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DISSERTATION

Inflammation, Metabolic Syndrome, & Early Life Stress in Major Depression – an Investigation into the Mind-Body Connection of Affective Disorders

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SUMMARY

INFLAMMATION, METABOLIC SYNDROME, & EARLY LIFE STRESS IN MAJOR DEPRESSION – AN INVESTIGATION INTO THE MIND-BODY CONNECTION OF AFFECTIVE DISORDERS

PREFACE (MOTIVATION & BACKGROUND):

In the following, a work in the field of psychoneuroimmunology will be presented.

Inspiration for this dissertation came from the doctoral candidate's fascination for the topic of mind-body-interactions. A fascination shared by philosophers and scientists alike for centuries. The question of whether the mind plays a role in physical illness and vice versa goes back to the earliest days of medicine, and ever since has been discussed, debated, and at times repudiated. Maybe most prominently, French philosopher René Descartes stated that the mind is distinct from matter, but nevertheless has an influence on it. According to Descartes, this interaction took place in the pineal gland (Descartes, 1637). But even long before that, the classic medical traditions of China and India were of holistic nature. The ancient Chinese believed that internal sources such as exaggerated or attenuated emotions (qiqing) cause changes in *qi* (the quality which needs to be distributed evenly across the body to sustain health). Chinese physicians used the prescription of "optimistic attitude" in order to treat poor health (Hafen et al., 1996). Similarly, the Indian healing tradition of Ayurveda assumed a dependence of the soul from the health of the body. Medicines would thus include not only substances, but "appropriate thoughts", as well. Ancient Greek medicine believed that the mind could affect the course of illness. It was assumed that disease resulted from an imbalance of the four humors (blood, phlegm, yellow bile, and black bile). These humors were also assumed to give rise to the human temperaments. An excess of black bile was thought to cause melancholia, for example. And treatment always included a psychological component. The first dramatic decrease of mortality from disease, however, was brought about by the sanitation movement in the 1840s, which still held the view that filth caused an imbalance in the bodily humors. Nevertheless, it pointed out an important direction: that despite the benefits of holistics, a good understanding of inflammatory processes is necessary for better disease outcomes. Interestingly, research on inflammatory diseases and the

discovery of antibiotics in the late 19th century neglected and then even rejected a mind-body-connection (Sternberg & Gold, 2002). This notion was only reversed a couple of decades ago with the identification of a network that exists between the immune system and the brain.

The field of *psychoneuroimmunology* has come into existence only in comparatively recent times. The term as such was first introduced by Robert Ader during his presidential lecture to the American Psychosomatic Society in 1980 (Ader, 1980). Thanks to serendipity, Ader discovered a possible link of communication between the brain and the immune system while he was conducting food aversion experiments. Ader and his colleague Cohen conditioned rats to a pairing of saccharin and a toxin with immunosuppressive properties (cyclophosphamide). When the rats were exposed solely to the saccharin solution thereafter, they showed significantly lower levels of antibodies, thus the immunosuppressive effect of the drug had been conditioned to the taste of saccharin (Ader & Cohen, 1975). After this first discovery, well-designed human studies began to appear documenting effects on the immune system by laboratory manipulations of the emotional state of the subjects, or the occurrence of life events. Janet Kiecolt-Glaser and Ronald Glaser, for example, demonstrated that a variety of stressors such as marital distress (e.g. Jaremka, 2013; Kiecolt-Glaser, 2005), caregiving to Alzheimer's disease patients (Damjanovic et al., 2007), or a cumulation of daily stressors (Gouin et al., 2012) had the potential to diminish the immunocompetence of those affected. Thus, psychoneuroimmunology is the most integrative scientific discipline capable of providing an understanding of health in all its complexity. It integrates the medical observations based on experience of the ancient times with modern high-standard research into the molecular mechanisms of inflammation. Professor of classics and history of science, Philip van der Eijk (2005), says that "The questions that are raised today are similar to those raised by Greek philosophers and medical writers." He interprets this as a sign for the importance of these questions and that they "are a part of our cultural self reflection."

The particular interest of the dissertation presented here lies in the connection between major depression and the immune system. The study of *melancholy* has already been of interest to the old Greeks. It has been regarded as a condition in which the mind as well as the body are disturbed, and in which cognitive and emotional malfunctioning are attributed to particular states of the body (the above mentioned humors) (Ephesus, 2008). Moreover, already the ancient Greeks believed that melancholic women were more prone to cancer (Solomon, 2002). Modern research generated scientific proof of a relation between major depression and somatic diseases: Meta-analyses consistently showed that depression is associated with an

increased risk of overall mortality (RR= 1.81) (Cujpers et al., 2013), the development of cardiovascular related outcomes (RR= 1.81) (Nicholson et al., 2006), diabetes (RR= 1.60) (Mezuk et al., 2004), hypertension (RR= 1.42) (Meng et al., 2012), stroke (RR= 1.34) (Dong et al., 2013), and obesity (RR= 1.58) (Lupino et al., 2011). Major depression also increases the risk of Alzheimer's disease (RR= 1.66) (Gao et al., 2013), and to a lesser extent even cancer (RR= 1.29) (Chida et al., 2008).

ABSTRACT:

Background: Research increasingly considers the immunological effects of major depression. The impact of the metabolic syndrome on inflammatory biomarkers is investigated in the first study. The second study assesses the impact of early life stress on inflammatory biomarkers in adulthood. The third study combines the results of the first two by determining the relationship between components of the metabolic syndrome, early life stress, and fibrinogen levels.

Methods: 70 depressed inpatients with or without the metabolic syndrome were assessed at admission to the clinic and after amelioration of depressive symptomatology for study 1. 25 of these provided data for studies 2 and 3. The metabolic syndrome was diagnosed via the International Diabetes Federation's criteria. Severity of depression was measured with the 17-item Hamilton Depression Rating Scale (HDRS). Early life stress and parental bonding were quantified with the Childhood Trauma Questionnaire (CTQ), and Parental Bonding Inventory (PBI). Biomarkers included adiponectin, resistin, serum amyloid A (SAA), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), soluble E-selectin (sE-selectin), interleukin-6 (IL-6), and CD40 ligand (CD40L) (the last two only in the first study). The relationships between the metabolic syndrome, early life stress, and fibrinogen were calculated with path analyses. Two main models were tested: the metabolic syndrome mediating/ not mediating between early life stress and fibrinogen levels.

Results: Study 1: A 2-factorial ANOVA (metabolic syndrome x time) revealed that the metabolic syndrome's presence affected adiponectin ($F_{43,1}$= 5.56; p< .05; η^2= .11) and IL-6 levels ($F_{25,1}$= 6.80; p< .05; η^2= .21) significantly. There was also a trend for effects on fibrinogen levels ($F_{47,1}$= 3.66; p= .06; η^2= .08). Study 2: Nearly all patients reported a parental style of "affectionless control". Physical neglect significantly predicted fibrinogen levels (R^2= .42, adjusted R^2= .27, ß= .56, p= .04). Study 3: The model without the mediating effect of the

metabolic syndrome provided an excellent fit for our data: (χ^2= 0.02, df= 1, p= .90, CFI= 1.00, NNFI= 2.71, RMSEA= 0.00).

Conclusion: For the first time, an additive effect of the metabolic syndrome on inflammatory biomarkers was demonstrated in depressed patients. Furthermore, inflammation might be an important mechanism mediating the unfavorable effects of adverse childhood experiences. The metabolic syndrome was not found to be a mediator between early life stress and fibrinogen levels in adulthood. Therapeutic options targeting the mood-inflammatory pathway in depression, such as psychotherapeutic treatment of early childhood adversities or novel psychopharmacologic interventions will be discussed.

GERMAN ABSTRACT (ZUSAMMENFASSUNG):

Hintergrund: Eine zunehmende Anzahl von Untersuchungen beschäftigt sich mit immunologischen Aspekten der Major Depression. Eingangs wird der Einfluss des metabolischen Syndroms auf Konzentrationen immunologischer Biomarker untersucht. Die zweite Studie beschäftigt sich mit dem Einfluss frühkindlichen Stresses auf inflammatorische Biomarker im Erwachsenenalter. In der dritten Studie werden die Ergebnisse aus den ersten beiden zusammengeführt und mögliche Zusammenhänge zwischen dem metabolischen Syndrom, frühkindlichem Stress und Fibrinogenwerten untersucht.

Methoden: Für die erste Studie wurden die Daten von 70 depressiven Versuchspersonen mit und ohne metabolischem Syndrom bei Aufnahme in die Klinik sowie nach Besserung der Symptomatik erfasst. An der zweiten und dritten Studie nahmen 25 Patienten von der Ursprungskohorte teil. Das metabolische Syndrom wurde nach den Kriterien der *International Diabetes Federation* diagnostiziert. Mittels der Hamilton-Skala (17-Itemform) wurde die Depressionsschwere bestimmt. Frühkindlicher Stress und Bindungserfahrungen wurden mit dem Childhood Trauma Questionnaire bzw. dem Fragebogen zur elterlichen Bindung eingeschätzt. Folgende Biomarker wurden erfasst: Adiponektin, Resistin, Serum Amyloid A (SAA), C-reaktives Protein (CRP), Tumornekrosefaktor-α (TNF-α), lösliches E-Selectin (sE-Selectin), Interleukin-6 (IL-6) und CD40L Ligand (CD40L) (letztere beide nur in der ersten Studie). Die Zusammenhänge zwischen dem metabolischen Syndrom, frühkindlichem Stress, Fibrinogen und Depression wurden mittels Pfadanalysen berechnet. Dabei wurden zwei Hauptmodelle getestet: mit bzw. ohne mediierenden Effekt des metabolischen Syndroms zwischen frühkindlichem Stress und Fibrinogenwerten.

Ergebnisse: Studie 1: Eine 2-faktorielle Varianzanalyse (metabolisches Syndrom x Zeitpunkt) ergab einen Zusammenhang zwischen metabolischem Syndrom und Adiponektinwerten ($F_{43,1}$= 5.56; p< .05; η^2= .11), sowie IL-6-Werten ($F_{25,1}$= 6.80; p< .05; η^2= .21) . Zudem gab es einen Trend für Effekte auf Fibrinogen ($F_{47,1}$= 3.66; p= .06; η^2= .08). Studie 2: Fast alle Patienten erinnerten einen Erziehungsstil „liebloser Kontrolle“ aus ihrer Kindheit und Jugend. Körperliche Vernachlässigung sagte Fibrinogenwerte signifikant voraus (R^2= .42, adjusted R^2= .27, ß= .56, p= .04). Studie 3: Die Ergebnisse der Pfadanalysen unterstützten das Model ohne mediierende Wirkung des metabolischen Syndroms (χ^2= 0.02, df= 1, p= .90, CFI= 1.00, NNFI= 2.71, RMSEA= 0.00).

Schlussfolgerung: Zum ersten Mal konnte ein additiver Effekt des metabolischen Syndroms auf inflammatorische Biomarker in depressiven Patienten nachgewiesen werden. Darüber hinaus könnte Inflammation einen wichtigen Mechanismus bei der Mediation ungünstiger Folgen frühkindlichen Stresses darstellen. Das metablische Syndrom erwies sich nicht als Mediator zwischen frühkindlichem Stress und Fibrinogenwerten im Erwachsenenalter. Psychoinflammatorisch wirksame Behandlungsstrategien, wie z.B. am frühkindlichem Stress ansetzende psychotherapeutische bzw. neuartige psychopharmakologische Interventionen, werden im Hinblick auf die Ergebnisse diskutiert.

INTRODUCTION:

The first paper *“Inflammatory biomarkers in 70 depressed inpatients with and without the metabolic syndrome”* introduces the reader to the concept of a *mind-body-connection*, presenting evidence for depression having the potential to affect physical health, with a particular focus on the metabolic syndrome. After a short overview depicting the metabolic syndrome and its putative relationship to major depression, a summary concerning the metabolic syndrome and inflammation is given – concentrating on chronic subclinical inflammation. Following this, the activation of the inflammatory system in relation to depression is reflected upon, especially familiarizing the reader with the macrophage theory of depression (Smith, 1991), a kynurenine pathway theory (Müller & Schwarz, 2007) and the possibility of a common genetic substrate shared by depression and inflammation (Vaccarino et al., 2008). After this comprehensive introduction, the study is outlined: Since the metabolic syndrome and major depression are both associated with immunity related changes, and given the fact that both conditions can increase the probability of a mutual appearance, this study

focuses on the examination of potential additive immunological effects that might arise due to their simultaneous appearance.

Similar to the first paper, the second study with the title *"Childhood maltreatment and adult proinflammatory status in patients with major depression"* also puts an emphasis on the topic whether depression itself causes immunoalterations. Whereas the focus of the first article is put on an additive effect of the metabolic syndrome and major depression, the second article addresses the possibility of early life stress being a potential cause for immunity related changes in major depression. An overview introduces the reader to the concept of early life stress, its different types, and relation to major depression as well as to the immune system. Subsequently, a thorough review of animal and human studies is presented with regards to different early life stressors such as early separation, bereavement, unsafe attachment, and abuse. The introduction concludes with the research question, whether there are effects of adverse childhood experiences on numerous different proinflammatory markers in adult inpatients with major depression.

The third paper *"Pathways linking early life stress, metabolic syndrome, and the inflammatory marker fibrinogen in depressed inpatients"* combines the findings of its two predecessors. Its aim is to generate hypotheses concerning the relationship between early life stress, the metabolic syndrome, and inflammation in a sample of depressed inpatients. After a concise introduction regarding these topics, the study describes the findings of the above mentioned first two papers. Both, the metabolic syndrome and early life stress proved to be important contributing factors to subclinical immunoactivation in depressed inpatients. In particular, the hepatically synthesized acute-phase protein fibrinogen was found to be influenced by the metabolic syndrome as well as early life stress, turning it into our target immunomarker for further calculations.

METHODS:

Originally, 71 patients of the Department of Psychiatry of the Charité University Medicine-CBF, Berlin were included into the study. Within its course one patient was excluded due to the development of a common cold, reducing the study sample to 70. All patients suffered from a depressive episode when admitted to the hospital (the individual diagnosis varied

within the range of the affective disorders spectrum). Data were assessed at two time points. T1: at admission to the clinic, and T2: four to five weeks after the beginning of inpatient treatment and amelioration of the symptomatology. Depression measurements, inflammatory biomarkers, metabolic syndrome criteria, and sociodemographic variables were all gathered at T1, the first two also at T2. Major Depression was diagnosed according to DSM-IV and ICD-10 criteria. Severity of depression was quantified with the 17-item Hamilton-Depression-Rating scale (HDRS) (Hamilton, 1960), and further characterized by the assessment of the lifetime number of depressive episodes, duration of the current episode, and past suicide attempts. Inflammatory biomarkers were: adiponectin, tumor necrosis factor-α (TNF-α), resistin, sE-selectin, serum amyloid A, C-reactive protein (CRP), fibrinogen, IL-6, and CD40L (the last two only in the first study). (A comprehensive description of all biomarkers is given in the first paper.) The metabolic syndrome was assessed at admission according to the criteria of the International Diabetes Federation (IDF, Alberti et al., 2005). Sociodemographic variables included were sex and age. The study followed a naturalistic design, i.e. all patients were treated according to their psychiatrist's choice of different kinds of antidepressant drugs, psychotherapy and adjunctive methods.

Statistical analysis:

First study: In order to examine the impact of the metabolic syndrome on levels of inflammatory biomarkers in a cohort of inpatients suffering from major depression, a two-factorial analysis of variance (ANOVA) was conducted at two measurement points after correcting for confounding variables and covariates. HDRS scores were also added as covariates into the regression analysis to assess their influence on the concentrations of the inflammatory biomarkers.

Second study: 58 of the original 71 patients were included. In addition to the earlier mentioned inclusion criteria of the first study, early life stress and parental bonding were assessed. Of these 58 patients, 25 handed in fully completed questionnaires. Early life stress was measured with the German version of the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1994; Wulff, 2007). It enables the retrospective assessment of early traumatic stress in the form of emotional, physical, and sexual abuse, emotional and physical neglect, and to check for minimization/denial. The German version of the Parental Bonding Instrument (PBI) (Lutz et al., 1995) was applied to quantify the attachment to the parents. It evaluates parental bonding for each parent during the first 16 years of life. Eventually, each

person's score can be assigned to one of four quadrants: 1. affectionate constraint (high care, low protection), 2. affectionless control (low care, high protection), 3. optimal parenting (high care, high protection), 4. neglectful parenting (low care, low protection). A multiple hierarchical regression analysis was carried out to estimate the relative contribution of early life stress, and other potentially relevant variables on immunological measures.

Third study: There were 25 fully completed data sets. In addition to the general methods, path analyses (using AMOS 18.0.2) were performed to examine the relations between early life stress, metabolic syndrome, and inflammation. Altogether, two models were tested: First a model with the metabolic syndrome cluster as a mediator between early life stress and inflammation; second, a model without the mediating effect of the metabolic syndrome. Severity of depression was added to each model to account for its possible impact on inflammation. The overall fits were examined using established goodness-of-fit-indices, with a non-significant χ^2-statistic as the primary criterion of model fit (Hayduk et al., 2007).

RESULTS:

Our first study found about one quarter of the patients to be affected by the metabolic syndrome, which is a finding that parallels previous research (Jakovljević et al., 2007). The main result consisted of the metabolic syndrome having an additive effect on some, but not all proinflammatory markers. Specifically, the metabolic syndrome had a significant effect on adiponectin ($F_{43,1}$= 5.56, p< .05, η^2= .11) and IL-6 levels ($F_{25,1}$= 6.80, p< .05, η^2= .21). There was also a striking trend for the influence of the metabolic syndrome on fibrinogen levels ($F_{47,1}$= 3.66, p= .06, η^2= .08).

In the second study, nearly all patients rated the parental styles of their caregivers as "affectionless control" (having experienced low levels of care, and high levels of protection). Highest childhood trauma scores were obtained on the two subscales referring to emotional maltreatment. Severity of early adverse events was highest for the neglect and emotional cruelty subscales. The CTQ subscale "physical neglect" proved to significantly predict fibrinogen scores ($F_{5,19}$= 2.80, p= .05). Severity of depression was not an influential factor ($F_{7,17}$ = 1.81, p= .15).

Concerning the third study, even though the first model (with the metabolic syndrome functioning as a mediator) was not supported by the data (χ^2= 7.02, df= 1, p= .008, CFI= .00, NNFI= -9.44, RMSEA= .50), the second model provided an excellent fit for the data (χ^2= 0.02, df= 1, p= .90, CFI= 1.00, NNFI= 2.71, RMSEA= .00). Including severity of depression into the models did not yield any good indices of fit.

DISCUSSION:

The first paper's main result consisted of adiponectin, trendwise fibrinogen, and IL-6 levels being influenced by the metabolic syndrome in depressed inpatients. Thus, previous findings of decreased adiponectin levels in patients with the metabolic syndrome (You et al., 2008; Natal et al., 2008), as well as previously depressed medicated patients (Narita et al., 2006) were complemented and expanded. Our finding of significantly increased IL-6 levels mirrors previous reports of increased blood-levels in major depression (Fernandez-Real et al., 2001; Schiepers et al., 2005; Sluzewska et al., 2005) and in the metabolic syndrome (Kressel et al., 2009). Similarly our observation of increased fibrinogen levels supports reports of elevated levels of this acute-phase reactant in major depression (Panagiotakos et al., 2004, Fernandez-Real et al., 2001) as well as in the metabolic syndrome (Kressel et al., 2009). The study stresses the importance of assessing (and keeping in mind) the metabolic syndrome in patients with major depression. Patients with both conditions are at a high risk of suffering from more frequent and more severe cardiovascular side effects than patients with only one of these two syndromes. This is highly relevant with respect to treatment strategies. Certain treatments (such as the application of atypical antipsychotics and mood stabilizers) can initiate or worsen metabolic symptoms. There are also a number of possible explanations as to why not all markers were affected by the metabolic syndrome, which are therefore discussed in detail: e.g. a subclinical metabolic syndrome (i.e. comprising one or two symptoms) or differences between the patient groups with and without the metabolic syndrome in terms of duration of the current episode, number of suicide attempts in the past, age, and treatment conditions.

The main findings of the second study depicted that neglect and emotional maltreatment were the most common types of early life stress in our sample. The previous finding of early emotional adverse events being of great importance to the development and maintenance of major depression (Chapman et al., 2004, Moskvina et al. 2007) was consistent with our

results. Furthermore nearly all patients reported having experienced a parental style of affectionless control. The paper also confirmed an association between increased fibrinogen levels and physical neglect, replicating and expanding previous research (Danese et al., 2007). The results are discussed in relation to investigations of chronic stress and burnout as indirect mirror images of adverse childhood experiences.

The third study showed that early life stress and metabolic syndrome associated factors each have an independent impact on immunity, meaning that in our sample the trajectory between early life stress and inflammation was not mediated by metabolic syndrome associated factors. Alternatively, the severity of childhood adversity could also have had an influence. Compared to previous research, which highlights the very severe forms of childhood adversity as an impact on distinct symptoms of the metabolic syndrome cluster (e.g. central obesity) in later life (Thomas et al., 2008), our sample was mainly exposed to emotional and physical neglect as well as to emotional abuse as opposed to physical and sexual abuse. The possibility of epigenetics having a higher influence on fibrinogen levels than on the metabolic syndrome is also discussed.

Strengths and limitations of these respective studies are described, and ideas for future research and clinical applications discussed. A common asset of all three investigations is the study population in terms of being 'real patients' who are very well characterized psychopathologically as well as medically. A shared critical point of all papers is the amount of confounding variables that accompany such participants, and the resulting caution that needs to be taken when interpreting the data. Importantly, recent research is pointing to innovative and novel therapeutic options targeting the mood-inflammatory pathway: Tumor-necrosis factor antagonists (e.g. infliximab) have been investigated as a possible treatment option for certain subgroups of treatment-resistant depression (Raison et al., 2013). Acetyl-salicylic acid (ASA) by irreversibly inhibiting COX-1 as well as COX-2, and thereby increasing prostaglandin as well as thromboxane levels, which in turn decreases TNF-α and IL-6 has a potential as an add-on to current SSRI therapy leading to higher remission rates compared to SSRI monotherapy (Mendlewitz et al., 2006). Celecoxib, a selective COX-2 inhibitor, influencing the production of prostaglandins and downstream cytokins could increase therapeutic efficacy of antidepressants (e.g. Abbasi et al., 2012). Minocycline, a tetracyclic antibiotic could be beneficial for treating mood disorders through simultaneously acting on multiple pathways of depression (Soczynska et al., 2012). Furthermore curcumin, an

old-age spice with a long tradition in complementary and alternative medicine has been recently reviewed for its potential use with MDD via its potential to decrease cytokines, stabilizing HPA-activity, and decreasing oxidative stress (Brietzke et al., 2013, Lopresti et al., 2012). Such new ideas of clinical applications are also discussed (according to their publishing dates). Psychotherapeutic treatment strategies that are particularly targeted at reversing the consequences of adverse childhood experiences, such as schema therapy (Young 2006), might also reverse the putative long lasting psychophysiological effects of early life stress.

Taken together the dissertation composed of these three here presented papers investigates different aspects that could give rise to subclinical immunoalterations in patients with major depression. In addition to this, it also assesses possible pathways between the metabolic syndrome, early life stress and immunological biomarkers. The psychoimmunologic aspects concerning major depression are vast and yet with many questions left unanswered, meaning that major depression is a complex disease with respect to different psychobiological systems, caused by and affecting multiple different networks. Assessment should be thorough, and treatment multidimensional with respect to all possible co-occurring and morbidity affecting conditions (e.g. psychotherapeutic treatment of childhood adversity, appropriate psychopharmacological strategies not causing or worsening the metabolic syndrome). We did find possible relationships between psychological, biographical and somatic factors in patients with major depression. Future projects should however, investigate the underlying molecular mechanisms in more detail.

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SELECTED PUBLICATIONS

Inflammatory Biomarkers in 70 Depressed Inpatients with and without the Metabolic Syndrome

Childhood Maltreatment and Adult Proinflammatory Status in Patients with Major Depression

Pathways Linking Early Life Stress, Metabolic Syndrome, and the Inflammatory Marker Fibrinogen in Depressed Inpatients

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Psychiatria Danubina, 2013; Vol. 25, No. 3, pp 227-235 *Original paper*

CHILDHOOD MALTREATMENT AND ADULT PROINFLAMMATORY STATUS IN PATIENTS WITH MAJOR DEPRESSION

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SUMMARY

Background: *An increasing body of research considers the immunological effects of major depression. It remains an open question, whether depression itself acts in an immunomodulatory fashion or whether other factors related to depression result in these immunological effects. Regardless, major depression is often the result of early life stress, the implications of which are not satisfactorily understood.*

Subjects and methods: *Early life stress was retrospectively evaluated in 25 depressed inpatients via the CTQ (Childhood Trauma Questionnaire). Its impact on immunological biomarkers (fibrinogen, SAA, CRP, adiponectin, TNF-α, resistin, and sE-selectin) in adulthood was assessed via multiple regression analyses. Parental bonding was assessed via the PBI (Parental bonding questionnaire), severity of depression with the HDRS-17 (Hamilton-Depression-Rating Scale).*

Results: *Nearly all patients had experienced a parental style of affectionless control. Physical neglect significantly predicted fibrinogen levels (R^2=0.42, adjusted R^2=0.27, β=0.56, p=0.04). Severity of depression was not associated with immune markers.*

Conclusion: *Childhood maltreatment was linked to fibrinogen levels in our sample. Thus, inflammation may be an important mechanism mediating the adverse effects of early life stress on adult health in patients with major depression.*

Key words: *childhood maltreatment - major depression - subclinical inflammation*

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INTRODUCTION

Various studies have demonstrated an association between major depression and immuno-activation in the form of mild inflammatory responses, possibly initiated by cytokines (Frodl et al. 2012, Maes et al. 1990, Kim et al. 2007, Sperner-Unterweger 2005, Zeugmann et al. 2010, Zeugmann et al. 2012), thereby putting patients at risk for serious comorbidities such as cardiovascular diseases with all their implications. What remains unclear, is, whether depression itself causes the immuno-response, or whether there are other variables associated with depression or with the immune system that are the actual triggering force.

It has been suggested elsewhere, that depression and cardiovascular disease might share one common underlying factor, i.e. that they are two possible outcomes that result from the same prior stress-related insult to the body (Miller & Blackwell 2006, Mosovich et al. 2008). More specifically, pro-inflammatory cytokines, released in response to stress, may reduce serotonin levels and subsequent platelet aggregation causing both depression and atherosclerosis.

The pathways for an intact immune system as well as a healthy mind are established early on in life and both develop in dialogue with environmental experiences. Given that the consequences of early adverse experiences can perpetuate into adulthood, it is important to investigate whether childhood maltreatment also affects the immunocompetence of patients with depression.

A history of childhood stress such as experiencing the world to be insecure, perceiving oneself as being unlovable or not valuable and regarding the future as not being trustworthy caused by emotional abuse and neglect, sexual and physical abuse as well as physical neglect all increase the risk of developing a depressive disorder in adulthood (Rojo-Moreno et al. 1999, Ritchie et al. 2009, Wright et al. 2009, Subic-Wrana et al. 2010).

The immunological sequelae of early adverse events have been investigated in animal studies, both in terms of their short-lived and prolonged effects. Reite and colleagues were the first group to demonstrate a disturbance in the immune system subsequent to the disruption of a peer-attachment bond in pigtailed monkey infants (Reite et al. 1981). Subsequent reports showed a suppressed immune response in infant bonnet macaques associated with maternal separation (Laudenslager 1982) and passive behaviours identical to behaviours triggered by the activation of the acute phase response (Hennessy et al 2004, Hennessy et al. 2010) during isolation of guinea pig pups.

Long term consequences of early life stress on the immune system were first considered by Laudenslager et al., who showed that monkeys with early separation experiences had lower proliferation responses to B and T cell mitogens as adults (Laudenslager 1985). Moreover, repeated separation from their dams can lead to enhanced cytokine response during influenza viral infection in adulthood in mice (Avitsur et al. 2006).

As far as human studies are concerned, severe stress in the form of bereavement has been found to produce

an abnormality in immune function in the following weeks and months (Bartrop 1977, Buckley et al. 2012, Schleifer et al. 1983). Short term laboratory marital interaction studies have demonstrated that negative and hostile behaviours during marital disagreements promote immune dysregulation (Kiecolt-Glaser et al. 2005). In an investigation of married couples that could be interpreted in terms of long-term consequences of rearing conditions, Gouin et al. found that individuals with higher levels of attachment avoidance had larger interleukin-6 (IL-6) responses to a marital disagreement compared to less avoidant individuals (Gouin et al. 2009). Attachment avoidance is a behaviour expressed in relationships that has been learned early on in life, mirroring that the persons considered have experienced attachment to be unsafe, unstable or unpredictable when they grew up. Thus the study indirectly showed that unfavourable rearing conditions can have an impact later on in life in stressful situations also on a physiological level.

In a more direct investigation of adverse childhood experiences and their role in acutely stressful situations in adulthood, Carpenter et al. and Gouin et al. report enhanced IL-6 responses to a stress task or daily stressors, respectively, in adults with a history of childhood abuse (Carpenter et al. 2010, Gouin et al. 2012). Similarly, Pace et al. provided evidence for an exaggerated inflammatory response to stress in depressed male patients with a history of early life stress during a stress challenge (Pace et al. 2006). However, early life stress was not associated with immune variables as such, but only with Hamilton Depression Rating Scale scores in this study.

Contrasting this, and shifting the focus away from acutely stressful situations, Danese et al. demonstrated that childhood maltreatment led to elevated pro-inflammatory markers at age 32 (Danese et al. 2007). In a different set of analyses from the aforementioned study, Danese et al. argued that the effects of childhood maltreatment significantly attenuated the association between depression and high levels of hsCRP in depressed 32-year olds in comparison to controls - thereby providing the first pieces of evidence for the hypothesis that depression itself is not the cause of immunoactivation in depressed patients, but possibly early life stress (Danese et al. 2008).

With the current study we aim to comprehensively assess the effects of adverse childhood experiences on numerous different proinflammatory markers in adult inpatients with major depression.

SUBJECTS AND METHODS

Subjects and study design

Subjects are members of the ”Endophänotypisierung affektiver Erkrankungen” (“endophenotyping of affective disorders”) study, part of which is presented here. 58 of the original study members (n=71) are included in the current study. Patients were recruited when referred to the Clinic for Psychiatry and Psychotherapy at the University Hospital Berlin – Charité, Campus Benjamin Franklin between 2005 - 2007. The study was approved by the ethics committee of the Charité and all patients gave their written informed consent to participate. All patients suffered from a depressive episode when admitted to the hospital; the individual diagnosis varied within the range of the affective disorders spectrum (F31, F32, F33 for ICD-10 diagnoses and 296.XX for DSM-IV diagnoses, respectively).

Patients with acute respiratory infections within the last two weeks before assessment or during the study, any active medical illnesses that could etiologically be related to the ongoing depression, immune and autoimmune diseases, anti-inflammatory medication, a history of drug or alcohol abuse within 1 year prior to admission, a schizophrenic or schizoaffective disorder were excluded from the study.

Data was assessed at two time points: at T1 within a few days after referral to the clinic and at T2, which was 4-5 weeks after the beginning of inpatient treatment. Sociodemographic variables were collected at T1.

Questionnaires concerning the assessment of early life stress and parental bonding were sent to the patients after they had been released from the clinic in order to avoid a negative bias when answering the questions due to severe depression (Murphy et al. 2010). Of the 58 patients that were initially approached, 25 returned all questionnaires and these were used for further analysis. Despite the comparatively low number of subjects, all patients were thoroughly investigated and the data set is complete. Statistical analyses were kept simple so that we could attain useful and meaningful results despite the small number of subjects. The results will be useful in the sense of “signal detection” in further studies.

Clinical and laboratory data were anonymous, and all ratings and interviews were performed either by a trained psychiatrist or clinical psychologist who was blind to the immunological results.

Early life stress

Early life stress experiences were quantified using the German version of the Childhood Trauma Questionnaire (CTQ) (Bernstein et al. 1994, Wulff 2007) which retrospectively assesses early traumatic stress during childhood and adolescence, examining five forms of maltreatment – emotional, physical and sexual abuse, and emotional and physical neglect as well as minimization/denial.

Parental bonding

Parental bonding was assessed using a German version of the Parental Bonding Instrument (PBI) (Lutz et al. 1995) which retrospectively evaluates parental bonding for each parent during to the first 16 years of life. Two scales termed “care” (depicting the dimension of care vs. indifference/rejection) and “overprotection”

(depicting the dimension of overprotection vs. allowance of autonomy and independence) operationalise parental styles from the child's perspective. The "care" and "overprotection" scores can be assigned to one of four quadrants:

- affectionate constraint (high care, low protection);
- affectionless control (high protection, low care);
- optimal parenting (high care, high protection);
- neglectful parenting (low care, low protection).

Adult inflammation

Antecubital venipunctures following an overnight fast took place in the early morning hours (always between 8:00 and 9:00 a.m.). The blood was then centrifuged at 3000g for ten minutes, immediately divided into aliquots, and frozen at –70°until analysis.

Inflammatory parameters were analyzed using standard ELISA for adiponectin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), resistin, soluble E-selectin (sE-selectin), and CD40ligand (CD40L) (all R&D Systems). Serum-amyloid A (SAA), C-reactive protein (CRP), and fibrinogen were analyzed as described earlier (Koenig 1999).

Due to technical difficulties in the laboratory assessing the inflammatory biomarkers, levels of IL-6 were only available for 13 and CDL40 only for 12 subjects. All markers with less than 20 samples were excluded from further analysis.

Major Depression

Severity of depression was quantified with the HDRS (Hamilton-Depression Rating Scale, 17-item version) (Hamilton 1969). Additionally, we also assessed the lifetime number of depressive episodes, duration of the current episode and suicidal attempts in the past.

Statistical analysis

All variables were tested for normality of distribution by means of Kolmogorov-Smirnoff tests. A drop-out analysis was conducted to test for differences between those patients that returned the questionnaires and those who did not. For this purpose, independent samples *t*-tests were carried out. Mann Whitney U-tests were used to assess group differences in variables that were not normally distributed. Chi-square tests were applied to dichotomous variables. Correlations between the immune markers at the two time points were carried out and average scores computed for further analysis. To estimate the relative contribution of early life stress as measured by the CTQ and other potentially contributing variables (HDRS scores, age, sex) on immunological measures, we performed a multiple hierarchical regression analysis. Demographic variables were entered into the first step, CTQ treatment domain (i.e. emotional neglect, physical neglect, emotional abuse) in the second step, and severity of depression (HDRS scores) were added in a third step. All statistical analyses were performed using the PASW software, version 18.0 for Macintosh.

RESULTS

Drop out analysis

Patients who returned their questionnaires did not differ on any of the immunological, clinical or demographic measures from the group that did not return the questionnaires concerning early life stress and parental bonding.

Socio-demographic and clinical characteristics of the current sample

The demographic and clinical characteristics of the study sample are summarized in Table 1 below.

Table 1. Socio-demographic and clinical variables

Variable		
Age (years)	47.80	(15.02)
Sex (male/female) (n)	8/17	
Episode (n)	4.00	(3.35)
Length of episode (weeks)	33.96	(55.79)
Suicide attempts in the past (n)	0.43	(0.84)
HDRS-score T1	21.64	(6.59)
HDRS-score T2	13.11	(4.81)

Values are depicted as means (± standard deviation), unless otherwise stated

Parental bonding

Seven patients did not complete a questionnaire regarding their father, as they had been raised by their mothers alone. Nearly all patients rated the parental styles of their parents as "affectionless control", both for their mothers and fathers as shown in Table 2.

Table 2. Parental bonding assessed with the PBI

Maternal parental style	n
affectionate constraint	1
affectionless control	18
optimal parenting	4
neglectful parenting	2
Paternal parenting style	
affectionate constraint	1
affectionless control	12
optimal parenting	1
neglectful parenting	4

Childhood trauma

Table 3 shows a summary of patients' scores on the CTQ's different subscales. Highest scores were obtainned on the two subscales considering emotional maltreatment. False negative trauma reports are negligible as depicted by the minimization/denial scale.

Table 3. Means ±standard deviations (SD) of CTQ-subscales

CTQ- subscale	mean	±SD
Emotional abuse	11.86	5.35
Physical abuse	7.46	3.24
Sexual abuse	6.50	4.18
Emotional neglect	14.92	5.60
Physical neglect	9.52	2.90
Minimization/denial	0.40	0.71

Grouping the patients according to severity of early adverse events across subscales of the CTQ revealed that most patients of our sample had experienced early stress in the form of neglect and emotional cruelty. Sexual and physical abuse were not very common (Table 4).

Immune markers

Immune markers' measures were correlated at the two time points assessed and an average score for each immune marker was calculated for further analyses (Table 5).

Table 5. Pearson's correlations of the immunological markers at T1 and T2

Marker	Pearson's correlation r
Fibrinogen	0.42*
SAA	0.65**
CRP	0.64**
Adiponectin	0.44*
TNF-α	0.46*
Resistin	0.33
sE selectin	0.62*

$p<0.05$, ** $p<0.001$

Multiple Regression Analyses

The first model of the regression analysis assessing the influence of age and sex on fibrinogen did not reach significance ($F_{2,22}=8.23$, $p=0.45$). The second model with the CTQ subscales included, significantly predicted (in the form of physical neglect) fibrinogen scores ($F_{5,19}=2.80$, $p=0.05$). When severity of depression was added to the third model (HDRS scores at T1 and T2) it did not prove to be significant ($F_{7,17}=1.81$, $p=0.15$) (Table 6).

Table 4. Severity of early adverse events across different subscales of the CTQ

Scale	None-minimal (n)	Low-moderate (n)	Moderate-severe (n)	Severe-extreme (n)
Emotional abuse	8	7	2	8
Physical abuse	17	3	3	2
Sexual abuse	19	2	3	1
Emotional neglect	3	10	4	8
Physical neglect	7	6	6	6

Table 6. Results of the multiple regression analysis for fibrinogen

	B	SE B	ß	p	R^2	adjusted R^2
Step 1					0.07	-0.02
constant	3.51	0.74		0.00		
age	0.00	0.01	0.01	0.95		
sex	-0.04	0.31	-0.26	0.22		
Step 2					0.42	0.27
constant	2.33	0.77		0.01		
age	-0.02	0.01	-0.32	0.17		
sex	-0.15	0.29	-0.10	0.61		
emotional abuse	-0.04	0.03	-0.31	0.23		
emotional neglect	0.05	0.03	0.36	0.18		
physical neglect	0.14	0.06	0.56	0.04		
Step 3					0.43	0.27
constant	2.13	0.93		0.04		
age	-0.02	0.01	-0.31	0.27		
sex	-0.20	0.33	-0.14	0.54		
emotional abuse	-0.04	0.04	-0.28	0.32		
emotional neglect	0.03	0.05	0.25	0.49		
physical neglect	0.14	0.07	0.55	0.07		
HDRS1	0.01	0.03	0.09	0.73		
HDRS2	0.02	0.05	0.11	0.70		

HDRS= Hamilton depression rating scale at T1 (1) and T2 (2)

Age and sex had a significant impact on SAA levels. In particular, the first model assessing the influence of age and sex reached statistical significance ($F_{2,22}$=4.40, p=0.03). The second and third models including CTQ subscales and severity of depression, respectively did not prove to be significant ($F_{5,19}$=1.90, p=0.14 and $F_{7,17}$=1.81, p=0.15) (Table 7).

Age as well as sex had a statistically significant impact on the average measure of resistin levels. ($F_{2,22}$=7.57, p=0.00 for the first model). The model as a whole remained significant when CTQ scores were added ($F_{5,19}$=1.90, p=0.14), without any particular variable reaching significance. When severity of depression was added to the equation, the model was not significant any more ($F_{7,17}$=1.76, p=0.16) (Table 8).

Table 7. Results of the multiple regression analysis for SAA

	B	SE B	ß	p	R^2	adjusted R^2
Step 1					0.29	0.22
constant	6.35	4.28		0.15		
age	0.11	0.06	0.36	0.06		
sex	-3.69	1.80	-0.37	0.05		
Step 2					0.33	0.16
constant	4.35	5.50		0.44		
age	0.10	0.08	0.33	0.19		
sex	-4.00	2.04	-0.40	0.07		
emotional abuse	0.17	0.24	0.19	0.49		
emotional neglect	-0.09	0.23	-0.11	0.69		
physical neglect	0.25	0.45	0.15	0.59		
Step 3					0.42	0.18
constant	2.96	6.15		0.64		
age	0.04	0.09	0.13	0.66		
sex	-3.94	2.18	-0.40	0.09		
emotional abuse	0.11	0.24	0.12	0.66		
emotional neglect	-0.20	0.31	-0.24	0.53		
physical neglect	0.43	0.46	0.26	0.37		
HDRS1	0.27	0.19	0.35	0.18		
HDRS2	-0.07	0.32	-0.06	0.84		

HDRS= Hamilton depression rating scale at T1 (1) and T2 (2)

Table 8. Results of the multiple regression analysis for resistin

	B	SE B	ß	p	R^2	adjusted R^2
Step 1					0.41	0.35
constant	12.27	2.93		0.00		
age	-0.11	0.04	-0.46	0.01		
sex	3.07	1.23	0.41	0.02		
Step 2					0.45	0.31
constant	14.76	3.75		0.00		
age	-0.10	0.05	-0.41	0.08		
sex	2.47	1.39	0.33	0.09		
emotional abuse	0.07	0.16	0.11	0.66		
emotional neglect	-0.17	0.16	-0.26	0.31		
physical neglect	-0.04	0.31	-0.03	0.90		
Step 3					0.47	0.25
constant	13.49	4.45		0.01		
age	-0.10	0.06	-0.41	0.14		
sex	2.14	1.58	0.29	0.19		
emotional abuse	0.09	0.18	0.13	0.62		
emotional neglect	-0.26	0.22	-0.40	0.27		
physical neglect	-0.05	0.34	-0.04	0.89		
HDRS1	0.07	0.14	0.13	0.60		
HDRS2	0.11	0.23	0.13	0.63		

HDRS= Hamilton depression rating scale at T1 (1) and T2 (2)

Analysis of the potential impacts on CRP levels did not yield any significant results in the first ($F_{2,22}$=0.78, p=0.47, R^2=0.07, adjusted R^2=-0.02), second ($F_{5,19}$=1.50, p=0.24, R^2=0.28, adjusted R^2=0.10), or third model ($F_{7,17}$=1.10, p=0.41, R^2=0.31, adjusted R^2=0.03) of the regression analysis.

None of the variables assessed had an impact on adiponectin levels: ($F_{2,22}$=0.34, p=0.72, R^2=0.03, adjusted R^2=-0.06 for the first model, $F_{5,19}$=1.46, p=0.25, R^2=0.28, adjusted R^2=0.09 for the second model, and $F_{7,17}$=1.70, p=0.18, R^2=0.41, adjusted R^2=0.17 for the third model).

Similarly, TNF- α did not prove to be influenced by any of the variables that were evaluated here: ($F_{2,22}$=0.87, p=0.43, R^2=0.07, adjusted R^2=-0.01 for the first model, $F_{5,19}$=1.09, p=0.40, R^2=0.22, adjusted R^2=0.02 for the second model, and $F_{7,17}$=1.76, p=0.16, R^2=0.42, adjusted R^2=0.18 for the third model).

None of the variables, that were entered into any of the three steps of the regression analysis had an influence on levels of sE-selectin: ($F_{2,22}$=0.16, p=0.85, R^2=0.01, adjusted R^2=-0.08 for the first model, $F_{5,19}$=0.59, p=0.71, R^2=0.14, adjusted R^2=-0.09 for the second model, and $F_{7,17}$=0.66, p=0.70, R^2=0.21, adjusted R^2=-0.11 for the third model).

DISCUSSION

This study addressed the relationship between early life stress and clinically relevant proinflammatory markers in an inpatient population with major depression. The main findings of our investigation were that neglect and emotional maltreatment (i.e. emotional abuse and neglect) were the types of early life stress most commonly present in our sample, which is consistent with previous findings that early emotional adverse experiences are highly important to the development and maintenance of major depression (Chapman et al. 2004, Moskvina et al. 2007). The assessment of parental bonding mirrored these findings as nearly all patients experienced a parental style of affectionless control.

Past research has indicated that major depression is associated with increased levels of proinflammatory cytokines (Maes et al. 1990, Kim et al. 2007, Sperner-Unterweger 2005, Zeugmann et al. 2010, Zeugmann et al. 2012) possibly due to the accompanying induction of indoleamine 2,3-dioxygenase (IDO), an enzyme degrading tryptophan into kynurenine at the cost of serotonin (Muller & Schwarz 2007).

There is also an association between depression and cardiovascular disease (Frasure-Smith et al. 1995, Frasure-Smith & Lesperance 2006), which is probably due to depression and cardiovascular disease sharing inflammatory pathways that are involved directly in the processes that generate, initiate, maintain and worsen atherosclerosis and plaque rupture (Sack 2002, Paraskevas et al. 2008).

Depression and cardiovascular disease might result from prior stress related insult to the body (Miller & Blackwell 2006). This, of course, should have implications for the treatment of the patient groups affected, i.e. that physiological changes disadvantageous for general health should not be overseen during the treatment of a mood disorder.

We found physical neglect to be significantly associated with increased levels of fibrinogen. This is the first study showing that this coagulation marker is significantly influenced by early life experiences in adults suffering from major depression.

Our finding confirms the findings of Danese et al. (2007) of increased adult fibrinogen levels in participants that had been maltreated as children and we expanded them to the field of major depression.

In support of our finding, fibrinogen has been reported to be positively related to vital exhaustion resulting from chronic stress in teachers (Kudielka et al. 2008), and burnout in women (Toker et al. 2005) thereby indicating a presumable association between prolonged periods of stress and increment of this acute-phase protein. According to the schema-focused model of occupational stress and work dysfunctions (Bamber 2006) it is individuals with early maladaptive schema that gravitate towards occupations with similar dynamics to the toxic environments that created them. Those affected subsequently re-enact these early maladaptive schemas and their associated coping styles (Bamber & McMahon 2008). Thus, in an indirect manner the above mentioned studies draw attention to the fact that adverse childhood experiences can affect fibrinogen levels in adulthood and our study confirmed this in a more direct manner.

Considering the meaning of mild proinflammatory activation, increased fibrinogen levels have been found to predict and contribute to cardiovascular mortality (Kop et al. 2010).

It would be advantageous to investigate whether treatment strategies that are particularly targeted at reversing the consequences of adverse childhood experiences, such as schema therapy (Young 2006), might also reverse the putative long lasting psychophysiological effects of early life stress. Furthermore, Cox-2 inhibitors have been suggested for the treatment of at least some patients with major depression (Muller et al. 2006) and it might be of interest whether subgroups of patients such as the sample studied here would benefit from these compounds.

An important limitation to the current study is that it was conducted under naturalistic conditions. Nearly all patients had been receiving psychopharmacological treatment at admission to the clinic. From admission onwards all patients medicated and received psychotherapy (group or individual). Evidence considering putative immunoregulatory properties of antidepressants is available (e.g. Bah et al. 2011, Chavda et al. 2011) but not unequivocal (Haastrup et al. 2012). Psycho-

therapeutic interventions have been found to influence concentrations of inflammatory markers (e.g. Thornton et al. 2009), but there is only a very limited body of research in this area. However, owing to our study design, no conclusions can be drawn considering putative anti-inflammatory effects of the treatments applied. Furthermore, had there been any immuno-regulatory effects of the therapies, they would have been evenly spread as all our patients were all treated at all times of data assessment.

CONCLUSIONS

Altogether the topic of early life stress and subsequent immunomodulation is quite new and not well investigated so far. Our findings will hopefully trigger further research in this area, which will provide larger samples with complementing data. Considering depressive disorders, experiences of disturbed and abusive attachment often play a pivotal role in the development of this disease, the consequences of which remain present up to adulthood (e.g. in the form of maladaptive core beliefs). Since the mind and body are not separate entities, it is very likely that the echo of the past does not only resound on a psychological domain but spreads also to somatic areas, some evidence for which was provided here.

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Conflict of interest:

S. Zeugmann: None declared. A. Quante: None declared. N.Buehrsch: None declared. M.Bajbouj: None declared. I.Heuser: Bayer Health Care – consultant, Astra Zeneca – consultant; Novartis – consultant, GE Health Care – consultant. I.Anghelescu: None declared.

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PATHWAYS LINKING EARLY LIFE STRESS, METABOLIC SYNDROME, AND THE INFLAMMATORY MARKER FIBRINOGEN IN DEPRESSED INPATIENTS

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SUMMARY

***Background**: Previous research has shown that metabolic syndrome as well as early life stress can account for immunoactivation (e.g. in the form of altered fibrinogen levels) in patients with major depression. This study aims at assessing the relationship between components of metabolic syndrome, early life stress and fibrinogen levels, taking the severity of depression into consideration.*

***Subjects and methods**: Measures of early life stress and signs of metabolic syndrome were collected in 58 adult inpatients diagnosed with depression. The relationships between the factors were assessed by means of path analyses. Two main models were tested: the first model with metabolic syndrome mediating between early life stress and fibrinogen levels and the second model without the mediating effect of metabolic syndrome.*

***Results**: The first model was not supported by our data (χ^2=7.02, df=1, p=0.008, CFI=0.00, NNFI=-9.44, RMSEA=0.50). The second model however provided an excellent fit for the data (χ^2=0.02, df=1, p=0.90, CFI=1.00, NNFI=2.71, RMSEA=0.00). Extending the models by introducing severity of depression into them did not yield good indices of fit.*

***Conclusions**: The developmental trajectory between early life stress and inflammation appears not to be mediated by metabolic syndrome associated factors in our sample. Possible reasons including severity and type of early life stress, as well as potential epigenetic influences are discussed.*

***Key words:** early life stress - metabolic syndrome – inflammatory – fibrinogen - depression*

* * * * *

INTRODUCTION

Depressive disorders are often preceded by a history of life adversities and commonly accompanied by various comorbidities, each of which possibly exerts its influence on the general health of the individual affected. Furthermore, a mild immunoactivation has been observed in patients with major depression (Kim et al. 2007, Sperner-Unterweger 2005). According to the 'cytokine hypothesis of depression', cytokines are involved in behavioral and neurochemical features of depression (Schiepers et al. 2005). Altered plasma cytokine levels have been reported in patients with affective disorders independent of a physical illness (Haack et al. 1999, Maes et al. 1999). E.g. associations between depression and TNF-α and its soluble receptors p55 and p75 (Himmerich et al. 2008, Piletz et al. 2009) or increased levels of IL-1β (Piletz et al. 2009) have been found. Furthermore, more and more studies provide evidence for an autoimmune tendency in depressed patients. It has been shown that $CD4^+$ and $CD25^+$ Treg cells may contribute to the immune imbalance in patients with major depression, as the $CD4^+$ and $CD25^+$ Treg cells decrease in patients' peripheral blood (Li et al. 2009). However, the putative causes and networks of such immuno-alterations are still not sufficiently understood.

Prenatal life, infancy, childhood, and adolescence are critical periods characterized by increased vulnerability to stressors (Chrousos 1996). The presence of unfavorable stressors, that are excessive and prolonged in nature, during these periods not only affect personality development and the behavior of those affected, but may also have adverse consequences on a physiological level, such as metabolism and immune response. This is due to the inability of the stress system to generate an appropriate response to these stressors in the long term. The body's stress response is generally meant to be of short and limited duration. The time-limited nature of this process renders its accompanying anti-growth, anti-reproductive, catabolic and immuno-suppressive effects temporarily as beneficial. However, chronic activation of the stress system, e.g. due to adverse and unfavorable rearing conditions, may entail a number of disorders following an increased and prolonged secretion of CRH and glucocorticoids. For example, individuals, who as undergraduate students rated the relationship with their parents as cold and detached had a fourfold greater risk of chronic illness including not only psychiatric diagnoses but also heart

disease and type II diabetes in midlife (Russek & Schwartz 1997).

One possible somatic consequence of a hyperactive stress system is the development of components of a metabolic syndrome (Vanitallie 2002).

In a primate study, Chrousos (Chrousos 2000) demonstrated the association between chronic stress, hypercortisolism and metabolic syndrome. Monkeys were exposed to chronic stress, which activated the HPA-axis permanently. The thus induced hypercortisolism lead to visceral obesity, insulin resistance and other biochemical manifestations of metabolic syndrome along with severe coronary atherosclerosis.

Moreover, several studies have shown that stressful emotional life experiences during childhood may lead to the development of obesity in later life (Alvarez et al. 2007, D'Argenio et al. 2009, Grilo et al. 2005), for example via a disordered eating behavior and a reduced capacity to implement nutrition and physical activity plans for the prevention of weight gain (Alvarez et al. 2007).

All relevant longitudinal studies suggest a higher incidence of metabolic syndrome and/or its components (high waist circumference, high triglyceride level, low HDL level, high blood pressure, and high glucose level) among those with depressive symptoms (Raikkonen et al. 2002, Raikkonen et al. 2007, Goldbacher et al. 2009, Pulkki-Raback et al. 2009, Vaccarino et al. 2008, Vanhala et al. 2009, Vanhala et al. 2009, Viinamaki et al. 2009). Associations between an activation of the cytokine system (e.g. TNF-α) and weight gain during psychotropic treatment of depressed patients have also been described (Kraus et al. 2002).

Recently we demonstrated that metabolic syndrome constitutes one important contributing factor for the subclinical immunoactivation in depressed inpatients (Zeugmann et al. 2010). In a further study we reported that early life stress constitutes a second factor contributing to levels of inflammatory biomarkers in a population of depressed inpatients (Zeugmann et al. Submitted). In both studies we found the hepatically synthesized acute-phase reactant fibrinogen to be affected by metabolic syndrome and by early life stress in a cohort of inpatients with major depression.

Previous research reports fibrinogen to be increased in depression and to be associated with metabolic syndrome. In recent years the role of plasma fibrinogen as an independent cardiovascular risk marker has been increasingly recognized (Mann 2002, Yan et al. 2010). Occlusive thrombi are commonly found in myocardial infarction, sudden cardiac ischemic death, or unstable angina pectoris. Thrombosis is recognized as the central mechanism underlying these atherosclerotic compilations (Davies 1996). Importantly, prospective epidemiological studies have revealed an association of fibrinogen with subsequent incidence of ischemic cardiovascular events up to 20 years in the future (Kannel et al. 1996). Because fibrinogen appears to be affected by different factors associated and co-occuring with major depression and because of its potential cardiovascular risk, it is important to gain a more profound understanding of the circuits associated with this inflammatory marker.

Metabolic syndrome and early life stress have been found to account for immunoactivation in depressed patients. Furthermore, early life stress has been reported to predispose those affected to develop a metabolic syndrome. To the authors' knowledge, there is no study, however, investigating both of the above simultaneously in major depression. Thus, in this pilot study with the aim of hypothesis generation under complex conditions, we wanted to assess the relations between early life stress, metabolic syndrome and inflammation in a sample of depressed inpatients in a real life setting.

SUBJECTS AND METHODS

Subjects

Subjects are members of the "Endophänotypisierung affektiver Erkrankungen" ("endophenotyping of affective disorders") study, part of which is presented here. Those 58 of the original study members (n=71) that agreed to participate in the current part of the study are included here. Patients were recruited when referred to the Clinic for Psychiatry and Psychotherapy at the University Hospital Berlin – Charité, Campus Benjamin Franklin between 2005 - 2007. The study was approved by the ethics committee of the Charité and all patients gave their written informed consent to participate. All patients suffered from a depressive episode when admitted to the hospital; the individual diagnosis varied within the range of the affective disorders spectrum (F31, F32, F33 for ICD-10 diagnoses and 296.XX for DSM-IV diagnoses, respectively).

Patients with acute respiratory infections within the last two weeks before assessment or during the study, an active medical illnesses that could etiologically be related to the ongoing depression, immune and autoimmune diseases, anti-inflammatory medication, a history of drug or alcohol abuse within 1 year prior to admission, a schizophrenic or schizoaffective disorder were excluded from the study.

Methods

Data were assessed within a few days after referral to the clinic. Questionnaires concerning the assessment of early life stress and parental bonding were sent to the patients after remission and after discharge from the clinic in order to avoid a negative bias when answering the questions due to severe depression. Of the 58 patients that were initially approached, 25 returned all questionnaires and these were used for further analysis. Statistical analyses were kept simple so that we could attain useful and meaningful results despite the small number of subjects.

Clinical and laboratory data were anonymous, and all ratings and interviews were performed either by a trained psychiatrist or clinical psychologist who were blind to the laboratory data.

The study followed a naturalistic design, i.e. all patients were treated according to their psychiatrist's choice with different kinds of antidepressant drugs (with the dosage adjusted according to clinical judgment and plasma levels where applicable), cognitive behavioral therapy and adjunctive methods.

Major depression

Severity of depression was quantified with the HDRS (Hamilton-Depression Rating Scale, 17-item version) (Hamilton 1969).

Metabolic syndrome

The individual components of metabolic syndrome were assessed at admission according to the criteria of the International Diabetes Federation – IDF, which state the following: central obesity defined as waist circumference =94 cm for Europid men and = 80 cm for Europid women. Furthermore, any two of the following four factors should be met: 1. raised triglycerides, i.e.=150mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality, 2. reduced HDL cholesterol, i.e. <40 mg/dL (1.03 mmol/L) in males and <50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality, 3. raised blood pressure, i.e. systolic blood pressure =130 or diastolic =85 mm/Hg or specific treatment of previously diagnosed hypertension, and 4. raised fasting plasma glucose, i.e. = 100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes.

A metabolic factor was calculated for each patient. Patients received a score according to the number of signs of the metabolic cluster they displayed (running from 0 to 5). This was done to quantitatively assess the putative impact of metabolic syndrome cluster, including subclinical cases, with for example only 2 out of the 5 possible risk factors.

Early life stress

Early life stress experiences were quantified using the German version of the Childhood Trauma Questionnaire (CTQ) (Bernstein et al. 1994, Wulff 2007) which retrospectively assesses early traumatic stress during childhood and adolescence, examining five forms of maltreatment – emotional, physical and sexual abuse, and emotional and physical neglect as well as minimization/denial.

Fibrinogen

Blood was drawn through antecubital venipuncture following an overnight fast in the early morning hours. The blood was then centrifuged at 3000g for ten minutes, immediately divided into aliquots, and frozen at –70° until analysis. Fibrinogen levels were analyzed using standard ELISA (R&D Systems).

Statistical analyses

All variables were tested for normality of distribution by means of Kolmogorov-Smirnov tests. A drop-out analysis was conducted to test for differences between those patients that returned the questionnaires concerning early life stress and those who did not. Because there were some non-normally distributed variables and in order to simplify the analysis, we used the nonparametric Mann-Whitnex-Wilcoxon-Test for all variables. All statistical analyses were performed using the PASW software, version 18.0 for Macintosh. Path analysis (AMOS 18.0.2) was performed to examine the relations between early life stress, metabolic syndrome and inflammation. Two models were tested. First, a model with metabolic syndrome cluster as a mediator between early life stress and inflammation. Second, a model without the mediating effect of metabolic syndrome. Severity of depression was added to each model to account for its possible impact on inflammation.

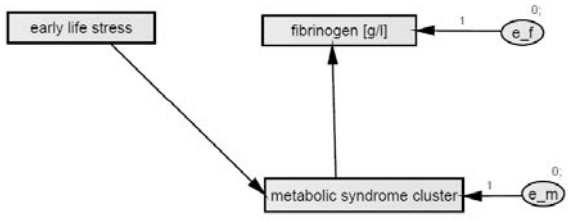

Figure 1. Model 1

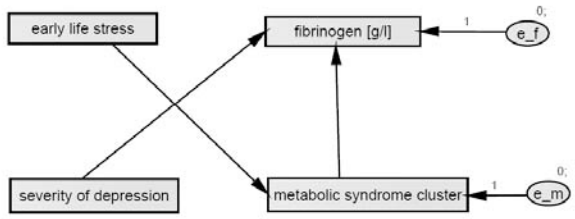

Figure 1b. Model 1b

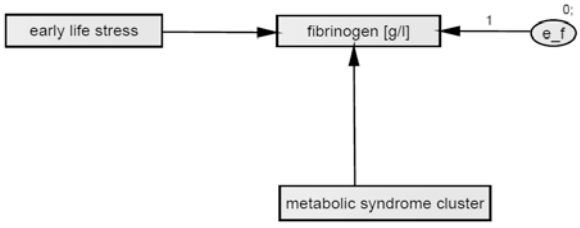

Figure 2. Model 2

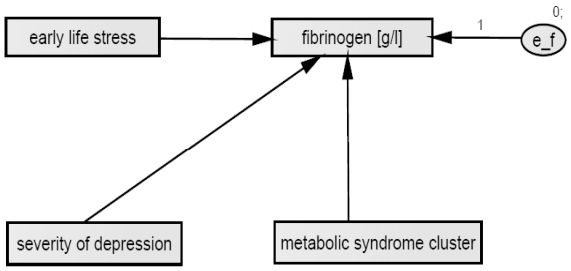

Figure 2b. Model 2b

As can be seen from the models (Figure 1) the exogenous variables were early life stress and severity of depression (the latter one only for models 1b and 2b (see Figure 1b and Figure 2b). The mediating variable was metabolic syndrome/factor. The dependent variable was inflammation (fibrinogen levels).

The overall model fits were examined using established goodness-of-fit-indices. A non-significant χ^2 statistic was used as the primary criterion of model fit (Hayduk et al. 2007). We included other recommended indicators such as the non-normed fit-index (NNFI) sometimes also called Tucker Lewis index (TLI), the comparative fit index (CFI), and the root mean-square error of approximation (RMSEA). Models were accepted as a satisfactory description of the observed data when CFI and NNFI values exceeded 0.90. For the RMSEA, values below 0.05 indicate an excellent model of fit, whereas values of 0.05 to 0.08 indicate a good fit (Bentler & Bonett 1980, Bentler 1990, Hu & Bentler 1999, Hu & Bentler 1995, Browne & Cudeck 1993).

RESULTS

Demographic variables

The demographic and clinical characteristics of the study sample are summarized in table 1 below.

Table 1. Socio-demographic and clinical variables

variable		
age (years)	47.80	(15.02)
sex (male/female) (n)	8/17	
episode (n)	4.00	(3.35)
length of episode (weeks)	33.96	(55.79)
suicide attempts in the past (n)	0.43	(0.84)
HDRS-score	21.64	(6.59)

Metabolic syndrome

Mean values for the components of metabolic syndrome are displayed in table 2.

Table 3 depicts the values of metabolic syndrome composite scores and their distribution.

Early life stress. Drop out analysis

Patients who returned their questionnaires did not differ on any of the immunological, clinical or demographic measures from the group that did not return the questionnaires concerning early life stress.

Table 2. Values for the components of the metabolic syndrome. Different values for males and females are given, where required according to the criteria of the IDF (see methods section)

	male male and female combined	female		min	max
waist circumference (cm)	94.75 (8.50)	83.00 (17.24)	male:	86.00	104.50
			female:	64.00	116.00
blood pressure (systolic) (mmHg)	112.29 (15.88)		90.00	150.00	
blood pressure (diastolic) (mmHg)	73.40 (10.48)		60.00	90.00	
HDL cholesterol (mg/dl)	62.30 (14.17)	61.94 (15.48)	male:	53.08	90.42
			female:	39.44	83.00
fasting glucose (mg/dl)	81.88 (23.72)		48.00	147.00	
triglycerides (mg/dl)	127.52 (99.84)		44.00	512.00	

Values are depicted as means (± standard deviation), unless otherwise stated

Table 3. Metabolic syndrome composite scores and their distribution.

number of factors of the metabolic syndrome cluster	n
0	9
1	6
2	5
3	2
4	3
5	0

Table 4. Means ±standard deviations (SD) of CTQ-subscales

CTQ- subscale	mean	±SD
emotional abuse	11.86	5.35
physical abuse	7.46	3.24
sexual abuse	6.50	4.18
emotional neglect	14.92	5.60
physical neglect	9.52	2.90
minimization/denial	0.40	0.71

Table 5. Severity of early adverse events across different subscales of the CTQ

scale	none-minimal (n)	low-moderate (n)	moderate-severe (n)	severe-extreme (n)
emotional abuse	8	7	2	8
physical abuse	17	3	3	2
sexual abuse	19	2	3	1
emotional neglect	3	10	4	8
physical neglect	7	6	6	6

Table 4 shows a summary of patients' scores on the CTQ's different subscales. Highest scores were obtained on the two subscales considering emotional maltreatment. False negative trauma reports are negligible as depicted by the minimization/denial scale.

Grouping the patients according to severity of early adverse events across subscales of the CTQ revealed that most patients of our sample had experienced early stress in the form of neglect and emotional cruelty. 8 (32%) patients were exposed to emotional neglect, 8 to emotional abuse (32%) and 6 (24%)to physical neglect, which is comparable to previous reports (Wiersma et al. 2009) who reported rates of 40.2% for regular emotional neglect and 25% for regular psychological abuse in a cohort of depressed patients. Sexual and physical abuse were not very common (Table 5).

Path analysis

Figure 3 (Model 1) displays the standardized path coefficients for a mediating model. This hypothesized model did not provide a good fit for the data: χ^2=7.02, df= 1; p=0.008, CFI=0.00; NNFI=-9.44; RMSEA=0.50.

Similarly (Figure 3b – Model 1b) the same model controlling for severity of depression seems not supported by the data: χ^2=9.51; df=3, p=0.023; CFI=0.00; NNFI=-4.69; RMSEA=0.30.

Figure 4 (Model 2) displays the standardized path coefficients for an independent model, i.e. a model in which early life stress and metabolic syndrome both independently influence fibrinogen levels in depressed patients. This model provided an excellent fit for the data: χ^2=0.02; df=1; p=0.90; CFI=1.00; NNFI=2.71; RMSEA=0.00.

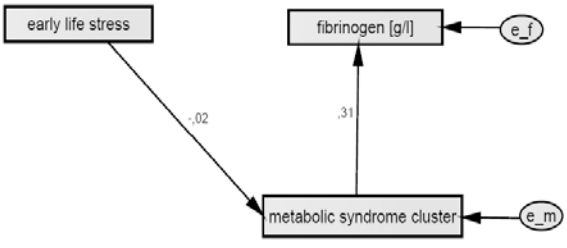

Figure 3. Model 1: numbers on arrows represent standardized regression weights

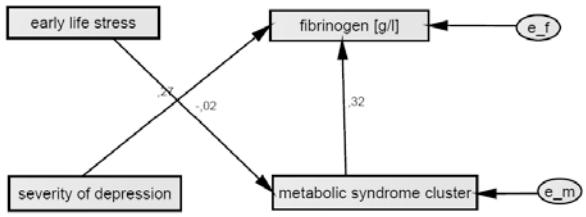

Figure 3b. Model 1b: numbers on arrows represent standardized regression weights

Extending the model by introducing severity of depression into it appears not supported by the data (see Figure 4b (Model 2b)): χ^2=4.18; df=3; p=0.24.

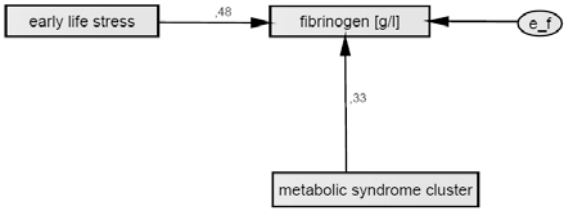

Figure 4. Model 2: numbers on arrows represent standardized regression weights

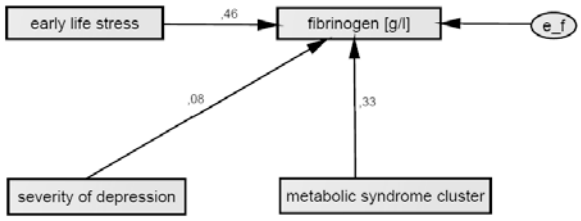

Figure 4b. Model 2b: numbers on arrows represent standardized regression weights

DISCUSSION

The results of the present study suggest that the developmental trajectory between early life stress and inflammation is not mediated by metabolic syndrome associated factors in our sample of depressed inpatients. Early life stress and metabolic syndrome associated factors each have an impact on immunity independent of each other.

The finding of early life stress's long-term impact on immune function later in life is in keeping with the current body of research. Previous investigations have demonstrated a longitudinal effect of early life-stress on adult immune functioning in rhodents and primates (Laudenslager et al. 1982, Laudenslager et al. 1985, Avitsur et al. 2006). Human studies parallel these findings: Activation of maladaptive psychological schema during marital disagreements, which mirror unfavorable rearing conditions, were shown to lead to increased cytokine responses (Gouin et al. 2009). Current study also parallels findings by Pace et al. (2006) and Danese et al. (2007, 2008, 2009) which showed that early life stress, particularly in the form of neglect or emotional maltreatment affects specific immunological parameters even later on in life.

Moreover, previous studies found fibrinogen to be positively related to vital exhaustion resulting from chronic stress in school teachers (Kudielka et al. 2008) and to burnout in women (Toker et al. 2005), thereby indicating a likely association between prolonged periods of stress and increment of this acute-phase protein. According to the schema-focused model of occupational stress and work dysfunctions (Bamber 2006), it is individuals with early maladaptive schema that gravitate towards occupations with similar dynamics and structures to the toxic environments and relationships that created them. Those affected subsequently re-enact these early maladaptive schemas and their associated coping styles at the workplace (Bamber & McMahon 2008). Thus, in an indirect manner the above mentioned studies draw attention to the fact that adverse childhood experiences can affect fibrinogen levels in adulthood and our study confirmed this in a more direct manner.

The link between metabolic syndrome and fibrinogen complements research in this area. A proinflammatory and prothrombic state (e.g. altered levels CRP, TNF-α, fibrinogen, IL-6, leptin, resistin, and adiponectin) has been linked to metabolic syndrome (Sutherland et al. 2004). Higher levels of acute-phase reactants, adhesion molecules and coagulation markers (including fibrinogen) were found in obese and overweight women (Wildman et al. 2011), and it has been suggested that waist circumference is the component of metabolic syndrome that most significantly influences the micro-inflammatory response including fibrinogen concentrations (Rogowski et al. 2010).

Fibrinogen is an important determinant of thrombogenicity and blood viscocity (Koenig 2003, Tracy 2003). As a nonspecific phase-reactant it is a downstream component of the inflammatory cascade and is implicated in the pathogenesis of atherosclerosis, myocardial injury and heart failure (Mann 2002), as well as constituting an independent risk to myocardial dysfunction (Yan et al. 2010). Since our sample consisted of patients that were not all suffering from a fully-developed metabolic syndrome, these were comparatively healthy individuals that might therefore benefit from preventive and prophylactic interventions, such as statin therapy (Ridker 2009). Moreover, considering the early life stress - fibrinogen pathway, therapies aiming at reducing the effects of early life stress into present life (such as schema therapy (Young et al. 2006) might also be beneficial considering the micro-inflammatory state of the patients affected, at least in certain subgroups which should be investigated in follow-up research.

We did not find a direct path between early life stress and the metabolic syndrome cluster.

There are no studies assessing the effect of child abuse on metabolic syndrome in later life. Most studies concentrate on one or more symptoms out of the cluster.

There is evidence that very severe forms of childhood adversity are more likely to be associated with an increased risk of central obesity (a leading symptom of metabolic syndrome) in mid-adulthood (Thomas et al. 2008) in comparison to less severely stressful emotional environments, where the effects on body composition appear to be weaker. Since our sample was mainly exposed to emotional and physical neglect and emotional abuse, as opposed to physical or sexual abuse, the degree of severity of early life stress could account for the missing association.

Although both metabolic syndrome and immune function, result from the interplay between genetic predisposition and a circumjacent environment, inflamematory biomarkers such as fibrinogen could be determined to a lesser extent by genetic effects and be more susceptible to epigenetic influences compared to metabolic syndrome components. Heritability of fibrinogen has been estimated to be at 34% (Best et al. 2004, Pankow et al. 1998). However, the components of metabolic syndrome appear to be even more strongly inherited: Commonly reported heritability values from family and twin studies range from 40-55% for abdominal obesity, 10-75% for fasting glucose, 25-60% for trigylcerides, 30-80% for HDL, 20-70% for systolic blood pressure and 10-50% for diastolic blood pressure (Teran-Garcia & Bouchard 2007). Immunological memory in general comprises of an important adaptive response to environmental challenges and adapting to them is vital for a well-functioning pathogen defense. Thus, ability to adapt is the core of a healthy immune system. Possibly, in comparison to immunological variables, metabolic syndrome related factors could be less prone to environmental influences.

Of course some limitations of this study have to be addressed. We had a return rate of approx. 50% for our questionnaires considering early life stress. Thus, we lost about half of our initial study population. However when the current literature is taken into account, we feel that our return rates for the questionnaires lies within the midrange, in comparison to other studies (e.g. Warner et al. 2004: response rate 60% for questionnaires distributed on the wards; Harrison-Woolrych & Ashton 2011: response rate of 42% for questionnaires

sent out retrospectively). Of course high drop-out rates should always be avoided. Even though we took great care to approach each patient personally via telephone when the questionnaires were sent out, 50% still did not return them. It has to be taken into account that the very nature of our questions could have contributed to the drop out rates as questions were tackling very intimate and sensitive data of the patients' pasts. Furthermore, patients were sent the questionnaires after they had been released from the clinic (in order to prevent response bias from depressive symptomatology) after which patients might not have felt comfortable to confront the topics in question, especially while not being in the intensive care of the hospital staff.

Even though the total number of cases that could be considered for analysis is relatively low, we took great care to keep the models very simple (compared to what is generally entered into structural equation models) in order to attain meaningful results, which we also did. Path analysis as a method is usually used in larger data sets, but it is justifiable to be used here, because we have only one or two equations (two equations for models 2 and 2b – see Fig. 3 and 3b, and one equation for models 1 and 1b – see Fig. 4 and 4b) and three and four variables, respectively in our model and therefore it is very simple.

Since this was a pilot study in a real-life setting under complex conditions aimed at generating hypotheses, our reported results should be replicated and verified in a larger sample. Future research should also include trials on differential treatment options adapted to the putative underlying pathways of patients' micro-inflammatory statuses.

CONCLUSIONS

In conclusion, our study is relevant for patients with metabolic syndrome who have experienced early life stress, as well as for depressed patients with a coexisting/simultaneous metabolic syndrome. The study highlights the impact of even milder forms of both, while also pointing out that metabolic syndrome does not seem to mediate the association between early life stress and fibrinogen concentrations in adulthood.

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Conflict of interest : None to declare.

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CURRICULUM VITAE

SARA ZEUGMANN

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

My resume is not published for privacy reasons in the electronic version of dissertation.

COMPLETE LIST OF PUBLICATIONS

Zeugmann S, Buehrsch N, Bajbouj M, Heuser I, Anghelescu I, Quante A.: Childhood maltreatment and adult pro-inflammatory status in patients with major depression. Psychiatr Danub 2013;25(3):227-35.

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AFFIDAVIT

I, Sara Zeugmann, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic: "*Inflammation, Metabolic Syndrome, & Early Life Stress in Major Depression – an Investigation into the Mind-Body Connection of Affective Disorders"*. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (see above) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date

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Signature

DECLARATION OF ANY EVENTUAL PUBLICATIONS

Sara Zeugmann had the following share in the following publications:

Publication 1: Zeugmann S, Quante A, Heuser I, Schwarzer R, Anghelescu I Inflammatory biomarkers in 70 depressed inpatients with and without the metabolic syndrome. J Clin Psychiatry 2010;71(8):1007-16.

Contribution in detail: recruitment of participants, data collection, statistical analyses, writing of the manuscript

Publication 2: Zeugmann S, Buehrsch N, Bajbouj M, Heuser I, Anghelescu I, Quante A.: Childhood maltreatment and adult pro-inflammatory status in patients with major depression. Psychiatr Danub 2013;25(3):227-35.

Contribution in detail: recruitment of participants, data collection, statistical analyses, writing of the manuscript

Publication 3: Zeugmann S, Quante A, Popova-Zeugmann L, Kössler W, Heuser I, Anghelescu I. Pathways linking early life stress, metabolic syndrome, and the inflammatory marker fibrinogen in depressed inpatients. Psychiatr Danub 2012;24(1):57-65.

Contribution in detail (recruitment of participants, data collection, statistical analyses, writing of the manuscript

Signature of the doctoral candidate

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