Cytokine levels in the blood may distinguish suicide attempters from depressed patients

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Conflict of Interest Statement

All authors declare that there are no conflicts of interest

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Abstract

Elevated plasma cytokines is a common finding in Major Depressive Disorder (MDD), although not consistent. It is currently not known whether the inflammatory changes are confined to any specific subgroup of depressive patients. We here analyzed three inflammatory markers in suicidal and non-suicidal depressive patients, as well as healthy controls.

Plasma Interleukin (IL)-2, IL-6 and tumor necrosis factor (TNF)-α were measured in 47 suicide attempters, 17 non-suicidal depressed patients and 16 healthy controls. Study participants were evaluated using the Comprehensive Psychopathological Rating Scale (CPRS) with subscales for anxiety and degree of depression, as well as the Suicide Assessment Scale (SUAS).

We found increased levels of IL-6 and TNF-α as well as decreased IL-2 concentrations in suicide attempters compared to non-suicidal depressed patients and healthy controls. The results were adjusted for potential confounders of cytokine expression, such as age, sex, body mass index (BMI), degree of depression, anxiety, personality disturbance, abuse and type of medication.

These results demonstrate for the first time that suicidal patients display a distinct peripheral blood cytokine profile compared to non-suicidal depressed patients. Thus, our study provides further support for a role of inflammation in the pathophysiology of suicidality.

Key Words: suicide, depression, inflammation, interleukin (IL)-6, interleukin (IL)-2, tumor necrosis factor (TNF)-α

Introduction

Recent evidence indicates that inflammation might be involved in the pathophysiology of psychiatric disorders. The expression of cytokines, chemokines, and other inflammatory markers is often altered in the blood of patients with depression, schizophrenia and bipolar disorder (Goldstein et al., 2009; Maes et al., 1995; Potvin et al., 2008; Raison et al., 2006). In experimental animals, systemically administered pro-inflammatory cytokines induce "sickness behavior", which closely resembles depressive symptoms, including social withdrawal, decreased appetite and motivation (Dantzer, 2001). Moreover, among patients receiving interferon (IFN)-α and interleukin (IL)-2 as treatment for hepatitis C or cancer, up to 50% develop depressive symptoms (Denicoff et al., 1987; Renault et al., 1987).

It is not clear why some psychiatric patients display elevated markers of inflammation, while others do not (Haack et al., 1999; Steptoe et al., 2003). One explanation could be that the diagnostic groups are symptomatically and biologically heterogeneous, and the inflammatory changes may be specific for certain cross-diagnostic features or subtypes. Such a subtype may be suicidal patients. Indeed, a post-mortem study indicated that microgliosis was present in the brains of both patients with schizophrenia and depression who had committed suicide, but not in patients from the same diagnostic groups who died from other causes (Steiner et al., 2008).

Only few other studies have investigated inflammatory changes associated with suicidal behavior (Gabbay et al., 2009). An initial study reported elevated concentrations of soluble IL-2 receptor (sIL-2R) in the blood of suicide attempters (Nassberger and Traskman-Bendz, 1993). We have reported high levels of IL-6 in the cerebrospinal fluid (CSF) of suicidal patients

(Lindqvist et al., 2009), and another recent study found elevated cytokine mRNAs in post-mortem brain tissue from suicide victims (Tonelli et al., 2008). However, it is not known if IL-6 or any other cytokines are altered in the blood of suicidal patients.

In the present study, we measured cytokine levels in the blood of suicidal patients, and compared them to those in non-suicidal, equally depressed subjects and healthy controls. We tested the hypothesis that peripheral inflammation in depression may be confined to suicidal patients.

Materials and Methods

Participants

This study was approved by the Ethical Review Board for human studies, Lund/Malmoe, Sweden. Fifty-four suicide attempters and 18 untreated patients with Major Depressive Disorder (MDD) were enrolled on admission to Lund University Hospital. Eighteen somatically healthy control subjects without any previous or ongoing psychiatric conditions were randomly selected from the municipal population register in Lund, Sweden. Individuals on antibiotics and anti-inflammatory medications were excluded from the study, leaving 47 suicide attempters (6 untreated and 41 treated), 17 untreated non-suicidal depressed patients and 16 healthy controls in the study. All study participants underwent a general physical examination which showed no evidence of ongoing infection. Demographic data and somatic diagnoses for the study participants are shown in table 1.

MDD patients were un-medicated for their depressive disorder for at least one month before sample collection. Forty-one suicide attempters were on medications at the time of sample collection: 9 were taking selective serotonin reuptake inhibitors (SSRI), 9 were taking serotonin–norepinephrine reuptake inhibitor (SNRI) and remaining 23 were taking a combination of different antidepressant drugs. Suicide attempt methods are shown in table 2.

Psychiatric ratings

All study participants were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) and evaluated using Montgomery and Asberg Depression Rating Scale (MADRS), the Suicide Assessment Scale (SUAS) and the Brief-Scale for Anxiety (BSA).

MADRS is a 10-item subscale with a maximum total score of 60 that has been derived from the Comprehensive Psychopathological Rating Scale (CPRS) (Montgomery and Asberg 1979). BSA is a CPRS-derived subscale for anxiety, also consisting of 10 items, as previously described (Tyrer et al., 1984). The Suicide Assessment Scale (SUAS) is an interview-based scale, consisting of 20 items assessing signs and symptoms related to suicidality and has a maximum score of 80 (Stanley et al., 1986).

Blood sampling procedures

Blood samples were collected in conjunction to the suicide attempt, between 7.30 and 8.00 am after a night of fasting and bed rest. The same conditions applied to all the samples. The blood was placed on ice and centrifuged (3000 r/minutes, at +4°C) within one hour. Plasma was collected and stored at -80°C. Samples had never been thawed prior to the cytokine analysis. The volume of some plasma samples was insufficient for analysis of all cytokines, and the final numbers of measurements for each cytokine are given in Fig. 1.

Blood samples were collected from all seasons throughout the year for both patients and controls. Blood samples from healthy controls / non-suicidal depressed patients were collected between 2001-2003 and from the suicide attempters between 2006-2008. All samples were frozen within 1 hour after blood collection and stored at -80°C until cytokine measurements. In our previous studies we did not find any correlation between cytokine levels and the sample storage time for over 15 years (Lindqvist et al., 2009).

Cytokine analysis

Cytokines were assayed on three 96-well plates with samples from healthy controls, non-suicidal depressed patients and suicidal patients distributed on all three plates. To avoid batch-to-batch variation, all the reagents used for the cytokine analysis were from the same kit.

IL-2, IL-6 and tumor necrosis factor (TNF)-α were measured in the plasma using ultra-sensitive electrochemiluminescence immunoassays according to the manufacturer's recommendations (Meso Scale Discovery, UK). Standards and samples were analyzed in duplicates. The detection limits for IL-2, IL-6 and TNF-α were 0.137, 0.050 and 0.075 pg/ml, respectively.

Statistical analysis

Data for all three cytokines were transformed into normal distribution using the natural logarithms before statistical analysis. Samples with zero cytokine levels (one sample from healthy control for TNF-α; one sample from healthy control and six samples from suicide attempters for IL-2) were assigned a value corresponding to one-tenth of the lowest detectable sample in the assay.

The potential effect of the confounding factors was tested in linear regression models. The first model consisted of age, sex, body mass index (BMI), degree of anxiety (BSA score), degree of depression (MADRS score) as well as a co-morbid diagnosis of personality disturbance or abuse. The second model consisted of different types of medication (entered as main group: SSRI, SNRI, neuroleptics, anti-epileptics, benzodiazepines, propiomazin and hydroxizin). Log-

transformed IL-2, IL-6 and TNF-α were each entered as dependents in the two models Data from the suicide attempters only were included in the regression analysis.

For the groupwise analysis of suicidality, the cytokines were analyzed using univariate general linear model (GLM). Suicidality was entered as the independent factor (suicidal, depressive and control), controlling for age, sex and BMI. For *post hoc* groupwise comparisons, simple contrast was used with the suicidal patients as a reference group.

To rule out the possibility of an effect of degree of depression as a confounder between the suicidal patients and depressive non-suicidal group, a linear regression model was generated with age, BMI, sex, MADRS score and group (suicidal vs. depressed non-suicidal) entered as independents.

Alpha-level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS 17.0 (SPSS, USA).

Results

Demographic data including age, sex, BMI, as well as MADRS and SUAS score for the study participants are shown in table 1. Log-transformed cytokine measurements for each individual are shown as a scatter plot in Fig.1.

Effects of potential confounders

Linear regression analysis revealed no significant effect of age, sex, BMI, degree of anxiety (BSA score), degree of depression (MADRS score), co-morbid diagnosis of personality disturbance or abuse on the cytokine levels in the suicide attempters (table 3).

The different types of medications used by the suicide attempters were entered in a second model, including sex, age, BMI, SSRI, SNRI, neuroleptics, anti-epileptics, benzodiazepines, propiomazin and hydroxizin. There was an effect of neuroleptics (n = 7) on IL-2 and TNF-α levels (table 4), but no other significant effects.

Impact of suicidality on cytokine levels

A general linear model was generated with sex, age and BMI entered as covariates. The cytokine levels were entered as dependents, and the effect of group based on suicidality studied. We found a significant main effect of suicidality on IL-2, IL-6 and TNF-α levels ($F(2,73) = 6.09$, $p = 0.004$ for IL-2; $F(2,70) = 4.58$, $p = 0.014$ for IL-6; $F(2,70) = 3.20$, $p = 0.047$ for TNF-α). There was no effect of age, BMI and sex and no significant interaction between suicidality and covariates (data not shown). *Post hoc* comparisons indicated that IL-6 and TNF-α

concentration was significantly higher, whereas IL-2 concentration was lower in the plasma of suicide attempters compared to depressed patients and healthy controls (table 5).

Eliminating confounding factors

There was a trend towards higher MADRS scores in suicide attempters compared to non-suicidal depressed patients (table 1). Therefore, a linear regression model was generated for each of the three cytokines with age, sex, BMI, MADRS score and suicidality (suicidal vs. depressed non-suicidal) as independents. The results were still that suicidality had a significant effect in elevating levels of IL-6 and TNF-α, and a significant effect in decreasing IL-2 levels (table 6).

Since we found that neuroleptics affected cytokine concentrations in the linear regression model, we performed a linear regression excluding suicide attempters treated with neuroleptics (n=7). The results remained statistically significant in the regression models for all three cytokines, demonstrating that the observed differences in cytokine levels were not due to neuroleptics treatment (table 7).

Medication used for intoxication in the suicide attempters is shown in table 2. Only two patients used paracetamol and NSAID, drugs with known anti-inflammatory properties. The result of the linear regression remained significant also after the exclusion of these two patients (data not shown).

Discussion

In this study, we found increased IL-6 and TNF-α as well as decreased IL-2 concentrations in the plasma of suicide attempters, compared to non-suicidal depressed patients and healthy controls. Although there was a trend towards higher MADRS scores in depressive, non-suicidal patients than in the suicide attempters (table 1), a clinical relevance of this is unlikely since both scores represent a depression of moderate severity. Importantly, we did correct for MADRS score in our statistical analysis, to eliminate any possible confounding effect of depression severity.

Our study thus suggests that inflammatory changes may not be present in all depressed patients, but may be confined to suicidal states. Further studies are warranted to analyze additional subgroups of depressive patients with respect to inflammation. As we did not include a group of treatment-resistant non-suicidal patients in this study, one possibility that must be investigated further is that the results of this study maybe pertinent to treatment-resistant patients in general, not only to suicide attempters.

It is interesting that we observed elevated levels of IL-6 and TNF-α levels in the suicidal patients, without any clinical signs of ongoing systemic or localized infection or inflammation. We recently reported elevated IL-6 levels in the cerebrospinal fluid of another cohort of suicide attempters (Lindqvist et al., 2009). Based on these data, we propose that the peripheral cytokine changes observed in the current study may mirror a low-grade inflammation in the central nervous system. In support of this hypothesis, two recent studies demonstrated microgliosis and elevation of cytokine mRNAs in post-mortem brain tissue from suicide victims (Steiner et al.,

2008; Tonelli et al., 2008). It is still unclear whether the central neuroinflammation is primary, or if the peripheral inflammation is the precipitating factor.

While IL-6 and TNF-α levels were elevated, IL-2 levels were reduced in suicidal patients. This phenomenon could possibly be caused by the elevated concentrations of sIL-2R previously observed in suicide attempters (Nassberger and Traskman-Bendz, 1993), since binding of SIL-2R leads to internalization of the sIL-2R/IL-2 complex and lysosomal degradation (Smith and Popmihajlov, 2008). The decreased IL-2 levels could also be due to a decreased production, which has been described in an *in vitro* study of lymphocytes from suicidal patients (Kim et al., 2008).

Some of the suicidal patients received anti-depressive medications. These patients were all treatment-resistant, as defined by MADRS scores and the occurrence of a suicide attempt in spite of ongoing treatment. Interestingly, we found an effect of neuroleptics on IL-2 and TNF-α levels, whereas there was no effect of the other treatment groups. Reduced serum IL-2 levels in response to risperidone and haloperidol treatment have indeed been previously reported in schizophrenia patients (Zhang et al., 2004). Importantly, the differences in cytokines remained statistically significant for all cytokines even after excluding the seven suicidal patients receiving neuroleptics from the analysis in our current study. Thus, the altered inflammatory parameters seem to be a state marker of suicidality irrespective of ongoing treatment. The majority of available studies on the effects of SSRI and SNRI treatment on immune markers suggest that if effective, such medication would generally lower the levels of plasma cytokines (Lanquillon et al., 2000; O'Brien et al., 2007; Yoshimura et al., 2009).

A potential limitation of the present study was that retrospective data, such as the duration of treatment and disease, were not registered. Furthermore, data on smoking habits was not available and could therefore not be included as a potential confounding factor in the regression analysis. Another potential confounder could be the suicide attempt method, which was intoxication in ninety-one percent of patients in our sample. However, only two patients used either paracetamol or NSAID for intoxication, and the results of the statistical analysis was the same when these two patients were excluded. The majority of patients used a benzodiazepine for intoxication. We do consider all patients free from the drug used for intoxication at the time of blood sampling, which occurred at a mean of 11 days after the intoxication. Nevertheless, we cannot completely rule out the potential role of the intoxication using the current study design.

Storage time between sample collection and cytokine analysis in our study was different for suicide attempters and depressed/healthy control subjects. Degradation of several cytokines has previously observed after 4 years of storage at -80°C (de Jager et al., 2009) thus indicating that the difference in storage time could represent a limitation of the present study. However, it should be noted that using sample handling conditions established in our laboratory, we found no correlation between cytokine levels and sample storage time (Lindqvist et al., 2009).

In summary, we demonstrate evidence of altered cytokine levels in the peripheral blood of suicidal patients, compared to non-suicidal depressed patients and healthy controls. The findings stand in treatment-resistant patients, irrespective of ongoing medication. Suicidal patients may thus be distinguished biologically from other groups of depressive patients. Future prospective

studies are warranted in order to evaluate cytokine levels in other subgroups of depressed patients, as well as the efficacy of cytokine measures in suicide risk-assessment.

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References

Dantzer, R., 2001. Cytokine-induced sickness behavior: where do we stand? Brain Behav Immun 15, 7-24.

de Jager, W., Bourcier, K., Rijkers, G.T., Prakken, B.J., Seyfert-Margolis, V., 2009. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. BMC Immunol 10, 52.

Denicoff, K.D., Rubinow, D.R., Papa, M.Z., Simpson, C., Seipp, C.A., Lotze, M.T., Chang, A.E., Rosenstein, D., Rosenberg, S.A., 1987. The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. Ann Intern Med 107, 293-300.

Gabbay, V., Klein, R.G., Guttman, L.E., Babb, J.S., Alonso, C.M., Nishawala, M., Katz, Y., Gaite, M.R., Gonzalez, C.J., 2009. A preliminary study of cytokines in suicidal and nonsuicidal adolescents with major depression. J Child Adolesc Psychopharmacol 19, 423-430.

Goldstein, B.I., Kemp, D.E., Soczynska, J.K., McIntyre, R.S., 2009. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. J Clin Psychiatry 70, 1078-1090.

Haack, M., Hinze-Selch, D., Fenzel, T., Kraus, T., Kuhn, M., Schuld, A., Pollmacher, T., 1999. Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. J Psychiatr Res 33, 407-418.

Kim, Y.K., Lee, S.W., Kim, S.H., Shim, S.H., Han, S.W., Choi, S.H., Lee, B.H., 2008. Differences in cytokines between non-suicidal patients and suicidal patients in major depression. Prog Neuropsychopharmacol Biol Psychiatry 32, 356-361.

Lanquillon, S., Krieg, J.C., Bening-Abu-Shach, U., Vedder, H., 2000. Cytokine production and treatment response in major depressive disorder. Neuropsychopharmacology 22, 370-379.

Lindqvist, D., Janelidze, S., Hagell, P., Erhardt, S., Samuelsson, M., Minthon, L., Hansson, O., Bjorkqvist, M., Traskman-Bendz, L., Brundin, L., 2009. Interleukin-6 is elevated in the

cerebrospinal fluid of suicide attempters and related to symptom severity. Biol Psychiatry 66, 287-292.

Maes, M., Meltzer, H.Y., Bosmans, E., Bergmans, R., Vandoolaeghe, E., Ranjan, R., Desnyder, R., 1995. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. J Affect Disord 34, 301-309.

Nassberger, L., Traskman-Bendz, L., 1993. Increased soluble interleukin-2 receptor concentrations in suicide attempters. Acta Psychiatr Scand 88, 48-52.

O'Brien, S.M., Scully, P., Fitzgerald, P., Scott, L.V., Dinan, T.G., 2007. Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. J Psychiatr Res 41, 326-331.

Potvin, S., Stip, E., Sepehry, A.A., Gendron, A., Bah, R., Kouassi, E., 2008. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. Biol Psychiatry 63, 801-808.

Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 27, 24-31.

Renault, P.F., Hoofnagle, J.H., Park, Y., Mullen, K.D., Peters, M., Jones, D.B., Rustgi, V., Jones, E.A., 1987. Psychiatric complications of long-term interferon alfa therapy. Arch Intern Med 147, 1577-1580.

Smith, K.A., Popmihajlov, Z., 2008. The quantal theory of immunity and the interleukin-2-dependent negative feedback regulation of the immune response. Immunol Rev 224, 124-140.

Stanley, B., Traskman-Bendz, L., Stanley, M., 1986. The suicide assessment scale: a scale evaluating change in suicidal behavior. Psychopharmacol Bull 22, 200-205.

Steiner, J., Bielau, H., Brisch, R., Danos, P., Ullrich, O., Mawrin, C., Bernstein, H.G., Bogerts, B., 2008. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. J Psychiatr Res 42, 151-157.

Steptoe, A., Kunz-Ebrecht, S.R., Owen, N., 2003. Lack of association between depressive symptoms and markers of immune and vascular inflammation in middle-aged men and women. Psychol Med 33, 667-674.

Tonelli, L.H., Stiller, J., Rujescu, D., Giegling, I., Schneider, B., Maurer, K., Schnabel, A., Moller, H.J., Chen, H.H., Postolache, T.T., 2008. Elevated cytokine expression in the orbitofrontal cortex of victims of suicide. Acta Psychiatr Scand 117, 198-206.

Tyrer, P., Owen, R.T., Cicchetti, D.V., 1984. The brief scale for anxiety: a subdivision of the comprehensive psychopathological rating scale. J Neurol Neurosurg Psychiatry 47, 970-975.

Yoshimura, R., Hori, H., Ikenouchi-Sugita, A., Umene-Nakano, W., Ueda, N., Nakamura, J., 2009. Higher plasma interleukin-6 (IL-6) level is associated with SSRI- or SNRI-refractory depression. Prog Neuropsychopharmacol Biol Psychiatry 33, 722-726.

Zhang, X.Y., Zhou, D.F., Cao, L.Y., Zhang, P.Y., Wu, G.Y., Shen, Y.C., 2004. Changes in serum interleukin-2, -6, and -8 levels before and during treatment with risperidone and haloperidol: relationship to outcome in schizophrenia. J Clin Psychiatry 65, 940-947.

Figure legends

Figure 1. Log-transformed cytokine measurements for each individual. Data are shown for groups of healthy controls, depressed patients and suicide attempters. A solid black line represents the mean of each group; n number of subjects in each group.

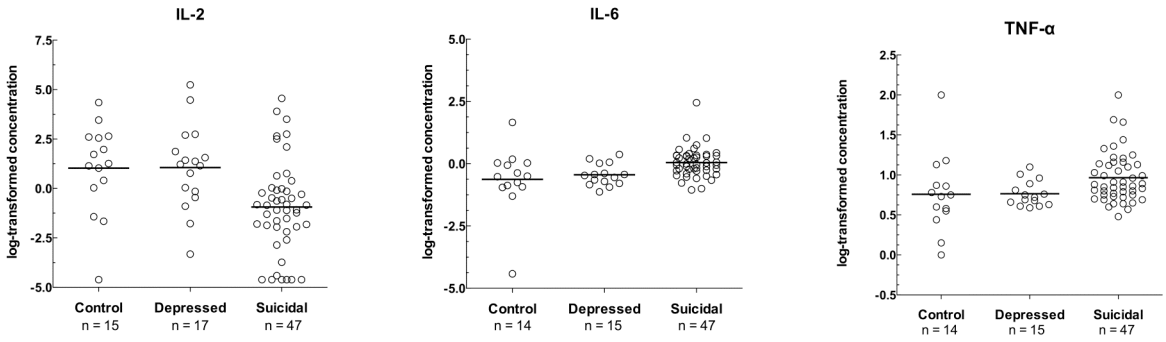

Figure 1

Table 1. Demographic data and somatic diagnoses for the study participants

Variable	Healthy subjects n = 16	Non-suicidal depressive patients n = 17	Suicide attempters n = 47
Age, *mean ± SD*	34.9 ± 11.5	33.8 ± 10.9	39.3 ± 15.0
Gender, *male/female*	8/8	9/8	21/26
BMI, *mean ± SD*	23.3 ± 3.4	24.9 ± 8.0	25.8 ± 4.2
MADRS, *median (IQR)*	0 (0 - 1)	27 (22 -32)	21 (12-31)
SUAS, *median (IQR)*	0 (0 - 0)	29 (25 - 34.5)	37.5 (26 - 55)
Somatic diagnosis *(n)*	Allergy (2)	Allergy (1) Asthma (1) Migraine (1) Ulcerative colitis, inactive (1)	Allergy (1) Arthrosis (2) Asthma (1) Diabetes (3) Fibromyalgia (1) High blood pressure (2) Hyperthyroid (1) Hypothyroid (1) Migraine (2) MS (1) Obesity (1) Polycystic ovaries (1) Tinnitus (1)

BMI, body mass index; IQR, interquartile range; MADRS, Montgomery and Asberg Depression Rating Scale; SD, standard deviation; SUAS, Suicide Assessment Scale

Table 2. Suicide attempt methods

Intoxication (single drug)	Intoxication (multiple drugs)	Violent suicide attempt
n = 20	n = 23	n = 4
Benzodiazepines [a]	Most common drugs:	Drowning (1)
	Benzodiazepine [a] (21)	Hanging (2)
	SSRI (12)	Car accident (1)
	SNRI (7)	
	Opiates (5)	
	Propiomazin (9)	
	Hydroxyzin (3)	
	Gaba-modulators [b] (2)	
	Paracetamol (1)	
	Acetylsalicylic acid (1)	
	Rat poison (1)	

SSRI, selective serotonin reuptake inhibitors; SNRI, serotonin–norepinephrine reuptake inhibitor

[a] Benzodiazepines also include the benzodiazepine derivates zolpidem and zopiclone.

[b] Includes gabapentin and pregabalin

Table 3. Linear regression model of the association between log-transformed cytokines and potential confounding factors

Independent variable	IL-2		IL-6		TNF-α	
	B	*p*	B	*p*	B	*p*
sex	1.004	.182	.049	.805	-.155	.142
age	-.021	.430	.009	.205	.002	.528
BMI	.034	.712	-.002	.951	.000	.972
personality disturbance	-.072	.927	.072	.734	.155	.167
abuse	.758	.362	.051	.819	-.127	.276
BSA	-.073	.292	-.009	.623	-.006	.550
MADRS	.010	.799	.002	.868	.001	.813

B Regression coefficient; BSA, Brief-Scale for Anxiety; BMI, body mass index; IL-2, interleukin-2; IL-6, interleukin-6; MADRS, Montgomery and Asberg Depression Rating Scale; TNF-α, tumor necrosis factor-α

Table 4. Linear regression model of the association between log-transformed cytokines and different types of treatment

Independent variable	IL-2		IL-6		TNF-α	
	B	*p*	B	*p*	B	*p*
neuroleptics	-2.582	**.014***	-.066	.813	.304	**.037***
SNRI	-.642	.387	-.041	.842	-.028	.786
SSRI	.531	.494	.093	.662	.053	.627
anti-epileptics	-1.654	.080	.349	.177	.138	.292
hydroxizin	.170	.854	-.191	.452	-.136	.294
propiomazin	.839	.258	.012	.954	-.170	.105
benzodiazepines	-1.110	.140	-.219	.286	-.030	.773

B Regression coefficient; IL-2, interleukin-2; IL-6, interleukin-6; SSRI, selective serotonin reuptake inhibitors; SNRI, serotonin–norepinephrine reuptake inhibitor; TNF-α, tumor necrosis factor-α

* Statistically significant *p* values are shown in bold

Table 5. Univariate general linear model testing the effect of suicidality on log-transformed cytokine levels (for groupwise comparisons, simple contrast was used with the suicidal patients as a reference group)

	IL-2		IL-6		TNF-α	
	Contrast estimate	***p****	Contrast estimate	***p****	Contrast estimate	***p****
Healthy controls *vs.* suicide attempters	1.813	**.010**	-.618	**.009**	-.202	**.052**
Depressed patients *vs.* suicide attempters	1.880	**.005**	-.448	**.048**	-.199	**.048**

IL-2, interleukin-2; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α

* *p* values are shown in bold

Table 6. Linear regression model testing the effect of sex, age, BMI, MADRS score and suicidality (suicidal vs depressed non-suicidal) on log-transformed cytokine levels

Independent variable	IL-2		IL-6		TNF-α	
	B	*p*	B	*p*	B	*p*
sex	.708	.217	.027	.853	-.091	.227
age	-.013	.533	.004	.405	-.001	.835
BMI	-.027	.609	.011	.409	.001	.850
MADRS	-.013	.626	-.002	.770	.000	.920
suicidality	1.066	**.002***	-.235	**.010***	-.106	**.022***

B Regression coefficient; BMI, body mass index; IL-2, interleukin-2; IL-6, interleukin-6; MADRS, Montgomery and Asberg Depression Rating Scale; TNF-α, tumor necrosis factor-α

* Statistically significant *p* values are shown in bold

Table 7. Linear regression model testing the effect of sex, age, BMI, and suicidality on log-transformed cytokine levels after exclusion of patients treated with neuroleptics

Independent variable	IL-2		IL-6		TNF-α	
	B	*p*	B	*p*	B	*p*
sex	.701	.249	.010	.948	-.083	.249
age	-.019	.369	.005	.323	.000	.883
BMI	-.027	.629	.016	.269	.002	.785
MADRS	-.002	.977	-.007	.645	.003	.653
suicidality	.887	**.014***	-.232	**.015***	-.089	**.038***

B Regression coefficient; BMI, body mass index; IL-2, interleukin-2; IL-6, interleukin-6; MADRS, Montgomery and Asberg Depression Rating Scale; TNF-α, tumor necrosis factor-α

* Statistically significant *p* values are shown in bold