive disorders and immunity: 20 years of progress and discovery.* Brain Behav Immun, 2007. **21**(4): p. 374-83.

20. Korschenhausen, D.A., et al., *Fibrin degradation products in post mortem brain tissue of schizophrenics: a possible marker for underlying inflammatory processes.* Schizophr Res, 1996. **19**(2-3): p. 103-9.
21. Muller, N. and M. Schwarz, *Schizophrenia as an inflammation-mediated dysbalance of glutamatergic neurotransmission.* Neurotox Res, 2006. **10**(2): p. 131-48.
22. Rapoport, S.I., *Blood-brain barrier in physiology and medicine*1976, New York: Raven Press. xii, 316 p.
23. Elovaara, I., et al., *CSF in Alzheimer's disease. Studies on blood-brain barrier function and intrathecal protein synthesis.* J Neurol Sci, 1985. **70**(1): p. 73-80.
24. Wallin, A., et al., *Blood brain barrier function in vascular dementia.* Acta Neurol Scand, 1990. **81**(4): p. 318-22.
25. Blennow, K., et al., *Blood-brain barrier disturbance in patients with Alzheimer's disease is related to vascular factors.* Acta Neurol Scand, 1990. **81**(4): p. 323-6.
26. Axelsson, R., E. Martensson, and C. Alling, *Impairment of the blood-brain barrier as an aetiological factor in paranoid psychosis.* Br J Psychiatry, 1982. **141**: p. 273-81.
27. Kirch, D.G., et al., *Abnormal cerebrospinal fluid protein indices in schizophrenia.* Biol Psychiatry, 1985. **20**(10): p. 1039-46.
28. Bauer, K. and J. Kornhuber, *Blood-cerebrospinal fluid barrier in schizophrenic patients.* Eur Arch Psychiatry Neurol Sci, 1987. **236**(5): p. 257-9.
29. Stefanopoulou, E., et al., *Cognitive functioning in patients with affective disorders and schizophrenia: a meta-analysis.* Int Rev Psychiatry, 2009. **21**(4): p. 336-56.
30. Bromet, E.J., et al., *Diagnostic shifts during the decade following first admission for psychosis.* Am J Psychiatry, 2011. **168**(11): p. 1186-94.
31. Lichtenstein, P., et al., *Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study.* Lancet, 2009. **373**(9659): p. 234-9.
32. Purcell, S.M., et al., *Common polygenic variation contributes to risk of schizophrenia and bipolar disorder.* Nature, 2009. **460**(7256): p. 748-52.
33. O'Dushlaine, C., et al., *Molecular pathways involved in neuronal cell adhesion and membrane scaffolding contribute to schizophrenia and bipolar disorder susceptibility.* Mol Psychiatry, 2011. **16**(3): p. 286-92.
34. Bayard-Burfield, L., et al., *Impairment of the blood-CSF barrier in suicide attempters.* Eur Neuropsychopharmacol, 1996. **6**(3): p. 195-9.
35. Bechter, K., et al., *Cerebrospinal fluid analysis in affective and schizophrenic spectrum disorders: identification of subgroups with immune responses and blood-CSF barrier dysfunction.* J Psychiatr Res, 2010. **44**(5): p. 321-30.
36. Zetterberg, H., N. Mattsson, and K. Blennow, *Cerebrospinal fluid analysis should be considered in patients with cognitive problems.* Int J Alzheimers Dis, 2010. **2010**: p. 163065.
37. Goodwin, F.K., K.R. Jamison, and S.N. Ghaemi, *Manic-depressive illness : bipolar disorders and recurrent depression*. 2nd ed2007, New York, N.Y.: Oxford University Press. xxvi, 1262 p.
38. Belmaker, R.H., *Bipolar disorder.* N Engl J Med, 2004. **351**(5): p. 476-86.
39. Burdick, K.E., et al., *Cognitive dysfunction in bipolar disorder: future place of pharmacotherapy.* CNS Drugs, 2007. **21**(12): p. 971-81.
40. Akiskal, H.S. and G.H. Vazquez, *[Widening the borders of the bipolar disorder: validation of the concept of bipolar spectrum]*. Vertex, 2006. **17**(69): p. 340-6.
41. Sachs, G.S., et al., *Rationale, design, and methods of the systematic treatment enhancement program for bipolar disorder (STEP-BD).* Biol Psychiatry, 2003. **53**(11): p. 1028-42.

42. Ryden, E., et al., *A history of childhood attention-deficit hyperactivity disorder (ADHD) impacts clinical outcome in adult bipolar patients regardless of current ADHD.* Acta Psychiatr Scand, 2009. **120**(3): p. 239-46.
43. Sheehan, D.V., et al., *The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10.* J Clin Psychiatry, 1998. **59 Suppl 20**: p. 22-33;quiz 34-57.
44. Saunders, J.B., et al., *Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II.* Addiction, 1993. **88**(6): p. 791-804.
45. Berman, A.H., et al., *Evaluation of the Drug Use Disorders Identification Test (DUDIT) in criminal justice and detoxification settings and in a Swedish population sample.* Eur Addict Res, 2005. **11**(1): p. 22-31.
46. Ganrot, K. and C.B. Laurell, *Measurement of IgG and albumin content of cerebrospinal fluid, and its interpretation.* Clin Chem, 1974. **20**(5): p. 571-3.
47. Tibbling, G., H. Link, and S. Ohman, *Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values.* Scand J Clin Lab Invest, 1977. **37**(5): p. 385-90.
48. Blennow, K., et al., *Protein analysis in cerebrospinal fluid. II. Reference values derived from healthy individuals 18-88 years of age.* Eur Neurol, 1993. **33**(2): p. 129-33.
49. Blennow, K., et al., *Protein analysis in cerebrospinal fluid. III. Relation to blood-cerebrospinal fluid barrier function for formulas for quantitative determination of intrathecal IgG production.* Eur Neurol, 1993. **33**(2): p. 134-42.
50. Saija, A., et al., *Ageing influences haloperidol-induced changes in the permeability of the blood-brain barrier in the rat.* J Pharm Pharmacol, 1992. **44**(5): p. 450-2.
51. Kornhuber, J., et al., *Alcohol consumption and blood-cerebrospinal fluid barrier dysfunction in man.* Neurosci Lett, 1987. **79**(1-2): p. 218-22.
52. Reiber, H. and J.B. Peter, *Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs.* J Neurol Sci, 2001. **184**(2): p. 101-22.
53. Sindic, C.J., M.P. Van Antwerpen, and S. Goffette, *The intrathecal humoral immune response: laboratory analysis and clinical relevance.* Clin Chem Lab Med, 2001. **39**(4): p. 333-40.
54. Horne, M., A. Sowa, and D. Isenman, *Philosophical assumptions in Freud, Jung and Bion: questions of causality.* J Anal Psychol, 2000. **45**(1): p. 109-21.

Figure legends

Figure 1.
Grouped scatter plot showing the sex distributions of albumin cerebrospinal/serum ratio in (a) bipolar patients, and (b) healthy controls. AlbQ equals cerebrospinal/serum albumin ratio. The red horizontal bars represent the medians.

Figure 2.
Scatter plot of age distributions and albumin cerebrospinal/serum ratio in (a) bipolar patients, and (b) healthy controls. AlbQ equals cerebrospinal/serum albumin ratio.

Figure 3.
Grouped scatter plot showing the subject group distributions of albumin cerebrospinal/serum ratio (a) before excluding subjects with abnormal MRI, and (b) after excluding subjects with abnormal MRI. AlbQ equals cerebrospinal/serum albumin ratio. The red horizontal bars represent the medians.

Figure 4.
Grouped scatter plot showing the subject group distributions of albumin cerebrospinal/serum ratio (a) before excluding subjects with abnormal MRI, and (b) after excluding subjects with abnormal MRI. AlbQ equals cerebrospinal/serum albumin ratio. The red horizontal bars represent the medians.

Figure 5.
Scatter plot of albumin cerebrospinal/serum ratio and (a) age at onset, and (b) duration of illness in bipolar patients. AlbQ equals cerebrospinal/serum albumin ratio.

Figure 6.
Grouped scatter plot showing the distributions of albumin cerebrospinal/serum ratio in patients with (yes) and without (no) prescribed antipsychotic drugs. AlbQ equals cerebrospinal/serum albumin ratio. The red horizontal bars represent the medians.

Table 1.

	Controls (N=86)	**Bipolar disorder** (N=134)
Sex (male/female)	38/48	54/80
Age (years)	34 (28-46)	36 (28-50)[c]
Diagnosis (BPD-I/II/III /V/NOS/Cycl/SDM/)[a]		64/53/7/1/6/1/2/
Age at onset		19 (15-25)[c]
Duration of illness		15 (9-26)[c]
Total number of episodes		11 (7-23)[c]
Mania		1 (0-2)[c]
Depression		10 (6-20)[c]
Psychosis (yes/no)		65/69
CGI		4 (4-5)[c]
Medications		
Lithium (yes/no)		79/55
Anticonvulsants (yes/no)		46/88
Antidepressives (yes/no)		63/71
Antipsychotics (yes/no)		35/99

[a] BPD I = bipolar disorder type I, BPD II = bipolar disorder type II, BPD III = bipolar disorder type III (hypomania induced by antidepressants), BPD V = bipolar disorder type V (patient with major depression and relatives with mania), NOS = Bipolar disorder not otherwise specified, Cycl = cyclothymia, SDM = schizoaffective disorder manic type

[b] CGI = clinical global impression

[c] Presented as median (interquartile range)

Demographics and clinical characteristics of the study population. Presented as median (interquartile range) or ratio.

Table 2.	
Children, 1,5 - 14 y/o	<5,0
Adults, 15 – 44 y/o	<6,8
Adults, 45 – 89 y/o	<10,2

Upper reference limits for CSF/serum albumin ratio, used by Clinical Neurochemistry Laboratory in Mölndal, Sweden

Figure 1.

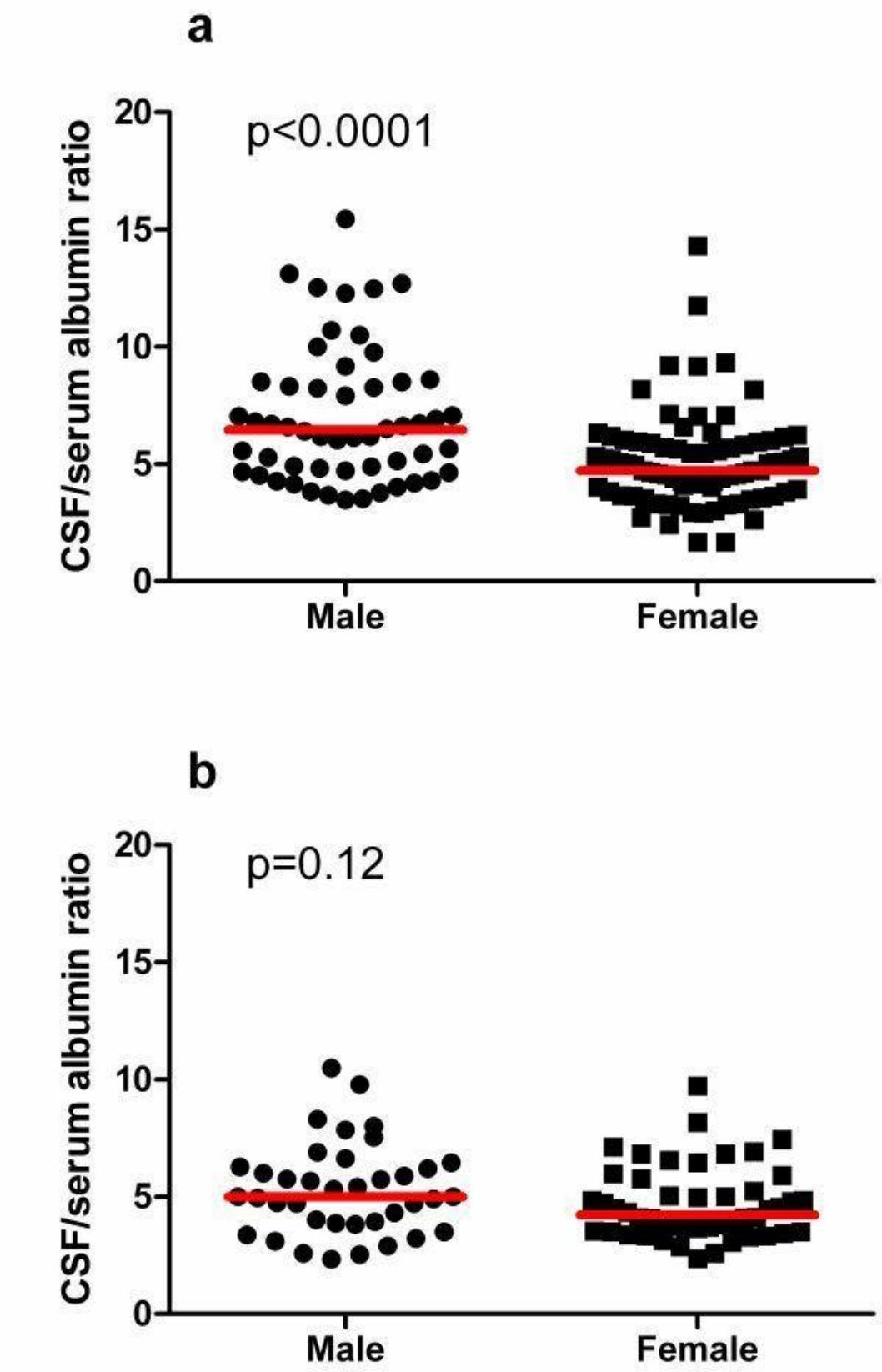

a
p<0.0001
CSF/serum albumin ratio
20
15
10
5
0
Male
Female
b
p=0.12
CSF/serum albumin ratio
20
15
10
5
0
Male
Female

Figure 2.

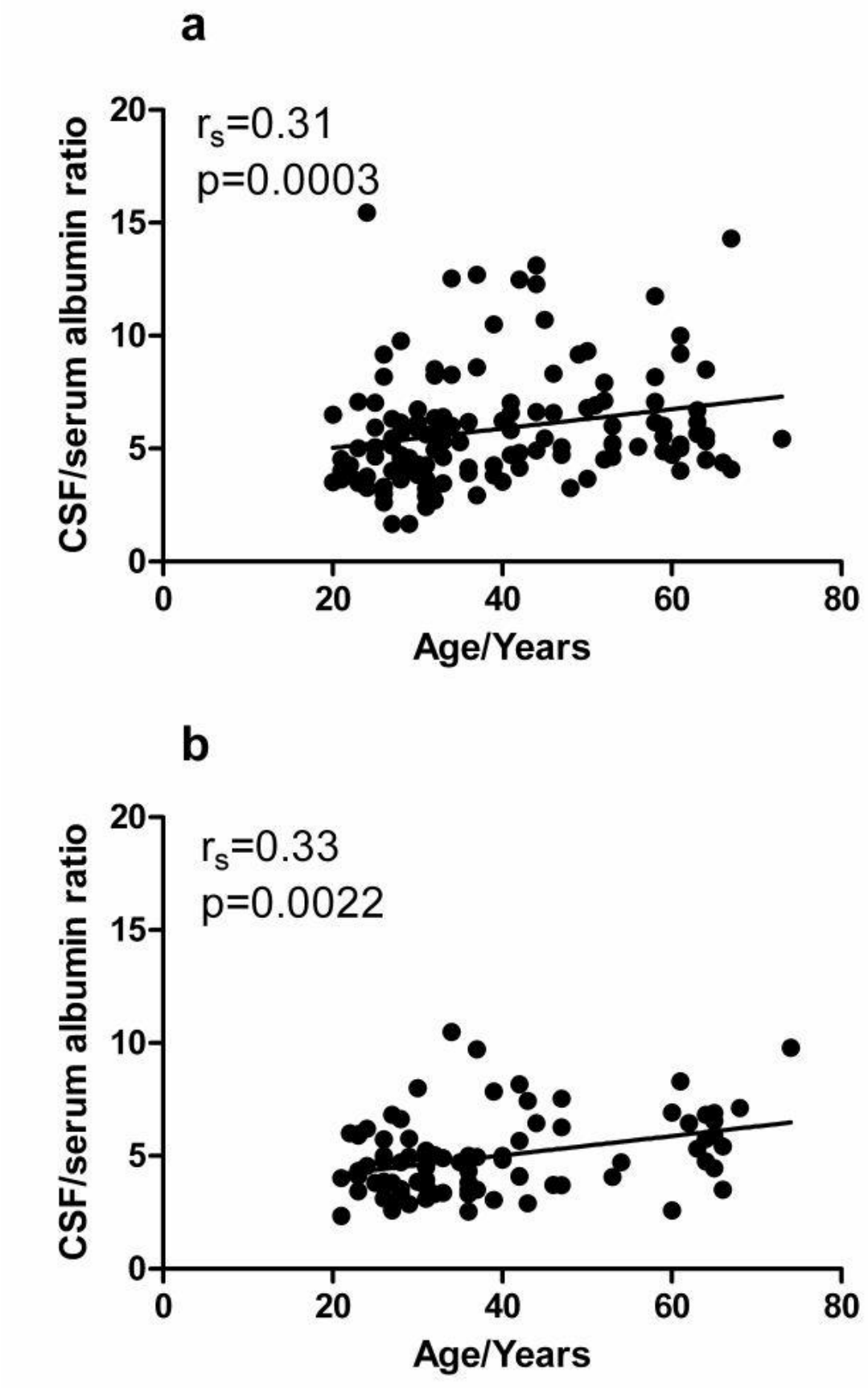

a
r_s=0.31
p=0.0003
CSF/serum albumin ratio
Age/Years
b
r_s=0.33
p=0.0022
CSF/serum albumin ratio
Age/Years

Figure 3.

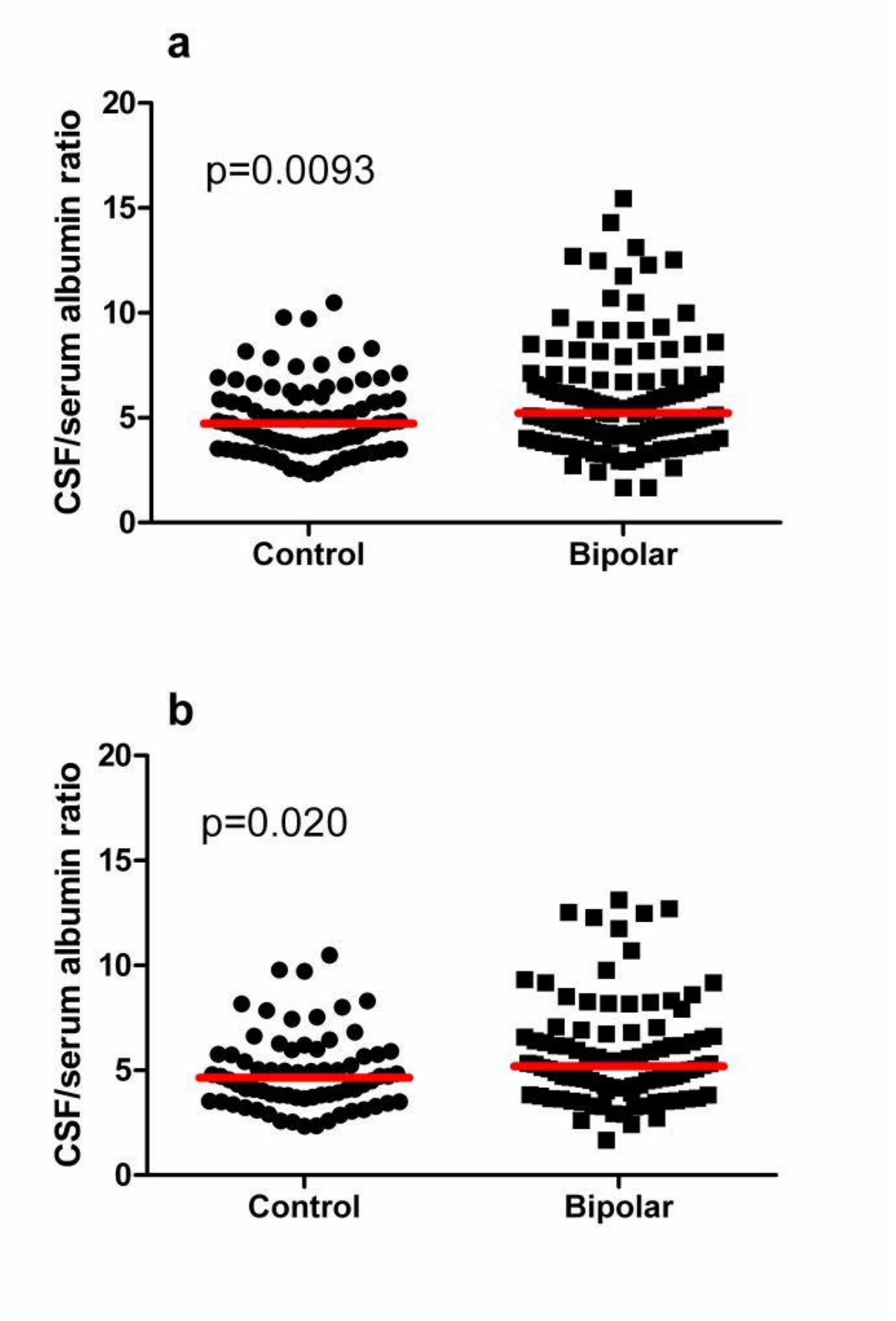

a
CSF/serum albumin ratio
20
15
10
5
0
p=0.0093
Control
Bipolar
b
CSF/serum albumin ratio
20
15
10
5
0
p=0.020
Control
Bipolar

Figure 4.

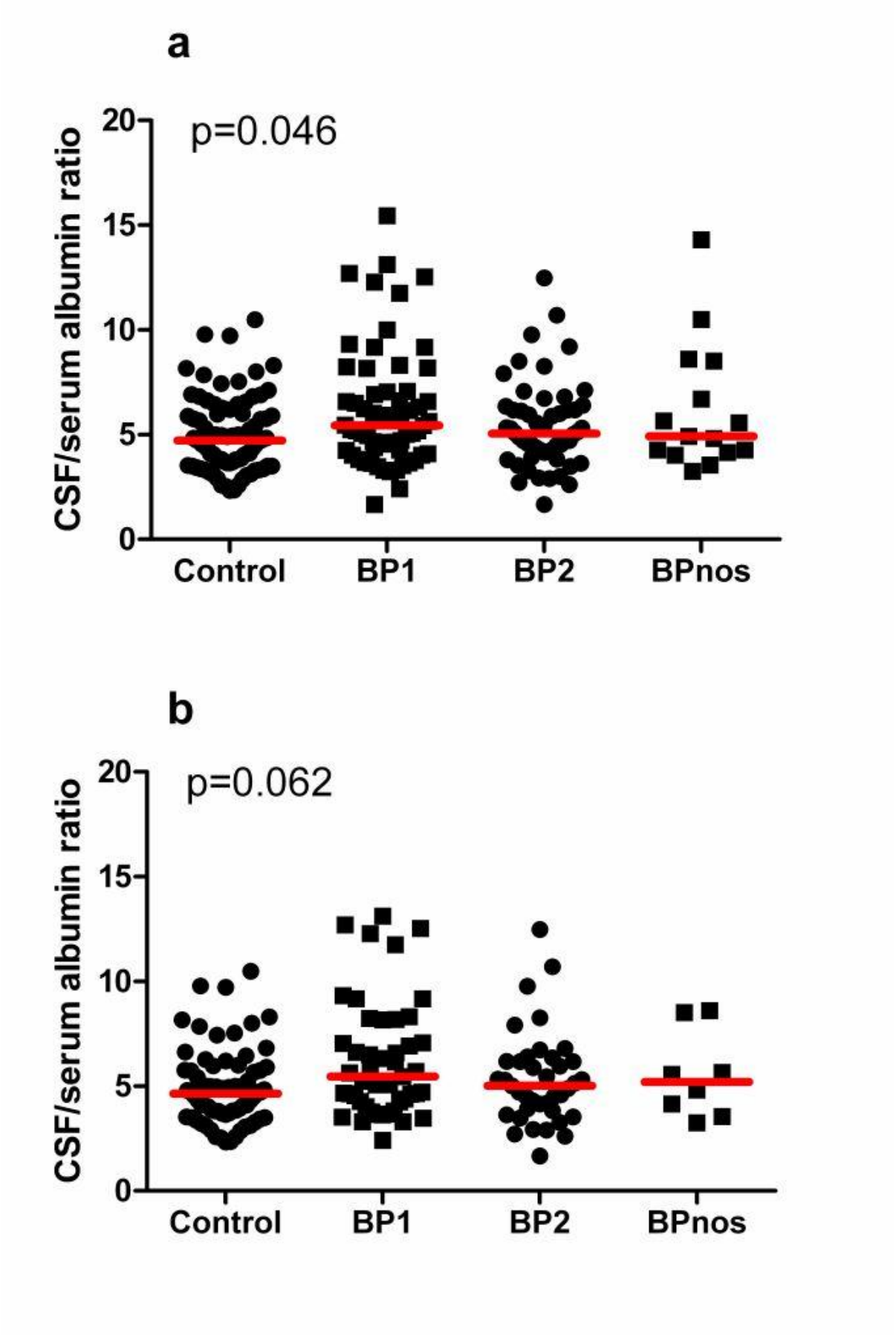

a
p=0.046
CSF/serum albumin ratio
20
15
10
5
0
Control
BP1
BP2
BPnos
b
p=0.062
CSF/serum albumin ratio
20
15
10
5
0
Control
BP1
BP2
BPnos

Figure 5.

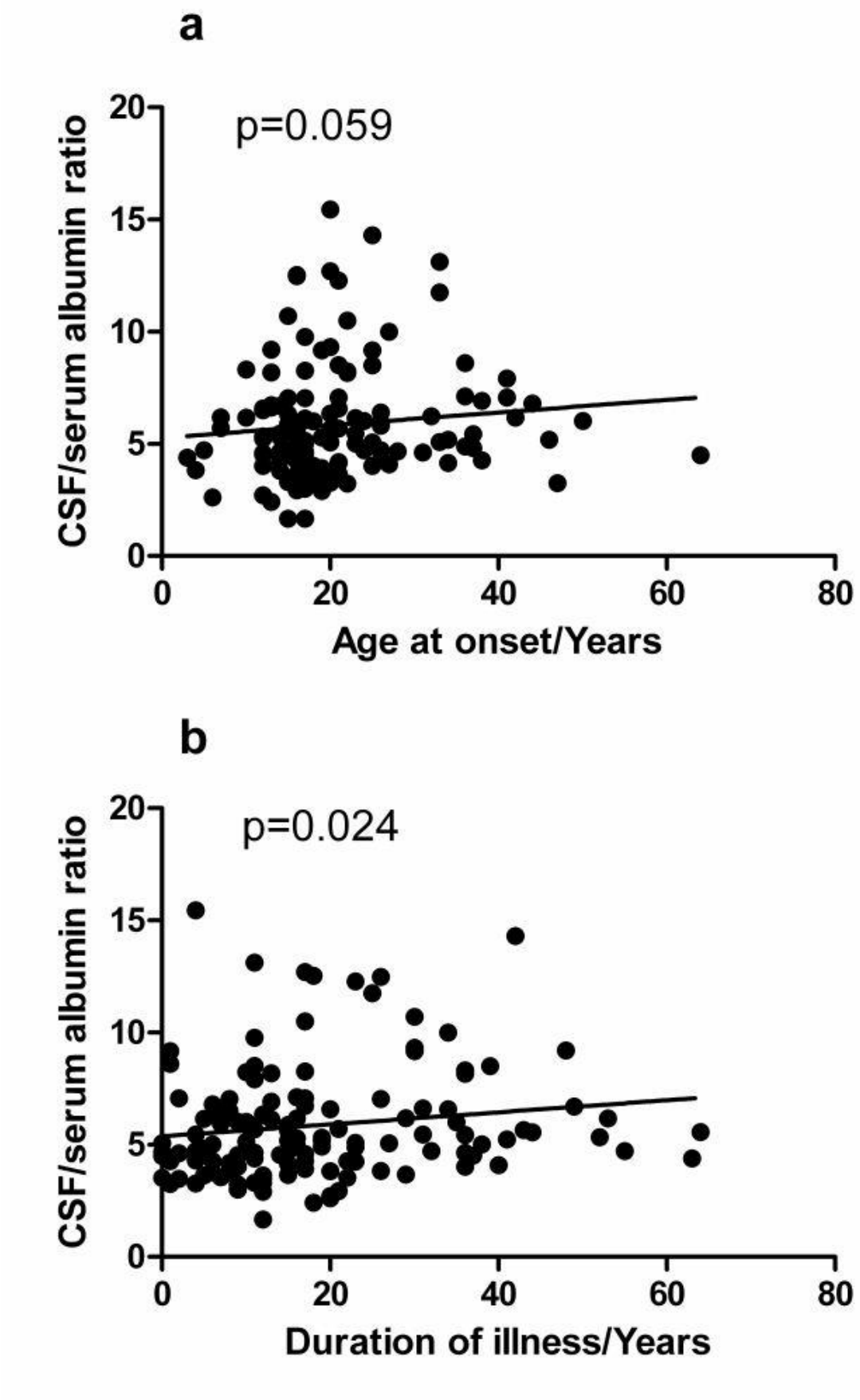
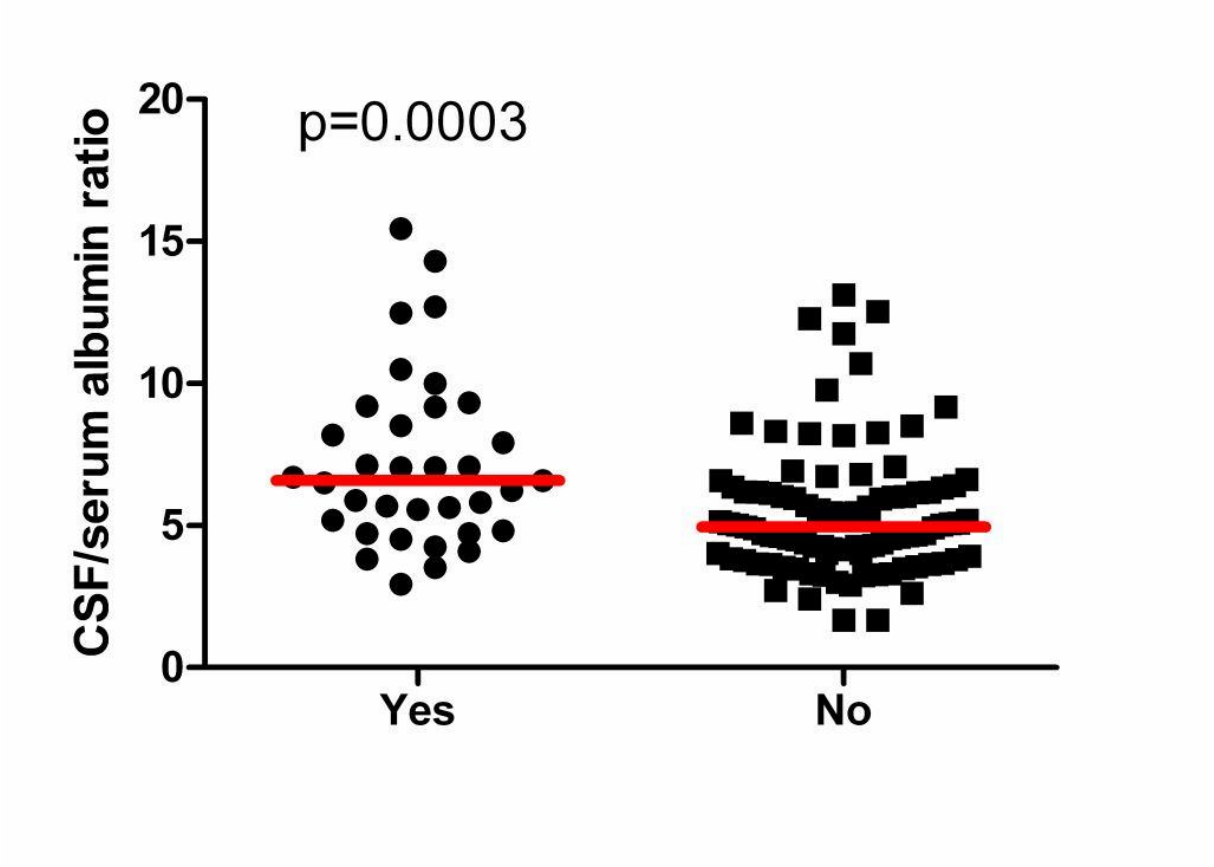

a
p=0.059
CSF/serum albumin ratio
Age at onset/Years
b
p=0.024
CSF/serum albumin ratio
Duration of illness/Years

Figure 6.



Populärvetenskaplig sammanfattning

Har patienter med bipolär sjukdom skadad blod-hjärnbarriär?

I ett samarbete mellan Sahlgrenska akademin och Karolinska Institutet har en studie gjorts med syftet att utreda om patienter som drabbats av bipolär sjukdom (tidigare kallad manodepressiv sjukdom), i större utsträckning än friska människor, har skador som ökar risken att blodburna ämnen kan ta sig till hjärnan. Studien visar att det finns ett statistiskt säkerställt samband mellan bipolär sjukdom och skador på, den så kallade, blod-hjärnbarriären.

Bakgrunden till studien är att ett flertal forskningsstudier i världen kunnat påvisa ett samband mellan flera psykiska sjukdomar och skador på blod-hjärnbarriären, vilken skall skydda hjärnan genom att fungera som ett filter mellan blod och hjärna. Exempel på tillstånd där dessa skador kunnat påvisas hos en större andel patienter, jämfört med andelen hos friska individer, är schizofreni, paranoid psykos och vissa affektiva sjukdomar, såsom depression. Dock har forskning om relationen mellan barriärskada och bipolär sjukdom varit begränsad och de studier som gjorts har innehållit få patienter, vilket gör att resultatens tillförlitlighet minskar.

Genomförandet utgick från att först, med hjälp av bl.a. specialister i psykiatri, psykiatriska tester och undersökningar med magnetkamera, tillse att studiens 134 patienter (rekryterade från en bipolär-enhet på en psykiatrisk klinik i Stockholm) fått korrekt diagnos och utesluta andra tillstånd som kan påverka resultatet av studien. Samtidigt utsågs 86 frivilliga, friska, ålders- och könsmatchade personer (genom ett slumpmässigt urval av statistiska centralbyrån), som alla genomgick tester och undersökningar, identiska med patienternas, för att utesluta sjukdomar och andra tillstånd som skulle göra dem olämpliga som jämförelse-(kontroll-)grupp. Därefter vidtog inhämtning av provmaterial från både patienter och

kontrollgrupp. Materialet bestod av dels blod (serum), och dels cerebrospinalvätska (även kallad ryggmärgsvätska) insamlad genom att en provtagningsnål införs mellan ländkota 3/4 eller 4/5 (så kallad lumbalpunktion) och en liten mängd vätska dras ut. Denna cerebrospinalvätska (CSV), som omger nerverna fram till deras utträde ur ryggraden, cirkulerar hela vägen upp till skallen och är samma vätska som omger hjärnan. På laboratoriet analyserades sedan serum och CSV, varvid koncentrationen av albumin (ett protein som levern producerar och frisläpper i blodet) i dessa båda noterades. Eftersom blod-hjärnbarriären agerar som ett filter mellan blod och CSV skall albuminkoncentrationen, hos en människa med fungerande barriär, vara ungefär 150 gånger högre i blodet jämfört med i CSV. För att avgöra om patienterna hade en fungerande blod-hjärnbarriär dividerades koncentrationen av albumin i CSV med koncentrationen i serum (CSV/serum), varpå dessa kvoter (resultaten av divisionerna) kunde jämföras med kvoterna från den friska kontrollgruppen. Slutligen beräknades om det förelåg någon statistiskt etablerad skillnad mellan CSV/serum-kvoterna hos patientgruppen jämfört med kontrollgruppen, samt hur statistiskt säker denna skillnad var.

Resultatet av studien är att man kan konstatera att en betydande andel av studiens patienter med bipolär sjukdom definitivt har ökad CSV/serum-kvot av albumin, vilket indikerar att funktionen i deras blod-hjärnbarriär är nedsatt. De bakomliggande orsakerna till bipolär sjukdom (liksom många andra psykiska sjukdomar) är idag okända och upptäckter i denna typ av studier är mycket viktiga. Genom identifiering av gemensamma defekter hos patienter med samma sjukdom ger man uppslag till var de bakomliggande orsakerna till sjukdomen kan hittas och kan därigenom styra framtida forskning på rätt väg.