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Pro-inflammatory cytokines and psychotherapy in depression: Results from a randomized clinical trial

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ABSTRACT

Depression is a serious condition that is associated with great psychic suffering and major impairments on the patient's general health, quality of life, and social and occupational activities. In some cases, it may lead to suicide. Regardless of the innumerous research works that have already addressed depression in wide and specific facets, there is still a lot to grasp in order to effectively help preventing and treating depression. This work presents data from a randomized clinical trial that sought to evaluate the effectiveness of two brief psychotherapeutic for Depression: Cognitive Behavioral Therapy (CBT) and Supportive-Expressive Dynamic Psychotherapy (SEDP). This was a convenience sample composed of 46 individuals that were evaluated using a structured diagnostic interview and then randomly allocated to the SEDP group. We examined baseline and post-intervention serum levels of the Interleukin-6 (IL-6) and the Tumor Necrosis Factor (TNF-α) in addition to the severity of depressive symptoms according to the Outcome Questionnaire - 45.2 (OQ-45.2) and the Beck Depression Inventory (BDI). Results show that serum IL-6 and TNF-α levels, as well as the scores from the OQ-45.2 and the BDI significantly decreased after 16 sessions of SEDP ($p < 0.001$), except for the Interpersonal Relationship domain from the OQ-45. Despite the reduction of serum cytokines levels and OQ-45 and BDI scores, they were only significantly correlated regarding the social role domain from the OQ-45. Nonetheless, our data suggests an effective role of brief psychodynamic psychotherapy in the reduction of depressive symptoms and serum inflammatory levels that are associated with depression.

1. Introduction

Depression is a common mental disorder that often starts at a young age, reduces people's functioning, it is often recurring, and can lead to suicide (WFMH, 2012). The World Federation for Mental Health states that Depression is estimated to affect 350 million people and lifetime prevalence rates range from approximately 3 percent in Japan to 16.9 percent in the United States, with most countries falling somewhere between 8 and 12 percent. Moreover, unipolar depressive disorders will be the leading cause of the global burden of disease by 2030 (WFMH, 2012). Population-based studies in Brazil found a general prevalence of depression of 17–20% (Andrade et al., 2012; Munhoz, 2012), while the prevalence found in clinical samples ranges from 22% to 47% (Fleck et al., 2003; Molina et al., 2012).

The nature and etiology of depression is subject of divided opinion regarding psychogenic and biological causes (Beck and

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Alford, 2009; Maes et al., 1995). Several investigations have demonstrated an association between inflammatory processes and major depressive disorder (Dantzer et al., 2008; Neto et al., 2011; Rot et al., 2009; Schneider and Prvulovic, 2013). In fact, several clinical studies and animal models pointed that inflammation increases the risk of occurrence of major depressive episodes (Bayramgürler et al., 2013; Dantzer, O'Connor, 2008; Heesch et al., 2013; Maes et al., 1995) even though the causal relation between them is still unclear.

Pro-inflammatory cytokines, such as the Interleukin-6 (IL-6) and the Tumor Necrosis Factor (TNF-α) may represent the key factor in the (central) mediation of the behavioral, neuroendocrine and neurochemical features of depressive disorders (Schiepers et al., 2005). They coordinate the local and systemic inflammatory response to microbial pathogens and act on the brain to cause behavioral symptoms of sickness, such as sleepiness, fatigue, loss of appetite and decreased libido (Berthold-Losleben and Himmerich, 2008; Dantzer, O'Connor, 2008). These symptoms have been described as "sickness behavior" and are related to the behavioral changes of depression (Berthold-Losleben and Himmerich, 2008). It is possible that depression represents a maladaptive version of cytokine-induced sickness, which could occur when activation of the innate immune response is exacerbated in intensity and/or duration, or that takes place in the context of an increased vulnerability to depression (Dantzer, O'Connor, 2008).

Studies have found increased serum levels of inflammatory biomarkers in depressed patients and decreased levels after antidepressant pharmacological intervention (Dowlati et al., 2010; Dunjic-Kostic et al., 2013; Himmerich et al., 2008; Lanquillon et al., 2000; Leonard, 2014). Other studies have associated non-pharmacological interventions (e.g. exercise, electroconvulsive therapy, sleep deprivation, relaxation) both with depressive symptoms and inflammatory markers (Branco et al., 2014; Buel et al., 2015; Carneiro et al., 2015; Irwin et al., 2015; Jr. et al., 2015; Kéri et al., 2014; Ranjbar et al., 2015). However, literature is yet to reveal if the reduction of depressive symptoms after psychotherapy is accompanied by a reduction in pro-inflammatory cytokines serum levels. One pilot study evaluated the changes in IL-6 levels after seven sessions of cognitive-behavioral psychotherapy and revealed a significant decrease in the IL-6 levels after the intervention (Gazal et al., 2013). Another study found a significant decrease in the severity of depressive symptoms and serum IL-6 and TNF—α after cognitive-behavioral psychotherapy, but not in narrative cognitive therapy (Moreira et al., 2015). Moreover, one study on cognitive therapy for depression and peripheral oxidative stress parameters found that cognitive psychotherapies were able to counteract peripheral oxidative stress in depressed patients, reducing thiobarbituric acid reactive species (TBARS) levels in the follow-up, nitric oxide in the post-treatment and follow-up, and increasing the total thiol content in the post-treatment and follow-up (Kaufmann et al., 2015).

In terms of psychotherapy, the key interventions for depression in primary care settings are treatment with generic antidepressant drugs and brief psychotherapy. Studies reveal a significant reduction of depressive symptoms after short-term psychodynamic psychotherapy at post-treatment and at follow-up (Bressi et al., 2010; Driessen et al., 2010; Leichsenring, 2001). It may strengthen the patient's abilities of realistic adaptation, insight, problem-solving, and rectification, through the elucidation of basic aspects of the patient's current situation (Fiorini, 2008). Moreover, there is a need for laboratorial measures to indicate treatment termination due to the limitation of self-report instruments.

Having considered this background, the aim of this study was to investigate the reduction of depressive symptoms and concomitant changes in pro-inflammatory cytokine levels in depressed patients before and after sixteen sessions of brief psychodynamic psychotherapy.

2. Methods

This was a quasi-experimental study that evaluated depressed patients who participated in a randomized clinical trial conducted in a mental health ambulatory (Pelotas/Brazil). The clinical trial tested the efficacy of two short-term psychotherapeutic models for depression (Supportive Expressive Dynamic Psychotherapy and Cognitive-Behavioral Psychotherapy). This work presents data from the dynamic psychotherapeutic model only. The clinical trial is nested within an outpatient research and mental health evaluation service from the Catholic University of Pelotas.

3. Recruitment

Public health facilities in the urban area of Pelotas, including primary care and mental health units, were contacted. Recruitment also included advertisements at local media and referral from other research works at the university.

4. Participants

This was a convenience sample. The participants were individuals who have voluntarily sought our psychology service (after reading or hearing about the research in the media) wishing to receive treatment; or had been referred from the public facilities where recruitment took place.

Every patient responded to a questionnaire about gender, age, schooling (years), socioeconomic status, current use of psychiatric medication, chronic clinical disorders (spinal problems, arthritis or rheumatism, cancer, diabetes, bronchitis or asthma, hypertension, cardiac problems, kidney insufficiency, tuberculosis, tendinitis or synovitis, cirrhosis, or other), current tobacco use/abuse, and current alcohol abuse. Major Depressive Disorder and comorbidities were evaluated by a Psychologist previously trained regarding evaluation methods and who attended to weekly supervision meetings. In case of any doubts concerning the diagnosis, patient was furtherly evaluated by a psychiatrist from the research team.

Patients who were diagnosed with MDD were included in the clinical trial if they had signed informed consent and if they fulfilled the following criteria: (1) MDD was the only or the most distressing current disorder; (2) the patient agreed to the treatment; (3) the patient was not currently using or had used any antidepressant medication in the two months prior to the treatment; (4) the patient did not present moderate or severe suicide risk; (5) there was no dependence of alcohol and/or abuse of illegal substances; (6) there were no psychotic symptoms. Thus, only patients with mild or moderate depression were included in the trial.

5. Data collection

Data collection occurred from July 2012 to June 2015. Blood samples were obtained at baseline and post-treatment (sessions 1 and 18) of psychotherapeutic treatment. Samples were obtained at a proper lab at the University, on the same Campus of the Psychological Clinic from the Catholic University of Pelotas, where psychotherapy sessions were carried out. Instruments on depressive symptoms were also administered at sessions 1 and 18.

6. Outcomes

The diagnosis of Depression and comorbidities was carried out using the Mini International Neuropsychiatric Interview (MINI)

Plus (Amorim, 2000). The ABEP (Brazilian Association of Research Companies (ABEP, 2003) was used to evaluate the socioeconomic status of the participants. The Outcome Questionnaire-45 (OQ-45) (Lambert et al., 2004) and the Beck Depression Inventory (BDI-II)) (Beck et al., 1996) were used to evaluate the severity of depressive symptoms.

7. Biochemical assay

For the biochemical analyses, 10 mL of blood were withdrawn from each subject by venipuncture into a anticoagulant-free vacuum tube after the interview, between 8:00 and 11:00 am; patients were required a minimum fasting period of 4 h. The blood was immediately centrifuged at 4000 × g for 10 min, and serum was kept frozen at −80 °C until analysis.

Serum levels of IL-6 and TNF-α were measured using a commercially available enzyme immunoassay kit (DuoSet ELISA Development, R&D Systems, Inc., USA). Briefly, the samples and standard curve were incubated in 96-well microplates coated with mouse anti-human antibodies for the cytokines of interest (IL-6 or TNF- α) for 2 h at room temperature. The plates were then washed 3 times with wash buffer, supplemented with biotinylated mouse anti-human probes for the cytokine of interest (IL-6 or TNF- α), and incubated for 2 h at room temperature. After washing, a third incubation with streptavidin conjugated to horseradish–peroxidase (diluted 1:200) was carried out for 20 min at room temperature. After 20 min, the plates were washed and incubated for 20 min with the substrate solution (R&D Systems) and stopped with 2 N H_2SO_4 (R&D Systems). The amount of IL-6 and TNF- α was determined by measuring the absorbance at 450 nm. The standard curve demonstrated a direct relationship between the optical density and IL-6 and TNF- α concentration. All samples and standards were measured in duplicate, and the coefficient of variation was less than 5%. The IL-6 and TNF- α levels were expressed in pg/mL.

8. Treatment protocol

The protocol was composed of eighteen sessions that included sixteen 50-min sessions of Supportive-Expressive (SE) Dynamic Psychotherapy of Depression (Luborsky et al., 1995), as proposed by Luborsky's theory. The first and eighteenth sessions were aimed at rapport and data collection (Fig. 1).

The SE Dynamic Psychotherapy of Depression is focused on the dynamic issues presented by depressed patients (sense of helplessness, vulnerability to disappointment and loss, states of anger turned inward rather than directed outward, vulnerability of the self-esteem, suicidal ideation and intention, pessimistic explanatory style, poor capacity to recognize the state of depression, poor capacity to notice events that trigger depression, inclination to expect negative responses from self and others). The four basic tasks of SE dynamic psychotherapy are: (1) attend to forming an alliance; (2) formulate the basic relationship pattern by means of the core conflictual relationship theme (CCRT) method; (3) help the patient to come to a generally higher morale and acquire ways of coping and mastering the conflicts in the CCRT; and (4) attend to the meanings of separation from the treatment so that they will not interfere with the patient's retention of the gains. Full other details may be obtained in the manual (Luborsky et al., 1995).

In order to determine the CCRT of each patient, the first three sessions of psychotherapy were transcribed by a neutral research member (graduate Psychology student). The transcribed session was sent to a randomized judge, who did not participate in supervisions or reunions with the research group. The judge would identify the CCRT of the patient, in accordance with the method's manual (Luborsky, 1984) and, afterwards, the therapist would receive the CCRT in order to be used as the focus of the treatment, alongside the other dynamic aspects of depression aforementioned.

9. Statistical analyses

Analyses were conducted in the software SPSS version 21 and the GraphPad Prism 6.0 for Windows. The Student's t test for paired-samples was used to compare the baseline and post-treatment mean scores of the OQ-45 (total score and subscales) and the BDI. The differences in the serum IL-6 and TNF-α levels at baseline and post-treatment were evaluated using the Wilcoxon signed-ranked test, and the Mann–Whitney and the Kruskal–Wallis test was used to verify the association between the independent variables and the cytokines levels. The results with p-values ≤ 0.05 were considered statistically significant.

10. Ethical aspects

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. This study design was approved by the ethics committee of the Catholic University of Pelotas, which is associated to the National Committee of Ethics in Research (National Counsel of Ethics in Research – CONEP) under the protocol number 66006 from July 2012. Also, informed consent of the participants was obtained after the nature of the procedures had been fully explained.

11. Results

A total of 322 individuals were initially enrolled in the clinical trial. From these, 87 participants were excluded for scheduling the first session and not attending it (for three times) or for fulfilling exclusion criteria after beginning treatment. The clinical trial was composed of 235 individuals (128 in the SEDP group and 107 in the CBT group). From these, 56 individuals concluded SEDP treatment and 56 participants concluded CBT. Ten blood samples from the individuals that concluded SEDP treatment could not be included in the analysis.

Thus, 46 participants were included in this report (Table 1). From these, 36 (76.1%) individuals were female, mostly (56.5%) from socioeconomic class B, with mean 33.85 ± 10.52 years of age and 11.17 ± 03.62 years of study. Thirty-one (67.4%) individuals presented some (or more than one) chronic clinical disorder (37.0% spinal problems, 06.5% arthritis or rheumatism, 04.3% cancer, 02.2% diabetes, 13.0% bronchitis or asthma, 13.0% hypertension, 10.9% cardiac problems, 02.2% kidney insufficiency, 02.2% tuberculosis, 19.6% tendinitis or synovitis, 04.3% cirrhosis, 19.6% other), 47.8% presented some comorbid anxiety disorder, 67.4% presented some comorbid personality disorder, 19.6% were smokers, 32.6% were alcohol users/abusers, and 8 (17.4%) individuals were on psychiatric medication (benzodiazepines). In addition, 43 (93.5%) individuals concluded the treatment protocol, and 3 (06.5%) people abandoned therapy.

The independent variables which were significantly associated ($p < 0.020$) with serum IL-6 and/or TNF-α levels at baseline and/or at post-intervention were considered as possible confounding factors. Baseline/post-intervention serum IL-6 levels were associated with age ($p = 0.191$; $p = 0.039$) and current use of psychiatric medication ($p = 0.126$; $p = 0.776$), but not with gender ($p = 0.256$; $p = 0.732$), socioeconomic status ($p = 0.849$; $p = 0.958$), schooling ($p = 0.940$; $p = 0.636$), chronic clinic disorders ($p = 0.930$; $p = 0.246$), presenting some anxiety disorder ($p = 0.688$; $p = 0.561$), presenting some personality disorder ($p = 0.494$; $p = 0.228$), current use of tobacco ($p = 0.699$; $p = 0.616$), and

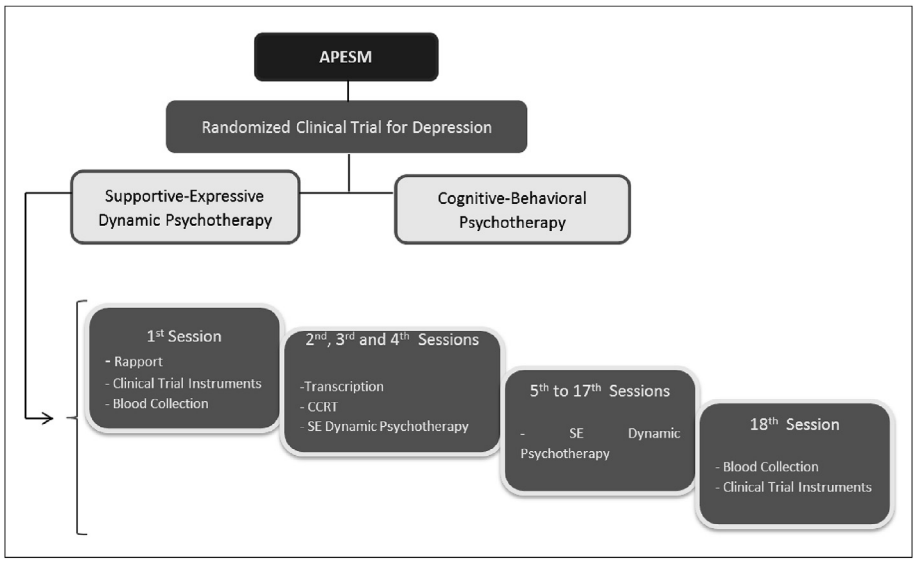

Fig. 1. Study design and treatment protocol.

Table 1
Sociodemographic characteristics of the sample.

Variables	N (%)/Mean ± SD
Gender[a]	
Male	11 (23.9)
Female	36 (76.1)
Age (years)[c]	33.85 ± 10.52
Schooling (years)[c]	11.17 ± 03.62
Socioeconomic classification[a]	
A	11 (23.9)
B	26 (56.5)
C	09 (19.6)
Chronic Clinical Disorder[a,b]	
No	13 (28.3)
Yes	31 (67.4)
Completed treatment[a]	
No	03 (06.5)
Yes	43 (93.5)
Current Use of Psychiatric Medication[a]	
No	38 (82.6)
Yes	08 (17.4)
Some Anxiety Disorder[a,b]	
No	23 (50.0)
Yes	22 (47.8)
Some Personality Disorder[a,b]	
No	08 (17.4)
Yes	31 (67.4)
Tobacco Abuse/Dependence[a,b]	
No	35 (76.1)
Yes	09 (19.6)
Alcohol Abuse[a,b]	
No	29 (63.0)
Yes	15 (32.6)
Total	46 (100)

[a] Variable contains missing values.
[b] Simple and relative frequencies (%) calculated per row.
[c] Mean and standard deviation.

current alcohol abuse ($p = 0.464$; 0.427). Baseline/post-intervention serum TNF-α levels were associated with gender ($p = 0.148$; $p = 0.501$), socioeconomic status ($p = 0.990$; $p = 0.115$), and presenting some anxiety disorder ($p = 0.671$; $p = 0.176$), but not with age ($p = 0.294$; $p = 0.995$), schooling ($p = 0.912$; $p = 0.667$), chronic clinic disorders ($p = 0.410$; $p = 0.328$), current use of psychiatric medication ($p = 0.530$; $p = 0.330$), presenting some personality disorder ($p = 0.892$; $p = 0.303$), current use of tobacco ($p = 0.989$; $p = 0.255$), and current alcohol abuse ($p = 0.913$; $p = 0.817$).

Baseline serum IL-6 levels were significantly correlated with the social role domain of the OQ-45 ($r = 0.403$; $p = 0.005$) and the initial BDI score ($r = 0.391$; $p = 0.007$). Serum TNF-α levels were significantly correlated with the social role domain of the OQ-45 ($r = 0.348$; $p = 0.018$) at baseline. The analysis revealed a significant decrease in severity of depressive symptoms according to the BDI ($p = 0.000$) and significantly reduced mean of total score and subscales scores in the OQ-45 ($p = 0.000$), except for the interpersonal relationships domain ($p = 0.137$). Nonetheless, mean OQ-45 scores remained above the cutoff scores in all domains.

Concerning the pro-inflammatory cytokines, serum IL-6 and TNF-α levels significantly decreased ($p = 0.000$) after short-term dynamic psychotherapy (Table 2). There was no significant association between serum IL-6 or TNF-α levels and severity of depressive symptoms at post-intervention. Nonetheless, the variation of serum IL-6 levels (Δ IL6) was significantly associated with score reduction in the social role domain (ΔOQ SR) of the OQ-45 ($p = 0.026$), but the correlation was not maintained after adjustment for age and current use of medication ($p = 0.178$). The variation of serum TNF-α levels (Δ TNF) was significantly associated with the social role domain of the OQ-45 (ΔOQ SR) even after adjusting for gender, socioeconomic status, and presenting some anxiety disorder ($p = 0.002$) (Fig. 2).

12. Discussion

This is the first study, to our knowledge, to present changes in serum cytokine levels correlated with the reduction of depressive symptoms after short-term dynamic psychotherapy. Up to the moment, most studies have addressed the association between inflammation and depression using pharmacological treatment (Lanquillon et al., 2000; Leonard, 2014; Marques-Deak et al., 2007) and little is known about such changes after psychotherapeutic interventions. In addition, the studies on psychotherapy and

Table 2
Serum IL-6 and TNF- α levels and severity of depressive symptoms (BDI and OQ-45.2) at baseline and after psychodynamic psychotherapy.

	Baseline		Post-intervention		p-value
	Median (Interquartile intervals)	Mean ± SD	Median (Interquartile intervals)	Mean ± SD	
BDI[a]		29.00 ± 10.24		19.91 ± 14.63	0.000
OQ-45.2[a]					
Total Score		82.20 ± 16.68		72.09 ± 24.38	0.000
SD score		45.15 ± 10.92		38.70 ± 15.18	0.000
IR score		20.64 ± 03.94		19.38 ± 05.96	0.137
SR score		15.47 ± 04.18		12.71 ± 05.06	0.000
IL-6 (pg/mL)[b]	05.44 (02.58; 06.38)		02.82 (02.20; 04.42)		0.000
TNF- α (pg/mL)[b]	11.60 (07.61; 16.27)		05.47 (04.42; 07.95)		0.000

BDI = Beck Depression Inventory; OQ-45 = Outcome Questionnaire 45.2; SD = Symptom Distress; IR = Interpersonal Relationship; SR = Social Role; IL-6 = Interleukin-6; TNF-α = Tumor Necrosis Factor −α.
[a] Student's t test.
[b] Wilcoxon signed-ranked test.

inflammation so far have used CBT models of treatment and there is no data regarding psychodynamic approaches of therapy.

Several studies have showed the involvement of the immune system in depression. However, the exact mechanisms that contribute for this disorder are not fully understood (Furtado and Katzman, 2015). The main pro-inflammatory cytokines involved in depression are IL-1β and TNF-α. Nevertheless, IL-6 signaling is necessary to regulate the release of these cytokines; thus, there is an increase of IL-6 promoting IL-1β and TNF-α release (Dantzer, O'Connor, 2008). One meta-analysis reported significantly higher concentrations of TNF-α and IL-6 in depressed patients, whereas there were no differences in the concentrations of the other pro-inflammatory cytokines investigated (IL-1 β, IL-2, IL-8 and IFN- γ). The authors suggest that these negative results are because the studies used considerable small population sizes, which may have made it difficult to observe associations (Dowlati et al., 2010). Interestingly, it has been suggested that the pro-inflammatory cytokines IL-6 and TNF-α are involved in the stimulation of corticotropin-releasing hormone activating the HPA axis and increasing the cortisol levels (Cowen, 2002). Dysregulation of the HPA axis is an important finding associated with depressive behavior, underscoring the direct clinical significance of elevations in pro-inflammatory cytokines (Dantzer, O'Connor, 2008). Another work (Howren et al., 2009) determined that CRP, IL-6, IL-1, and soluble IL-1 receptor levels are increased in depressed patients. On the other hand, other works suggest that the inhibition of anti-inflammatory cytokines promotes an increase in intensity and duration in sickness behavior (Dantzer, O'Connor, 2008). In animal models, central administration of IL-10 attenuates behavioral sickness signals induced by LPS and IL-10-deficient mice have an exacerbated sickness behavior when LPS is administered (Bluthé et al., 1999; Leon et al., 1999). Moreover, recent findings of our laboratory showed that higher IL-10 levels were observed in depressed patients with later disease onset when compared with controls or early-onset patients, and these levels were negatively correlated with illness duration (Gazal et al., 2015). Thus, these changes in pro- and anti-inflammatory cytokines in the brain support the idea that the immune balance regulates several cytokines functions in depression.

Pro-inflammatory cytokines are essential for normal brain development and homeostatic regulation of synaptic scaling. It is the disturbance of this intricate equilibrium between physiological and pathophysiological levels of cytokines in the brain that affects synaptic plasticity and plays a critical role in the pathophysiology of MDD (Khairova et al., 2009). Concerning inflammation and psychotherapy, one important study (Kéri et al., 2014) evaluated the pro-inflammatory pathways related to the "leaky gut" hypothesis of depression and CBT. Their results indicate a significant reduction of 16S rDNA level, Toll-Like Receptors-4 expression and factor Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells (NF-κβ) expression, but there was no significant decrease of IL-6 levels. Another study (Kaufmann et al., 2015) investigated the oxidative-antioxidative systems in depression in response to CBT and narrative cognitive therapy. Both psychotherapies were able to reduce thiobarbituric acid reactive species (TBARS) levels in the follow-up, nitric oxide at post-treatment and follow-up, and increasing the total thiol content at post-treatment and follow-up.

One pilot study (Gazal et al., 2013) indicated that IL-6 levels and depressive symptoms were significantly reduced after therapy. Even though there was no correlation of IL-6 levels and symptom severity, the variation of IL-6 levels and the remission rate were significantly correlated, which is in accordance to the present investigation. Another study (Moreira et al., 2015) showed significantly reduced IL-6 and TNF-α levels after CBT. Nonetheless, there was no correlation between the remission of depressive symptoms and the decrease of serum IL-6 and TNF-α levels. Our results reveal that serum IL-6 and TNF-α levels significantly decreased after intervention ($p \leq 0.001$), which corroborates the findings from other studies, including important reviews and meta-analysis studies (Dantzer, O'Connor, 2008; Dowlati et al., 2010; Gazal et al., 2013; Howren et al., 2009; Liu et al., 2012; Moreira et al., 2015), and add on to the specific field of psychotherapy (and SEPD) and inflammation.

The present study also found that the social role domain from the OQ-45 was significantly correlated with serum IL-6 and TNF-α levels at baseline and with serum TNF-α levels at post-treatment. This is a very important result that corroborates the association between sickness symptoms, such as social withdrawal, in depression and inflammation (Einseberger et al., 2010) and the results linking social ties to aspects of inflammation (Chiang et al., 2012). The social role domain focuses on the patient's level of dissatisfaction, conflict, distress, and inadequacy in tasks related to their employment, family roles, and leisure life (Lambert et al., 2004). Studies show that lonelier individuals present increased levels of pro-inflammatory cytokines in response to an acute psychological stressor (Moieni et al., 2015a). Moreover, recent studies have found increased TNF-α levels in response to endotoxin, which is a laboratory stressor (Chiang et al., 2012; Einseberger et al., 2010; Moieni, Irwin, 2015a; Moieni et al., 2015b; Song et al., 2015).

Short-term dynamic psychotherapy has been effective in reducing depressive symptoms, which is in accordance with other studies (Bressi et al., 2010; Driessen et al., 2010). Psychological interventions can profoundly alter patients' sets of beliefs, ways of thinking, affective states and patterns of behavior. Nevertheless, the putative mechanisms and underlying changes in the brain still need elucidation (Linden, 2006). There is evidence that synaptic

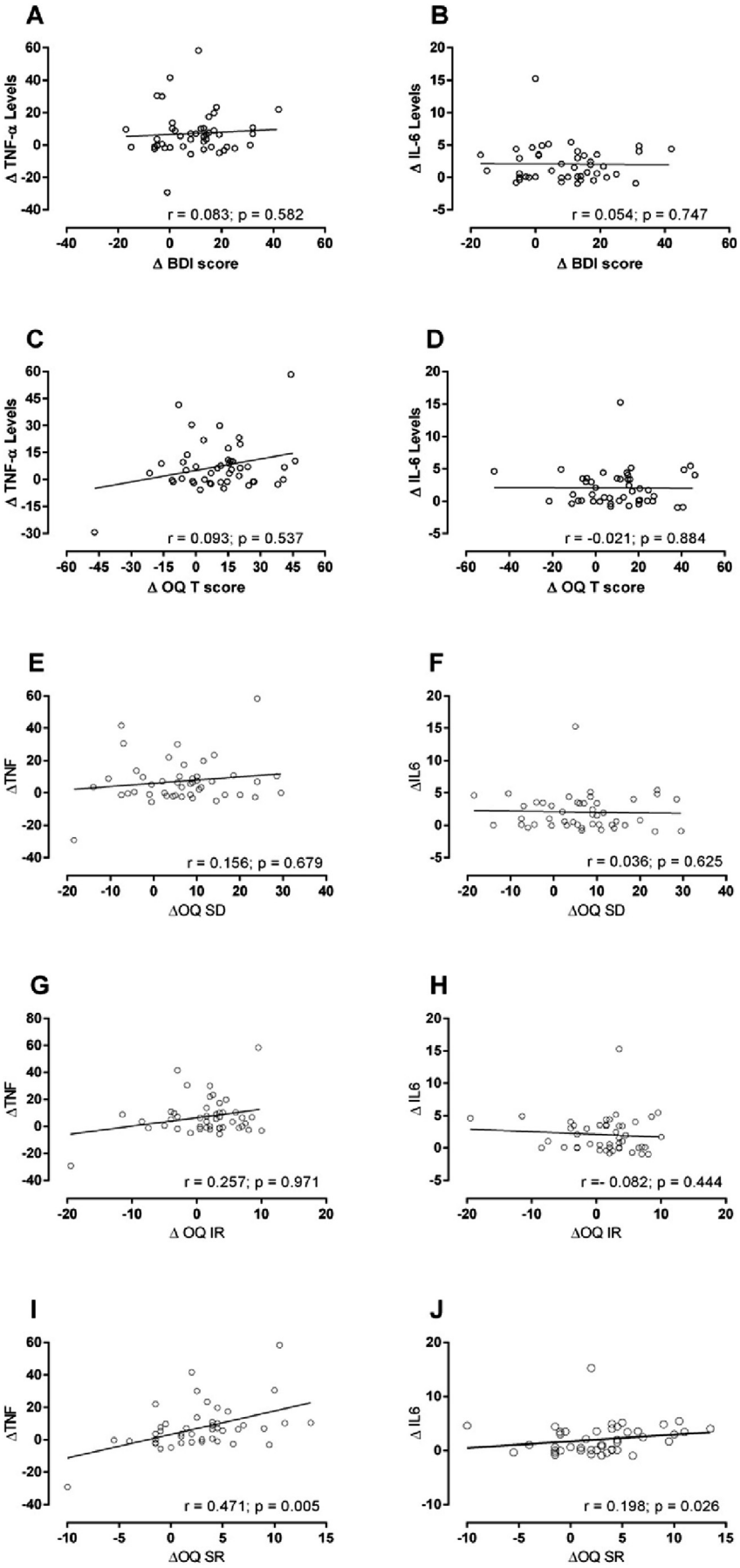

Fig. 2. Correlation between TNF-α, IL-6, BDI and OQ-45 scores from baseline to post-treatment.

plasticity is impaired in mood disorders, and that activation of the immune system network may be related to at least some aspects of the complex pathophysiology of depressive disorders (Khairova et al., 2009). Studies with antidepressant medication indicate that inhibiting pro-inflammatory cytokine signaling is a potential strategy for treating depressive disorders, especially in patients with evidence of increased inflammatory activity before therapy, who might be less likely to respond to conventional agents (Khairova et al., 2009).

The Beck Depression Inventory and the Outcome Questionnaire are widely used instruments in clinical and research practice and their use in this randomized clinical trial add reliability to our results. Nonetheless, scores remained above the cutoff scores indicating mild to moderate depression in the BDI (score $\geq$ 12 indicates mild depression; score $\geq$ 20 indicates moderate depression; and score $\geq$ 35 indicates severe depression) and clinically significant distress in the OQ-45 (Total Score $\geq$ 63; Symptom Distress $\geq$ 36; Interpersonal Relationship $\geq$15; Social Role $\geq$ 12). However, this does not invalidate our results since there are innumerous variables that may play an important role on therapy outcome (Lambert, 2013).

The present study found age and current use of medication to be possible confounding factors in the associations with IL-6 levels, which is in accordance to other studies (Glaus et al., 2014; Howren et al., 2009; Liu et al., 2012; Ramirez et al., 2016). In fact, the association between the variation of serum IL-6 levels and symptom remission (social role) was not maintained after adjusting for these variables. Gender, socioeconomic status and anxiety disorders were found to be possible confounding factors in the associations with TNF-α levels, which is also in accordance with literature (Fraga et al., 2015; Glaus et al., 2014; Martin et al., 2015; Wagner et al., 2015). Nevertheless, the association between the decrease of serum TNF-α levels and the improvement of the scores in the social role domain from the OQ-45 persisted meaningful even after adjusting for these variables.

Our study has some limitations. First, this was a convenience sample and we had a relatively small sample size, which precluded the stratification of cytokine levels (i.e. markedly low or elevated levels) in order to maintain statistical power. These factors could prevent the generalization of our results; however, the main goal of this study was to understand the relationship between depressive symptoms and cytokine levels before and after psychotherapy regardless of inflammatory cut-off points, since it has not been quite described in literature. Another possible limitation is that we did not stratify the results according to types of depression, as other studies have presented (Dunjic-Kostic et al., 2013; Marques-Deak et al., 2007; Miller et al., 2013). However, there is evidence that the sub-classification of depression may not predict differences in cytokine levels (Marques-Deak et al., 2007) and that some types of depression are not associated with differences in the concentrations of cytokines (Rudolf et al., 2014).

Literature shows that the field of depression, depressive symptoms and psychotherapeutic approaches for depression has been widely studied. Nonetheless, the continued increasing incidence and recurrence of depressive episodes urge for research works aimed at understanding its physiopathology, its psychodynamics, and at developing low-cost, brief, and effective treatment strategies for Depression to be implemented in primary health care. This study is a contribution on the field of inflammation and depression and on the effectiveness of short-term psychotherapy for depression.

Contributors

All authors participated in the study design and writing of the manuscript. Giovanna Silva participated in the coordination of the clinical trial, patient treatment, and statistical analyses. Luana Barbosa participated in the coordination of the clinical trial and patient treatment. Jaciana Araújo evaluated patients, and Pedro San Martin helped in the organization of data. Luciano Souza and Ricardo Silva coordinated the evaluation service and the clinical trial, supervised therapist training and activities regarding the intervention models. Karen Jansen participated in the coordination of the evaluation service and data analyses. Mariane Molina coordinated the evaluation service. Carolina Wierner and Jean Oses coordinated laboratory staff, conducted the ELISA essays and analyzed data. All authors approved the final version of the manuscript.

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Conflict of interest statement

All authors declare that they have no conflicts of interest.

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