Peripheral metabolites of the kynurenine pathway are decreased in serious mental illness and show phase-specific differences in bipolar disorder patients

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Research Article

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Abstract

Background

Previous studies have linked disturbances in the kynurenine pathway, responsible for the main catabolism of tryptophan and a key regulator of the immune system, to mental disorders such as major depressive disorder (MDD), bipolar disorder (BD) or schizophrenia (SCZ). However, the relationship between tryptophan catabolism and the presentation of psychiatric disorders seems to be rather complex, as up to now results have mostly been inconsistent or even contradictory. In this study, we measured plasma levels of tryptophan catabolites (TRYCATs: tryptophan, kynurenine, kynurenic acid and quinolinic acid) in a sample of in total 175 participants consisting of individuals suffering from an acute disease episode seeking inpatient treatment as well as healthy controls (HC) to investigate whether individual metabolites could serve as a biomarker for differential diagnosis.

Results

Significantly decreased levels of tryptophan, kynurenine, kynurenic acid and quinolinic acid were found in the patient group as a whole. This was mainly driven by the difference between BD patients and HC. Specifically, the manic symptom domain in manic and mixed phase BD patients displayed significantly lower kynurenine and kynurenic acid levels. We could not find significant differences between the psychiatric disorders disqualifying TRYCATs as biomarkers for differential diagnosis. None of the assessed potential demographic or pharmaceutical confounding factors revealed a significant correlation to TRYCAT concentrations. Upon reaching (partial) remission, the changes in TRYCAT levels partially normalized in the patient group.

Conclusions

Our data suggests an involvement of the kynurenine pathway in mental disorders, especially BD. Although we cannot prove a causal relationship, underlying mechanisms might include pro-inflammatory states in the central nervous system and/or increased neurotoxicity contributing to the immune assault. Also considering the manifold, but inconsistent previous analyses regarding TRYCAT concentrations in psychiatric disease, larger, cross-sectional and longitudinal studies will be needed for detangling the mystery about the role the tryptophan catabolism plays in the pathophysiology of mental disorders and for answering the burning question if it might constitute a possible therapeutic target in the future.

1. Background

Serious mental illness (SMI) is defined as a mental, behavioral or emotional health condition resulting in significant functional impairment in at least one aspect of everyday life (Sanchez et al., 2020). Most prevalent disorders include major depressive disorder (MDD), bipolar disorder (BD) or schizophrenia

(SCZ), which are a major contributor to global burden of disease and a frequent cause of disability (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). Even though a majority of patients at least in developed economies can be efficiently treated with psychopharmacotherapy as well as non-medication treatment, there are still more than 20% of difficult-to-treat patients whose condition cannot be sufficiently improved with the available treatment options (Howes et al., 2021). The development of novel psychotropic drugs is a costly and time-consuming process with only a minority of the compounds actually reaching the market. This can mainly be attributed to our still rather inadequate knowledge regarding the neurobiological pathomechanisms underlying mental disorders. Additionally, biomarkers for differential diagnosis and identification of subgroups or endophenotypes of specific disorders are not yet available in clinical routine.

In recent years, the existence of inflammatory or autoimmune related subtypes of mental disorders is increasingly recognized. Amongst others, disturbances in the kynurenine pathway, which is responsible for the main catabolism of tryptophan and constitutes a key regulator of the immune system, have been associated with SMI. See Fig. 1 for a simplified overview of the kynurenine pathway.

Many of the metabolites of the kynurenine pathway (tryptophan catabolites = TRYCATs) are neuroactive – with some showing neurotoxic properties (Guillemin, 2012, Kaindl et al., 2012) – and interfere with neurotransmitter systems exerting effects on NMDA receptor signaling and glutamatergic neurotransmission (Carpenedo et al., 2001, Stone and Perkins, 1981) that in turn are extensively studied in the etiology and treatment of psychiatric disorders (Ohgi et al., 2015). Several studies have linked altered levels of specific TRYCATs to patients suffering from different mental disorders, as recently reviewed by Savitz (Savitz, 2020). In BD disorder patients specifically, correlations between TRYCAT concentrations and particular features such as sex, severity of depressive symptoms and presence of manic or psychotic symptoms have been shown before (Platzer et al., 2017, Myint et al., 2007, Olsson et al., 2010, Mukherjee et al., 2018). However, the relationship between the kynurenine pathway and BD symptoms seems to be rather complex, as up to now results have mostly been inconsistent or even contradictory. The essential confounding factors responsible for the heterogeneity of results investigating this relationship remain unclear (Zhang et al., 2021).

The aim of this study was to investigate whether individual metabolites of the kynurenine pathway could serve as a biomarker for the differential diagnosis of SMI. Therefore, we analyzed plasma concentrations of several TRYCATs in an acute episode and after (partial) remission of patients with MDD, different phases of BD, and/or SCZ in comparison to healthy controls. Additionally, we assessed and controlled for different demographic, clinical and pharmaceutical features that could possibly influence TRYCAT concentrations.

2. Methods

2.1 Participants

The sample consisted of 175 participants in total: 56 suffering from BD (11 in a manic or hypomanic episode, 15 in a mixed episode and 30 in a depressed episode), 40 suffering from MDD in an acute episode, 39 from SCZ and 40 healthy controls (HC). For demographic and clinical details see Table 1. The patients were recruited upon their admission for inpatient treatment suffering from an acute episode of their illness at the Department of Psychiatry, Psychotherapy and Psychosomatic Medicine, University Hospital of Frankfurt. All patients fulfilled ICD-10 criteria for the respective disorder (evaluated by two specialists independently, SKS and AR). The severity of symptoms was measured using MADRS (Montgomery-Åsberg Depression Scale) (Schmidtke et al., 1988), YMRS (Young Mania Rating Scale) (Muehlbacher et al., 2011), as well as PANSS (Positive and Negative Syndrome Scale) (M. J. Mueller et al., 2000).

Table 1

Group differences in demographic data were calculated between HC, MDD, BD and SCZ regarding age, gender, educational level (university degree), BMI, smoking status, alcohol and illegal drug use using parametric or non-parametric statistical tests as appropriate. Corrected level of significance $p \leq 0.01$. SD: standard deviation; m: male; f: female; BMI: body mass index; AUDIT: Alcohol Use Disorders Identification Test; HC: healthy controls; MDD: major depressive disorder; BD: bipolar disorder; SCZ: schizophrenia.

Item	Diagnosis	N	Mean (SD)	p
Age	HC	37	42.86 (13.45)	0.361
	MDD	40	45.50 (14.23)	
	BD	56	41.00 (12.41)	
	SCZ	39	40.85 (14.69)	
Sex (m/f)	HC	22/18	0.260	
	MDD	15/25		
	BD	23/33		
	SCZ	21/18		
University degree	HC	14 (40.0%)	< 0.0001	
	MDD	7 (17.5%)		
	BD	17 (30.1%)		
	SCZ	4/39 (10.3%)		
BMI (kg/m^2)	HC	38	24.31 (3.26)	0.051
	MDD	40	27.03 (6.03)	
	BD	56	26.37 (4.61)	
	SCZ	39	27.18 (5.92)	
Nicotine use	HC	3 (7.8%)	< 0.0001	
	MDD	9 (22.5%)		
	BD	30 (53.6%)		
	SCZ	25 (64.1%)		
Alcohol use (AUDIT sum score)	HC	40	1.50 (0.75)	0.257
	MDD	40	2.20 (3.22)	
	BD	51	2.98 (4.20)	
	SCZ	39	3.08 (6.22)	

Item	Diagnosis	N	Mean (SD)	p
Illegal drug use	HC	1 (0.3%)	< 0.0001	
	MDD	14 (35.0%)		
	BD	20 (37.7%)		
	SCZ	26 (66.7%)		

For the patient groups, but not for HC, a second assessment was performed shortly before they were discharged; the mean duration of inpatient treatment was 30 days. During treatment and between the two measured time points (T1 and T2), all diagnostic groups showed significant improvement of symptoms as measured by MADRS, YMRS and PANSS (see Table 2). Patients were classified as responders to treatment if symptom severity scores were decreased by at least 50% at time of blood draw of T2 which was shortly before discharge (1–2 days before discharge). MDD and BD patients were further classified as remitters if the respective scores were ≤ 10 (MADRS, MDD and BD) or ≤ 12 (YMRS, BD only) at T2. None of the SCZ patients were remitted at T2. Controls were mainly recruited from hospital staff or relatives of hospital staff and were screened for psychiatric symptoms using the MINI-DIPS (MINI Diagnostic Interview for Psychiatric Disorders) (Sheehan et al., 1998).

Table 2
Symptom severity was measured in the respective diagnostic subgroups by MADRS, YMRS and PANSS, compared between T1 and T2 and analyzed by Wilcoxon tests. Corrected level of significance was set at $p \leq 0.01$. T1: time point 1 (admission); T2: time point 2 (shortly before discharge); SD: standard deviation; MADRS: Montgomery-Åsberg Depression Scale; YMRS: Young Mania Rating Scale; PANSS: Positive and Negative Syndrome Scale, subscales G: General Psychopathology, P: Positive Scale, N: Negative Scale; MDD: major depressive disorder; BD: bipolar disorder; SCZ: schizophrenia.

Scale	Diagnosis	T1		T2		p
		N	**Mean (SD)**	**N**	**Mean (SD)**	
MADRS	MDD	40	31.20 (7.61)	39	11.92 (7.17)	< 0.0001
	BD total	55	23.11 (12.59)	55	10.15 (6.45)	
	BD depressed	30	29.76 (10.03)	30	10.87 (7.29)	< 0.0001
YMRS	BD total	48	7.90 (8.30)	48	1.83 (4.00)	< 0.0001
	BD (hypo)manic	11	16.45 (7.95)	11	5.18 (6.79)	0.006
PANSS_G	SCZ	39	46.15 (10.23)	39	31.23 (7.20)	< 0.0001
PANSS_P			26.23 (5.57)		13.87 (5.29)	
PANSS_N			22.79 (9.99)		15.72 (7.24)	
PANSS_ Total Score			95.18 (20.79)		60.82 (17.08)	

Exclusion criteria for patients and HC were acute or chronic infectious diseases, severe neurological or autoimmune disorders and other severe somatic diseases. Further exclusion criteria for HC were a history of mental disorders, as well as first-degree relatives with mental disorders. All participants gave written informed consent to participate in the study.

2.2 Biomaterial sampling

Blood was taken from the participants by venous puncture after fasting for 10–13 hours in the morning (between 8–10 am). For kynurenine (KYN), tryptophan (TRY), kynurenic acid (KYNA) and quinolinic acid (QA) analysis, plasma was separated by centrifugation at 4°C for 10 minutes at 2,300 rpm and aliquoted in 500 µl. After that, plasma was stored at -80°C until further use.

2.3 Mass Spectrometry procedure

Quantification of plasma levels of TRY and its catabolites KYNA, KYN and QA was performed by validated assays based on liquid chromatography tandem mass spectrometry (LC-MS/MS), similar to previous publications (Küster et al., 2017, Doolin et al., 2018). KYNA, TRY and QA are quantified together in one assay, while KYN is quantified separately. Both assays comprise sample clean-up by protein precipitation followed by reversed-phase chromatography and mass spectrometric detection in the

positive ion multiple reaction monitoring (MRM) mode using the deuterated analogues of the analytes, namely [D5]tryptophan, [D5]kynurenic acid, [D4]kynurenine and [D3]quinolinic acid as internal standards. LC-MS/MS (Agilent 1290 Series; API 6500™ AB Sciex) was used with an Atlantis® T3 column (3 µm, 50 x 2.1 mm; Waters). Mobile phase A consisted of 0.2% formic acid in water, and mobile phase B consisted of 0.2% formic acid, in acetonitrile. Plasma samples were prepared by addition of ice-cold methanol for protein precipitation. Subsequently, samples were centrifuged and supernatant was evaporated to dryness under a gentle stream of nitrogen and reconstituted in mobile phase A. Improved lower limits of quantification (LLOQ) were 100.0 nmol/l (KYN), 2.0 µmol/l (TRY), 50.0 nmol/l (QA) and 5.0 nmol/l (KYNA). Quinolinic acid/kynurenic acid ratio (QA/KYNA) was calculated.

2.4 Statistical analysis

Statistical analysis was performed using SPSS (version 28, IBM, Armonk, USA). Data were tested for normal distribution and parametric (KYN and TRY levels; t-test, ANOVA, Pearson's correlation) or non-parametric tests (QA, KYNA levels and QA/KYNA ratio; Mann-Whitney U test, Kruskal-Wallis test, Spearman's Rho correlation) calculated as appropriate. As we analyzed differences in four metabolites and the QA/KYNA ratio, we corrected our data for the primary outcome for multiple testing by Bonferroni correction and set the level for significance accordingly at $p = 0.05/5 = 0.01$.

3. Results

3.1 Demographic data

Patients and healthy controls did not significantly differ in age, sex ratios and body mass index (BMI) (Table 1). However, there were significantly more smokers and users of illicit drugs among the patients compared to HC. Also, HC as well as BD patients had a university degree significantly more often than patients with MDD and SCZ.

Symptom severity was measured using standard instruments (MADRS, YMRS and PANSS) and compared between acutely ill patients at T1 and the same patients shortly before discharge from the hospital at T2 (Table 2). Regarding symptom severity, there were statistically significant differences between the two time points. 55.8% of the initially depressed MDD and BD patients had a MADRS sum score of 10 and lower and were therefore considered to be remitted at T2. 96.0% of the initially manic BD patients showed an YMRS score of 12 and lower at T2 and were therefore considered to be remitted at discharge. Those were also showing MADRS < 10. Similarly, the patients suffering from SCZ described a clinical and statistically significant improvement of symptom severity at T2: all patients were below a sum score of 54 in the general psychopathology scale (maximum score 112), below 31 in the positive symptom scale (maximum score 49) and below 37 in the negative symptom scale (maximum score 49). For further information see Supplementary Table 1.

As expected, different psychopharmacological medications were administered during inpatient treatment. For a simplified overview we assigned the medication to four groups: antidepressants (AD), lithium

carbonate (Li), antipsychotics (AP), anticonvulsants (AC) and the respective combinations. The majority of patients received a combination of substances from at least two different groups, with BD patients taking medication from different substance groups most frequently (see Supplementary Tables 2 and 3).

3.2 Longitudinal analysis of peripheral TRYCAT levels in patients with SMI

We investigated whether there was a correlation between age, sex, BMI, smoking or use of illicit drugs and the measured metabolites (using either Pearson's or Spearman's correlation). We found differences between men and women when analyzing the whole sample, showing that females had lower levels in kynurenine (T1: $p = 0.026$; T2: $p = 0.089$), tryptophan (T1: $p = 0.025$; T2: $p = 0.072$) and kynurenic acid (T1: $p < 0.0001$; T2: $p = 0.047$). Consecutively, also the QA/KYNA ratio was higher in females (T1: $p = 0.002$; T2: $p = 0.04$). After correction for multiple comparison, only the results for kynurenic acid and the QA/KYNA at T1 differed significantly between females and males. When comparing the different medication groups, there was a difference regarding kynurenine at T1, however, this was not statistically significant after correcting for multiple comparison. Furthermore, in post-hoc tests, there was not a single substance or specific combination that was associated with lower or higher kynurenine levels (see Supplementary Table 4).

Regarding differences between the diagnostic groups, the HC group showed significantly higher levels of kynurenine (ANOVA, $p = 0.002$), tryptophan (ANOVA, $p = 0.004$), kynurenic acid (Kruskal-Wallis test, $p < 0.0001$) and quinolinic acid (Kruskal-Wallis test, $p = 0.002$) (Fig. 2). The difference in the QA/KYNA ratio was not significant when applying correction for multiple testing.

Post-hoc analysis showed that the significant difference in kynurenine at T1 was mainly driven by differences between HC and BD patients ($p = 0.001$) and, to a lesser extent, SCZ patients ($p = 0.045$). Tryptophan, by contrast, was significantly different only in BD patients when compared to HC (post-hoc test, $p = 0.002$). Regarding quinolinic acid, we could not detect a significant difference after correction for multiple comparison similar to the QA/KYNA ratio. However, kynurenic acid was significantly higher in HC when compared to each of the diagnostic groups in the post-hoc tests (all $p < 0.0001$). There was no significant difference between disorders when analyzing the sample without healthy controls neither at T1 nor T2.

Comparing T2 of the patient groups with T1 of HC (we only measured one time point in HC), only kynurenic acid remained significantly increased in healthy controls (Kruskal-Wallis test, $p < 0.0001$), whereas the results of higher kynurenine and reduced QA/KYNA ratio in HC did not withstand correction for multiple comparison. There was no significant difference between T2 in patients and T1 in healthy controls in tryptophan or quinolinic acid.

Paired t-test or Wilcoxon tests revealed that in the whole patient sample, there was a significant increase of kynurenine (paired t-test, $p = 0.004$), kynurenic acid (Wilcoxon test, $p < 0.0001$) and quinolinic acid (Wilcoxon test, $p = 0.01$) between T1 and T2, which also withstood correction for multiple comparison.

Tryptophan levels were numerically increased at T2, whereas the QA/KYNA ratio did not change significantly between the two time points.

In an exploratory ANOVA taking the diagnosis as between-subjects factor and sex, age, BMI, medication, smoking status, substance abuse and educational level as covariates into account, there was a significant difference in T1 for kynurenine concentrations in the whole sample ($p = 0.022$) with higher concentrations in HC compared with patients. From the included covariates, only the diagnosis had a significant between-subjects effect ($p = 0.03$).

Regarding tryptophan, only BD and MDD patients showed a marginally significant increase between T1 and T2, but not SCZ patients, implying a diagnosis x time point interaction for this group ($p = 0.01$).

For kynurenic acid levels, there was an increase towards the normal range in BD, but no difference between T1 and T2 for MDD and SCZ patients. The QA/KYNA ratio did not significantly change in either group.

3.3 TRYCAT levels of patients in different BD phases

As we included BD patients in depressed, as well as manic and mixed episodes, we additionally analyzed these patients regarding their mood state (Fig. 3). The overall reduction of kynurenine and kynurenic acid in BD was more prominent in manic and mixed BD patients when compared to depressed BD patients (ANOVA, $p = 0.007$; Kruskal-Wallis test, $p = 0.007$ respectively). Concentrations of the other TRYCATs did not differ significantly between the different BD phases. Additionally, there was a marginally significant negative correlation between kynurenic acid levels and YMRS scores in T1 ($p = 0.012$) and a positive correlation between kynurenic acid and MADRS scores in T1, which however did not withstand correction for multiple comparison. The QA/KYNA ratio at T2 was significantly positively correlated with MADRS scores at T1 ($p = 0.01$). PANSS total score at T2 was marginally significantly negatively correlated with tryptophan at T1 ($p = 0.019$).

In a next step, we compared the subgroups of depressed MDD vs. BD patients ($n = 40$ vs. $n = 30$) to investigate if one of the measured TRYCATs could prove useful to distinguish between MDD and bipolar depression (see Supplementary Fig. 1). However, we could not show a significant difference between these groups.

Finally, we wanted to investigate if patients classified as remitters had higher TRYCAT concentrations than non-remitters at T2 and if therefore TRYCAT levels can be considered as an marker for therapy response. However, we could only find a numerical, but not a significant trend toward higher concentrations in kynurenine, kynurenic acid and quinolinic acid for patients who remitted regarding their depressive symptoms (see Supplementary Table 2).

4. Discussion

In our study, we investigated tryptophan, kynurenine, kynurenic acid and quinolinic acid levels as well as the QA/KYNA ratio in patients with MDD, BD and SCZ in comparison to HC. We measured the metabolites in an acute episode and after clinical improvement, shortly before discharge from inpatient treatment. Intriguingly, we could show a significant decrease of all four TRYCATs when comparing SMI patients to HC in the whole sample. This finding confirms results from previous similar analyses (Cathomas et al., 2021, Birner et al., 2017) and was even corroborated for several metabolites in two very recent meta-analyses (Hebbrecht et al., 2021, Marx, McGuinness, et al., 2021). Although decreased tryptophan levels will most probably also lead to a decrease in all downstream metabolites, the nominally and statistically most pronounced reduction could be shown for kynurenic acid levels, which suggests a shift in tryptophan catabolism from the kynurenic acid branch toward the 3-hydroxykynurenine and quinolinic acid branch (see also Fig. 1). This hypothesis is further supported by an increase of QA/KNYA ratio in SMI patients, even if it did not withstand statistical correction for multiple testing. While kynurenic acid is considered rather neuroprotective (Foster et al., 1984), the alternative metabolites 3-hydroxykynurenine and quinolinic acid constitute free radical generators and lead to neuronal dysfunction or even cell death by several independent mechanisms (Guillemin, 2012). A shift towards the generation of neurotoxic compounds in the kynurenine pathway supports the hypothesized role for TRYCAT alterations in the pathophysiology of SMI and a possible contribution to their unspecific morphological brain abnormalities (Hibar et al., 2018, van Erp et al., 2016, Fang et al., 2015). Our results partially support this hypothesis, showing a lower protective kynurenic level in patients vs. healthy controls, which then increases at least in the BD patients after reaching (partial) remission. However, quinolinic acid was not increased in patients as expected. Compared with neurological diseases, mental disorders do not show degeneration or irreversible damage to neuronal cells. Therefore it seems conceivable that the shift towards neurotoxicity is less marked in mental illnesses (Lovelace et al., 2017). This is also in line with the hypothesis of phasic low-grade inflammation playing a role in at least subgroups of SMI patients (Bauer and Teixeira, 2021).

In our study, tryptophan was increased in healthy controls compared with SMI subgroups at T1 and accordingly also the other catabolites downstream with hints to a shift towards less neuroprotection. Furthermore, tryptophan, the main starting product of the kynurenine pathway, also constitutes the endogenous precursor for serotonin, which in turn plays a key role in the monoamine hypothesis in the pathophysiology of SMI. Though only a small percentage of tryptophan is metabolized into serotonin, a decreased bioavailability of tryptophan as indicated in our sample seems compatible with a serotonin deficit in SMI patients. On the other hand, reduced tryptophan levels will probably also lead to reduced kynurenine and therefore kynurenic acid and quinolinic acid levels in its downstream metabolism. Most interestingly, Marx and colleagues postulated that the serotonin deficit might not only originate from decreased tryptophan availability, but also from a shift in the tryptophan metabolism away from serotonin and toward the kynurenine pathway in SMI patients (Marx, McGuinness, et al., 2021).

There were no significant differences between diagnostic groups, suggesting that the metabolites we assessed cannot be used as biomarkers for differential diagnosis. Comparing patients to HC, we found least significant differences in TRYCAT levels for the MDD group. In fact, only the kynurenic acid

concentration was significantly decreased when compared to HC. All demographic factors we assessed for our participants (sex, age, BMI, medication, smoking status, substance abuse and educational level) did not significantly correlate with TRYCAT concentrations.

A number of different other studies had to face similar problems lacking statistical significance (Milaneschi et al., 2021, Maes et al., 2011), while meta-analyses with a much larger sample size showed significance also for lowered kynurenine and tryptophan levels in MDD patients (Marx, McGuinness, et al., 2021). However, studies still show contradictory results regarding the question in which direction and to what extent TRYCAT levels are disturbed in patients with specific mental disorders, which might also suggest the existence of different subgroups of SMI patients with a different degree or kind of alteration in the kynurenine pathway. Most interestingly, we could show a significant difference between BD patients in a depressed episode vs. a manic or mixed episode, which suggests that disturbances in the kynurenine pathway might correlate with symptom dimensions (here: manic symptoms) rather than disease severity or categorical diagnosis. This was partly strengthened by correlations between YMRS, MADRS and PANSS scores in kynurenic acid, QA/KYNA ratio and tryptophan.

While most previous analyses focusing on TRYCAT alterations in mental disorders were designed as cross-sectional studies, we chose a longitudinal approach to allow a second assessment of the whole sample before discharge from inpatient treatment. At T2, all four TRYCATs were numerically or significantly increased as compared to T1, indicating a partial normalization of tryptophan metabolism. The partial reversal went along with a reduction of clinical symptoms (measured by MADRS, YMRS or PANSS, respectively), which raises the question to what extent psychopharmacological interventions during inpatient treatment may have also induced this increase in TRYCAT levels. An association of particular medication groups (antidepressants, lithium carbonate, antipsychotics or anticonvulsants) with TRYCAT levels at T2 could not be shown in this study, which might be due to rather small numbers in the individual medication subgroups.

TRYCATs are proposed to be mediators of autoinflammation and have a close connection to the HPA axis and to cytokines such as interferon-α, TNF-α or interleukins, which facilitate activation of tryptophan metabolism via the kynurenine pathway (Mandi and Vecsei, 2012). Cytokines themselves have gained increasing attention in psychiatric research and the fact that several of these pro-inflammatory markers are elevated in SMI patients (Goldsmith et al., 2016) supports the hypothesis that neuroinflammation may be involved in the pathogenesis of mental disorders, or at least subgroups of patients. Treatment with anti-inflammatory substances such as cyclooxygenase(COX)-inhibitors, statins or monoclonal antibodies is proposed to have a positive effect on psychiatric symptoms in MMD and SCZ patients implying possible immunomodulatory interventions for SMI patients in the future as recently reviewed in (N. Mueller, 2019).

Interpreting the data obtained during this study, one always has to keep in mind that we analyzed plasma levels of TRYCATs, not local concentrations in the central nervous system. A handful of studies have conducted investigations of central TRYCAT levels obtained from cerebrospinal fluid (CSF) in the context

of psychiatric disorders: Linderholm and colleagues for example found increased levels of kynurenine and kynurenic acid in the CSF of patients with SCZ (Linderholm et al., 2012). This observation was confirmed in euthymic BD patients with a lifetime history of psychosis compared with bipolar patients without a history of psychosis. However, no healthy controls were included here (Olsson et al., 2012). Interestingly, these results are contradictory to our data with higher blood levels of kynurenine and kynurenic acid in healthy controls compared with patients. Regarding the correlation between peripheral and central TRYCAT levels in MDD, a recent meta-analysis by Skorobogatov and colleagues proposed a strong correlation only for kynurenine and 3-hydroxykynurenine, while for the other metabolites concordant data was missing (Skorobogatov et al., 2021). Interestingly, the few published studies argued for inverse changes of central and peripheral tryptophan metabolism in SMI patients: while serum or plasma TRYCAT levels appear decreased, as also shown in this study, concentrations of kynurenine and/or kynurenic acid seem elevated in CSF samples (Wang and Miller, 2018, Cao et al., 2021, Plitman et al., 2017, Sellgren et al., 2019). The reasons for this may be numerous: considering the whole organism, tryptophan is mainly catabolized in the gastrointestinal system, where serotonin fulfils different sensory and motoric functions (Mawe and Hoffman, 2013). Notably, an even smaller percentage of tryptophan seems to contribute to serotonin concentrations in the gut when compared to the brain, indicating variations in the use of the different catabolic branches between brain and periphery. Additionally, alterations in the microbiome appear to play a role in mental disorders, so the peripheral measured TRYCATs might also hint at reduced microbiome functions in SMI (Ortega et al., 2022). Furthermore, TRYCATs themselves seem to be regulated brain-specifically (Badawy, 2017) and vary in their ability to pass the brain-blood barrier (Fukui et al., 1991, Marx, Lane, et al., 2021). Recently, in a secondary analysis of the SCZ dataset of the Psychiatric Genomic Consortium (PGC), an interaction (rs13265509, $p = 1.1\times10^{-7}$) in a locus containing the *IDO2* (Indoleamine 2,3-dioxygenase 2) gene, which also plays a role in the kynurenine pathway, was reported to be associated with gene-sex interaction in SCZ (Blokland et al., 2022). Therefore, the contribution of the kynurenine pathway to pathomechanisms in mental illnesses seems to be complex and as well risk gene variants as different organ systems might be involved.

5. Limitations And Conclusions

In summary, we were able to demonstrate that metabolites of the kynurenine pathway show altered plasma concentrations in patients with SMI. Whether this might hint at a causal pathomechanism or rather is a byproduct of potential low-grade inflammation or altered microbiome activity cannot be answered with our study design. Since we found a significant difference in acutely ill and remitted patients, TRYCAT levels might additionally function as objective biomarkers for therapeutic response. However, the time points were not standardized as this was a naturalistic clinical sample recruited during their hospitalization. Nearly all the patients were medicated as studies with drug-naïve patients are ethically not easily justifiable. Therefore, we cannot completely rule out the effect of medication in the measured metabolites compared with unmedicated healthy controls. Subgroups for the medication difference analysis were very small, which might have prevented the detection of significant differences.

We could not find statistically significant differences between particular diagnostic groups except for manic or mixed phase vs. depressed BD patients, putatively because the number of patients in the diagnostic groups differed and was partly rather small.

Our study further strengthens the hypothesis of the kynurenine pathway as a possible key regulator in the interaction of an immune imbalance and altered neuronal signaling in SMI patients, even though no other markers of potential inflammation like C-reactive protein or cytokines were assessed, which could have helped to further contextualize our findings. Phase-specific differences in BD patients hint toward a link between TRYCAT levels and symptom dimensions, indicating that future studies should further investigate subgroups of patients rather than classify merely according to established diagnostic systems. Larger and possibly also multicentric, cross-sectional and longitudinal studies will be needed for elucidation of the role the tryptophan catabolism plays in the pathophysiology of mental disorders and for answering the burning question if it might constitute a possible therapeutic target in the future.

Abbreviations

AC: anticonvulsant; AD: antidepressant; AP: antipsychotic; BD: bipolar disorder; BMI: body mass index; CSF: cerebrospinal fluid; COX: cyclooxygenase; HC: healthy control; HK: 3-hydroxykynurenine; HPA axis: hypothalamic-pituitary-adrenal axis; KYN: kynurenine; KYNA: kynurenic acid; LC-MS/MS: liquid chromatography tandem mass spectrometry; Li: lithium carbonate; LLOQ: lower limits of quantification; MADRS: Montgomery-Åsberg Depression Scale; MDD: major depressive disorder; MINI-DIPS: MINI Diagnostic Interview for Psychiatric Disorders; MRM: multiple reaction mode; PANSS: Positive and Negative Syndrome Scale; PGC: Psychiatric Genomic Consortium; QA: quinolinic acid; SCZ: schizophrenia; SMI: serious mental illness; TRY: tryptophan; TRYCAT: tryptophan catabolite; YMRS: Young Mania Rating Scale.

Declarations

Ethics approval and consent to participate: All participants gave written informed consent to participate in the study. The study was approved by the Ethics Committee of the Goethe University of Frankfurt (Approval No. 425/14) and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Consent for publication: Not applicable.

Availability of data and materials: The datasets used in this study are available from the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no known competing financial interests or personal conflicts of interest that could have appeared to influence the work reported in this paper.

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Authors' contributions: MB recruited the patients and healthy controls and performed the statistical analysis and wrote the manuscript, MB wrote the manuscript and drafted the figures and tables, CK recruited SCZ patients and healthy controls and collected blood and phenotypic data, KK recruited BD and MDD patients and healthy controls and collected blood and phenotypic data, NBK recruited BD and MDD patients and healthy controls and collected blood and phenotypic data, SE recruited SCZ, BD and MDD patients and healthy controls and collected blood and phenotypic data, KAA organized the mass spec measurements and drafted the manuscript, RB recruited SCZ patients and drafted the manuscript, DS designed the study and organized the mass sprec measurements, RMcN finalized the manuscript bd edited the language, AR and SKS designed the study, organized the recruitment, SKS supervised the data analysis and drafted and finalized the manuscript. All authors reviewed the manuscript.

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Figures

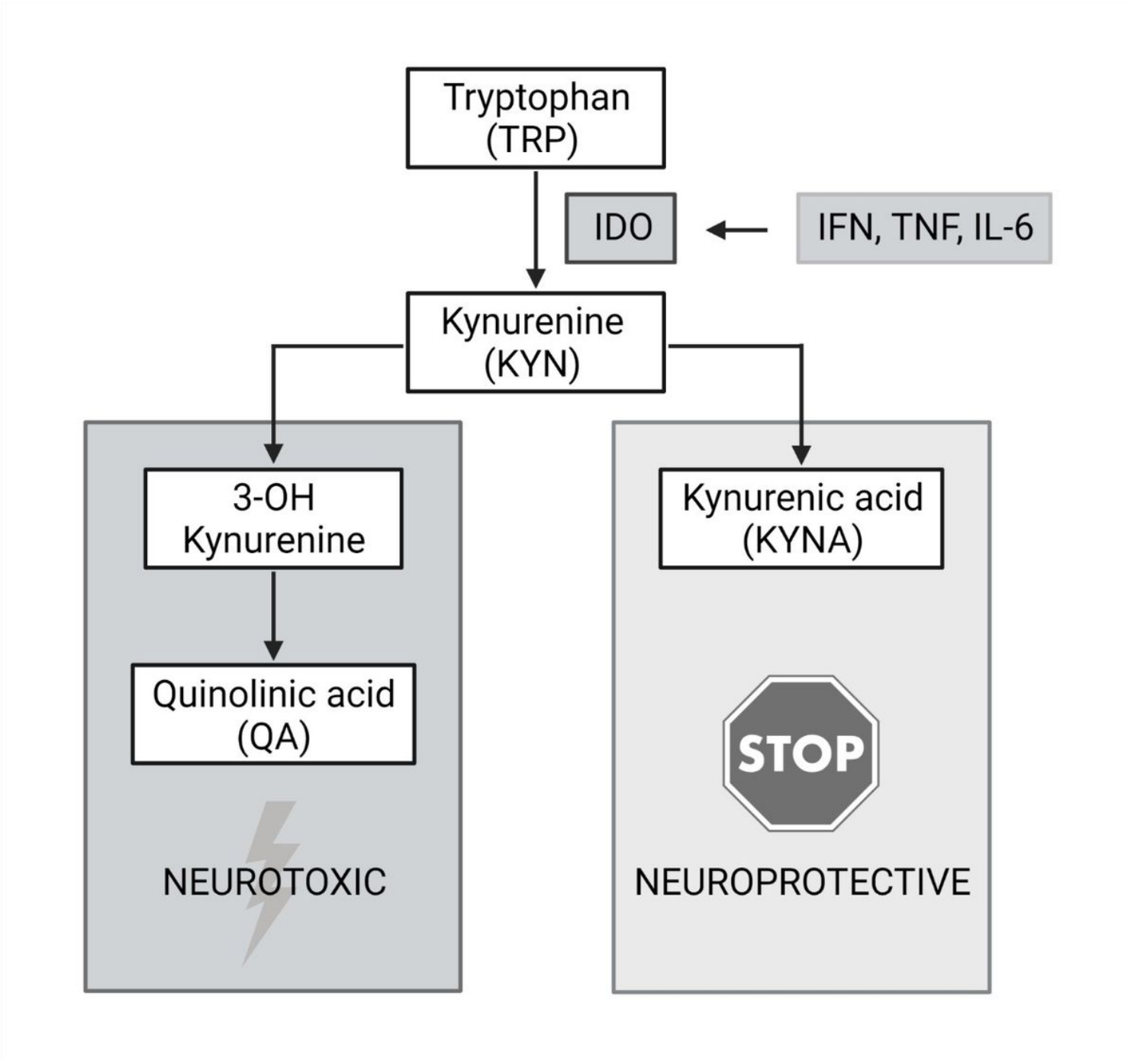

Figure 1

Selected details from the different branches of tryptophan catabolism are depicted in a simplified way to emphasize possible metabolism into neurotoxic quinolinic acid and neuroprotective kynurenic acid. IDO: Indoleamine 2,3-dioxygenase; IFN: interferon; TNF: tumornecrosis factor; IL-6: interleukin 6. Created with BioRender.

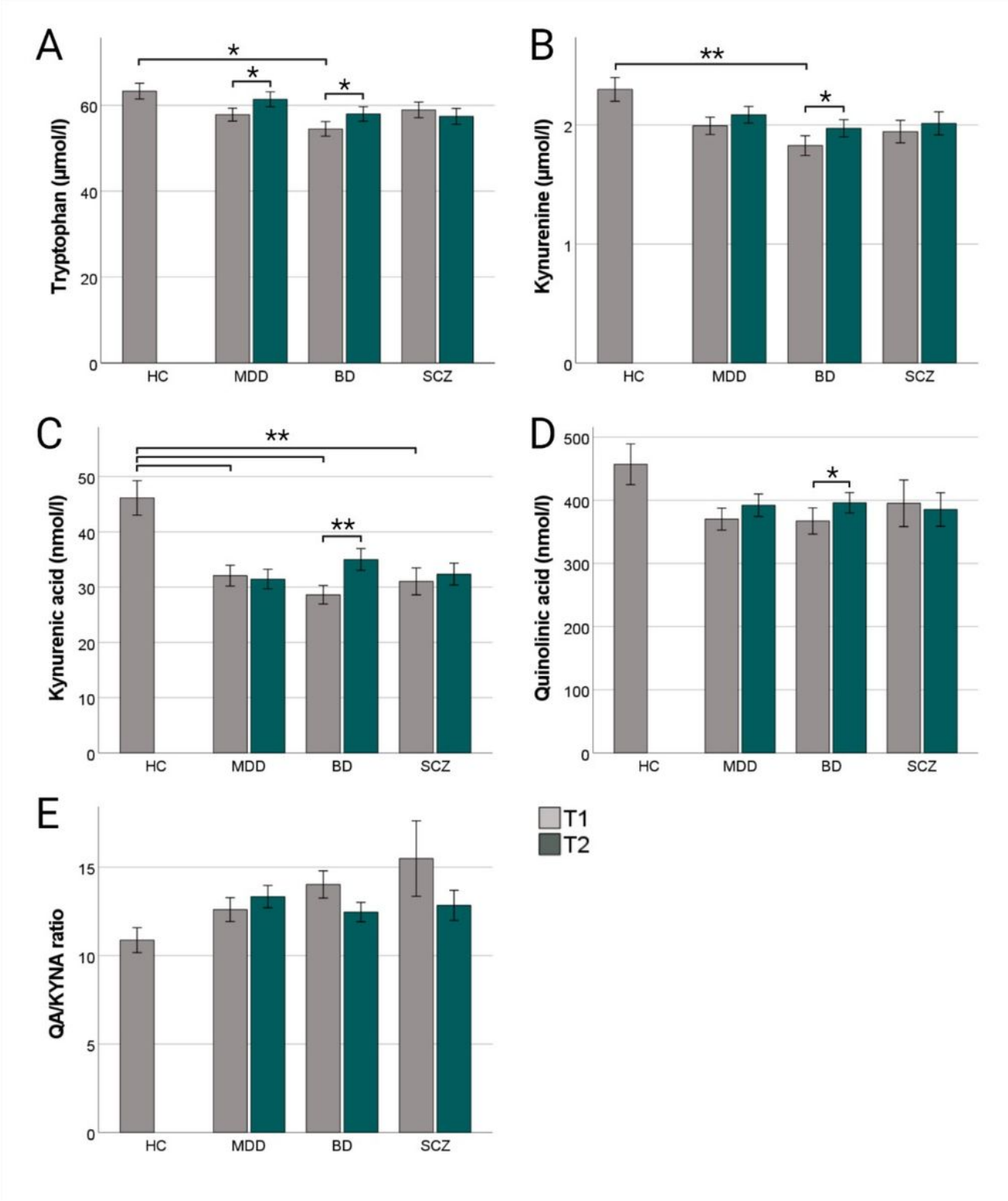

Figure 2

Concentrations of kynurenine, tryptophan, kynurenic acid and quinolinic acid measured at T1 and T2 are depicted among the different diagnostic groups of SMI. The HC group showed a significantly higher concentration of all four metabolites, but no significant differences could be observed between MDD, BD and SCZ groups neither at T1, nor at T2. Interestingly, the reduction of kynurenine, kynurenic acid and quinolinic acid levels was partially reversed at T2. Concentrations are displayed as mean with standard

deviation. SMI: serious mental illness; HC: healthy control; MDD: major depressive disorder; BD: bipolar disorder; SCZ: schizophrenia; T1: time point 1; T2: time point 2; corrected *p≤0.01, **p≤0.001.

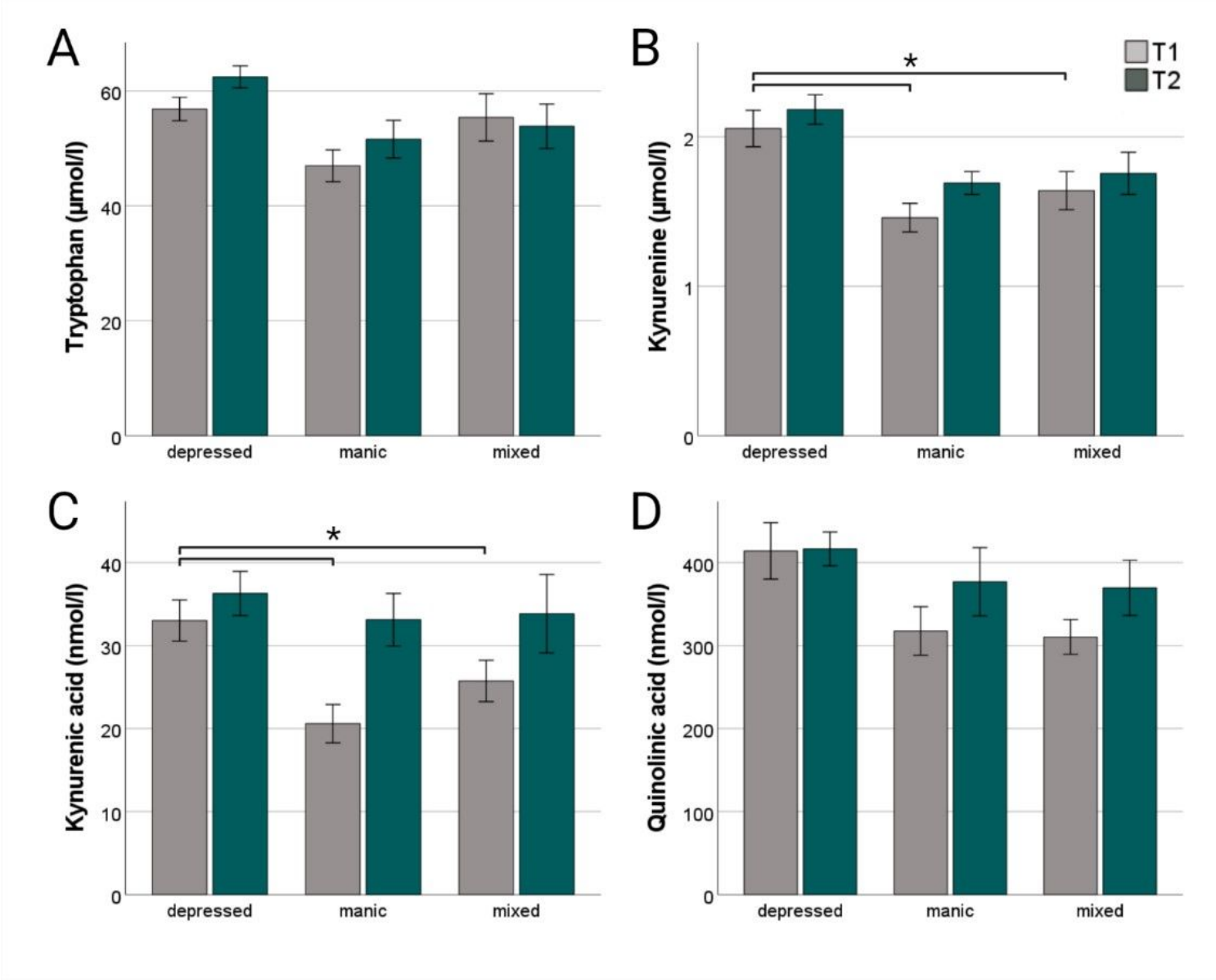

Figure 3

Manic and mixed phase BD patients show significantly lower kynurenine and kynurenic acid levels in an acute episode when compared to depressed BD patients. Concentrations are displayed as mean with standard deviation. BD: bipolar disorder; T1: time point 1; T2: time point 2; *p≤0.01.

Supplementary Files

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