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Circulating cytokine levels in the treatment of comorbid anxiety disorders

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Abstract

Psychotherapy research aims to investigate predictors and moderators of treatment outcome, but there are few consistent findings. This study aimed to investigate cytokines in patients undergoing treatment for anxiety disorders and whether the level of cytokines moderated the treatment outcome. Thirty-seven patients with comorbid and treatment-resistant anxiety disorders were investigated using multilevel modelling. Serum cytokine levels were measured three times: pretreatment, in the middle of treatment, and at the end of treatment. Anxiety and metacognitions were measured weekly throughout treatment by self-report. The levels of monocyte chemoattractant protein-1, tumour necrosis factor-alpha, and interleukin-1 receptor antagonist did not change during therapy or were not related to the level of anxiety. Metacognitive beliefs predicted anxiety, but the relationship between metacognitions and anxiety was not moderated by cytokines. Limitations of the study include that the patients were not fasting at blood sampling, and we did not assess body mass index, which may affect cytokine levels. The lack of significance for cytokines as a predictor or moderator may be due to a lack of power for testing moderation hypotheses, a problem associated with many psychotherapy studies. Cytokines did not predict the outcome in the treatment of comorbid anxiety disorders in our sample. Furthermore, cytokines did not moderate the relationship between metacognitions and anxiety.

Significant outcomes

- Cytokines did not predict the outcome in the treatment of anxiety disorders
- Level of cytokines did not moderate the relationship between metacognitions and anxiety.

Limitations

- Patients were not fasting at blood sampling.
- Body mass index was not assessed.
- Sample size indicates reduced power to detect significant moderators.

Introduction

Anxiety disorders are among the most common mental disorders (Kessler *et al.*, 2005), and there is evidence that psychological treatments are effective (Hans & Hiller, 2013). The disorders often co-occur (Kessler *et al.*, 2012) with substance use disorders, especially alcohol, and researchers have, therefore, started to investigate processes that are common across different anxiety disorders, so-called transdiagnostic processes (Harvey *et al.*, 2004). Irrespective of whether anxiety disorders are studied separately or as a syndrome, there is no single treatment effective for everyone. Thus, there is an interest to investigate what works for whom, enabling a form of more personalised medicine (Insel, 2009). Knowledge about predictors and moderators of treatment outcome are thus needed (Kraemer *et al.*, 2002). Predictor variables, clinical or biological, provide information on the associated dependent variable. Moderator variables are identified when the relationship between two variables depends on a third variable.

In psychotherapy research, metacognitions (Wells, 2009) have been found to be an important time-varying process predictor called a within-person effect (Johnson *et al.*, 2018). Typically, in psychotherapy research, individuals are compared with other individuals, called a between-person effect (Molenaar, 2004). However, a within-person effect isolates how a patient changes over the course of therapy related to their usual level. Thus, in the study by Johnson *et al.* (2018), the following clinical implication could be drawn based on a within-person finding: If a clinician targets metacognition in therapy, reduction in anxiety will likely follow.

According to metacognitive therapy (MCT) (Wells, 2009), the use of specific strategies to regulate emotions is dependent on metacognitions. Metacognition was originally defined as knowledge or beliefs about thinking and strategies used to regulate and control thinking processes (Flavell, 1979). Metacognitions are crucial for the development and maintenance of psychological disorders (Wells, 2009). In therapy, two types of metacognitive beliefs are targeted, positive and negative. Positive metacognitive beliefs are beliefs about the usefulness of worry, ruminations, and threat monitoring (e.g. 'If I worry, I will be prepared', 'If I ruminate, I will find a solution'). Negative metacognitive beliefs concern the uncontrollability of thoughts and their danger (e.g. 'I cannot control my thoughts', 'Worry can damage my mind'). In therapy, the therapist has to identify the specific strategies to regulate emotions (e.g. worry and rumination) and the metacognitive beliefs that give rise to the use of this strategy and challenge this. Previous studies from our group have shown that metacognition, in general, and positive metacognitions, specifically, predict anxiety on a within-person level (Hoffart *et al.*, 2018; Johnson *et al.*, 2018).

It is an open question whether the within-person effect of metacognitions on outcome is moderated by specific characteristic. Thus, an important research goal is to find characteristics that explain variability in within-person relationships between meta-cognitions and subsequent symptoms. A recent systematic review stated that many studies found associations between psychological treatment and inflammation (Moraes *et al.*, 2018). Further, it was mentioned that little is known about the impacts of psychoneuroimmunological interventions and the effects on disease progression (Moraes *et al.*, 2018). Aiming to fill this gap in the literature, the current study explores the potential moderating role of cytokines on psychological treatment in anxiety patients.

Findings regarding predictors of the outcome in anxiety disorder treatment are inconclusive. For example, some studies find associations between the degree of comorbidity or personality problems and the outcome (Bohart & Wade, 2013; Goddard *et al.*, 2015), while other studies do not (Hoffart *et al.*, 2015; Johnson & Hoffart, 2019). One reason for the lack of consistent findings may be that the predictors and moderators of treatment outcome investigated have been psychological constructs (Schneider *et al.*, 2015), and these psychological constructs are often poorly defined, limiting construct validity (Fried, 2017). Anxiety disorders should be understood in a biopsychosocial framework (Engel, 1977). It is, therefore, of clinical interest to investigate possible biological predictors of their outcome. Such biological predictors could be, for example, the immunological molecules cytokines. Cytokines constitute a group of small messenger molecules, which commonly are divided into two families, pro- and anti-inflammatory cytokines. The research efforts for investigating the association between cytokines and anxiety disorders are increasing (Vogelzangs *et al.*, 2013).

Inflammatory cytokines may be associated with behavioural change in many ways. They may alter the metabolism of neurotransmitters such as serotonin, dopamine, and glutamate (Moron *et al.*, 2003) and also influence the hypothalamic–pituitary–adrenal axis through their actions towards the release of corticotrophin-releasing hormone, adrenocorticotropic hormone, and cortisol (Raison *et al.*, 2010). Pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and tumour necrosis factor-alpha (TNF-α), enhance the immune response to help speed the elimination of pathogens and the resolution of the inflammatory challenge (Kronfol & Remick, 2000; Dalgard *et al.*, 2017). Also, levels of anti-inflammatory cytokines have been reported to be altered in generalised anxiety disorders when compared to healthy controls, contributing to an altered cytokine balance (Hou *et al.*, 2017). Therefore, the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1RA) was also assessed in the current study. Elevated levels of cytokines have been found for panic disorder and post-traumatic stress disorder (PSTD) (Hoge *et al.*, 2009) and also for anxiety disorders in general (Vogelzangs *et al.* 2013). Furthermore, an association between inflammatory dysregulation and anxiety – for example, for TNF-α – has been reported (Renna *et al.*, 2018). However, the association between TNF-α and anxiety disorders is not found in a recent paper (Glaus *et al.*, 2018).

Thus, the evidence concerning specific cytokines and the relationship with anxiety disorders is inconclusive. To our knowledge, no studies have investigated cytokines in relation to treatment outcome in anxiety disorders.

Aims of the study

In this exploratory study, we firstly wanted to investigate if the pre-treatment level of anxiety correlated with the levels of some selected cytokines. Then we examined how cytokines developed over the course of therapy for comorbid anxiety disorders and whether the mean level of cytokines predicted anxiety. Thirdly, we investigated whether the mean level of cytokines moderated the within- and between-person relationship between a therapy process (meta-cognitions) and anxiety over the course of therapy.

Materials and method

Participants

The patients were referred to the Department of Anxiety Disorder at Modum Bad because they had not benefited sufficiently from outpatient treatment and were undergoing an 8-week inpatient treatment program for treatment resistance. Patients enrolled at the Department of Anxiety Disorders have, on average, 3.7 diagnoses at intake (Johnson *et al.*, 2017). Thus, the terms comorbid anxiety and treatment-resistant anxiety disorders are used. The study included 37 patients who were undergoing treatment. The average duration of anxiety disorder problem was 16 years, and 85% of the patients were either disabled, out of work, or on sick leave when entering treatment (Johnson *et al.*, 2017). Primary diagnoses treated at the department are PTSD, social phobia, panic disorder with and without agoraphobia, generalised anxiety disorder (GAD), and specific phobia. The term comorbid anxiety disorders refers to patients which is homogeneous in terms of having anxiety disorders and possible common processes underlying them but heterogeneous in terms of the composition of anxiety disorders. Thus, the patients represent a typical clinical sample. Some patients used medications which are known to have immunomodulatory properties. There were 10 patients who used Paracetamol, five patients used Paracetamol and Ibuprofen in combination, three patients used Ibuprofen, three patients used Escitalopram, one

patient used Diclocil antibiotics, and one patient used Diclofenac. Since few patients used medications at all, and only five patients used Paracetamol and Ibuprofen for more than a single-occurring event, we did not take the use of medication into consideration in the analyses.

There were 17 male and 20 female patients in the study. The mean age was 43.6 years (SD = 11.0 years). The following primary diagnoses were present: 14 patients had PSTD, 7 patients had social phobia, 5 patients had panic disorder with agoraphobia, 1 patient had GAD, and 1 had a specific phobia. Nine patients did not have any specific registered diagnosis, but had either PTSD, SAD, PDA, or GAD, since that is the inclusion criteria for treatment at the Department of Anxiety Disorder.

The patients were part of a larger study investigating cytokines in psychological disorders (Toft *et al.*, 2018). The study was approved by the Norwegian Regional Ethics Committee prior to data collection (reference number 2014/2189), and the participants gave their written consent.

Treatment

At the Department of Anxiety Disorders, MCT and cognitive behavioural therapy (CBT) are administered. The MCT ($N = 15$) consists of a manualised treatment protocol for the generic MCT model (Wells, 2009). The CBT ($N = 22$) is based on disorder-specific models for panic disorder (Wells, 1997), social phobia (Clark & Wells, 1995), and PSTD (Foa *et al.*, 2007). Both treatments have in common that they are highly structured, are based on established manuals, and have shown efficacy in the treatment of anxiety disorders. In the current study, we did not separate between the two treatments; thus, the results encompass the treatment of anxiety disorders using either CBT or MCT.

Self-report measures

Anxiety and metacognitions were measured weekly throughout treatment through self-report. *Beck Anxiety Inventory* (BAI; Beck *et al.*, 1988) measures anxiety symptoms the last week with 21 items. The items are rated on a Likert scale from 0 to 3 with a maximum score of 63. The psychometric properties of the BAI are satisfactory (e.g. Steer *et al.*, 1993). *The Meta-Cognitions Questionnaire 30* (MCQ-30; Cartwright-Hatton & Wells, 1997) measures metacognitive beliefs. The items are rated on a 4-point Likert scale, and the total score varies from 30 to 120. MCQ-30 has been found to have adequate psychometric properties (Wells & Cartwright-Hatton, 2004).

Serum preparation and cytokine measurements

Peripheral circulating cytokines were measured pretreatment, in the middle of treatment, and at the end of treatment. The length of stay, and thus the number of weeks between each blood sample, varied between the various enrolments due do holiday seasons and other treatment-related events. Thus, the average amount of time from baseline to T_1 was 4 weeks, and from baseline to T_2 was 8 weeks. The blood samples were taken at the local hospital laboratory between 08:00 a.m. and 09:00 a.m. Samples were collected in Vacuette 8 ml serum tubes, immediately turned upside down 8–10 times, and set to rest between 30 and 60 min, and lastly centrifuged in a Kubota 2420 swing-out centrifuge at room temperature for 10 min at 1917 *g*. The separated serum was stored in two 2 ml Nunc tubes in a −80°C freezer until assay.

Before analysis, samples were thawed on ice, vortexed, and spun down at 14 000 × *g* for 10 min at 4°C. Cytokines were measured by using Bio-Plex xMAP technology (Bio-Rad, Austin, TX, USA) with a Luminex IS 100 instrument (Bio-Rad, Hercules, CA, USA), powered using Bio-Plex Manager (version 6.0.1) software. The StatLIA software package (version 3.2, Brendan Scientific, Carlsbad, CA, USA), incorporating a weighted, five-parameter logistic curve-fitting method, was used to calculate sample cytokine concentrations. We present data on MCP-1, TNF-α, and IL-1RA because they are within the detectable range. Inter-assay coefficients of variability (CV) were 6.7% for MCP-1, 7.4% for TNF-α, and 10.2% for IL-1RA, all within the 21% acceptability limit. All zero values were replaced with the limit of detection (LOD). At T_0, the median level of MCP-1 was 23.29, and the LOD was 6.48. There were 3 zero values which were replaced with the LOD. The median level in TNF-α was 0.18, and the LOD was 0.08. There were 15 zero values which were replaced with the LOD. The IL-1RA median level was 32.25, and the LOD was 7.87. There were no zero values. At T_1, the MCP-1 median level was 24.24, and the LOD was 3.56. There was one zero value which was replaced with the LOD. The TNF-α median level was 0.42, and the LOD was 0.36. There were 13 zero values which were replaced with the LOD. The IL-1RA median level was 29.65, and the LOD was 8.32. There were no zero values. At T_2, the MCP-1 median level was 23.54, and the LOD was 4.33. There were four zero values replaced with the LOD. The TNF-α median level was 0.26, and the LOD was 0.02. There were 15 zero values which were replaced with the LOD. The IL-1RA median level was 31.95. The LOD was 6.69. There were no zero values. The cytokine, which was omitted from the study, is presented together with its median values and the LODs. At T_0, the IL-1β median level was 0.06, and the LOD was 0.03. At T_1, the IL-1β median level was 0.08, and the LOD was 0.01. At T_2, the IL-1β median level was 0.07, and the LOD was 0.03.

Statistical analysis

The data used in the process outcome analyses were nested in a two-level structure (weeks nested within patients) thus longitudinal multilevel modelling (MLM) was used for the analysis (Fitzmaurice *et al.*, 2004; Curran & Bauer, 2011).

MLM makes use of all available data in the estimation of model parameters. Thus, a participant with missing data can be included in the analysis and affect the estimation of model parameters (Kwok *et al.*, 2008). In this study, the process variable, metacognition, was disaggregated in both within and between effects according to the criteria outlined by Wang and Maxwell (2015), using person-mean centering. Thus, two variables were entered into the prediction analysis, a between-patient and a within-patient effect. Disaggregation is important because the raw score reflects two sources of variance: how an individual differs from other individuals (between-person effect) and how the individual differs from their usual level (within-person effect).

Random intercepts and random slope were added to the empty models if the model fit was improved. The data were modelled for heteroscedastic residual variance over time, and quadratic time was tested for improved model fit. Covariance structure like AR1 was tested. The most parsimonious model was selected using log-likelihood tests on model fit. A fixed linear time with a heteroscedastic residual variance over time was chosen. An AR1 covariance structure of the residuals gave the best model fit for anxiety and MCP1. The covariance structure for TNF-α and IL-1RA was homoscedastic. TNF-α, IL-1RA, and MCP1 were modelled without random slope.

Maximum likelihood (ML) was used as the estimation method (Fitzmaurice *et al.*, 2004).

First, we examined whether anxiety, metacognition, and cytokines changed as a function of time and if anxiety and cytokines correlated at pretreatment. Second, we investigated whether the mean level of cytokine levels *predicted* the slope of anxiety. Third, we examined whether the between- and within-person effect of metacognitions predicted anxiety as was found in a recent paper (Johnson *et al.*, 2018). Finally, we investigated if the within-person effect and between-person effect of metacognitions on anxiety over the course of therapy was *moderated* by the mean level of cytokines.

The mean level of cytokines was used since the cytokines did not change over the course of therapy. We established a temporal sequence between metacognitions and anxiety to ensure that the predictor was measured before the outcome. The predictor scores were lagged and thus related to the anxiety scores 4 days later. SPSS version 25.0 (IBM, 2017) was used for all analysis. We used a p-level of 0.05.

Results

Correlations at pretreatment

The mean anxiety level at baseline was 28.7, SD = 13.4. Anxiety level did not correlate with TNF-α, $r = -0.09$, $p = 0.59$, MCP1 levels, $r = 0.25$, $p = 0.15$, or IL-1RA, $r = 0.19$, $p = 0.26$.

Change over time

First, we investigated whether the cytokines TNF-α, MCP1, IL-1RA, and anxiety changed over the course of therapy. MCP1 [β = 2.77, SE = 3.61, $t(69.3) = 0.8$, $p = 0.44$, CI (−4.4, 9.8)] did not change significantly over the course of therapy, and the same was the case for TNF-α [β = −0.09, SE = 0.20, $t(66.6) = -0.5$, $p = 0.65$, CI (−0.49, 0.31)] and IL-1RA [β = 5.56, SE = 6.02, $t(70.3) = 0.9$, $p = 0.36$, CI (−6.44, 17.56)]. However, anxiety decreased [β = −1.19, SE = 0.19, $t(35.6) = -6.09$, $p = <0.001$, CI (−1.57, −0.79)] significantly during therapy. Since the cytokines did not change over the course of therapy, the mean level from the three time points was used in the subsequent analysis.

Cytokines as predictor of outcome

We investigated whether the mean level of cytokines during treatments predicted outcome. TNF-α, MCP1 or IL-1RA did not predict anxiety over the course of therapy (Table 1).

Within-person effects of metacognitions on anxiety

Table 2 shows that metacognitions predicted anxiety over the course of therapy both on a within-person level and on a between-person level.

Cytokines as predictors of within-person- and between-person effects

The cytokines (MCP1, TNF-α, IL-1RA) did not moderate the within-person effects or between-person effects of metacognitions on anxiety (Table. 2). A higher level of metacognitions predicted a higher level of anxiety through treatment (between-person effects), but this relationship was not dependent on cytokines. A lower metacognition in 1 week predicted a lower anxiety the next week (within-person effect), but this relationship was also not dependent on cytokines.

Discussion

Multiple cross-sectional studies have investigated the relationship between immune markers and anxiety disorders; we tested the hypothesis that mean levels of cytokines may predict the outcome in comorbid anxiety disorders, and also whether the level of cytokines moderated the relationship between a therapy process (metacognitions) and anxiety. No such relationships were found, although the results replicated the finding that metacognitive beliefs predicted anxiety disorders as found in Johnson *et al.* (2018). Several studies have used psychological construct to predict outcome in psychotherapy, but this is the first study to investigate whether cytokines affects the outcome for comorbid anxiety disorders.

The levels of circulating cytokines did not change significantly over time, even though the anxiety symptoms subsided. One reason for the lack of change might be that the sample consisted of comorbid and previously treatment-resistant anxiety disorder patients. It might be that these patients had a more chronically increased cytokine level (e.g. Toft *et al.*, 2018) due to the duration of their problems, hence not displaying a trajectory related to treatment outcome. Furthermore, some of the patients had PTSD, and it has previously been shown that patients with PTSD increase their cytokine level during treatment (Toft *et al.*, 2018). The results from the current study of treatment-resistant patients indicate that cytokine levels in patients with anxiety disorders in general do not change over the course of treatment, even if they report fewer symptoms.

Although TNF-α, MCP-1, and IL-1RA were unrelated to the outcome in the present sample with anxiety disorder, there might be subgroups of patients where cytokines are important. For example, immune dysregulation is especially found in persons with a late-onset anxiety disorder, suggesting the existence of a specific late-onset anxiety subtype with a distinct aetiology (Vogelzangs *et al.*, 2013). This subgroup could not be analysed in the current sample due to low sample size.

Metacognition predicted the outcome, both on a within-person level and a between-person level. At the within-person level, a decrease of a patient's metacognitive beliefs in a given week was associated with reduced anxiety in the subsequent week. At the between-person level, the lower level of metacognitions predicted lower overall level of anxiety. Thus, the importance of metacognition as a key process in psychotherapy was corroborated (Wells, 2009; Johnson *et al.*, 2018). However, the level of cytokines did not moderate this relationship.

Limitations

This study has several strengths. First, the sample consisted of comorbid anxiety disorders, which is typical in clinical practice. Moreover, cytokines were measured three times and anxiety and metacognition weekly during treatment. Limitations regarding this study should be acknowledged. We cannot rule out cytokine of other classes that may have a bearing on metacognition and treatment outcome. Even if the samples were taken at the same time of the day and were handled swiftly in accordance with recommendations (Altara *et al.*, 2015; Aziz *et al.*, 2016), we had little control over the patients' food intake (Zhou *et al.*, 2010) and body mass index. Due to sample size and power limitations, we could not meaningfully separate between MCT and CBT in the analysis, making possible interactions with treatment unknown.

Table 1. TNF-α, MCP1, and IL-1RA as predictors of anxiety among patients undergoing an inpatient treatment for treatment-resistant anxiety disorders

Variable	TNF-α	MCP1	IL-1RA
		Fixed effects	
Intercept	29.44*** (2.34), [24.7, 34.2]	26.28*** (2.89), [20.4, 32.2]	26.34*** (2.99), [20.2, 32.4]
Time	−1.13*** (0.21), [−1.6, −0.71]	−1.34 ***(0.27), [−1.89, −0.79]	−1.14*** (0.28), [−1.7, −0.5]
TNF-α	−0.16 (0.36), [−0.9, 0.6]		
MCP1		0.08 (0.06), [−0.04, 0.21]	
IL-1RA			0.06 (0.05), [−0.04, 0.16]
Time*TNF-α	−0.02(0.04), [−0.09, 0.05]		
Time*MCP1		0.004 (0.005), [−0.01, 0.01]	
Time*IL-1RA			−0.001 (0.004), [−0.01, 0.01]
		Random effects	
Intercept	139.1*** (41.9)	131.3*** (39.9)	132.9*** (40.5)
Covariance	−0.27 (0.26)	−0.32 (0.24)	−0.26 (0.26)
Time	0.62 (0.33)	0.64* (0.32)	0.64 (0.33)
−2 LL	2460.82	2464.61	2468.18

*$p < 0.05$. ***$p < 0.001$. In brackets, 95% confidence intervals.

Table 2. Metacognitions as predictor of anxiety and MCPI, TNF-α, and IL-1RA as moderators of metacognitions on anxiety

Variable	Metacognition	MCP1	TNF-α	IL-1RA
		Fixed effects		
Intercept	−5.89 (4.9), [−15.9, 4.2]	−15.1 (8.4), [−30.8, 2.0]	−7.0 (6.0), [−19.2, 5.1]	−10.46 (6.9), [−24.4, 3.5]
Time	−0.75*** (1.9), [−1.1, −0.3]	−0.8*** (0.2), [−1.2, −0.3]	−0.8*** (0.2), [−1.2, −0.3]	−0.74*** (0.2), [−1.2, −0.3]
Mcq_bp	0.51*** (0.07), [0.4, 0.7]	0.63***(0.11), [0.4, 0.9]	0.54***(0.09), [0.4, 0.7]	0.59*** (0.10), [0.4, 0.8]
Mcq_wp	0.25 ***(0.05), [0.16, 0.34]	0.25***(0.08), [0.10, 0.40]	0.26***(0.05), [0.15, 0.36]	0.30*** (0.08), [0.2, 0.5]
Cytokine		0.32 (0.24), [−0.16, 0.82]	0.54 (1.4), [−2.4, 3.4]	0.09 (0.12), [−0.15, 0.34]
Mcq_wp*cytokine		−0.001 (0.001), [−0.003, 0.003]	−0.003 (0.007), [−0.018, 0.012]	−0.001 (0.001), [−0.004, 001]
Mcq_bp*cytokine		−0.003 (0.002), [0.009, 0.002]	−0.01 (0.03), [−0.06, 0.04]	−0.002 (0.002), [−0.05, 0.01]
		Random effects		
Intercept	135.4*** (41.5)	134.9***(41.8)	142.1*** (44.2)	139.7***(43.9)
Covariance	−0.84***(0.09)	−0.84*** (0.1)	−0.85*** (0.1)	−0.85*** (0.3)
Time	0.62*(0.31)	0.61 (0.32)	0.62* (0.32)	0.64* (0.32)
−2 LL	2125.05	2148.41	2138.09	2150.23

MC, Metacognitions. In brackets, 95% confidence intervals; Mcq_bp, Metacognitions between-person effects; Mcq_wp, Metacognitions within-person effects.
*$p < 0.05$. ***$p < 0.001$.

Some somatic diagnoses are likely to exert a chronic inflammatory effect, for instance, hepatitis, cancer, HIV, or cardiovascular disease, but unfortunately, we did not acquire any somatic diagnostic information. Further, we did not run any tests for excluding patients with ongoing somatic infection, for instance, C-reactive protein analysis. Further, this study lacks a group of healthy controls. Comparing cytokine levels between healthy controls and patients could have provided interesting information about differences between mentally ill and healthy people. However, the aims of the study were met regardless of such limitations. The lack of significance for cytokines as a predictor or moderator may be due to a lack of power for testing moderation hypotheses, a problem associated with many psychotherapy studies (Cuijpers *et al.*, 2016). However, as Arntz *et al.* (2015) argue, predictors could be accumulated across different studies to build an empirical knowledge base. In future research, a larger sample of patients with anxiety disorders should be investigated, separating late or early onsets of anxiety disorders. Furthermore, it should be investigated whether the inflammatory levels depend on what kind of mechanisms are targeted in therapy (Lasselin *et al.* 2016)

Conclusion

The clinical implication from this study is that variability in cytokines is not related to the outcome in the treatment of anxiety disorders.

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Author contributions. SJ and AH designed the study. HT collected the data. SJ wrote the first draft. All authors (SJ, AH, HT, TT, SPN, LL, and JB) participated in analysis and interpretation of data and revised the manuscript. All authors approved the final version of the manuscript.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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