Alzheimer's disease related biomarkers in bipolar disorder - A longitudinal one-year case-control study

Running title: Biomarkers for neurodegeneration in bipolar disorder

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ABSTRACT

Introduction: Bipolar disorder (BD) is a heterogeneous mental disorder characterized by recurrent relapses of affective episodes: Subgroups of patients with BD have cognitive deficits, and an increased risk of dementia.

Methods: This prospective, longitudinal, one-year follow-up, case-control study investigated biomarkers for AD and neurodegenerative diseases, namely: cerebrospinal fluid (CSF) amyloid beta (Aβ) isoforms and ratios (Aβ42, Aβ40, Aβ38), CSF soluble amyloid precursor protein (sAPP) α and β, CSF total (t-tau) and phosphorylated tau (p-tau), CSF neurofilament-light (NF-L), CSF neurogranin (NG), plasma-isoforms Aβ42 and Aβ40, plasma-tau, plasma-NF-L, and serum S100B, in patients with BD (N = 62, aged 18-60) and gender-and-age-matched healthy control individuals (N = 40). CSF and plasma/serum samples were collected at baseline, during and after an affective episode, if it occurred, and after a year. Data were analyzed in mixed models.

Results: Levels of CSF Aβ42 decreased in patients with BD who had an episode during follow-up (BD-E) (N = 22) compared to patients without an episode (BD-NE) (N = 25) during follow-up (P = 0.002). Stable levels were seen for all other markers in BD-E compared to BD-NE during the one-year follow-up. We found no statistically significant differences between patients with BD and HC at T0 and T3 for Aβ42, Aβ40, Aβ38, Aβ42/38, Aβ42/40, sAPPα, sAPPβ, t-tau, p-tau, p-tau /t-tau, NF-L, NG in CSF and further Aβ40, Aβ42, Aβ42/40, t-tau, NF-L in plasma, S100B in serum, and APOE-status. Furthermore, all 18 biomarkers were stable in HC during the one-year follow-up from T0 to T3.

Conclusion: A panel of biomarkers of Alzheimer's and neurodegeneration show no differences between patients with BD and HC. There were abnormalities of amyloid production/clearance during an acute BD episode. The abnormalities mimic the pattern seen in AD namely decreasing CSF Aβ42 and may suggest an association with brain amyloidosis.

Keywords: Cerebrospinal fluid, bipolar disorder, amyloid, tau, longitudinal, case-control, neurofilament light

INTRODUCTION

Bipolar disorder, illness progression and cognitive impairment

Bipolar affective disorder (BD) is a disabling mental illness with a prevalence of 1-2% corresponding to 40.000 individuals in Denmark, a high risk of recurrence of manic and depressive episodes, a lifelong elevated risk of suicide (Kessing & Andersen, 2005), a substantial heritability of 60-80% (Miret, Ayuso-Mateos, Sanchez-Moreno, & Vieta, 2013), and a 10-15 years shorter life expectancy than the general population (Kessing, Vradi, & Andersen, 2015; Kessing, Vradi, McIntyre, & Andersen, 2015; Laursen, 2011). BD may be conceptualized as a progressing disorder with increasing risk of recurrence for every new affective episode, progressive shortening of inter-episode intervals with each recurrence (Kessing & Andersen, 2017). Long-term population based studies suggest that patients with BD have a higher risk of developing age-related dementia as compared with the background population as revealed by our group (Kessing & Andersen, 2004; Kessing & Nilsson, 2003) and others (Cooper & Holmes, 1998), and confirmed in an independent meta-analysis (da Silva, Goncalves-Pereira, Xavier, & Mukaetova-Ladinska, 2013). An association

between BD and cognitive impairment during euthymia has repeatedly been described (Bourne et al., 2013). Global or selective cognitive impairments are prevalent among 50-70% of patients with BD during clinical remission (Bora, 2018; Bora & Ozerdem, 2017; Jensen, Knorr, Vinberg, Kessing, & Miskowiak, 2016; Russo et al., 2017; Szmulewicz, Valerio, & Martino, 2019). The cognitive impairments emerge early, among younger newly diagnosed adults (Kjaerstad et al., 2019) and middle-aged patients with BD (Arts, Jabben, Krabbendam, & van Os, 2008; Cullen et al., 2019), and they are robustly associated with impaired functioning (Jimenez-Lopez et al., 2019; Mora, Portella, Forcada, Vieta, & Mur, 2013; Sparding et al., 2015).

Although considerable progress has been made over the past two decades, our understanding of the biological nature of illness progression in BD remains limited (Burdick et al., 2019).

Amyloid beta (Aβ)42 in cerebrum, cerebrospinal fluid (CSF), and plasma in Alzheimer's disease (AD)

Severe cognitive deficits are characteristic for AD, which is the most common cause of dementia. The neuropathology of AD is characterized by abnormal accumulation of Aβ peptides in plaques and neurofibrillary tangles in cerebrum as well as neurodegeneration that affect several neurotransmitter systems (Obrocki et al., 2020). Core AD biomarkers are low levels of CSF Aβ42 and high levels of CSF total tau (t-tau) and hyperphosphorylated tau (p-tau) (Jack et al., 2013; Lleo et al., 2019; Simonsen et al., 2017). Interestingly, the level of CSF Aβ decreases with age even among healthy individuals between the age of 18 and 80 years (Paternico et al., 2012; Popp et al., 2010). Accordingly, as indicated by amyloid imaging, Aβ42 load even at levels below those seen in typical AD has been associated with neural alterations in younger and middle-aged healthy individuals (Kennedy et al., 2012; Rieck, Rodrigue, Kennedy, Devous, & Park, 2015; Rodrigue et al., 2012). Thus, among healthy individuals, a dose-response relationship of Aβ42 load to neural

function has been found beyond the effects of age (Kennedy et al., 2012). Although Aβ plaques may play a key role in AD pathogenesis, the severity of cognitive impairment correlates better with CSF tau levels and the burden of neocortical neurofibrillary tangles (Roda, Montoliu-Gaya, & Villegas, 2019) that are mainly composed of hyperphosphorylated tau (p-tau) in patients with AD (Ross & Poirier, 2004). The pathological process has not yet been clarified, although dysfunctional transport of Aβ across the blood-brain barrier appears to be integral to disease development (Versele et al., 2020; Zetterberg et al., 2014).

The biological nature of cognitive impairment in bipolar disorder

As a possible link between AD and affective disorders pathophysiology, several studies have investigated CSF Aβ42 and plasma Aβ42 levels in patients suffering from major depression, as recently reviewed (Abbasowa & Heegaard, 2014). Despite inconsistencies that are partly ascribed to the application of different assay formats (Mattsson et al., 2013), study results indicate a potentially altered Aβ metabolism in affective disorder. Only two cross sectional studies have investigated Aβ42 in patients with BD versus healthy control (HC) individuals in younger adults finding higher ratios of CSF isoforms Aβ42/38 and Aβ42/40 in patients with BD in remission compared to HC individuals (Jakobsson et al., 2013) and lower plasma Aβ42 and higher Aβ40/42 ratio in patients with bipolar depression compared to HC, which are similar to findings in AD (Piccinni et al., 2012). A recent review with network analyses of AD-related biomarkers found lower biomarker levels in AD, compared to intermediary levels in patients with depression and higher levels in control individuals (Tang et al., 2019).

Cerebrospinal fluid biomarkers of neurodegeneration in patients with bipolar disorder

A recent systematic review from our group found that a line of biomarkers related to neurodegeneration, including p-tau and t-tau, neurofilament light (NF-L) (Jakobsson et al., 2014; Kern et al., 2018), and S100B (Jakobsson et al., 2014; Kroksmark & Vinberg, 2018), showed statistically significant differences between patients with BD and healthy control individuals (HC) in cross-sectional studies (Knorr et al., 2018).

NF-L is a nerve cell body synthesized protein subunit of neurofilaments that provide stability to axons. Increased NF-L levels in CSF and plasma has been proposed as a marker of neuroaxonal injury (Osborn et al., 2019; Petzold, 2005). NF-L relates to cognition among non-demented older adults albeit with small to medium effects (Murray et al., 2012) and plasma NF-L shows promise as an accessible biomarker with relevance to cognition in mild cognitive impairment (Osborn et al., 2019).

Neuronal protein neurogranin (NG) has been associated with changes in cognitive function in patients with mild cognitive impairment without AD. High CSF NG concentrations likely reflect synaptic dysfunction and CSF NG is associated with Aβ plaque pathology (Kvartsberg et al., 2018; Portelius et al., 2018). However, studies of CSF NG in patients with BD are lacking.
S100B is a calcium-binding protein located in glial cells that is related to blood-brain-barrier damage (Loftis et al., 2018). Several studies found elevated serum S100B in patients with acute affective episodes in comparison to HC (Kroksmark & Vinberg, 2018).

Finally, the ε4 allele of apolipoprotein E (*APOEε4*) is a well-established risk factor for dementia in AD (Kerr et al., 2016). However, *APOE* isoforms possible contribution to the risk of cognitive deterioration in BD has only been sparsely evaluated in older patients with BD (Kerr et al., 2016).

These studies are compelling and to unravel possible risk relations and causalities between affective disorder and AD and to determine how biomarkers for dementia-related disorders and

neurodegeneration change over time, and are associated with affective symptomatology, we need a prospective, longitudinal study, where central and peripheral biomarker levels are quantified.

The aims of the study

We present a prospective, longitudinal study with repeated measures of CSF and blood biomarkers during initial euthymia (T0), a subsequent affective episode (T1), during post-episode euthymia (T2) and after a one-year follow-up (T3) in patients with BD and HC individuals. This design allows monitoring of the dynamic changes in biomarkers according to disease-specific states of BD that is euthymic, depressive or (hypo)manic states.

The study aimed at investigating the effect of a new affective episode on biomarkers for dementia and neurodegeneration in CSF and blood in patients with BD. Furthermore, the study aimed to explore possible differences in biomarkers for dementia and neurodegeneration in CSF and blood in patients with BD compared to HC individuals.

Hypotheses

The levels of CSF Aβ42 decrease in patients with BD with (BD-E) compared to patients without (BD-NE) a new affective episode during a one-year follow-up (T0-T3).

When euthymia re-occurs after an affective episode (T2) the levels of CSF Aβ42 tend to normalize compared to pre-episode levels (T0), however not fully in BD-E.

METHODS AND MATERIALS

The ABETA study, conducted from April 2014 through April 2017, included adult patients with BD and HC individuals in a prospective, longitudinal case-control study with repeated measurements of dementia disorder-related biomarkers during a one-year follow-up.

The study includes a total of 86 patients with BD in a remitted state, aged 18-60 years and, at the time of inclusion admitted to The Copenhagen Mood Disorder Clinic, which covers an area of 1.6 million people an all psychiatric centers in the Capital Region of Denmark. The diagnoses of BD were confirmed according to Schedules for Clinical Assessment in Neuropsychiatry interview (Wing et al., 1990). Remission was defined as scores below 8 on both the Hamilton Depression Rating Scale 17-items (HAMD) and the Young Mania Rating Scale (YMRS) (Bech, Kastrup, & Rafaelsen, 1986). Further, 44 healthy, age and gender matched control individuals, were recruited via the Danish Donor Register, Frederiksberg Hospital.

Standardized fasting tests were assessed for biomarkers in CSF, plasma and serum. Patients were followed prospectively for a year with accurate assessment of mood episodes on a weekly basis. As expected, 50 % of the patients experienced a moderate to severe (HAMD or YMRS > 13 for two weeks) affective episode during the follow-up period and these patients have given repeated CSF, plasma and serum samples. Additionally, CSF, plasma and serum sampling were repeated following remission (T2) and finally after a year (T3) in both patients with BD and HC.

The study complies with the Helsinki Declaration and was approved by the local ethics committee (H6-2014-006) and The Danish Data Protection Agency (J.nr: 2014-58-0015). The study is reported according to the STROBE Statement (von Elm et al., 2014).

Biochemical analyses

All biochemical analyses were performed at the Clinical Neurochemistry Laboratory in Mölndal, Sweden, by experienced and board-certified laboratory technicians who were blind with respect to the clinical information. The CSF concentrations of Aβ42, Aβ40, Aβ38, sAPPα and sAPPβ, and were determined using the MSD sAPPα/sAPPβ Multiplex Assay and MSDHuman/Rodent (4G8) Abeta-Triplex Assay, respectively, as described by the manufacturer (Meso Scale Discovery, Gaithersburg, MD, USA). CSF concentrations of p-tau, t-tau, and Aβ1-42 were measured simultaneously by the Luminex xMAP technology using the Inno-Bia AlzBio3 kit (Innogenetics, Zwijndrecht, Belgium). The inter-assay coefficient of variability was 8% (sAPPα), 20% (sAPPβ), 2% (Aβ38), 15% (Aβ40), and 13% (Aβ42). The intra-assay coefficient of variability was below 10% for all biomarkers.

CSF Aβ42, 40 and 38 concentrations were measured using V-plex Peptide Panel 1 Kits Aβ38, Aβ40, Aβ42 (Meso Scale Discovery system, Rockville, MD, USA) according to the manufacturer's protocol. In short, 60 mL of CSF was diluted 2-fold in diluent provided with the kit and calibrators and controls were prepared according to protocol. On a pre-coated 96 well MSD plates, 25 mL of sample, calibrator or controls were added followed by addition of 25 mL of detection antibody and incubated at room temperature for two hours. Lastly, the plates were washed in washing buffer provided with the kit and read in an MSD imager at relevant wavelength.

CSF sAPPα and sAPPβ were measured using Human sAPPα and Human sAPPβ kits, respectively (IBL, Japan) according to the manufacturer's protocol. In short, 100 mL of 1:40 diluted CSF, controls and standards were added to wells of anti-human sAPPα or sAPPβ antibody pre-coated microtiter plates and incubated over night at 4°C. Following incubation, 100 mL HRP-conjugated detection antibody was added to the wells. The reaction was developed with TMB chromogen solution, and subsequently stopped with 1M sulfuric acid. The plate was quantified at 450 nm in a microplate reader.

CSF t-tau was measured by the hTAU Ag ELISA assay (INNOTEST, Fujirebio, Japan) according to the manufacturer's protocol. In short, to an anti-human tau antibody pre-coated 96 well microtiter plate 25 mL samples, controls and standards ranging from 50-2500 pg/mL were added followed by incubation with a biotinylated detection antibody to tau followed by peroxidase-conjugated streptavidin. The reaction was developed with tetramethyl benzidine (TMB) chromogen solution and stopped with 0.9M sulfuric acid. The plate was quantified at 450 nm in a microplate reader.

CSF p-tau was measured by the PHOSPHO-TAU (181p) ELISA assay (INNOTEST, Fujirebio, Japan) according to manufacturer's protocol. In short, on an anti-human p-tau antibody pre-coated 96 wells microtiter plate 75 mL samples, controls and standards ranging from 15.6-1000 pg/mL were added followed by incubation with a biotinylated detection antibody to p-tau and addition of peroxidase-conjugated streptavidin. The reaction was developed with TMB chromogen solution, and subsequently stopped with 0.9M sulfuric acid. The plate was quantified at 450 nm in a microplate reader.

CSF NF-L concentration was measured using NF-light ELISA kit (IBL international, Hamburg, Germany) by following the manufacturer's protocol. In short, to an anti-human NF-L antibody pre-coated 96 well microtiter plate 100 mL samples, controls and standards ranging from 100-10000 pg/mL were added followed by incubation with a biotinylated detection antibody to NF-L followed by peroxidase-conjugated streptavidin. The reaction was developed with TMB chromogen solution and stopped with stop reagent included in the kit. The plate was quantified at 450 nm in a microplate reader.

CSF NG concentration was measured using a previously published in-house Meso Scale Discovery assay (De Vos et al., 2015). In short, a 96 well QUCIKPLEX microtitter plates (MSD, Rockville, MD, USA) was coated with Ng7 antibody diluted in PBS over night at room temperature.

Following, the plates was blocked with MSD blocking solution A (MSD), and washed in PBS. To the plates 50 ul of samples, controls and standards ranging from 31.3-4000 pg/mL was added in addition to 50 ul/well of primary antibody (ab23570, Upstate Biotechnology, NY, USA). After washing a MSD detection Antibody (Goat anti-rabbit sulfotag, MSD) was added, and the plate was lastly read with MSD Read Buffer T with surfactant in a QUICKPLEX SQ 120 reader (MSD).

Plasma Aβ42 and Aβ40 concentrations were measured using a commercial Single molecule array (Simoa) assay on an HD-1 Analyzer according to instructions from the kit manufacturer (Quanterix, Billerica, MA). Plasma t-tau concentration was measured using a commercial Single molecule array (Simoa) assay on an HD-1 Analyzer according to instructions from the kit manufacturer (Quanterix, Billerica, MA). Plasma NF-L concentration was measured using an in-house Single molecule array (Simoa) assay on an HD-1 Analyzer (Quanterix, Billerica, MA), as previously described in detail (Gisslen et al., 2016). Serum S100B was measured using a commercial kit with electrochemiluminescence detection on an Elecsys instrument (Roche Diagnostics, Penzberg, Germany) (Jakobsson et al., 2014). APOE*4 carrier status of the participants was established by analyses of 2 µL DNA, as described elsewhere (Koch et al., 2002).

Statistical analyses

Data was analyzed according to a preestablished statistical analysis plan. All analyses were conducted with SAS software, version 9.4, (Copyright © 2013, SAS Institute Inc., Cary, NC, USA). The primary endpoint was the effect of an affective episode on CSF Aβ42 in patients with BD measured as differences in changes in CSF Aβ42 between patients with BD with and without an affective episode during a one-year follow-up. Secondary outcomes were differences in changes in Aβ38, Aβ40, Aβ 42/38, Aβ42/40, sAPPα, sAPPβ, t-tau, p-tau, p-tau /t-tau, NF-L, NG in CSF and

further Aβ40, Aβ 42, Aβ42/40, t-tau, NF-L in plasma, and finally in S100B in serum between patients with BD with and without an affective episode during a one-year follow-up. The primary outcome was defined as statistically significant if the Bonferroni-adjusted p-value was < 0.05 in analyses corrected for potential confounders. All *p*-values for secondary outcomes were corrected for multiple testing using the Benjamini & Hochberg procedure (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001). We applied a conservative cut-off for the false discovery rate at 0.05, which limits the rate of false positives among the reported findings to one in twenty, so that an adjusted *p*-value ≤ 0.05 was considered statistically significant. Biomarkers CSF NF-L, CSF NG, CSF t-tau, plasma NF-L and serum S100B were found to have a skew distribution and were therefore log-transformed prior to analysis.

The flowchart of the study has been reported elsewhere (Knorr et al., 2019).

Demographic and clinical data

Demographic and clinical data at timepoints T0, T2 and T3 were summarized in numbers and percentages (categorical data), means and standard deviations (normally distributed continuous data) and medians and quartiles (non-normally distributed continuous data). Comparisons of BD and HC at T0 and T3 was made using Fisher's exact test, Welch' t-test or the Mann-Whitney U-test, whichever was most appropriate.

Biomarkers in BD and HC at baseline and at the one-year follow-up

To compare biomarker levels between BD and HC a linear mixed model was applied with time (T0 or T3) and group (BD or HC) as fixed effects and with an unstructured covariance to account for correlation between the repeated measurements on the study participants. The analyses were

performed in three versions: Version 1: No adjustment for potential confounders, Version 2: Adjusted for gender, age, *APOE*-status, Version 3: Adjusted for gender, age, *APOE*-status, alcohol, smoking, and years of education. Estimated differences between BD and HC are reported for biomarker levels at T0, biomarker levels at T3, and change in biomarker level from T0 to T3.
The analyses were repeated with further stratification of BD into the participants who either had or had not experienced an episode during follow-up.
Internal validity of the measured biomarkers was evaluated by comparing their levels at T0 and T3 in HC, hence investigating with-person trajectories of CSF and blood biomarkers for Alzheimer's disease in the sample during a one-year follow-up.

Patients with BD, who had an affective episode during follow-up

A subgroup analysis was performed to evaluate changes in biomarker levels in patients with BD who had experienced an affective episode during follow-up. To this end a linear mixed model with timepoint (T0, T1, T2, T3) as fixed effect and an unstructured covariance was applied. Estimates were reported for changes between the time-points. The analysis was performed in two versions. Version 1: No adjustment for potential confounders, Version 2: adjusted for gender, age, alcohol, smoking, years of education, *APOE*-status, and the three mood-stabilizers lithium, quetiapine, and lamotrigine.

Analyses of co-variates

Potential predictors (gender, age, *APOE* status (carriers of the *APOEε4* allele yes/no), daily units of alcohol, numbers of cigarettes smoked a day, years of education, lithium (yes/no), antipsychotics (yes/no), antiepileptics (yes/no)) of the 18 biomarkers in BD-E at follow-up were evaluated in a

linear mixed model, first in univariate analyses with adjustment for time (T0-T3) and in a multivariate analysis with mutual adjustment.

Sensitivity analyses

All analyses were repeated including and excluding outliers. This did not alter the results to any significant extent. We report data including outliers.

RESULTS

Patients with BD and the HC individuals were well matched for age and gender and there were no statistically significant differences between the groups regarding years of education, BMI, and any of the dementia and neurodegeneration-related biomarkers both at baseline (T0) and follow-up (T3), Table 1. At T0 alcohol consumption did not differ between patients with BD and HC but at T3 the patients with BD consumed less alcohol compared to HC individuals. Smoking was more frequent among patients with BD compared to HC individuals at both T0 and T3. Figure 1 presents the flowchart of the study.

Insert Figure 1 somewhere around here

Insert Table 1 somewhere around here

We found no statistically significant differences between patients with BD and HC individuals at T0 and T3 for any of the 18 outcomes in mixed models: Aβ42, Aβ40, Aβ38, Aβ42/38, Aβ42/40, sAPPα, sAPPβ, t-tau, p-tau, p-tau /t-tau, NF-L, NG in CSF and further Aβ40, Aβ42, Aβ42/40, t-tau,

NF-L in plasma, S100B in serum, and *APOE*-status. Furthermore, all 18 biomarkers were stable in HC individuals during the one-year follow-up from T0 to T3, Table 2 and Figure 2.

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During the one-year follow-up (T0-T3) we found statistically significant *decreasing* levels of biomarkers in BD-E compared to BD-NE regarding the primary outcome CSF Aβ42 (-70.57, 95% CI -114.97 - -26.16, unadj. *p*-value 0.002, adj. p-value 0.018). Similar trends were found in CSF Aβ40, CSF Aβ38, CSF sAPPβ, and CSF t-tau, although none of these findings were significant after adjustment for multiple testing, Table 3. Furthermore, levels of CSF p-tau, and CSF NG *increased* in BD-E compared to BD-NE. Levels in BD-E compared to BD-NE during the one-year follow-up were stable for all other biomarkers: CSF Aβ42/38, CSF Aβ42/40, CSF sAPPα, CSF p-tau/t-tau, CSF NF-L, plasma Aβ42, plasma Aβ40, plasma Aβ42/40 ratio, plasma tau, plasma NF-L, and serum S100B. In subgroup analyses of patients with BD with (BD-E) or without (BD-NE) an affective episode during follow-up the mean plasma NF-L levels tended to be higher in BD-E compared to HC at both T0 and T3.

Insert Table 3 somewhere around here

In patients with an episode during follow-up CSF t-tau increased from T2 to T3, although results were not statistically significant after adjustment for multiple testing (18.68, *p*-value 0.018 (adj. *p*-value 0.491)). We found no statistically significant differences between any of the timepoints T0, T1, T2 or T3 regarding all other CSF, plasma and serum markers. We found no significant

differences in biomarker levels at the four timepoints and no differences in changes between the four timepoints in patients with BD that had a (hypo)manic relapse compared to patients with a depressive relapse, regarding any of the 18 markers in both corrected and adjusted analyses.

Regarding covariates, CSF NF-L increased significantly with age in both univariate and multivariate analyses (adjusted-*p*-value <0.0001 in both analyses). Otherwise, no effect on any of the biomarkers was found for the investigated predictors: gender, age, *APOE* status, duration of illness, number of months in either depression or (hypo)mania, bipolar type 1 or 2, antiepileptics, antipsychotics, lithium, prior psychosis, smoking or alcohol consumption. Notably, none of the potential predictors had a statistically significant effect on CSF Aβ42.

Finally, levels of plasma Aβ42 showed no statistically significant correlation (Pearson) with levels of CSF Aβ42.

DISCUSSION

This is the first longitudinal study ever with repeated CSF measures among patients with BD with and without a prospectively assessed mood episode and healthy control individuals. The study followed international consensus recommendations for CSF collection and analyses (del Campo et al., 2012; Hansson et al., 2018) . Levels of CSF amyloid beta (Aβ)42 *decreased* in patients with BD who had an episode during follow-up (BD-E) and *increased* in patients without an episode (BD-NE) during the one-year follow-up. Levels of CSF phosphorylated at amino acid 181 (p-tau) *increased* in BD-E and *decreased* in BD-NE.

Firstly, these results show, biological underpinning of the increased risk of Alzheimer's disease (AD) in patients with BD. We found differences in changes in biomarkers between patients who

developed an episode during follow-up and patients with BD who continued to be in a euthymic state throughout the one-year follow-up that mimic the pattern seen in AD, hence decreasing CSF Aβ42 and increasing CSF p-tau.

Secondly, we found no trait markers since we saw no statistically significant differences between patients with BD and HC regarding 18 biomarkers related to dementia and neurodegeneration although plasma NF-L was borderline statistically significantly increased in patients with BD compared to HC in models not adjusted for multiple testing. Higher levels of plasma NF-L in BD are in line with previous findings (Al Shweiki et al., 2019). We found no statistically significant differences in any biomarker levels in patients with BD between the four timepoints (T0, T1, T2 and T3).

Notably, the median age in our study was 33 years (quartiles (26; 42) and our study confirmed prior findings of no differences between younger patients with BD compared to HC regarding CSF markers of Aβ42, Aβ40, Aβ38, APPα, t-tau and p-tau (Jakobsson et al., 2013). CSF levels of t-tau, p-tau and NF-L have recently been shown to increase 2% per age year among cognitively unimpaired control individuals (25). Thus, these findings may suggest that possible age-related changes do not follow the same pace during the human lifespan. Thus, an Alzheimer signature does not apply to younger patients with BD as such. However, in a sample of patients with late life-bipolar disorder with mild cognitive impairment higher levels of p-tau and lower levels of CSF Aβ42 were reported in patients compared to HC (Forlenza et al., 2016). Our finding of decreased CSF Aβ42 and increased p-tau related to relapse of a new affective episode implies a link between BD and the increased risk of AD in patients with BD. During an affective episode there might be an increased influx of Aβ42 from CSF to the brain which reflects brain Aβ accumulation (Mattsson et al., 2014).

We did not confirm prior findings of higher levels of CSF NF-L in patients with BD compared to HC (Jakobsson et al., 2014), which may be related to the risk of false discovery due to several outcomes in that study too. Additionally, CSF NG levels did not separate between patients with BD and HC individuals in our study.

The main strengths of the study are the highly systematic design, the well characterized groups of patients and HC including stringent diagnoses, and the intense follow-up by a specialist of psychiatry with weekly assessments of mood states that captured new affective episodes. The patients with BD and HC individuals were well matched. Furthermore, all biochemical analyses were performed in a well estimated laboratory with the same assay lots and technicians with experience in handling CSF and blood biomarkers of neurodegeneration. Additionally, internal validity of the biomarkers was high since all biomarkers were stable during the one-year follow-up in HC individuals. The statistical analyses were reported with respect to the false discovery rate.

Limitations were the relative short time of follow up. With a longer follow up period the effect of multiple relapses could have been estimated. The patients were appointed to a specialty clinic regarding treatment of bipolar disorders and the close follow up may have prevented more relapses. However, the dropout rate was low.

In conclusion, decreasing CSF Aβ42 and increasing CSF p-tau during an affective relapse may be a biological underpinning of the progressive nature of bipolar disorder with increased risk of Alzheimer's disease, eventually.

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Disclosures

Authors UK, AHS, CSJ, MA, JF and SGH declare no conflicts of interests. HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.KB has served as a consultant or at advisory boards for Alzheon, BioArtic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Pfizer, and Roche Diagnostics. HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. LVK has within the preceding three years been a consultant for Lundbeck.

LEGENDS AND FOOTNOTES

Table 1

Legend: Clinical characteristics for patients with bipolar disorder and healthy control individuals.

Footnotes: Abbreviations: BD: Patients with bipolar disorder. HC: Healthy control individuals. QR = Quartiles 0, 25, 50, 75, 100%. HAMD[17] = Hamilton Depression Rating. DUI: Duration of bipolar disorder. [1]Fisher's exact test, [2]data log-transformed, *p*-values are t-tests of medians are the same. [3]Mann-Whitney U-test. [4]Welch t-test.

Table 2.

Legend: Biomarkers of dementia and neurodegeneration in patients with bipolar disorder and healthy control individuals.

Footnotes: Abbreviations: BD: Patients with bipolar disorder. HC: Healthy control individuals. QR = Quartiles 0, 25, 50, 75, 100%. [1]Fisher's exact test. [2]data log-transformed, *p*-values are t-tests of medians are the same. [4]Welch t-test.

Table 3.

Legend: Levels of cerebrospinal fluid biomarkers during a one-year follow-up in patients with bipolar disorder that have a new affective episode compared to patients that do not and healthy control individuals.

Footnote: Version 1 is un-corrected, Version 2 is corrected for gender, age and APOE* status (no change from V1 therefore not reported), Version 3 is corrected for gender, age, smoking, alcohol consumption and years of education. The number in any of the *b*-columns is an estimate of the difference between the difference in median outcome between follow-up and baseline among the groups compared; E: Patients with BD with an episode, NE: Patients with BD with no episode, HC: Healthy control individuals.

An example of the interpretation of a row of one of the log-transformed outcomes: '(NE T3/T0)/(HC T3/T0)' concerns the HC's and NE's at T0 and T3. Because the outcome is log-transformed, the interpretation is the following: the number in any of the b-columns is an estimate of [how much larger the median outcome among NE's is at T3 relative to T0] divided by [how much larger the median outcome among HC's is at T3 relative to T0]. I.e. it is an estimate of how much larger the rise in outcome-level is among NE's relative to among HC's.

Figure 1.

Legend: Flowchart for the Bipolar ABETA Follow-up Study

Figure 2.

Legend: Visualization of trends regarding mean values of 18 biomarkers for dementia and neurodegeneration in patients with (BD-E) and without (BD-NE) an episode, and healthy control individuals (HC) at four timepoints during a one-year follow-up.

Table 1	BD baseline (T0)	HC baseline (T0)	*p*-value	BD after an episode (T2)	BD follow-up (T3)	HC follow-up (T3)	*p*-value
N (% female)	85 (50%)	44 (53%)	0.85[1]	33 (45%)	70 (52%)	39 (53%)	1[1]
Age median (Q1; Q3)	33 (26; 42)	30 (25; 42)	0.71[4]	34 (26; 42)	35 (26; 42)	30 (25;42)	0.65[4]
Educational years, mean (SD)	6.09 (2.9)	5.98 (2.32)	0.64[3]	5.81 (2.81)	6.21 (2.9)	5.95 (2.28)	0.29[3]
BMI mean (SD)	25.4 (4.6)	24.9 (3.36)	0.46[4]	24.9 (3.9)	25.8 (4.9)	25.7 (3.7)	0.9[4]
Daily alcohol consumption, N units, median (Q1; Q3)	0.2 (0; 1)	0.5 (0; 1)	0.44 [3]	0.1 (0; 0.57)	0.22 (0.02; 1)	1 (0.25; 1.3)	0.02[3]
Smokers, N, (%)	28 (34%)	8 (18%)	0.096[1]	14 (42%)	25 (34%)	8 (20%)	0.132[1]
Daily cigarettes, median (min; max)	14.5 (0.2;20)	3.5 (0.5;30)	0.026[2]	16 (5;20)	12 (0.5;35)	2.5 (0.1;20)	0.032[2]
Bipolar type I, N, (%)	48 (58%)			19 (58%)	42 (60%)		
Bipolar type II, N, (%)	35 (42%)			14 (42%)	28 (40%)		
DUI, N years, median (Q1; Q3)	26 (12; 60)			36 (18; 60)	27.5 (12; 60)		
Prior psychosis, N (%)	36 (43%)			13 (39%)	30 (42%)		

Clinical global impression median (Q1; Q3)	5 (4;5)		
Lithium, N (%)	41 (49%)	18 (54%)	38 (54%)
Antipsychotics, N (%)	34 (39%)	19 (57%)	25 (35%)
Anticonvulsants, N, %	39 (46%)	23 (69%)	35 (50%)
Antidepressants, N, %	2 (2.5%)	1 (3%)	3 (4%)
Benzodiazepines N, %	6 (7%)	7 (21%)	9 (13%)

Table 2	BD baseline (T0)	HC baseline (T0)	*p*-value	BD after an episode (T2)	BD follow-up (T3)	HC follow-up (T3)	*p*-value
CSF Aβ42 mean (SD)	599 (177)	631 (157)	0.34[4]	544 (142)	589 (192)	636 (154)	0.26[4]
CSF Aβ40 mean (SD)	5665 (1602)	5888 (1340)	0.46[4]	5093 (1161)	5600 (1623)	5965 (1433)	0.33[4]
CSF Aβ38 mean (SD)	2212 (676)	2291 (589)	0.54[4]	1986 (516)	2193 (735)	2329 (589)	0.4[4]
CSF Aβ42/Aβ38 mean (SD)	0.27 (0.02)	0.28 (0.02)	0.37[4]	0.27 (0.01)	0.27 (0.03)	0.27 (0.02)	0.52[4]
CSF Aβ42/Aβ40 mean (SD)	0.11 (0.01)	0.11 (0.01)	0.54[4]	0.11 (0.01)	0.1 (0.01)	0.11 (0.01)	0.4[4]
CSF sAPPα mean (SD)	272 (109)	295 (112)	0.33[4]	220 (62)	268 (124)	298 (117)	0.32[4]
CSF sAPPβ mean (SD)	569 (191)	596 (207)	0.51[4]	468 (130)	552 (219)	613 (216)	0.26[4]
CSF t-tau mean (SD)	205 (82)	195 (73)	0.55[4]	174 (61)	201 (87)	195 (73)	0.78[4]
CSF p-tau mean (SD)	35 (11)	33 (8)	0.36[4]	29 (7)	32 (10)	33 (9)	0.72[4]
CSF p-tau/t-tau mean (SD)	0.18 (0.05)	0.18 (0.04)	0.66[4]	0.18 (0.04)	0.17 (0.04)	0.18 (0.03)	0.91[4]
CSF NF-L median (Q1; Q3)	332 (246; 479)	354 (214; 566)	0.65[2]	341 (245; 439)	341 (230; 524)	375 (243; 612)	0.78[2]
CSF NG median (Q1; Q3)	168 (139; 194)	171 (125; 216)	0.9[2]	149 (135; 179)	152 (121; 201)	165 (118; 214)	0.72[2]

Plasma Aβ42 mean (SD)	10.8 (2.5)	10.2 (2.1)	0.16[4]	11.2 (2.4)	10.6 (2.1)	10.5 (2.2)	0.79[4]
Plasma Aβ40 mean (SD)	224 (56)	215 (51)	0.37[4]	236 (49)	229 (49)	214 (40)	0.09[4]
Plasma Aβ42/Aβ40 mean (SD)	0.04889 (0.01013)	0.04808 (0.00834)	0.91[4]	0.04771 (0.00732)	0.04667 (0.00718)	0.04872 (0.00854)	0.3[4]
Plasma t-tau mean (SD)	3.04 (0.95)	2.82 (0.88)	0.19[4]	3.36 (0.99)	3.03 (0.99)	3.08 (0.99)	0.8[4]
Plasma NF-L median (Q1; Q3)	6.81 (4.97; 9.07)	5.73 (4.5; 7.84)	0.08[2]	7.66 (5.7; 10.35)	6.21 (5.25; 10.15)	6.81 (4.4; 8.42)	0.32[2]
Serum S100B median (Q1; Q3)	0.04 (0.03; 0.05)	0.04 (0.03; 0.05)	0.61[2]	0.04 (0.03; 0.05)	0.04 (0.03; 0.05)	0.04 (0.03; 0.06)	0.64[2]
APOE*4 N (%)	34 (41%)	11 (24%)	0.08[1]	12 (36%)	24 (34%)	11 (28%)	0.67[1]

Table 3	*B*	Version 1 *b1*	P-value (adj.)	Lower Upper	Version 2 *b2*	P-value (adj.)	Lower Upper	Version 3 *b3*	P-value (adj.)	Lower Upper
CSF Aβ42	(E T3-T0) - (NE T3-T0)	-66.61	0.004 (0.036)	-110.50 -22.71	-67.60	0.003 (0.027)	-111.44 -23.75	-70.57	0.002 (0.018)	-114.97 -26.16
	(E T3-T0) - (HC T3-T0)	-25.47	0.234 (1.00)	-67.79 16.86	-25.47	0.221 (1.00)	-68.44 16.10	-27.93	0.197 (1.00)	-70.76 14.90
	(NE T3-T0) - (HC T3-T0)	41.14	0.031 (0.279)	3.88 78.41	41.43	0.03 (0.27)	4.20 78.67	42.63	0.024 (0.519)	5.77 79.50
CSF Aβ40	(E T3-T0) - (NE T3-T0)	-556.29	0.002 (0.214)	-904.71 -207.86	-563.45	0.002 (0.214)	-911.26 -215.64	-599.90	0.001 (0.214)	-936.28 -263.52
	(E T3-T0) - (HC T3-T0)	-163.95	0.333 (0.855)	-499.80 171.91	-168.93	0.318 (0.847)	-507.07 166.21	-185.25	0.259 (0.826)	-509.90 139.40
	(NE T3-T0) - (HC T3-T0)	392.34	0.01 (0.339)	96.44 688.24	394.52	0.01 (0.339)	98.97 690.07	414.65	0.004 (0.266)	135.83 693.47
CSF Aβ38	(E T3-T0) - (NE T3-T0)	-244.62	0.002 (0.214)	-395.82 -93.41	-246.99	0.002 (0.214)	-397.62 -96.36	-255.69	0.001 (0.214)	-408.91 -102.47
	(E T3-T0) - (HC T3-T0)	-91.76	0.213 (0.826)	-237.50 53.97	-92.89	0.206 (0.826)	-238.02 52.23	-95.84	0.200 (0.826)	-243.60 51.91
	(NE T3-T0) - (HC T3-T0)	152.85	0.02 (0.503)	24.39 281.31	154.10	0.019 (0.491)	26.06 282.13	159.84	0.015 (0.423)	32.66 287.03
CSF t-tau	(E T3-T0) - (NE T3-T0)	-33.94	0.002 (0.214)	-54.63 -13.25	-33.98	0.002 (0.214)	-54.66 -13.31	-34.20	0.002 (0.214)	-55.43 -12.98
	(E T3-T0) - (HC T3-T0)	-10.58	0.294 (0.833)	-30.52 9.37	-10.48	0.297 (0.833)	-30.41 9.45	-10.40	0.314 (0.847)	-30.87 10.06
	(NE T3-T0) - (HC T3-T0)	23.36	0.01 (0.339)	5.77 40.96	23.50	0.01 (0.339)	5.93 41.08	23.80	0.009 (0.339)	6.16 41.44
CSF p-tau	(E T3-T0) - (NE T3-T0)	0.90	0.008 (0.339)	0.83 0.97	0.89	0.007 (0.339)	0.83 0.97	0.89	0.005 (0.314)	0.82 0.97
	(E T3-T0) - (HC T3-T0)	0.96	0.268 (0.826)	0.89 1.03	0.96	0.248 (0.826)	0.88 1.03	0.96	0.252 (0.826)	0.89 1.03
	(NE T3-T0) - (HC T3-T0)	1.07	0.052 (0.697)	1.00 1.14	1.07	0.05 (0.678)	1.00 1.14	1.07	0.0037 (0.585)	1.00 1.15
CSF NG	(E T3-T0) - (NE T3-T0)	0.88	0.035 (0.567)	0.79 0.99	0.88	0.034 (0.563)	0.79 0.99	0.88	0.027 (0.519)	0.79 0.99

(E T3-T0) - (HC T3-T0)	0.93	0.202 (0.826)	0.83 1.04	0.93	0.201 (0.826)	0.83 1.04	0.93	0.181 (0.826)	0.83 1.04
(NE T3-T0) - (HC T3-T0)	1.05	0.29 (0.833)	0.96 1.16	1.05	0.282 (0.826)	0.96 1.16	1.06	0.252 (0.826)	0.96 1.16

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