**Markers of neuroinflammation and neuronal injury in bipolar disorder: Relation to prospective clinical outcomes**

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**Abstract**

Background: Neuroimmune mechanisms have been linked to the pathophysiology of bipolar disorder based on studies of biomarkers in plasma, cerebrospinal fluid (CSF), and postmortem brain tissue. There are, however, no longitudinal studies investigating if CSF neuroimmune markers predict clinical outcomes and relate to markers of neuronal injury in patients with bipolar disorder. Here, we test the hypothesis that CSF markers of neuroimmune mechanisms are associated with neurochemical signs of neuronal injury and worse clinical outcome during long-term follow-up of patients with bipolar disorder.

Methods: CSF concentrations of IL-8, MCP-1, YKL-40, and NF-L from 77 euthymic patients with bipolar disorder were analyzed at baseline. Associations to clinical outcomes assessed 6-7 years after baseline were investigated, and adjusted for confounding factors.

Results: MCP-1 concentrations at baseline were positively associated with manic/hypomanic episodes and inpatient care during follow-up. YKL-40 concentrations were negatively associated with manic/hypomanic episodes and with occurrence of psychotic symptoms. Only the negative association between YKL-40 and manic/hypomanic episodes survived multiple testing correction.

Conclusions: High concentrations of CSF markers of neuroimmune mechanisms were not related to biomarker signs of neuronal injury at baseline and not consistently associated with poor clinical outcomes as assessed after 6-7 years in this prospective

study. These findings speak against a causal link between these proteins and disease progression. However, longitudinal studies of more neuroimmune markers across different mood states are needed to establish the role of neuroimmune mechanisms in bipolar disorder.

## 1. Introduction

Bipolar disorder is a severe psychiatric condition characterized by recurrent episodes of elevated (mania or hypomania), depressed, or mixed mood (Belmaker 2004). Several lines of evidence indicate that the neuroimmune system and neuroinflammation play a role in the pathophysiology of bipolar disorder (Rosenblat et al. 2014). Neuroinflammation is a wide concept involving central nervous system (CNS) innate immunological responses (O'Callaghan et al. 2008). Microglia are the key cellular mediators of the intrinsic brain immune system and microglia activation is a central component of neuroinflammation. Microglia activation has been suggested to affect neuronal growth, differentiation, and function, and abnormal interactions between microglia and neurons might lead to a variety of psychiatric symptoms (Beumer et al. 2012). Activated microglia produce cytokines and chemokines, compounds that have been shown to impact synaptic plasticity, neurotransmitter metabolism, and neurocircuits relevant to mood regulation (McAfoose and Baune 2009; Beumer et al. 2012; Haroon et al. 2012). Chronic exposure to elevated cytokines is further hypothesized to contribute to the development of mental disorders (Kraneveld et al. 2014).

Although many studies show *peripheral* inflammation in psychiatric disorders, this is not synonymous with neuroinflammation or microglial activation in the CNS (Bhattacharya et al. 2016). Due to the relative impermeability of the blood–CSF (cerebrospinal fluid) barrier, concentrations of cytokines and other proteins in serum or plasma differ from the concentrations in CSF (Maier et al. 2005; Bromander et al. 2012; Isgren et al. 2015). This means that altered concentrations of CSF proteins might be more sensitive and specific to CNS processes than equivalent blood

alterations. Studies investigating neuroinflammation markers in CSF from patients with bipolar disorder are, however, scarce. In two previous studies, we found higher concentrations of interleukin-1β (IL-1 β) and interleukin-8 (IL-8) in euthymic patients with bipolar disorder compared with controls (Söderlund et al. 2011; Isgren et al. 2015). For IL-8, there was a strong association to lithium- and antipsychotic treatment. In another study, we found higher concentration of two CSF markers of monocyte and microglia activation in bipolar disorder patients compared with controls: monocyte chemoattractant protein 1 (MCP-1; also called CCL-2) and chitinase-3-like protein 1 (CHI3L1; also called YKL-40) (Jakobsson et al. 2015). We further investigated CSF markers reflecting damages in brain cells and subcellular structures. Here, we found higher levels of neurofilament light chain (NF-L), a marker of axonal damage, in patients compared with controls, with a positive association to treatment with atypical antipsychotics (Jakobsson et al. 2014).

Interestingly, cross-sectional studies have found associations between markers of neuroinflammation or neuronal injury on the one hand, and brain imaging findings, clinical features, cognition, and suicidality on the other. Thus, one recent study found that the inflammation-related cytokines TNF-α, IL-8, IFN-γ, and IL-10 measured in serum were associated with changes related to structural connectivity in cortico-limbic networks in bipolar disorder (Benedetti et al. 2016). Another study found a possible relationship between manic symptomatology and pro-inflammatory gene expression in bipolar disorder (Haarman et al. 2014). We recently reported that cognitive impairment (executive function) in bipolar disorder was associated with higher levels of neuroinflammatory markers in CSF (Rolstad et al. 2015). In the same vein, CSF markers of neurodegeneration were associated with cognitive performance

in patients with bipolar disorder, including a negative association between high NF-L concentrations and decreased performance in tests of verbal function and memory (Rolstad et al. 2015). Finally, lower plasma and CSF levels of IL-8 (Janelidze et al. 2015), as well as lower CSF levels of MCP-1 (Janelidze et al. 2013), have been found in suicide attempters compared with healthy controls.

Studies assessing prospective associations between neuroinflammation and clinical outcomes are, however, lacking (Barbosa et al. 2014). The question remains open whether neuroinflammation is associated with disease progression. Further, nonclassical immune actions of immune mediators and cells in the CNS have been shown in the absence of CNS pathology, stressing that homeostatic and adaptive immune processes can be mistaken for pathological processes (Estes and McAllister 2014). Hence, although neuroinflammation might constitute a pathological mechanism in a causal model, it could also represent a reparative response to pathological mechanisms (Stertz et al. 2013; Barbosa et al. 2014; Reus et al. 2015; Bhattacharya et al. 2016). To address these questions, longitudinal studies are needed that investigate the relationship between markers of neuroinflammation and clinical outcomes.

The aim of this study was to investigate if CSF markers of neuroinflammation and neuronal injury in patients with bipolar disorder predict important clinical outcomes during a 6-7 year follow up period.

## 2. Methods

### *2.1. Study population*

The study included 77 patients with bipolar disorder. The work-up procedures for patients at baseline have been described in detail previously (Ryden et al. 2009). Patients were recruited from the St. Göran Bipolar Project, enrolling patients from the Northern Stockholm psychiatric clinic, Stockholm, Sweden, between October 2005 and April 2008. Inclusion criteria were an age of at least 18 years old and meeting the DSM-IV-TR criteria for any bipolar disorder spectrum diagnosis (bipolar disorder type 1, type 2, or not otherwise specified). The Affective Disorder Evaluation (ADE) was used to establish the diagnosis. The ADE is a semi-structured interview that includes adapted versions of the mood and psychosis modules of the Structured Clinical Interview for DSM-IV, and was developed for the Systematic Treatment Enhancement Program of Bipolar Disorder (STEP-BD) project (Sachs et al. 2003). Co-morbid psychiatric disorders were screened for by utilizing the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al. 1998). The ADE and M.I.N.I. interviews were conducted by board certified psychiatrists or residents in psychiatry. A best-estimate diagnostic decision (Leckman et al. 1982; Roy et al. 1997) was made by a consensus panel of experienced board-certified psychiatrists specialized in bipolar disorder treatment with access to patients records, ADE, M.I.N.I. and interviews with next of kin when possible. The Montgomery-Åsberg Depression Rating Scale (MADRS) and the Young Mania Rating Scale (YMRS) were used to assess depressive and manic symptoms in patients. Function and symptom severity were measured using the Clinical Global Impression (CGI) rating scale, and the Global Assessment of Functioning (GAF) Scale divided into function (GAF-f) and symptom (GAF-s).

After approximately 6-7 years, patients were contacted and re-scheduled for all follow-up visits. Patients who had undergone a lumbar puncture at baseline, and who had completed the follow-up visit were eligible for this study. At the follow-up visit, all patients were interviewed by board certified psychiatrist (C.S. & C-J. E) using a structured interview that included a detailed review of mood episodes and events (suicide attempts, inpatient care) that had occurred during the follow-up period. The interviewers had access to patients' electronic medical journals. The change in GAF score during the follow-up time (ΔGAF) was calculated by subtracting GAF scores at baseline from GAF scores at follow-up.

The study was approved by the Regional Ethics Committee in Stockholm and carried out in accordance with the Declaration of Helsinki. All participants gave oral and written consent to participate in the study.

*2.2. Lumbar puncture*

At baseline, CSF was obtained by lumbar puncture that occurred between 0900 and 1000 hours following an overnight fast. Patients were in a stable euthymic mood as judged by a physician at the time of CSF sampling. A total volume of 12 ml was collected, inverted to avoid gradient effects, divided into aliquots and stored at -80ºC pending analyses. All samples in this study were thawed and refrozen once before analysis.

*2.3. CSF analyses*

IL-8 was analyzed together with IL-1β, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, TNF-α, and IFN-γ using the MSD 96-well multi-array and multi-spot human cytokine assay

(Human Cytokine Assay Ultra-Sensitive kit, Meso Scale Discovery). MCP-1 concentration was measured using a commercial electrochemiluminescence enzyme-linked immunosorbant assay (ELISA; Human MCP-1 Ultra-Sensitive Kit, Meso Scale Discovery). YKL-40 concentration was measured using a commercial colorimetric ELISA (Human chitinase-3 quantikine ELISA kit, R&D systems Inc.). NF-L concentration was measured with a commercial ELISA assay (NFLight, UmanDiagnostis AB, Umeå, Sweden).

All analyses were performed according to the manufacturers' instructions at the Clinical Neurochemistry Laboratory in Mölndal, Sweden. Intra-assay coefficients of variation were below 10% for all assays. The staff performing the analyses was blinded to all phenotype information.

*2.4. Analysis of CSF/serum albumin ratio*

Serum and CSF concentrations of albumin were analyzed by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA), using a method accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). Intra- and inter-assay coefficients of variation were below 10%. The ratio between the albumin concentration in CSF (mg/L) and serum (g/L) was calculated and used to assess blood-CSF barrier function (Andersson et al. 1994).

*2.5. Statistical analyses*

*2.5.1. Rationale for selecting covariates to main regression analyses*

In order to select potentially confounding variables to adjust for in the main regression analyses (investigating associations between CSF analytes at baseline and clinical outcomes at 6-7 year follow-up), we first performed bivariate regression analyses testing possible associations between CSF analytes at baseline and clinical parameters at baseline. These parameters were age, sex, BMI (body mass index), CSF/serum albumin ratio, and pharmacological treatments (lithium, antipsychotic drugs, anticonvulsant drugs, antidepressant drugs, and benzodiazepines). Variables with a univariate correlation with a p-value <0.25 were included in ensuing multiple regression analyses (one for every CSF analyte). In a second step, we performed bivariate regression analyses testing possible associations between the main clinical outcomes and important clinical parameters at baseline. These were age, sex, BMI, CSF/serum albumin ratio, and pharmacological treatments (lithium, antipsychotic drugs, anticonvulsant drugs, antidepressant drugs, and benzodiazepines). Variables with a univariate correlation with a p-value <0.25 were included in ensuing multiple regression analyses (one for every clinical outcome). Variables with a p-value <0.05 in the multiple regression analyses (either associated with the CSF analyte, with the clinical outcome or with both) were finally added as covariates in the main regression analyses. Dependent variables not showing a normal distribution (as tested by one-sample Kolmogorov-Smirnov test) were log10 transformed prior to linear regressions to make the residuals more close to normal distribution.

*2.5.2. Main regression analyses*

For every CSF analyte (IL-8, MCP-1, YKL-40, NFL), five logistic regression analyses were performed, testing the associations between the analyte and the clinical outcomes manic/hypomanic episodes, depressive episodes, suicide attempts, inpatient

care, and psychotic symptoms during mood episodes. To make the odds ratios more interpretable, the analytes were divided by their standard deviations prior to the logistic regressions. While evaluating goodness-of-fit for the regression analysis by the Hosmer-Lemeshow test, the models fitted the data best when using log10 transformed values; therefore CSF analytes were log 10 transformed prior to regression analyses. Further, two linear regression analyses were performed for every CSF analyte, testing the associations between the analytes and the change in GAF-symptom and GAF-function during the follow-up time. Variables identified by the selective procedure described above were used as covariates in all regression analyses to adjust for potential confounders. Level of significance was set at $p<0.05$. SPSS Statistics version 22 (IBM Corporation) was used for all statistical analyses. Because of the multiple statistical tests performed in this study, correction for the false discovery rate (FDR) was applied, using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995). To this end, an online utility was used (SDM-ProjectWeb 2010).

### *2.6. Description of lost to follow-up*

The clinical features of the population available for this follow-up study were compared with the study populations previously used for studying patient-control differences in CSF-markers at baseline (Jakobsson et al. 2014; Isgren et al. 2015; Jakobsson et al. 2015).

## 3. Results

### *3.1. Demographics*

Demographic and clinical characteristics of the study population are presented in table 1. The clinical data from the follow-up refers to clinical events that had occurred between baseline and the 6-7 year follow-up. At baseline, all patients undergoing lumbar puncture were euthymic as judged by a physician. The results from the MADRS and YMRS assessments at the time of lumbar puncture showed that 80% of the patients had MADRS scores below 12 and 100% of the patients had YMRS scores below 12.

We compared individuals eligible for this study with the populations used for analyzing patient-control differences in CSF analytes at baseline (Jakobsson et al. 2014; Isgren et al. 2015; Jakobsson et al. 2015). This comparison showed that patients in this study had had a longer disease duration at baseline (median 17 years vs. 12 years). The other clinical features were similar. Supplementary table S1 shows a comparison between the follow-up study cohort and the cohort from the study of markers of monocyte and microglia activation (Jakobsson et al. 2015). The characteristics of the other two study baseline cohorts (Jakobsson et al. 2014; Isgren et al. 2015) were very similar to the Jakobsson (2015) study (data not shown).

*3.2. Associations between CSF analytes and clinical parameters at baseline*

At baseline, IL-8 was significantly associated with age ($p=0.002$; $\beta=0.302$), CSF/serum albumin ratio ($p=0.006$; $\beta=0.295$), and lithium treatment ($p=0.006$; $\beta=0.294$). MCP-1 was significantly associated with age ($p=0.002$; $\beta=0.348$). YKL-40 was significantly associated with age ($p=0.000$; $\beta=0.722$), CSF/serum albumin ratio ($p=0.027$; $\beta=0.168$) and lithium treatment ($p=0.013$; $\beta=0.184$). NF-L was significantly associated with age ($p=0.001$; $\beta=0.696$) and CSF/serum albumin ratio ($p=0.008$;

β=0.221). The multiple regression analyses are shown in detail in supplementary table S2.

*3.3. Associations between baseline variables and clinical outcomes at follow-up*

Manic/hypomanic episodes during the follow-up period was negatively associated with age (p=0.003; OR=0.928). Depressive episodes during the follow-up period was positively associated with antipsychotic (p=0.038; OR=10.3) and antidepressant (p=0.008; OR=5.46) treatments. Inpatient care was positively associated with the use of benzodiazepines (p=0.014; OR=6.06). Psychotic symptoms during mood episodes was negatively associated with age (p=0.014; OR=0.923) and positively associated with lithium treatment (p=0.014; OR=14.6). Suicide attempts or change in GAF scores during the follow-up time were not significantly associated with any of the clinical parameters.

*3.4. Associations between baseline markers of neuroinflammation and brain injury and clinical outcomes at follow-up*

There were no significant associations between IL-8 or NF-L and clinical outcomes after adding covariates to the analyses. MCP-1 concentrations were positively associated with having manic/hypomanic episodes and with inpatient care during the follow-up period. YKL-40 concentrations were negatively associated with having manic episodes and with having psychotic symptoms during mood episodes. When adjusting the p-values using an FDR-method, one significant finding remained: the negative association between YKL-40 and relapse into mania/hypomania. See table 2 for a summary of the regression analyses and supplementary tables 3-6 for statistical details.

## 4. Discussion

This is the first prospective study investigating if CSF markers of neuroinflammation and brain injury predict clinical outcomes in a long-term follow-up. We found that higher concentrations of YKL-40 were associated with lower risk of relapse into mania/hypomania. This finding was significant after correcting for multiple comparisons. We also found that higher concentrations of YKL-40 were associated with not having psychotic symptoms during mood episodes, and that higher concentrations of MCP-1 were associated with higher risk for manic/hypomanic episodes and inpatient care during the follow-up period. However, these three associations were not significant after adjusting the p-values for multiple testing. When inspecting the other statistically non-significant associations, of which some theoretically could be affected by low statistical power, neither of these suggest that high markers of neuroinflammation and neuronal injury would be associated with a poor prognosis. We therefore conclude that high levels of the CSF markers of neuroinflammation and neuronal injury - which we previously have shown to be elevated in patients with bipolar disorder - are not consistently associated with poor clinical outcomes.

Our findings should be viewed in the context that it is as yet undecided if neuroinflammation and microglial activation reflect a pathological mechanisms in bipolar disorder, a reparative response to pathological mechanisms, or a general state of vulnerability for mood disorders (Mesman et al. 2015; Bhattacharya et al. 2016). Our results speak against an important role for these proteins in the progression of bipolar disorder, at least in a dose-dependent matter. The findings are partly in line

with a prospective Dutch study that found higher serum levels of MCP-1 in bipolar offspring than in controls, together with higher expression of immune genes in monocytes (including MCP-1 gene). These aberrations were present irrespective of lifetime or future mood disorders. The authors suggested that the aberrant neuroimmune state in bipolar offspring reflects a general state of vulnerability for mood disorders rather than being of direct predictive value for development of a mood disorder (Mesman et al. 2015).

There are no previous prospective study of CSF-biomarkers and course of illness in bipolar disorder, but a few studies have prospectively investigated the relationship between serum markers of neuroinflammation or peripheral inflammation and clinical outcomes in bipolar disorder. One study found that CRP (C-reactive protein) levels were significantly increased in depressed men who developed manic symptoms during two years of follow-up (Becking et al. 2013). Another study found that MCP-1 and the pro-inflammatory cytokine IL-1α measured in serum predicted depressive relapse during a 12-month period after measurement (Bond et al. 2016). By contrast, we found no association between depressive relapses during the follow-up period and MCP-1 concentration in CSF.

Previous cross-sectional studies have found lower CSF levels of IL-8 in suicide attempters with anxiety than in healthy controls (Janelidze et al. 2015), and lower CSF MCP-1 in suicide attempters than in healthy controls (Janelidze et al. 2013). In our prospective study, we found no significant associations between these analytes and suicide attempts during the follow-up period. This could, however, be due to the

fact that only a few patients in this study attempted suicide during the follow-up period.

It should be noted that the baseline CSF samples in this study were collected from euthymic bipolar disorder patients. It is possible that the results would have been different if CSF from patients with mania or depression would have been studied, since neuroimmune activation might be more prominent during mood episodes. For instance, a Danish meta-analysis of cytokines, cytokine receptors, and receptor antagonists found more prominent differences between patients with bipolar disorder and control subjects during mania and depression compared to during euthymia (Munkholm et al. 2013).

Our study has limitations that should be considered. First, although we investigated the effects of many known potential confounders, there might be other factors that could not be controlled for in this study, e.g., physical activity and unrecorded medical conditions associated with inflammation. Second, although our study is the largest cohort of bipolar patients that has been followed-up after CSF collection, the sample size is yet limited which leads to power issues, especially when analyzing rare outcomes like suicide attempts. Third, the markers examined are proxies for, not direct measures of, neuroinflammation, microglial activation, and neuronal injury. Fourth, there is a risk of selection bias since some patients from baseline were lost to follow-up. However, the clinical characteristics of the study cohort were similar to those of the baseline cohorts. Finally, a limitation concerns correcting for the multiple tests performed in this study. Interpreting unadjusted p-values may lead to type I

errors. But applying corrections for multiple testing as we did, might instead lead to type II errors.

The strengths of this study include that protein concentrations were measured in CSF instead of in blood in contrast to the majority of previous studies in this area. This reduces the risks of measuring processes unrelated to the CNS. Another strength is that the cohort is well characterized with data on several important confounding factors, including the CSF/serum albumin ratio, and psychotropic drugs. The diagnostic procedures, including decisions by experienced board-certified psychiatrists specialized in bipolar disorder, ensures good diagnostic accuracy.

In conclusion, our study shows that high levels of CSF markers of neuroinflammation and neuronal injury are not consistently associated with poor clinical outcomes in a long-term follow-up of bipolar disorder patients. Tentatively, the assessed proteins may be involved in adaptive immune processes or reflect a general state of vulnerability for mood disorders rather than being of predictive value for disease progression. Since bipolar disorder most certainly involve complex neuroimmune related mechanisms, along with complex nonimmune mechanisms, longitudinal studies across different mood states are needed to obtain a more complete picture of the neuroimmune mechanisms of bipolar disorder.

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## 6. Disclosure

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**Table 1**. Clinical characteristics of the study population.

| | **Patients with bipolar disorder** (N=77)<br>Number (%) or median [IQR] |
|---|---|
| *Baseline data* | |
| Sex (m/f) | 33 (57.1%) / 44 (42.9%) |
| Age (years) | 37.0 [29.5-52.5] |
| BMI ($kg/m^2$)[a] | 25.6 [22.7-28.3] |
| Bipolar diagnosis [a] | |
| I | 38 (49.4%) |
| II | 28 (36.4%) |
| Other | 11 (14.3%) |
| Illness duration (years)[a] | 17.0 [11.0-29.3] |
| Rating scales | |
| CGI[b] | 4.0 [4.0-5.0] |
| MADRS[c] | 3.0 [1.0-10.5] |
| YMRS[d] | 0.0 [0.0-2.0] |
| GAF-symptom[a] | 68 [60-80] |
| GAF-function[a] | 68 [60-80] |
| Comorbid diagnoses (M.I.N.I.) | |
| Anxiety disorders[b] | 18 (23.3%) |
| Alcohol dependence or abuse[f] | 9 (12.5%) |
| Drug dependence or abuse[f] | 3 (4.2%) |
| Medications | |
| Lithium | 50 (64.9%) |
| Antipsychotics | 16 (20.8%) |

| | |
|---|---|
| Anticonvulsants | 24 (31.2%) |
| Antidepressants | 34 (44.2%) |
| Benzodiazepines | 19 (24.2%) |
| CSF proteins (pg/mL) | |
| IL-8 | 34.0 [26.8-45.8] |
| MCP-1[e] | 504 [415-635] |
| YKL-40 [e] | 85.8 [52.8-122] |
| NFL | 395 [232-607] |
| *6-7 year follow up data* | |
| Time between baseline LP and follow-up (years) | 6.4 [5.9-7.3] |
| Any manic/hypomanic episode [a] | 34 (44.2%) |
| Any depressive episode [e] | 53 (68.8%) |
| Any suicide attempt | 7 (9.1%) |
| Any inpatient care | 34 (44.2%) |
| Any psychotic symptoms [e*] | 16 (20.8%) |

[a] Data missing for three patients [b] Data missing for four patients [c] Data missing for 14 patients [d] Data missing for 17 patients [e]Data missing for one patient [f] Data missing for 5 patients * Psychotic symptoms during mood episodes

Abbreviations: IQR, interquartile range. BMI, body mass index. CGI, clinical global impression. LP, lumbar puncture. MADRS, Montgomery-Åsberg depression rating scale. M.I.N.I., Mini international neuropsychiatric interview. YMRS, young mania rating scale. GAF, global assessment of functioning.

**Table 2**. Results from regression analyses of concentrations of IL-8, YKL-40, MCP-1, NF-L at baseline and clinical parameters at 6-7-year follow-up in patients with bipolar disorder.

| **Clinical parameters** | **IL-8** | **MCP-1** | **YKL-40** | **NFL** |
|---|---|---|---|---|
| ***Manic/hypomanic episodes*** | OR=0.68 | **OR=1.83** | **OR=0.20** | OR=1.14 |
| | p=0.286 | **p=0.041** | **p=0.006** | p=0.770 |
| ***Depressive episodes*** | OR=0.81 | OR=1.22 | OR=0.83 | OR=1.19 |
| | p=0.590 | p=0.525 | p=0.706 | p=0.735 |
| ***Suicide attempts*** | OR=1.31 | OR=0.98 | OR=0.17 | OR=0.97 |
| | p=0.693 | p=0.969 | p=0.091 | p=0.970 |
| ***Inpatient care*** | OR=1.09 | **OR=1.95** | OR=0.57 | OR=1.32 |
| | p=0.826 | **p=0.022** | p=0.216 | p=0.539 |
| ***Psychotic symptoms*** | OR=1.34 | OR=1.12 | **OR=0.20** | OR=0.85 |
| | p=0.484 | p=0.751 | **p=0.015** | p=0.756 |
| ***ΔGAF-symptom²*** | β=0.07 | β=-0.14 | β==0.28 | β=-0.25 |
| | p=0.685 | p=0.285 | p=0.195 | p=0.240 |
| ***ΔGAF-function²*** | β=-0.24 | β=-0.17 | β=0.114 | β=-0.29 |
| | p=0.142 | p=0.201 | p=0.599 | p=0.173 |

Values in bold were significant at the 0.05 level (uncorrected p-values). Green color represents a positive association and red color represents a negative association.

Abbreviations: β, standardized beta coefficients (linear regressions). GAF, global assessment of functioning. OR, odds ratio (logistic regressions).

Covariates and statistical details in supplementary tables S3-6.