Faster speed of onset of the depressive episode is associated with lower cytokine serum levels (IL-2, -4, -6, -10, TNF-α and IFN-γ) in patients with major depression

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Short Communication

Faster speed of onset of the depressive episode is associated with lower cytokine serum levels (IL-2, -4, -6, -10, TNF-α and IFN-γ) in patients with major depression

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Abstract

Introduction: Cytokines might play a key role in the pathophysiology of major depressive disorder (MDD). The speed of onset of depressive episodes has been discussed as an important clinical parameter in MDD. The aim of this study was to investigate a potential influence of the speed of onset of the depressive episode on cytokine serum levels.

Method: Serum level of the cytokines interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ) granulocyte and monocyte colony stimulating factor (GM-CSF) were measured in a total of 92 patients with MDD that did not respond to at least one previous antidepressant treatment. Patients were retrospectively divided in two groups: Faster (≤ 4 weeks) and slower (> 4 weeks) onset of the depressive episode defined as the time passing from the first depressive symptoms to a full-blown depressive episode by using information from a clinical interview.

Results: We found significantly lower serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ in patients with a faster onset compared to patients with a slower onset of the depressive episodes. Furthermore, lower cytokine serum levels of IL-2, IL-8, IL-10 and IFN-γ were found in patients with a shorter duration (less than 6 months) compared to a longer duration (6-24 months) of the current depressive episode. This effect on cytokines was independent from the effect of the speed of onset of the depressive episode.

Conclusions: Patients with faster onset of the depressive episode might represent a biological subtype of MDD with lower serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ.

Keywords

Major depressive disorder

Onset of the depressive episode

Cytokine

Highlights

- Cytokines might play a key role in the pathophysiology of depression.
- Serum level of cytokines were measured in 92 patients with MDD.
- Faster onset of the depressive episode was associated with lower serum levels for IL-2, -4, -6, -10, TNF-α and IFN-γ.
- Depressive episodes with a faster onset might represent a biological subtype of depression.

1. Introduction

The cytokine hypothesis of depression postulates that cytokines play a key role in the pathophysiology of depression (Bauer and Teixeira, 2019). A mechanism that (among others) seems to be involved in the pathophysiology of depression is a neuroendocrine pathway that is mediated by a dysfunction of the hypothalamic pituitary adrenal (HPA) axis and leads to elevated levels of cortisol (Galecki and Talarowska, 2018; Jeon and Kim, 2016). Furthermore, anti-inflammatory treatment strategies have been shown to be effective in the treatment of depression (Bai et al., 2020).

Major depressive disorder (MDD) is a heterogeneous disease. Different subtypes such as atypical, anxious, melancholic and seasonal depression with differences in duration of the episode, severity of depression and treatment outcome, have been described (Musil et al., 2018; Schmidt et al., 2011). Specific treatments are recommended based on clinical subtypes: For

example, MAO-inhibitors are recommended in atypical depression (Ricken et al., 2017) while light therapy is recommended for seasonal depression (DGPPN, 2015).

Studies investigating the association of cytokine-changes (IL-6 and TNF-α) in melancholic or atypical depression revealed evidence of differential clinical subtypes on a neurobiological level (Dunjic-Kostic et al., 2012; Patas et al., 2013; Yoshimura et al., 2013). For example, Dunjic-Kostic et al. found IL-6 concentrations significantly elevated in melancholic depressive patients, but not in patients with atypical depression (Dunjic-Kostic et al., 2012). Patas et al. explored the underlying pathophysiology further in a large sample (n=1070) of patients with MDD, and discovered a robust positive statistical association between IL-6 and brain-derived neurotrophic factor (BDNF) concentrations in patients with melancholic, but not with non-melancholic MDD (Patas et al., 2013). This might explain why IL-6 levels may have a predictive value regarding the response to selective serotonin reuptake inhibitors (SSRIs) which was described in a report of a longitudinal study in 118 patients taking paroxetine or sertraline for eight weeks (Yoshimura et al., 2013). These exemplary studies suggest that cytokines like IL-6 may have some value as biomarkers on a pathophysiological, clinical and therapeutic level.

A large meta-analysis confirmed evidence for a pro-inflammatory state with elevated cytokine levels in patients with MDD compared to healthy controls (Osimo et al., 2020). Furthermore, recent evidence suggested that alterations in cytokine serum levels are associated with antidepressant treatment outcomes (Liu et al., 2020; Yang et al., 2019), however to date no biomarker has proven to be sufficiently specific, sensitive and robust for routine clinical use (Mora et al., 2018). We previously reported that we found no significant changes in cytokine serum levels during lithium augmentation (LA) in treatment resistant MDD (Ricken et al., 2018).

The speed of onset of depressive episodes can vary greatly, with some patients experiencing a rapid onset while others experience a prolonged progression of symptoms. As such, it is likely

that differences at a neurobiological level are correlated with the clinical parameter “onset of depression”. The onset of the depressive episode has been discussed as an important clinical parameter in affective disorders (Hegerl et al., 2008), Furthermore, the duration of the current episode over 24 months can be considered as a promising clinical predictor for treatment response (Riedel et al., 2011). However, studies investigating these parameters at a (neuro)biological level are notably missing.

To bridge this gap, the aim of this study was to investigate a potential influence of the speed of onset of the depressive episode on cytokine serum levels in patients with treatment resistant MDD.

2. Methods

2.1. Patients

The study population consists of a subsample of a naturalistic cohort study investigating treatment response and side effect burden of LA in MDD. In this subsample a total of 92 patients were included, when at least one baseline or endpoint cytokine serum levels was available (due to missing data the range of included measurements varies; for more details see Table 1). Inclusion criteria were: MDD, above 18 years of age, indication for an antidepressant pharmacotherapy, insufficient response to at least one adequate antidepressant pre-treatment and clinical indication for LA, Hamilton Depression Rating Scale (HDRS-17) (HAMILTON, 1960) score $\geq$ 12 and written informed consent. Diagnosis of MDD was confirmed by the Mini-International Neuropsychiatric Interview (Sheenan et al., 1998). Patients were recruited between December 2008 and December 2012 in 12 psychiatric departments of the Berlin Research Network on Depression, Berlin, Germany. Local ethics committees approved the study, and written informed consent was obtained from all subjects.

2.2. Procedure

Serum concentrations of the cytokines interleukin IL-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), granulocyte and monocyte colony stimulating factor (GM-CSF) were measured first in medicated patients before lithium augmentation (baseline) and again after 4-6 weeks of lithium use (endpoint). Severity of depression (measured using the HDRS-17) was measured on both occasions. Body mass index (BMI) was measured at study baseline. All patients received individual doses of lithium carbonate adapted to the lithium serum levels. Review of subjects' medical history revealed an absence of acute or chronic infectious diseases, and no use of anti-inflammatory or immunosuppressive medication. Severity of somatic comorbidity was assessed according to the updated version of the Charlson Comorbidity Score (Quan et al., 2011).

Patients were retrospectively divided in two groups: Faster ($\leq$ 4 weeks) and slower (> 4 weeks) onset of the depressive episode defined as the time passing from the first depressive symptoms to a full-blown depressive episode by using information from a clinical interview. The duration of the current episode was divided into the following three groups: less than 6 months, less than 2 years, and more than 2 years. The mean duration of the previous episode was divided into the following three groups: less than 6 months, less than 1 years, and more than 1 years.

Peripheral venous blood was drawn and centrifuged at 4000 revolutions per minute (r.p.m.) for 10 min at room temperature to separate the blood components. Serum was subsequently frozen at -80° C and rethawed prior to analysis. For quantification of cytokines IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF, TNF-α and IFN-y, bead array flow cytometry (Bio-Plex Pro™ Human Cytokine Assay, Bio-Rad Laboratories GmbH München) was used. The detection range for all measured cytokines was below 10 pg/ml (IL-2 = 1.6 pg/ml, IL-4 = 0.7 pg/ml, IL-6 = 2.6 pg/ml, IL-8 = 1.0 pg/ml, IL-10. 0,3 pg/ml, GM-CSF = 2.2 pg/ml, IFN-γ = 6.4 pg/ml, TNF-α = 6.0 pg/ml). The intra-assay and inter-assay coefficients were below 15% and 9%.

As mentioned above, previous analyses revealed no effect of LA on any of the measured cytokine levels during the treatment (Ricken et al., 2018) which allowed us to use baseline and endpoint cytokine serum levels for analysis. Additionally, we corrected the current analyses for the effect of LA (see statistical analysis).

2.3. Statistical analysis

We used a linear mixed model for statistical analysis to investigate the effect of the speed of onset of the depressive episode as an explanatory factor for differences in cytokine levels (dependent variable). Linear mixed-effects models have the advantage of allowing the investigation of variability between patients (heterogeneity) and simultaneously adjusting for the within-subject correlation. In the present analysis, random effects were permitted for the intercepts. We entered time (= effect of LA) and the covariates gender, age, BMI, duration of the current episode and severity of depression at both timepoints as fixed effects into the model. Model regression coefficients are reported together with their 95% Confidence Interval (CI). Kolmogorov-Smirnov test and the evaluation by a statistician were used to check whether the sample distributions were normal. Model regression coefficients are reported together with their standard error estimates (SE) and a 95% Confidence Interval (CI). We used the Statistical Analysis System (SAS) software, Version 9.4 for statistical analysis with the linear mixed model and SPSS (Version 21) for descriptive statistics. We applied Chi-Square and Mann Whitney-U tests where appropriate. A significance level of $p < 0.05$ was set for all analyses and $p < 0.1$ was considered a trend.

3. Results

For descriptive statistics, see Table 1. We found significantly lower serum levels of IL-2 (estimated differences: -0.16 pg/ml; 95%-CI: -0.29 to -0.04; $F_{1.82} = 7.19$; $p < 0.01$), IL-4 (estimated differences: -0.05 pg/ml; 95%-CI: -0.10 to 0.01; $F_{1.78} = 4.07$; $p = 0.047$), IL-6 (estimated differences: -0.15 pg/ml; 95%-CI: -0.27 to -0.02; $F_{1.82} = 6.06$; $p = 0.02$), IL-10

(estimated differences: -0.15 pg/ml; 95%-CI: -0.27 to -0.03; $F_{1.82} = 6.30$; $p = 0.01$), TNF-α (estimated differences: -0.09 pg/ml; 95%-CI: -0.15 to -0.02; $F_{1.81} = 7.56$; $p < 0.01$) and IFN-γ (estimated differences: -0.12 pg/ml; 95%-CI: -0.19 to -0.05; $F_{1.81} = 11.66$; $p < 0.01$) as well as a trend to lower levels of GM-CSF (estimated differences: -0.10 pg/ml; 95%-CI: -0.20 to 0.01; $F_{1.79} = 3.59$; $p = 0.06$) in patients with a faster onset compared to patients with a slower onset of the depressive episode. We found no significant effect of faster speed of onset of the depressive episode on IL-8 levels (estimated differences: -0.05 pg/ml; 95%-CI: -0.16 to 0.06; $F_{1.81} = 0.79$; $p = 0.38$). Regarding the confounding covariates in these analyses described above, we found significantly lower serum levels of TNF-α and GM-CSF in males compared to females. Age was associated with a lower serum level in IL-2 and a higher serum level in IL-4. The duration of the current episode was a significant confounding covariable for IL-2, IL-8, IL-10 and IFN-γ ($p < 0.05$, data shown in the Appendix). We found lower cytokine serum levels of IL-2, IL-8, IL-10 and IFN-γ in patients with a shorter duration (less than 6 months) compared to a longer duration (6-24 months) of the current depressive episode. In an exploratory analysis we tested the interaction of the variables ‘duration of the current episode’ and ‘speed of onset of the depressive episode’ and did not find any significant interaction effect ($p > 0.05$, data not shown).

We did not find a significant effect of any other covariate on any cytokine level besides the effects described above ($p > 0.05$, data shown in the Appendix).

Table 1: Comparison of demographic and clinical characteristics: Patients with faster versus slower speed of onset of the depressive episode measured at baseline

n* (%)	**All Participants**	**Faster onset**	**Slower onset**	**p-value[#]**
Responder[a]				0.24
Yes	41 (44.6)	16 (53.3)	25 (40.3)	
No	51 (55.4)	14 (46.7)	37 (59.7)	
Remitter[b]				0.23
Yes	32 (34.8)	13 (43.3)	19 (30,6)	
No	60 (65.2)	17 (56.7)	43 (69.4)	
Type of MDD				0.50
Single depressive episode	28 (37.3)	8 (32.0)	20 (40.0)	
Recurrent depressive episode	47 (62.7)	17 (68.0)	30 (60.0)	
Duration of the current episode				0.87
Less than 6 months	34 (40.0)	12 (42.9)	22 (38.6)	
Less than 2 years	36 (42.4)	12 (42.9)	24 (42.1)	
More than 2 years	15 (17.6)	4 (14.3)	11 (19.3)	
Mean duration of previous episodes				0.16
Less than 6 months	39 (48.1)	11 (40.7)	28 (51.9)	
Less than 1 years	17 (21.0)	4 (14.8)	13 (24.1)	
More than 1 years	25 (30.9)	12 (44.4)	13 (24.1)	
Gender				0.51
Male	35 (38.0)	10 (33.3)	25 (40.3)	
Female	57 (62.0)	20 (66.7)	37 (59.7)	
Psychotropic co-medication (stable during lithium augmentation)**				
Antidepressants				
SSRI	51 (57.3)	15 (53.6)	36 (59.0)	0.63
SNRI	21 (23.6)	8 (28.6)	13 (21.3)	0.45
TCA	10 (11.2)	3 (10.7)	7 (11.5)	0.92
NDRI	5 (5.6)	1 (3.6)	4 (6.6)	0.57
Agomelantine	2 (2.2)	1 (3.6)	1 (1.6)	0.42
Mirtazapine	12 (13.5)	3 (10.7)	9 (14.8)	0.60
MAO-I	1 (1.1)	0 (0)	1 (1.6)	0.47
Atypical antipsychotics	22 (24.6)	6 (21.5)	16 (26.2)	0.35
Antiepileptic drugs	8 (8.9)	3 (10.8)	5 (8.2)	0.27
Benzodiazepines	20 (22.7)	8 (28.6)	12 (20)	0.37
Low-potency antipsychotics	3 (3.4)	2 (7.1)	1 (1.7)	0.19
Mean [standard deviation]; n*				
Age in years	49.07 [15.0]; 92	43.57 [12.4]; 30	51.73 [15.5]; 62	**0.01**
HDRS-17 baseline score	21.14 [4.4]; 92	20.63 [5.0]; 30	21.39 [4.1]; 62	0.31
HDRS-17 study endpoint score	12.43 [7.1]; 92	11.43 [7.1]; 30	12.92 [7.5]; 62	0.42
BMI baseline in kg/m^2	25.42 [5.9]; 88	25.50 [6.1]; 30	25.38 [5.8]; 58	0.89
Charlson comorbidity score	0.20 [0.79]; 92	0.10 [0.40]; 30	0.24 [0.63]; 62	0.61
Lithium level at study endpoint in mmol/l	0.71 [0.15]; 92	0.71 [0.16]; 30	0.71 [0.15]; 62	0.89
Cytokine serum level in pg/ml				
IL-2	53.85 [117.7]; 91	33.10 [28.8];30	64.06 [141.7]; 61	**0.01**
IL-4	25.83 [23.9]; 90	20.90 [4.6]; 30	28.29 [28.8]; 60	**0.01**
IL-6	55.42 [111.4]; 91	30.57 [14.9]; 30	67.64 [134.4]; 61	**0.01**
IL-8	80.82 [100.2]; 92	77.70 [146.4]; 30	82.33 [69.2]; 62	**0.01**
IL-10	101.86 [178.1]; 91	59.55 [24.9]; 30	122.67 [214.3]; 61	**0.03**
GM-CSF	65.15 [43.3]; 91	61.90 [44.6]; 30	66.75 [43.0]; 61	0.38
IFN-γ	28.97 [16.1]; 91	23.57 [8.2]; 29	31.49 [18.2]; 62	**0.01**
TNF-α	28.32 [15.1]; 92	24.20 [5.9]; 30	30.31 [17.6]; 62	0.08

* Number of included patients varies due to missing data; N = 92 (faster onset n = 30; slower onset n = 62)
** Please note that some patients received more than one medication
# Result of t test or Mann-Whitney U test for metric parameters; Chi-square test for categorical parameters

a. Clinical response defined as HDRS-17 decrease of
≥ 50%; b. Remission defined as HDRS-17 score at study endpoint ≤ 7

MDD= *Major Depressive Disorders****; HDRS-17*** *= Hamilton Depression Rating Scale;* ***BMI****=Body mass index;* ***IL*** *= Interleukine;* ***GM-CSF*** *= Granulocyte and Monocyte Colony Stimulating Factor;* ***IFN-γ*** *= Interferon-gamma;* ***TNF-α*** *= Tumor Necrosis Factor alpha;* ***SSRI*** *= Serotonin Reuptake Inhibitors;* ***SNRI*** *= Serotonin and Norepinephrine Reuptake Inhibitor;* ***TCA*** *= Tricyclic Antidepressant;* ***NDRI*** *= Norepinephrine and Dopamine Reuptake Inhibitor (Bupropione);* ***MAO-I*** *= Mono Amine Oxidase Inhibitor (Tranylcypromine);* ***Atypical antipsychotics:*** *Olanzapine, Quetiapine, Risperidone, Aripriprazole and Clozapine;* ***Antieplileptic drugs:*** *Pregabalin, Valproat, Carbamazepine and Lamotrigin;* ***Benzodiazepines:*** *Lorazepam, Diazepam, Zolpidem and Zolpiclon;* ***Low-potency antipsychotics:*** *Melperone, Pipamerone and Chlorprothixene*

4. **Discussion**

This study was designed to investigate if the clinical parameter ‘speed of onset of the depressive episode’ is associated with cytokine profiles on a neurobiological level. We found significantly lower serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ as well as a trend of lower IL-4 and GM-CSF levels in those who experienced a depressive episode with a faster speed of onset. Our finding suggests that a subgroup of patients might be characterized by relatively low cytokine serum levels and a faster speed of onset of the depressive episode.

The speed of onset of depressive episodes is a clinical aspect of MDD that has been identified as a stable individual phenotype in depressive episodes (Hegerl et al., 2008) but investigations on cytokines or other neurochemical parameters are lacking.

Cytokines have been found to be altered under various stress paradigms in humans (Heinz et al., 2003; Steptoe et al., 2007) and animals; it could be demonstrated that acute as well as chronic stress leads to changes of IL-4, IL-6, IL-10 and TNF-α in rats (Himmerich et al., 2013). Accordingly, various forms of stress have been linked to cytokine alterations and the development of depression (Yang et al., 2015; Zunszain et al., 2011).

When interpreting cytokine levels in depressed patients, effects of the HPA axis on the immune system have to be considered. A sudden stress-related increase of the HPA axis activity with the quick release of glucocorticoids alongside a sudden onset of depression will suppress cytokine production as glucocorticoids have an anti-inflammatory potential (Refojo et al., 2001). In line with this it has, for example, been found that the elevated HPA axis activity in acute depression suppresses TNF-α production (Himmerich et al., 2006). Thus, we can assume that during a faster onset of a depressive episode alongside an acute stress reaction, glucocorticoid receptors remain sensitive and dampen cytokine secretion by immune cells.

If the speed of onset of the depressive episode is slower, cortisol receptor resistance may develop over time, which in turn might reduce the glucocorticoid-induced suppression of

cytokine production and thus lead to increased cytokine serum levels despite hypercortisolism (Pace et al., 2007). Therefore, this condition will lead to an increase in cytokine production and an activation of inflammatory reactions (Raison et al., 2006). A correlation between glucocorticoid receptor function (Pace et al., 2007) and glucocorticoid receptor resistance (Webster et al., 2001) is well known and plays a crucial role in the pathophysiology of depression.

Interestingly, we found lower cytokine serum levels of IL-2, IL-8, IL-10 and IFN-γ in patients with a shorter duration (less than 6 months) compared to a longer duration (6-24 months) of the current depressive episode. This effect on cytokines was independent from the effect of the speed of onset of the depressive episode. Our findings are in line with the previous described cortisol receptor resistance which leads to increased cytokine serum levels despite hypercortisolism in patients with a slower speed of onset of the depressive episode. It seems that the duration and the speed of onset of the current episode of depression might be two independent predictors for the cytokine profile, and might in fact both contribute to a specific (neuro)biological characteristic in MDD patients. Patients with a depressive episode duration of more than two years might constitute an additional subtype of chronically-depressed patients. In these patients, counter-regulatory mechanisms within the immune system might play a role for the long-term control of cytokine production. Those potential mechanisms include the mutual antagonism of cytokines, their pleiotropy, and the activity of regulatory T cells which are able to control and suppress cytokine production and immune response (Himmerich et al., 2019).

A limitation of our study pertains to the fact that we did not access cytokine levels at episode onset. Further, the time from onset of the episode until cytokine measurement varies in our sample. To address this, we controlled for the duration of the current episode, and noted that this did not change our findings in regard to the significant effect of faster onset on the cytokine levels in IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ. As the observation period for our study was

rather short (four weeks), we cannot exclude completely that hidden bipolarity (patients that will develop (hypo-) mania or a mixed episode during the course of the illness in the future) might have affected the speed of onset of the depressive episode. However, all patients were diagnosed as unipolar major depressive episode by senior psychiatrists in psychiatric departments and we confirmed diagnosis by the structured Mini-International Neuropsychiatric Interview (M.I.N.I.) that rules out patients with previous or current (hypo-) mania or mixed episodes. A further limitation is the lack of HPA axis parameters. Therefore, we cannot verify our HPA axis-related explanation for the differences in cytokine patterns between patients with faster and slower onset of the depressive episode. In addition to the aforementioned limitations, somatic comorbidities, comedication, antidepressant agents and lithium itself also influence the serum concentrations of numerous cytokines and immunomodulators (Adzic et al., 2018; Himmerich et al., 2019; Robertson et al., 2019; Wang et al., 2019). To address this, we controlled at least for the severity of somatic comorbidity measured with the Charlson Comorbidity Score as well as for the antidepressant groups and found no significant difference in the group of faster versus slower onset of the depressive episode (see Table 1). The strengths of our study include the use of a clinically well characterized sample and inclusion of a relatively large sample size. The repeated cytokine measurement after four weeks allowed for an evaluation of cytokine patterns over time and over different stages of depression. Interestingly, we observed the same result in six out of eight cytokines, which suggests a completely different immunological state of the subgroups and implies that a random effect is unlikely.

Our study builds upon existing evidence by being the first study to investigate neurobiological correlates of the clinical parameter ‘onset of the depressive episode’. This may contribute to a better specification of the heterogeneous group of MDD patients.

The study results may contribute to the identification of diagnostically relevant subgroups of depression. Different biological profiles of depression might require different and

individualized treatments. A meta-analysis found a significant antidepressant effect of anti-inflammatory agents (Bai et al., 2020). One might speculate that patients with a slower onset of the depressive episode, which is associated with a distinct immune signature with relatively high cytokine levels, might particularly benefit from such an anti-inflammatory treatment.

Previous studies have found association of elevated cytokine levels with specific depressive symptoms such as social withdrawal, suicidality, reduced appetite, cognitive deficits and sleepiness; for a review see (Lichtblau et al., 2013). Additionally, we have already mentioned in the Introduction that previous studies found elevated IL-6 concentrations associated with melancholic depression (Dunjic-Kostic et al., 2012). and response to treatment with SSRIs (Yoshimura et al., 2013). In contrast, we report that relatively low IL-6 levels seem to be associated with a fast onset of depression. Future studies might explore further and in a more comprehensive way whether IL-6 and other cytokines are associated with specific clinical characteristics and their time course. Our results indicate that not only elevated concentrations of a certain cytokine, but also relatively low cytokine levels within a sample of depressed patients may be of clinical and scientific significance.

In conclusion, the clinical parameter ‘onset of the depressive episode’ might be associated with lower serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ and may represent a biological subtype of MDD. This biological subtype might be an interesting target for a personalized treatment approach that should be addressed in future studies. Our results support the role of cytokines in the pathophysiology of MDD.

Authors statement

Contributors

Author P. Buspavanich, recruited study subjects, revised the manuscript until the final draft, undertook the statistical analysis and managed literature searches.

Author M. Adli designed the study, wrote the protocol and recruited study subjects, supervised the project and provided critical feedback.

Author M. Berger, M. Busche and S. Bopp recruited study subjects, undertook parts of statistical analysis, provided literature searches and critical feedback.

Author P. Schlattmann supervised the statistical analysis.

Authors C. Hindinger, S. Meyer and K. Hoffmann recruited study subjects, provided literature searches and analysis.

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Authors F. Lang and H. Fakhri performed the quantification of cytokines and provided critical feedback.

Author R. Ricken designed the study, wrote the protocol, recruited study subjects, supervised the project, undertook the statistical analysis and wrote the first draft of the manuscript.

All authors contributed to and have approved the final manuscript.

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Conflicts of interest

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Table 3. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine IL-2 serum levels (logarithmized, in pg/ml); number of observations = 172

	Estimated differences	Standard error	95%- Confidence-interval		t-value	p-value
			Lower	Upper		
Speed of onset of the depressive episode						
Fast onset	-0.16	0.06	-0.29	-0.04	-2.68	**0.01**
Slow onset (Ref)	-	-	-	-	-	-
Duration of LA						
Before LA	-0.03	0.03	-0.09	0.03	-0.86	0.39
After at least 4 weeks (Ref)	-	-	-	-	-	-
Gender						
Male	-0.06	0.06	-0.17	0.06	-1.00	0.32
Female (Ref)	-	-	-	-	-	-
Age	-0.01	0.01	-0.01	-0.01	-2.19	**0.03**
BMI	0.01	0.01	-0.01	0.01	-0.51	0.61
HAMD-17	0.01	0.01	-0.01	0.01	1.36	0.18
Duration of the current episode						
Less than 2 year	0.12	0.06	0.01	0.25	2.03	**0.046**
More than 2 years	-0.08	0.08	-0.24	0.09	-0.92	0.36
Less than 6 months (Ref)	-	-	-	-	-	-

***IL** = Interleukine; **Ref =** Reference; **LA** = Lithium augmentation; **BMI**=Body mass index; **HDRS-17** = Hamilton Depression Rating Scale*

Table 4. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine IL-4 serum levels (logarithmized, in pg/ml); number of observations = 171

	Estimated differences	Standard error	95%- Confidence-interval		t-value	p-value
			Lower	Upper		
Speed of onset of the depressive episode						
Fast onset	-0.05	0.03	-0.10	-0.01	-2.02	**0.047**
Slow onset (Ref)	-	-	-	-	-	-
Duration of LA						
Before LA	0.01	0.02	-0.04	0.06	0.40	0.69
After at least 4 weeks (Ref)	-	-	-	-	-	-
Gender						
Male	-0.06	0.02	-0.11	-0.01	-2.40	**0.02**
Female (Ref)	-	-	-	-	-	-
Age	0.01	0.01	-0.01	0.01	1.93	0.06
BMI	-0.01	0.01	-0.01	0.01	-1.41	0.16

HAMD-17	-0.01	0.01	-0.01	0.01	-0.58	0.56
Duration of the current episode						
Less than 2 year	0.01	0.03	-0.05	0.05	0.05	0.96
More than 2 years	-0.02	0.03	-0.08	0.05	-0.47	0.64
Less than 6 months (Ref)	-	-	-	-	-	-

IL *= Interleukine;* ***LA*** *= Lithium augmentation;* ***Ref*** *= Reference;* ***BMI****=Body mass index;* ***HDRS-17*** *= Hamilton Depression Rating Scale*

Table 5. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine IL-6 serum levels (logarithmized, in pg/ml); number of observations = 172

	Estimated differences	**Standard error**	**95%- Confidence-interval**		**t-value**	**p-value**
			Lower	**Upper**		
Speed of onset of the depressive episode						
Fast onset	-0.15	0.06	-0.27	-0.03	-2.46	**0.02**
Slow onset (Ref)	-	-	-	-	-	-
Duration of LA						
Before LA	-0.02	0.05	-0.11	0.07	-0.50	0.62
After at least 4 weeks (Ref)	-	-	-	-	-	-
Gender						
Male	-0.08	0.06	-0.20	0.03	-1.45	0.15
Female (Ref)	-	-	-	-	-	-
Age	-0.01	0.01	-0.01	0.01	-0.59	0.56
BMI	-0.01	0.01	-0.01	0.01	-0.35	0.73
HAMD-17	0.01	0.01	-0.01	0.01	0.77	0.44
Duration of the current episode						
Less than 2 year	-0.06	0.06	-0.06	0.18	1.01	0.31
More than 2 years	-0.05	0.08	-0.21	0.11	-0.62	0.54
Less than 6 months (Ref)	-	-	-	-	-	-

IL *= Interleukine;* ***LA*** *= Lithium augmentation;* ***Ref*** *= Reference;* ***BMI****=Body mass index;* ***HDRS-17*** *= Hamilton Depression Rating Scale*

Table 6. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine IL-8 serum levels (logarithmized, in pg/ml); number of observations = 173

	Estimated differences	**Standard error**	**95%- Confidence-interval**		**t-value**	**p-value**
			Lower	**Upper**		
Speed of onset of the depressive episode						
Fast onset	-0.05	0.06	-0.16	0.06	-0.89	0.38
Slow onset (Ref)						

	-	-	-	-	-	-
Duration of LA Before LA After at least 4 weeks (Ref)	0.01 -	0.05 -	-0.10 -	0.09 -	-0.15 -	0.88 -
Gender Male Female (Ref)	-0.02 -	0.05 -	-0.13 -	0.08 -	-0.44 -	0.66 -
Age	0.01	0.01	-0.01	0.01	1.01	0.32
BMI	-0.01	0.01	-0.01	0.01	-0.63	0.53
HAMD-17	-0.01	0.01	-0.01	0.01	0.28	0.78
Duration of the current episode Less than 2 year More than 2 years Less than 6 months (Ref)	0.13 0.01 -	0.06 0.07 -	-0.02 -0.15 -	0.24 0.15 -	2.33 0.00 -	0.02 1.00 -

IL *= Interleukine;* ***LA*** *= Lithium augmentation;* ***Ref*** *= Reference;* ***BMI****=Body mass index;* ***HDRS-17*** *= Hamilton Depression Rating Scale*

Table 7. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine IL-10 serum levels (logarithmized, in pg/ml); number of observations = 170

	Estimated differences	**Standard error**	**95%- Confidence-interval**		**t-value**	**p-value**
			Lower	**Upper**		
Speed of onset of the depressive episode Fast onset Slow onset (Ref)	-0.15 -	0.06 -	-0.27 -	-0.04 -	-2.51 -	**0.01** -
Duration of LA Before LA After at least 4 weeks (Ref)	-0.06 -	0.04 -	-0.14 -	0.02 -	-1.41 -	0.16 -
Gender Male Female (Ref)	-0.05 -	0.06 -	-0.16 -	0.06 -	-0.90 -	0.37 -
Age	0.01	0.01	-0.01	0.01	0.05	0.96
BMI	-0.01	0.01	-0.01	0.01	-0.19	0.85
HAMD-17	0.01	0.01	-0.01	0.01	1.61	0.11
Duration of the current episode Less than 2 year More than 2 years Less than 6 months (Ref)	0.20 0.02 -	0.06 0.08 -	0.08 -0.14 -	0.32 0.18 -	3.30 0.22 -	**0.01** 0.83 -

IL *= Interleukine;* ***LA*** *= Lithium augmentation;* ***Ref*** *= Reference;* ***BMI****=Body mass index;* ***HDRS-17*** *= Hamilton Depression Rating Scale*

Table 8. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine IFN-γ serum levels (logarithmized, in pg/ml); number of observations = 172

	Estimated differences	Standard error	95%- Confidence-interval		t-value	p-value
			Lower	Upper		
Speed of onset of the depressive episode						
Fast onset	-0.12	0.04	-0.19	-0.05	-3.41	**0.01**
Slow onset (Ref)	-	-	-	-	-	-
Duration of LA						
Before LA	-0.01	0.03	-0.06	0.06	-0.09	0.94
After at least 4 weeks (Ref)	-	-	-	-	-	-
Gender						
Male	-0.08	0.03	-0.14	-0.01	-2.40	**0.02**
Female (Ref)	-	-	-	-	-	-
Age	-0.01	0.01	-0.01	0.01	-0.25	0.80
BMI	-0.01	0.01	-0.01	0.01	-0.08	0.93
HAMD-17	0.01	0.01	-0.01	0.01	0.12	0.91
Duration of the current episode						
Less than 2 year	0.11	0.04	0.04	0.18	3.24	**0.01**
More than 2 years	0.01	0.05	-0.09	0.10	0.17	0.87
Less than 6 months (Ref)	-	-	-	-	-	-

IFN-γ *= interferon-gamma;* ***LA*** *= Lithium augmentation;* ***BMI****=Body mass index;* ***HDRS-17*** *= Hamilton Depression Rating Scale*

Table 9. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine TNF-α serum levels (logarithmized, in pg/ml); number of observations = 173

	Estimated differences	Standard error	95%- Confidence-interval		t-value	p-value
			Lower	Upper		
Speed of onset of the depressive episode						
Fast onset	-0.09	0.03	-0.15	-0.02	-2.75	**0.01**
Slow onset (Ref)	-	-	-	-	-	-
Duration of LA						
Before LA	-0.03	0.03	-0.09	0.02	-1.16	0.25
After at least 4 weeks (Ref)	-	-	-	-	-	-
Gender						
Male	-0.08	0.03	-0.14	-0.02	-2.74	**0.01**
Female (Ref)	-	-	-	-	-	-
Age	-0.01	0.01	-0.01	0.01	-0.18	0.87
BMI	-0.01	0.01	-0.01	0.01	-1.86	0.07

HAMD-17	0.01	0.01	-0.01	0.01	0.84	0.41
Duration of the current episode						
Less than 2 year	0.07	0.03	0.01	0.13	2.17	0.03
More than 2 years	0.03	0.04	-0.05	0.11	0.72	0.48
Less than 6 months (Ref)	-	-	-	-	-	-

TNF-α *= tumor necrosis factor alpha;* ***LA*** *= Lithium augmentation;* ***BMI****=Body mass index;* ***HDRS-17*** *= Hamilton Depression Rating Scale*

Table 10. The effect of patients with faster versus slower onset of the current depressive episode and covariates on cytokine GM-CSF serum levels (logarithmized, in pg/ml); number of observations = 172

	Estimated differences	**Standard error**	**95%- Confidence-interval**		**t-value**	**p-value**
			Lower	**Upper**		
Speed of onset of the depressive episode						
Fast onset	-0.10	0.05	-0.20	0.01	-1.90	0.06
Slow onset (Ref)	-	-	-	-	-	-
Duration of LA						
Before LA	-0.03	0.03	-0.10	0.04	-0.85	0.40
After at least 4 weeks (Ref)	-	-	-	-	-	-
Gender						
Male	-0.18	0.05	-0.28	-0.08	-3.73	**0.01**
Female (Ref)	-	-	-	-	-	-
Age	-0.01	0.01	-0.01	0.01	-1.10	0.28
BMI	0.01	0.01	-0.01	0.01	0.61	0.54
HAMD-17	-0.01	0.01	-0.01	0.01	-0.17	0.87
Duration of the current episode						
Less than 2 year	0.07	0.05	-0.04	0.17	1.29	0.20
More than 2 years	0.06	0.07	-0.08	0.20	0.85	0.40
Less than 6 months (Ref)	-	-	-	-	-	-

GM-CSF *= granulocyte and monocyte colony stimulating factor;* ***LA*** *= Lithium augmentation;* ***BMI****=Body mass index;* ***HDRS-17*** *= Hamilton Depression Rating Scale*