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Increased circulatory IL-6 during 8-week fluoxetine treatment is a risk factor for suicidal behaviors in youth

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ABSTRACT

Objective: Selective serotonin reuptake inhibitors (SSRIs) are commonly used to treat anxiety and/or depression in pediatric populations. However, the response rates are low (approximately 50%). Moreover, SSRI use is frequently associated with adverse events (AE). Currently there are no available biomarkers for treatment response/AE. Identification of biomarkers predicting early response and/or AE could help maximize the benefit-risk ratio for the use of SSRIs, and accelerate matching of treatments to patients. Pro-inflammatory cytokines were proposed as potential biomarkers.

Method: Ninety-two patients (35 boys and 57 girls) with major depressive disorder and/or anxiety disorders, aged 13.90 ± 2.41 years, were treated with fluoxetine (FLX) for 8 weeks. Plasma concentrations of TNFα, IL-6, and IL-1β were measured by enzyme linked immunosorbent assays before and after FLX treatment. Clinical response and AE were measured using several clinical scales, including the Clinical Global Impression – improvement, Children's Depression Rating Scale–Revised, the Beck Depression Inventory, the Screen for Child Anxiety Related Emotional Disorders, the Columbia suicide severity rating scale, and the Suicide Ideation Questionnaire.

Results: IL-6 levels increased after treatment only in the group of children who developed FLX-associated suicidality.

Conclusion: An increase in IL-6 levels during treatment may be a risk factor for the emergence of FLX-associated suicidality (OR = 1.70). Further studies are necessary to clarify the role and mechanism(s) of this cytokine in the pathogenesis of this life-threatening AE.

1. Introduction

Mood and anxiety disorders are the most common childhood psychiatric disorders. Selective serotonin reuptake inhibitors (SSRIs) are generally considered first-line treatment for depression and anxiety disorders in this age group. However, 30–40 percent of patients who receive the recommended dose and duration of treatment fail to respond (Maalouf and Brent, 2012). Moreover, some children and adolescents will respond to SSRI treatment with adverse events (AE). Particularly worrisome is the association of SSRIs with psychiatric adverse events (PAE), and especially SSRI-induced suicidal ideation and behaviors (Bridge et al., 2007; Cheung et al., 2005). This led to the "black box" warning, published in 2004, stating that in children and adolescents, treatment with anti-depressants (ADs) may pose a risk of suicidal behaviors (SBs), violence, aggression, mania, and other abnormal behavioral changes (Dantzer et al., 2008). Despite years of research and various evidence-based theories regarding molecular processes, the mechanisms leading to treatment response and emergence of AE remain unclear. Moreover, there are no biomarkers for prediction of either response to SSRIs or AE in children or adolescents treated for depression and/or anxiety disorders.

Pro-inflammatory cytokines have been proposed as possible

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biomarkers for treatment response, based on the inflammatory hypothesis suggesting pro-inflammatory cytokines as major contributors to the emergence of depressive symptoms (Miller and Raison, 2016; Rosenblat et al., 2014; Black and Miller, 2015) and suicidality (Ducasse et al., 2015; Ganança et al., 2016; Erhardt et al., 2013; Miná et al., 2015; Pandey, 2015; Więdłocha et al., 2018). Numerous studies report significant influence of ADs on pro-inflammatory/anti-inflammatory cytokines balance, but the available data is often inconsistent regarding specific cytokines and pharmacological intervention (Köhler et al., 2018; Manoharan et al., 2016). Moreover, most of the data regarding the effects of ADs on circulatory cytokine levels were documented in adults (Lanquillon et al., 2000; Yoshimura et al., 2013; Hannestad et al., 2011; Gabbay et al., 2009), with few publications in pediatric populations (Pandey et al., 2012; Amitai et al., 2016; Zohar et al., 2018; Pérez-Sánchez et al., 2018; Keaton et al., 2019).

Several studies suggest dysregulation of the immune system and cytokines in SBs. In order to examine if cytokine dysregulation is associated with suicide, several investigators assessed cytokine levels in plasma, cerebrospinal fluid (CSF), and postmortem brain of suicidal adult patients. In general, these studies indicate abnormalities of several cytokines in suicide (Więdłocha et al., 2018), with IL-6 being implicated most often (Pandey et al., 2018; Marini et al., 2016; Eftekharian et al., 2018; Shanee et al., 1997). To our knowledge, there are no studies on the contribution of pro-inflammatory cytokines to SSRI-induced AE, and specifically to SSRI-associated SBs.

In a previous study, we showed that FLX treatment in depressed/anxious children and adolescents significantly reduced plasma TNFα levels, and that levels of the pro-inflammatory cytokines TNFα, IL-6 and IL-1β were elevated in children refractory to FLX compared to responders (Zohar et al., 2018). The purpose of this study was to expand our research to a larger cohort, and test whether: (1) FLX treatment of children and adolescents with anxiety and/or depression disorders influence the levels of the pro-inflammatory cytokines TNFα, IL-6 and IL-1β, (2) plasma levels of these pro-inflammatory cytokines can predict response to FLX treatment, and (3) these circulatory pro-inflammatory cytokines can predict AE to FLX treatment, with special emphasis on SSRI-associated SBs. Our hypothesis states that FLX will demonstrate anti-inflammatory properties, as reflected by a decrease in pro-inflammatory cytokines, and that such an immunomodulatory effect will be associated with a favorable treatment response. Moreover, we hypothesized that patients with increased circulatory inflammatory cytokines will exhibit resistance to FLX treatment.

According to the available data in the literature regarding the relationship between IL and 6 and suicidality, we specifically a priori hypothesized that elevated plasma IL-6 levels would predict the emergence of FLX-associated SBs.

2. Method

2.1. Study design

We approached patients with consecutive admissions to the psychiatric outpatient department at a university-affiliated pediatric medical center, where severity of depressive/anxiety disorders justified pharmacological monotherapy with FLX. Our inclusion criteria were 6–18 years of age; and a diagnoses of major depression (MDD) or anxiety disorder were established according to the *Diagnostic and Statistical Manual of Mental Disorders*, 5th (DSM-5) criteria ed) (Bernstein and Shaw, 1997) following an interview according to the Kidi Schedule for Affective Disorders and Schizophrenia for School-aged Children, Present and Lifetime (K-SADS-PL) guidelines Add reference Shanee N, Apter A, Weizman A. A Psychometric properties of thr K-SADS-PL in an Israeli adolescent clinical population. Isr J Psychaitry Relat Sci. 1997; 34:179–186.

(30) All diagnoses were in the level of at least moderate severity, with a Clinical Global Impressions–Severity (GCI-S) score of ≥4 (consistent with accepted guidelines for the use of ADs in children and adolescents) (Birmaher et al., 1998; Clinical, 1976). Exclusion criteria included major neurological disorder or medical illness, mental retardation, organic brain syndrome, autism spectrum disorder (ASD); and a history of hypomania or mania, psychosis, or substance abuse. Children on psychiatric medications were excluded, except for stimulants or benzodiazepines received as a steady dose over a time period of more than 12 weeks. We did not exclude children who had a previous trial of SSRI if at least 6 months had passed since cessation of previous treatment. Presence of comorbid disorders and psychological treatment were allowed. Patients with anorexia nervosa or any other eating disorder were not excluded, unless exhibiting abnormal BMI (at least 15% underweight).

2.2. Evaluation

Response was measured with the Clinical Global Impressions–Improvement (CGI-I) scale (Poznanski et al., 1984). Continuous measures of depression and anxiety were conducted using the Children's Depression Rating Scale–Revised (CDRS-R) (Smucker et al., 1986), the Beck Depression Inventory (BDI) (Birmaher et al., 1999), and the Screen for Child Anxiety-Related Emotional Disorders (SCARED) (Birmaher et al., 1997; Kronenberg et al., 2007).

Adverse events were evaluated according to several measures: the SSRI Side-effect Profile Inventory (SEPI), a questionnaire designed to detect 24 of the most common possible AEs known for SSRIs and their severity, as previously described (Table S1, Supplementary Data) (Amitai et al., 2016; Amitai et al., 2015). Each AE was rated on a 5-point scale from 0 (none) to 4 (very severe). The SEPI form was completed at baseline, and administered biweekly by reading aloud the checklist, and asking patients additional open-ended questions regarding any AE they reported in order to determine its severity. For previously reported AEs, the investigators inquired whether that AE had improved, worsened or remained stable. The SEPI was administered prior to the administration of FLX, to insure patients reported emergence of new AEs and were not merely reporting on symptoms related to the disorder. Nonetheless, a substantial increase in previous complaints was considered a new AE.

A special emphasis was given to PAEs, which were divided into four clusters: (1) suicidality, (2) manic/hypomanic (including elevated mood), (3) activation syndrome (including agitation, hyperactivity, akathisia, nervousness, restlessness, irritability, hypersensitivity, worsening of anxiety symptoms and bursts of anger) (Reynolds, 1987), and (4) other complaint (including emotional numbness tremor, feeling spacy, tics, bruxism and psychotic symptoms). All PAEs were recorded if spontaneously reported by the participants or their parents during the clinical follow-up. Further, PAEs were systematically evaluated during the 8-week follow-ups using several clinical tools.

Suicidality cluster symptoms were evaluated using the: (1) SIQ-SV - Suicide Ideation Questionnaire, Short Version, completed by the child for the evaluation of suicidal ideation (SI) (Posner et al., 2011), where decreasing scores by a magnitude of more than 10% was considered worsening of SI; (2) Columbia Suicide Severity Rating Scale (C-SSRS), designed to standardize the assessment of a broad range of suicide-relevant behaviors, including the severity and intensity of SI, suicidal attempt (SA), and SA lethality (any elevation of score was considered a worsening in suicidality) (Young et al., 1978); and (3) question #13 in the CDRS questionnaire (any elevation of score after 8 weeks of treatment was considered a worsening in SI. Suicidal events during the follow-up period were classified into three categories: (1) actual SA – all self-injurious behaviors or harmful acts with an intent to die; (2) self-harm behaviors with no intent to die; and (3) new SI or worsening of SI. Category 2 coincides with the recent proposal to include non-suicidal self-injury (NSSI) as a nosological entity in the DSM-5 Research Criteria (Bernstein and Shaw, 1997). *Manic cluster symptoms* were assessed using the Young Mania Rating Scale (YMRS) (Mössner et al., 2007) after

8 weeks of treatment.

Activation syndrome cluster symptoms and all other AEs were determined according to the SEPI questionnaire (Amitai et al., 2016; Amitai et al., 2015).

PAEs were divided to three groups according to the FDA criteria for severity of AEs: mild, moderate or severe. Mild AE were AEs reported by the patients, or AEs reported in the clinical questionnaires that were not associated with functional impairment or demanding urgent intervention. Moderate AEs caused functional impairment or demanded intervention (including immediate discontinuation of the drug or addition of a psychotropic drug, e.g. antipsychotic treatment). Severe AEs were either life-threatening, required hospitalization, disabling, or caused death.

2.3. Procedure

The study was approved by the Helsinki committee of our institute, and informed consent was obtained from the subjects and their parents. After a confirmatory diagnostic assessment, all subjects received a starting FLX dosage of 10 mg/d for one week, subsequently increased to 20 mg/d through week 4. If the degree of improvement was minimal (CGI-I $\geq$ 3), dosage was increased from 20 to 40 mg/day in week 5. A follow-up meeting every two weeks assessed improvement of symptoms and AEs. At the end of week 8, each child was considered a responder or a non-responder according to CGI-I (CGI-I = 1/2 vs. CGI-I $\geq$ 3, respectively).

2.4. Plasma cytokine assessment

Peripheral blood (20 mL) was collected by venipuncture from all subjects between 9:00–11:00, prior to starting treatment and after 8 weeks of FLX treatment. Blood samples were centrifuged immediately (1200 × rpm) at 4 °C for 10 min to obtain plasma. Plasma samples were separated into aliquots and stored at −80 °C. During analysis, duplicate samples were analyzed to determine levels of human cytokines TNFα, IL-6 and IL-1β in the same run, to avoid inter-assay variability. Cytokines were assayed on seven 96-well plates with samples from all groups distributed evenly. All cytokines were assessed with a sandwich enzyme linked immunosorbent assay (ELISA). We used high sensitivity kits (R and D systems, Minneapolis, USA), following the manufacturer's protocol. The laboratory staff was blinded to the clinical data and the clinical team was blinded to the laboratory data.

2.5. Statistics

The Statistical Package for the Social Sciences (SPSS), version 17 for Windows (SPPS Inc., Chicago, IL) was used to create a database and conduct the statistical analyses. Data for all three cytokines were transformed into normal distribution using natural logarithm and all cytokine levels were scaled to z-scores. Samples with cytokine levels below detection limit were assigned a value corresponding to the lowest detectable value in the assay. Paired and unpaired t-tests and general linear models were used with repeated measures; associations between demographic and clinical variables with laboratory values were analyzed using Spearman's correlation or chi square test, as appropriate. A logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for emergence of suicidality during SSRI treatment and responsiveness. Several known risk factors for suicidality were examined as covariates. All analyses were two tailed and results expressed as Mean ± SD, with the level of significance set at 5%. P-values were corrected for multiple tests using FDR where applicable.

3. Results

3.1. Subjects

Ninety-five subjects were recruited, and 92 completed the 8-week follow-up: 35 (38%) boys and 57 (62%) girls aged 13.90 ± 2.41 years, 7 (8%) had a diagnosis of MDD alone, 67 (72.8%) had combined diagnoses of MDD and anxiety disorders, and 18 (20%) had a diagnosis of anxiety disorders alone (general anxiety disorder, social anxiety disorder, panic disorder with or without agoraphobia, separation anxiety disorder, specific phobia or a combination of these). The mean number of anxiety disorders was 1.85 ± 1.13 per patient. Of subjects with MDD, 17 (19%) had a diagnosis of double depression as main diagnosis, 15 (16%) children had obsessive–compulsive disorder (OCD), 14 (15.2%) had eating disorders, and 47 (51%) had attention deficit/hyperactivity disorder (ADHD). The average pretreatment BMI was 20.62 ± 4.07 Kg/m (Bridge et al., 2007). Six (7%) children had a previous trial with an SSRI, 59 (67%) had a first-degree family relative with unipolar MDD, and 9 (10%) had a second-degree family relative with unipolar MDD. Four children (4%) had a first-degree family relative with bipolar disorder (BPD), and 10 (11%) had a second-degree family relative with BPD.

3.2. Clinical results

Thirty-five (38%) children were identified as responders and received a rating of "very much improved" or "much improved" on the CGI-I. Supplementary Table S2 presents the demographic and clinical data. There were no significant differences between responders and non-responders in baseline characteristics, including age, gender, BMI, main diagnosis, type of depression, history of SSRI treatment, presence of family relatives with MDD or BPD, or pretreatment scores of anxiety and depression levels (p = NS for all).

Table 1 presents changes in psychometric scores from baseline to follow-up after 8 weeks of treatment. There was a statistically significant difference between responders and the non-responders in change in CDRS-R scores (responders vs. non-responders: –32.85 ± 15.45 vs. −16.28 ± 16.52, t = 4.71, df = 87, $p < 0.001$) and CGI-I scores (responders vs. non-responders: 1.97 ± 0.17 v.s. 3.58 ± 0.82, t = 14.25, df = 63, $p < 0.001$). No other significant differences were detected among groups.

Table 1
Comparison between Responders and Non-responders in the changes (from baseline to week eight) in the various ratings following fluoxetine treatment.

Characteristics	Responders (n = 35)	Non-responders (n = 57)	p value unpaired *t*-test
Change in BMI (kg/m^2)	0.05 ± 0.94	−0.11 ± 0.76	t = −0.77, df = 65, p = NS
CGI-I	1.97 ± 0.17	3.58 ± 0.82	t = 14.25, df = 63, p < 0.001*
Change in BDI scores	−8.55 ± 9.17	−5.94 ± 9.18	t = 1.30, df = 88, p = NS
Change in CDRS-R scores	–32.85 ± 15.45	−16.28 ± 16.52	t = 4.71, df = 87, p < 0.001*
Change in SCARED scores	−9.90 ± 11.75	−6.63 ± 11.77	t = 1.28, df = 89, p = NS

BDI, Beck Depression Inventory; BMI, body mass index; CDRS-R, Children's Depression Rating Scale – Revised; CGI-I, Clinical Global Impressions – Improvement; SCARED, Screen for Child Anxiety Related Emotional Disorders.

3.3. Adverse events

Eighty (87%) children developed an AE of any severity: 43 (47%) mild, 32 (35%) moderate, and 5 (5.4%) severe. Of these, 55 (60%) reported an AE spontaneously during a clinical visit, while AE were detected only in questionnaires for 25

(7%) children. No death related to AE occurred. Of the five children with serious AEs, one switched to a manic episode and four committed a serious SA that led to hospitalization.

3.4. Suicidality

Twenty-three (25%) children had a history of SA prior to starting treatment, as evaluated by the C-SSRS. No differences were detected between the group of children who had a previous SA and all others regarding age, gender or diagnosis (data not shown). Emergence of clinically meaningful SBs (worsening in SI that demanded urgent intervention, NSSI or SA) were reported by 18 (20%) of the FLX-treated children, predominantly in children with a previous SA (chi-square: χ (Bridge et al., 2007) = 4.51, df = 1, p = 0.034). Worsening in SI according to the clinical questionnaires (SIQ, CDRS and C-SSRS) was recorded in 30 (33%) of the children, with no relation to a history of a previous SA.

3.5. Activation syndrome

Clinically meaningful activation (agitation, aggression, or hyperactivity that demanded intervention) was observed during follow-up in 26 (28.3%) children under FLX treatment. A strong positive correlation was found between clinically meaningful suicidal behaviors and aggressive behaviors (chi-square: χ (Bridge et al., 2007) = 11.91, df = 1, p = 0.001). Subjective or objective activation symptoms according to the SEPI questionnaire were documented in 51 (56%) children.

3.6. Manic symptoms

Seven (8%) children developed clinically meaningful AEs from the manic cluster, including manic switch (N = 1), hypomanic episode (N = 1), or elevated mood (N = 5).

Thirty-five (38%) children developed emotional numbness during the 8-week follow-up, according to the SEPI.

3.7. Cytokines' measurements

No correlation was found between pretreatment cytokine levels and age, gender, or diagnosis (p greater than 0.1 for all). A positive correlation was found between pretreatment IL-6 levels and pretreatment BMI (Spearman correlation: r = 0.33, p = 0.002). No correlation was found with the two other cytokines, or between pretreatment cytokine and anxiety or depression levels, as measured by the scores of the corresponding scales at baseline (p greater than 0.1).

Table 2 and Fig. 1 describe the Z scores of log of the levels of the three measured cytokines, at the two time points. After 8 weeks of treatment, TNFα and IL-6 did not change significantly [paired t test, p = NS]; IL-1β increased significantly (paired t-test: z scores: −0.15 ± 0.96 vs. 0.05 ± 0.81, t = 2.14, df = 88, p = 0.035), but the significance was lost after FDR correction (p = 0.11). No significant differences were observed between the responders and non-responders groups in pretreatment cytokine levels (Supplementary Table S3), or between the differences in the before and after levels in the log of the levels of the three cytokines measured (unpaired t test: p = NS for all, see Fig. 2). For the raw levels of the cytokines in the whole group as well as in the responders and non-responders see Supplementary Tables S4 and S5.

Table 2
Cytokine levels (Z scores of the natural log values) before and after fluoxetine treatment in all the study population (N = 92).

Cytokine levels (z Scores)	Pretreatment	After 8 weeks of treatment	Paired t-test
TNFα levels	−0.008 ± 1.05	− 0.03 ± 0.88	t = 0.16, df = 87, p = NS
IL-6 levels	−0.20 ± 1.11	− 0.12 ± 0.91	t = −0.56, df = 86, p = NS
IL-1β levels	−0.15 ± 0.96	0.05 ± 0.81	t = −2.14, df = 88, p = 0.035, after FDR: 0.11

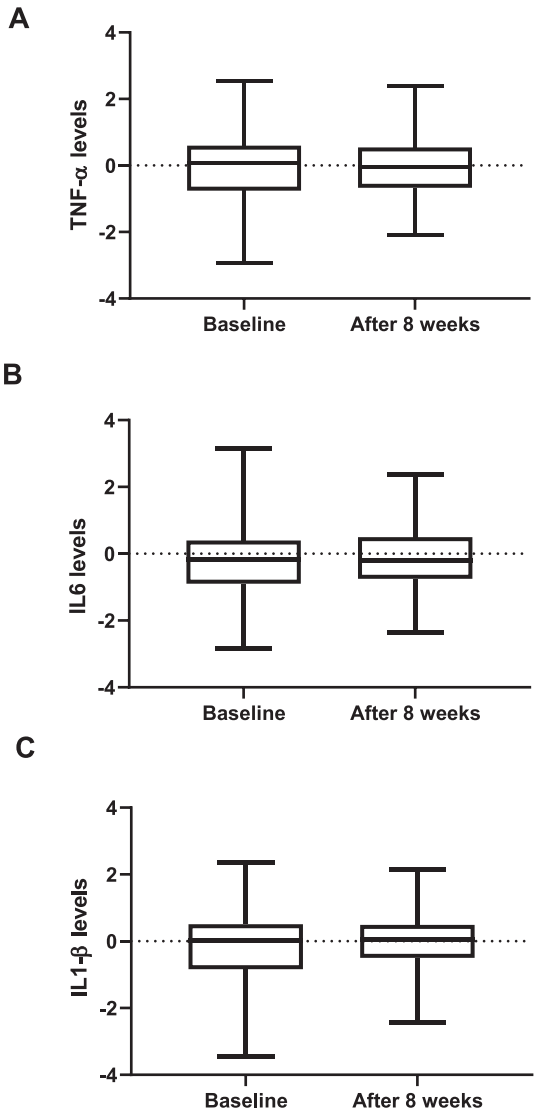

Fig. 1. Z scores of the log levels of the three measured cytokines in the whole sample. After 8 weeks of treatment, TNFα, IL-6 and IL-1β did not change significantly (data is plotted as a box plot with bars representing the standard error of the mean).

3.8. Adverse events

Lower baseline IL-6 levels were found in children who developed clinically meaningful suicidality (independent sample t-test: z scores: −0.75 ± 0.96 vs. −0.07 ± 1.09, t = 2.37, df = 87, p = 0.02),

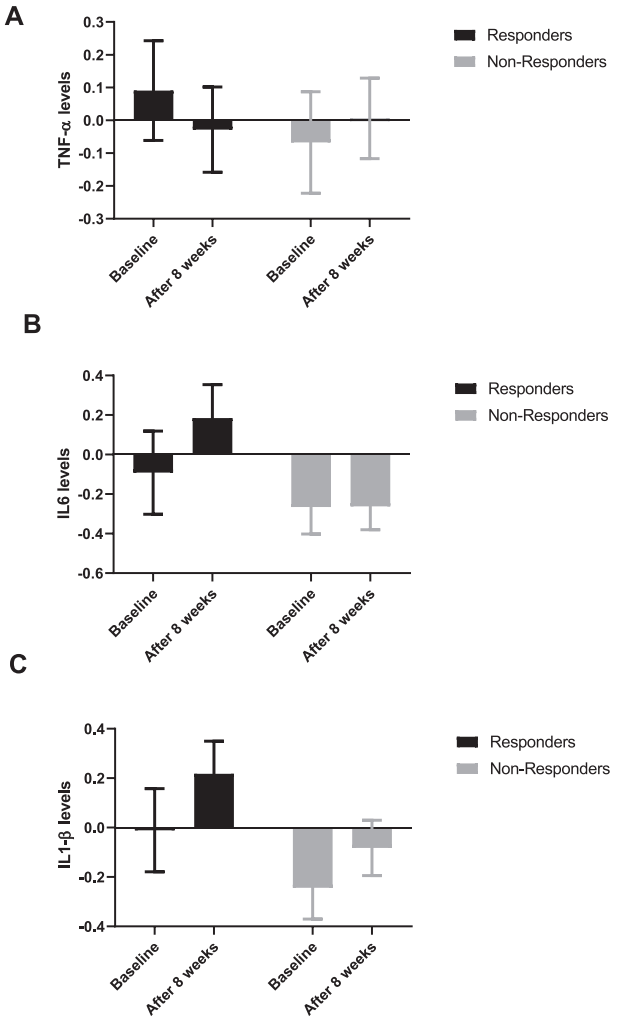

Fig. 2. Z scores of log levels of the three measured cytokines between responders and non-responders). No differences were observed between the differences in the before and after levels in the log of the levels of the three cytokines measured (p = NS for all). Data is plotted as a box plot with bars representing the standard error of the mean.

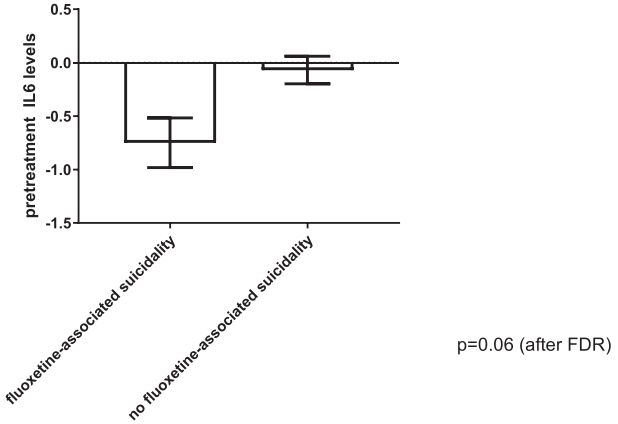

Fig. 3. Z scores of log of IL-6 pretreatment levels in children/adolescents who developed fluoxetine-associated suicidality vs. those who did not. Lower baseline IL-6 levels were found in children who developed clinically meaningful suicidality (independent sample t-test: -0.75 ± 0.96 vs. -0.07 ± 1.09, t = 2.37, df = 87, p = 0.02), however, significance was lost after FDR correction (p = 0.06) (data is plotted as a box plot with bars representing the standard error of the mean).

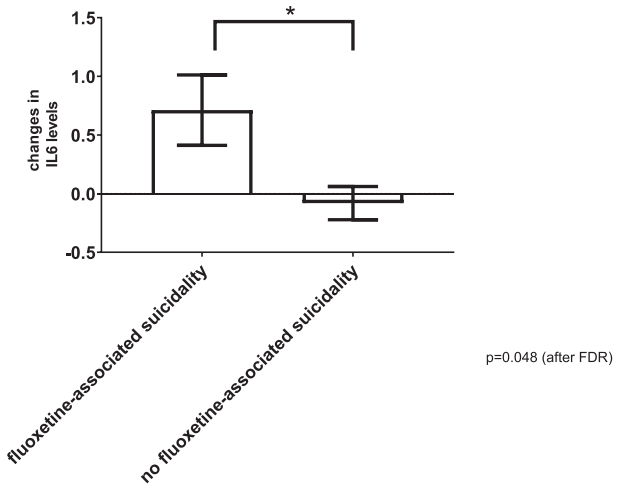

Fig. 4. Difference in z scores of log of IL-6 levels in children who developed fluoxetine-associated suicidality vs. those who did not. A significantly larger increase in the log of plasma IL-6 levels was detected following FLX treatment in children with FLX-associated suicidality vs. those who did not develop suicidality (p = 0.048). (Data is plotted as a box plot with bars representing the standard error of the mean).

however, significance was lost after FDR correction (p = 0.06) (Fig. 3). A significantly larger increase in the log of plasma IL-6 levels was detected following FLX treatment in children with FLX-associated suicidality vs. those who did not develop suicidality (independent sample t-test: z scores: -0.71 ± 1.24 vs. -0.08 ± 1.18, t = -2.46, df = 85, p = 0.016, after FDR correction (p = 0.048) (Fig. 4).

A backward stepwise logistic regression was performed to identify factors predicting the risk of FLX-associated suicidality. All known risk factors for SBs were included in the model, including gender, age, diagnosis, previous SA (yes/no) and differences in the log of the levels of all three cytokines measured. Table 3 describes the final model including gender, previous SA, and changes in IL-6 levels (χ (Bridge et al., 2007) = 11.604, DF = 3, P = 0.009). The strongest factor in predicting suicidality was the difference in the log of IL-6 levels, increasing the risk for FLX-associated suicidality with an OR of 1.70 (p = 0.032). All other variables did not contribute significantly.

No other differences were observed regarding other AEs and cytokine levels (data not shown).

4. Discussion

Given the relatively low rates of response for SSRI treatment in pediatric depression, it is important to identify biomarkers that predict response to therapy. Ability to predict biomarkers for AE is also important in efforts to personalize the treatment (Maes et al., 1992) especially in the pediatric population, due to concerns regarding SSRI-induced suicidality that led to the black-box warning. At present there are no valid clinical markers to personalize currently available ADs, all of which have similar mechanisms targeting monoamine neurotransmission. Thus, the current practice of AD prescription is by trial-and-error.

Circulatory inflammatory markers have been suggested to have the potential to personalize AD prescription, based on the theory that neuro-immuno-endocrinological interactions could be related to both psychiatric symptoms and medication-induced-AE. As far back as 1992, Maes proposed that dysregulated immune response contributes to depressive symptoms (Hiles et al., 2012). Evidence shows inflammatory cytokines contribute to depressive symptoms, and ADs attenuate the effects of inflammatory cytokines on the brain (Gabbay et al., 2009). Thus, it is reasonable to screen patients prior to AD treatment to identify subgroups of patients with increased inflammation, or

Table 3
Logistic regression predicting fluoxetine-associated suicidality (N = 88).

	B	SE	Wald χy^2 (df = 1)	OR (95% CI)	p value
Constant	−1.51	0.43	12.61	0.22	p < 0.001
Gender	−1.13	0.70	2.60	0.32 (0.08, 1.28)	p = 0.107
Previous SA	1.00	0.63	2.54	2.72 (0.79, 9.35)	p = 0.111
Change in IL-6 levels from baseline to week 8	0.53	0.25	4.61	1.70* (1.05, 2.76)	p = 0.032

SA: suicide attempt
*$p < 0.05$, remains significant following FDR correction

vulnerability to inflammation, in an attempt to reduce the heterogeneity of treatment response. Indeed, identification of such subgroups with a disordered inflammatory profile might be expected to increase the response rates, while precluding unnecessary exposure to AD treatments in those unlikely to benefit. Moreover, such biomarkers may prove beneficial in identifying and excluding patients likely to respond to treatment with AEs.

In our previous study, higher levels of three pro-inflammatory cytokines at admission were found to predict poor response to treatment, and TNFα levels significantly decreased following FLX treatment (Zohar et al., 2018). In this study, we expanded our cohort to 95 treated children and adolescents in order to validate our previous results; we also added clinical information regarding AEs, specifically SBs and activation symptoms.

In contrast to our previous report the levels of all three pro-inflammatory cytokines did not change significantly following FLX treatment. Transformed levels of IL-1β levels did increase after 8 weeks of treatment, but was not significant after FDR correction.

Studies on changes in levels of pro-inflammatory cytokines following SSRI administration in adults are inconsistent, and seem non-reproducible. Some studies found that pro-inflammatory cytokines significantly dropped after AD treatment. Hiles et al. reported that pharmacotherapy with AD drugs decreased concentrations of IL-6 (Serafini et al., 2013), while Hannestad et al. (Gabbay et al., 2009) demonstrated that AD therapy decreased IL-1β without having significant effect on IL-6 and TNFα. The decrease in IL-6 levels was, however, significant with a small effect size specifically for studies with the use of SSRIs.

Other studies failed to show this effect. A recent *meta*-analysis revealed significant decreases of IL-4, IL-6, and IL-10 in MDD subjects after AD treatment. In cases of IL-1 β, the decrease was significant exclusively for SSRI drugs, with no significant effect of AD medications on IL-2, TNFα, IFN-γ and CRP (Köhler et al., 2018). This suggests AD treatment affects the levels of cytokines in depression, however, the immunological dysregulation in MDD is complex and seems to be mediated by additional factors yet to be elucidated. It is important to note that the few pediatric studies also showed heterogeneous results. For instance, Pérez-Sánchez et al. (Keaton et al., 2019) showed that in adolescents treated for depression, the pro-inflammatory cytokines IFN-γ, IL-1β, TNFα, IL-6, IL-12, and IL-15 were decreased only at week 4 whereas IL-2 was increased only at week 8; the anti-inflammatory protein IL-1 receptor antagonist (IL-1Ra) was also reduced only at week 4, while IL-4 and IL-5 increased at week 8 (Pandey et al., 2018). In contrast, our previous study, also in a pediatric population, showed a significant decrease only in TNFα levels (Pérez-Sánchez et al., 2018).

In contrast to our previous study, pretreatment levels of the three cytokines did not correlate with psychopathological improvement and are, therefore, without predictive value. We did find differences between responders and non-responders in the behavior of the three cytokines measured, however, these did not reach statistical significance: TNFα levels decreased only in the responders group and increased in the non-responders group, consistent with the literature (Yoshimura et al., 2013). However, this finding did not reach statistical significance due to the large variability in cytokine levels. A non-significant increase in IL-6 levels was observed only in the responder group, while IL-1β levels increased in both groups. However, all these changes did not reach statistical significance.

Most of the studies on cytokines as possible biomarkers for treatment response were also conducted in adults (Lanquillon et al., 2000; Yoshimura et al., 2013; Hannestad et al., 2011). The different methodological approaches, such as type of the quantification of the psychopathological response or effects of psychopharmacological treatments on the immune parameters, led to diverse results, which are not conclusive with regard to the clinical course of depression. Nonetheless, the majority of studies suggest that activation of immunological parameters may be relevant for the outcome of depressive illness. Unfortunately, our study joins the inconsistent findings regarding the putative role of cytokines as biomarkers to predict response.

The most important finding was related to FLX-associated AE and specifically FLX-associated suicidality. Our study revealed that a significant elevation in IL-6 levels following 8-week FLX treatment appeared only in the group that developed FLX-associated SBs. A logistic regression performed on all known risk factors for suicidality showed that an increase in IL-6 following 8-week FLX treatment was the strongest factor significantly associated with risk for suicidality (OR = 1.70). Thus, it could be stated that, at least in our sample, the increase in IL-6 levels during treatment seemed to be a risk factor for the emergence of FLX-associated suicidality. These data underscore the relevance of IL-6 for this life-threatening AE.

Immune system dysregulation has already been associated with suicidal symptomatology, in adults as well as in adolescents. Pro-inflammatory cytokines have been implicated in the pathophysiology of SB (Ganança et al., 2016; Erhardt et al., 2013; Miná et al., 2015; Pandey, 2015; Więdłocha et al., 2018). There is growing evidence that inflammation, as manifested by increased levels of pro-inflammatory cytokines and inflammatory chemokines, is present in patients with SB and ideation. However, the results are inconclusive. The most consistent finding is elevated IL-6 in subjects with suicidality, as compared to patients without suicidality or healthy controls. The inflammatory changes can be detected in the periphery, CSF and brain parenchyma of affected patients. In one study, Pandey et al. showed that mRNA and protein expression levels of IL-1β, IL-6, and TNFα were significantly increased in Brodmann area 10 of teenage suicide victims compared with normal control subjects (Amitai et al., 2016). In another study, the same group showed that mRNA and protein levels of IL-1β, IL-6, TNFα, and lymphotoxin A were significantly increased, while levels of anti-inflammatory cytokine IL-10, and of IL-1Ra were significantly decreased in the prefrontal cortex of depressed individuals who died by suicide, compared with controls (Marini et al., 2016). Bay-Richter et al. (2015) showed a significant association between high CSF IL-6 levels and more severe suicidal symptoms in suicide attempters (Lindqvist et al., 2009). Black and Miller showed significant increases in in-vivo blood levels of IL-6 distinguished psychiatric patients with suicidality, both from psychiatric patients without suicidality and healthy control subjects (Ducasse et al., 2015). Lindqvist et al. found elevated CSF IL-6 levels in patients who attempted suicide compared with healthy controls. Higher levels of CSF IL-6 were associated with increasing severity of depression (Janelidze et al., 2011). Janelidze et al. showed that plasma IL-6 levels are increased in suicide attempters, compared with non-suicidal depressed patients and healthy controls (Nimmo et al.,

2013). Keaton et al. showed that a unique immuno-biological profile, including elevated IL-6 levels, was linked to increased suicide risk (Pandey et al., 2018).

Our study is the first to report an association between FLX-associated suicidality and immunological profile. It seems that in children prone to suicidality associated with FLX treatment, there is an inability of FLX to suppress elevation in IL-6 synthesis, and accumulation of the cytokine may lead to SBs. A possible explanation for this is that uncontrolled inflammation activates the kynurenine pathway, with a down-stream production of metabolites that affects the excitatory glutamate neurotransmission, which may eventually lead to symptom generation. Additionally, cytokines induce site-specific effects on behavior and emotion in different brain areas. Thus, it is possible that when FLX succeeds in restraining inflammation, children are protected from this AE; if FLX fails in diminishing inflammatory activation, children are more prone to the symptoms generated by the inflammatory processes and specifically to suicidality. Another possible explanation is that that FLX actually increases inflammation in some patients

This is consistent with the general IL-1 β rise with treatment that is reported in the literature, and consistent with other paradoxical reports of ADs increasing inflammatory biomarkers. (Hernández ME, Mendieta D, Martínez-Fong D, Loría F, Moreno J, Estrada I, Bojalil R, Pavón L. Variations in circulating cytokine levels during 52 week course of treatment with SSRI for major depressive disorder. Eur. Neuropsychopharmacol. 2008 Dec;18(12):917–24;

One important issue is the suitability of circulatory cytokine measures to reflect brain cytokines. Studies conducted in the CSF and postmortem brain of suicide victims suggest similar changes for IL-6, indicating that at least cytokines at the CSF may be a good measure of cytokine levels in the brain (Więdłocha et al., 2018). Although cytokines are also synthesized in the brain, bidirectional movements of cytokines between periphery and the CNS through several mechanisms have been suggested (Więdłocha et al., 2018). Thus, peripheral cytokines may not only be candidate biomarkers for suicide, but may also reflect similar changes in the brain. However further research is needed in order to validate this option.

There are some limitations and weaknesses in this study. Firstly, it is unlikely that a single biomarker represents a complex and heterogeneous phenomenon such as SSRI-induced suicidality. Antidepressants improve depressive symptoms only in a subset of patients and exert AE in others. Subsets of patients could respond to AD with various trajectories due to different pathophysiological mechanisms. Owing to the heterogeneity of the phenomenon, more pharmaco-metabolomic studies are required to identify key pathways involved in response to treatment/AE, and validate markers derived from different and common pathways. Studying an array of biomolecules that will be modulated on drug administration in different subsets of patients could facilitate finding potential biomarkers. Secondly, there are numerous other cytokines of interest that we did not investigate, which may also induce or inhibit production of other cytokines; it is important that future studies consider larger cytokine “networks” to obtain a broader picture of immune trajectories in patients. Also, we did not assess biomarkers from other pathophysiological pathways (for example: miRNAs). Thirdly, our treatment group consisted of children and adolescents with depression and/or anxiety disorders. Even though the pharmacological treatment for these disorders is the same (usually SSRIs), there might be different neurobiological mechanisms responsible for the different clinical phenotypes, which may confound our findings. Different diagnoses may have different mechanisms leading to FLX-associated suicidality. Fourthly, our study lacks a corresponding group of control subjects, such as an untreated depressed/anxiety group or a non-depressed/non-anxious healthy cohort.

Different methods for suicide attempt may also reflect variations in intent and lethality, and be associated with distinct inflammatory patterns. Due to the relatively small sample size in this study, suicide methods were not controlled for. Also, interactions with other biological markers associated with suicidal behaviors, such as monoamine or HPA axis mediators that might create potential confounds or contribute to SBs were not evaluated. We also have no measures of physical activity, which may well characterize adolescents with depression, and has been reported as influencing IL-6 levels (Hernández et al., 2008 Dec). Therefore, further research is needed to substantiate our preliminary results. However, the strength of the present study lies in its longitudinal nature and the wide panel of clinical and immunological parameters evaluated in all the young participants.

To conclude, in recent years, a number of studies have been performed in order to identify biological marker(s) associated with response to SSRI treatment; however, no biomarker has proven as a predictor of response. Moreover, no biomarkers for SSRI-induced AE were evaluated. Identification of potential biomarkers of patients at increased risk for iatrogenic SBs is of significant clinical importance. In the current study increases in IL-6 levels following 8-week FLX treatment were associated with high risk for emergence of SBs. It is possible that targeting the inflammatory system can provide a novel therapeutic approach in the pharmacotherapy of depression and SBs. For the goal of improved detection and treatment of patients at risk for SB, a detailed profound understanding of the origin, mechanisms, pathways and outcomes of response to SSRI treatment and AE is needed. The data are of clinical relevance and importance and support IL-6 as an accessible peripheral candidate biomarker with predictive value for the emergence of suicidality during FLX treatment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2019.12.017.

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