Association of inflammation markers in young adult patients with Obsessive-compulsive disorder

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1. Abstract

Background: Previous studies have shown that patients with obsessive-compulsive disorder (OCD) have elevated interleukin and chemokine levels in plasma. The purpose of this study was to investigate and validate whether a group of cytokines and chemokines are elevated in a cohort of young adult OCD patients.

Methods: A total of 43 patients (11 male/32 female) and 45 controls (15 male/30 female) with OCD were included in the study. The subjects were assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders- Clinical Version or Mini-International Neuropsychiatric Interview. The control group was screened with the Alcohol Use Disorders Identification Test. Proximity extension assay (PEA) was used to measure plasma levels of IL-6, IL-8, MIP-1α, and IL-10.

Results: A factor analysis for the cytokines was performed and logistic regression analysis revealed that the cytokines as a group have a significant association for OCD (P=0.031, OR: 2.2) and IL-8 was the cytokine with the highest significance (P=0.007) for the patient group. 

Conclusion: These findings suggest that this group of cytokines are associated with OCD diagnosis and strengthens previous findings of immune activity in the etiology of OCD. Therefore cytokines and chemokines could have an active role in the etiology of OCD and PEA could be useful in the search for biomarkers.
2. Introduction

Biological research into mental disorders is still relatively new in psychiatry, most mental disorders have a multifactorial etiology and pose a challenge for obtaining a diagnosis since mental health relies mainly on clinical data. Psychiatry is a medical area that detects and treats mental disorders through diagnostic manuals, clinical scales and clinical data. Therefore, it is a challenge diagnose patients accurately, since mental disorders evolve and change through time. Often, diagnoses change, which ultimately influences the treatment and outcome of each patient.

Since the beginning of the 1980s, several clinicians and scientists started to investigate diverse biomarkers to aid in the diagnosis of mental disorders, such as inflammation markers, hormones, neurotransmitters and in some cases antibodies. Therefore psychiatric research began to expand into areas that were previously not thought to influence mental disorders like neurobiology, immunology, and endocrinology. The objective of this thesis is to provide insight into young adult patients with obsessive-compulsive disorder (OCD) and how the influence of ongoing peripheral inflammation is associated with the disease and other factors that influence inflammation as well as validate previous findings on cytokine markers and find novel markers that could aid in the diagnosis and treatment of OCD. Understanding the evolution of the disease along with biological evidence can provide new information about the etiology of OCD and describe specific phenotypes that can make treatment choices more effective.

2.1 Neurobiology of obsessive-compulsive disorder

OCD is a psychiatric disorder characterized by symptoms consisting of compulsions, ritual actions, intrusive thoughts, and an overwhelming preoccupation. The obsessions or compulsions are recurrent and cause significant distress to the person, consume time and alter the person’s routine, social activities, occupation or social relationships. Sometimes a person with OCD will have both obsessions and compulsions or just a compulsion or an obsession. Obsessions are recurrent intrusive thoughts, which can also be ideas, sensations or even feelings. Compulsions, on the other hand, are conscious behaviors, which are methodic, standardized, and recurrent. Patients with OCD realize that these obsessions and compulsions are irrational and cause anxiety, sometimes the compulsions may be used to diminish anxiety caused by the obsessions, however, the compulsions may not reduce anxiety, and can, in fact, increase it (American Psychiatric Association 2013; Sadock and Sadock 2011).

Diverse biological factors have a role in the etiology of OCD. Neurotransmitters have a role in the pathology of OCD, for example, serotonin dysregulation is one of the hypotheses involved in the production of OCD symptoms (Sadock and Sadock 2011). Clinical drug trials support this hypothesis with data that shows how serotonergic drugs effectively treat OCD
symptoms compared to drugs that affect other neurotransmitter systems, however it is still not clear whether dysregulation of serotonin is the cause of OCD and the data is considered debatable by different scientists and clinicians (Sadock and Sadock 2011; Zohar, Greenberg, and Denys 2012). New hypothesis suggests that dopamine dysregulation also plays a part in the formation of OCD symptoms, and pharmacological and functional imaging studies with animals and humans show that dopamine dysregulation is present in OCD patients and could have an important role in the manifestation of symptoms (Klanker, Feenstra, and Denys 2013).

Heritability is a major risk factor for OCD since studies report that transmission within families is high, particularly in relatives of adolescents or children with OCD (Pauls 2010). Higher rates of OCD are present in families of children with OCD compared with families of adult patients with OCD, however, the etiology of childhood-onset OCD may be different from adults with OCD (Nakatani et al. 2011). Differences between adult onset and childhood-onset is seen in other psychiatric disorders such as schizophrenia and bipolar disorder (Rapoport and Inoff-Germain 2000; Mick and Faraone 2009) suggesting that genetic and/or epigenetic factors influence the appearance of the symptoms of each individual and these factors can be present in their relatives (Pauls et al. 2014).

2.2 The cortico-striato-thalamo-cortical pathway

With functional imaging studies of patients with OCD, the most mentioned model for the neurobiological and pathological basis of OCD involves the cortico-striato-thalamo-cortical (CSTC) pathway (Saxena and Rauch 2000). The model integrates the concept of a direct and indirect pathway in the frontostriatal circuitry and based on animal and human studies, the model suggests that a lowering of the activation threshold of this system augments activity of the direct pathway, therefore, bypassing the indirect pathway and resulting in hyperactivation of the orbitofrontal-subcortical pathway (Saxena and Rauch 2000).

The functional basis of the CTSC pathway is by glutamatergic signals from the frontal cortex, particularly the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) which control excitation of the striatum. The direct pathway activates the striatum and increases inhibitory GABA signals to the globus pallidus (GP) and substantia nigra (SN), decreasing inhibitory GABA output from the GP and SN to the thalamus, thus causing a release of glutamate from the thalamus to the OFC. The direct pathway works by positive-feedback, while in the indirect pathway the striatum inhibits the GP and decreases the inhibition of the subthalamic nucleus. This causes excitation of the GP and SN by the subthalamic nucleus and as a consequence inhibition of the thalamus. In OCD, the imbalance of both pathways causes excessive activation of the direct pathway over the indirect pathway (Pauls et al. 2014; Saxena and Rauch 2000).
Since the orbitofrontal cortex (OFC) controls awareness of danger, harm or hygiene, in a hyper activated model, such as the one mentioned, may result in obsessions and secondarily the appearance of compulsions that try to mitigate the anxiety caused by the over-awareness produced by the OFC, (Saxena and Rauch 2000; Milad and Rauch 2012) and ultimately leading to a reinforced and repetitive conduct when obsessions once again, resurface (Saxena and Rauch 2000).

With functional imaging studies of patients with OCD, results show that both adult and pediatric patients have augmented activation of the OFC (Menzies et al. 2008; Fitzgerald et al. 2011). Other studies also show that in adult and pediatric OCD patients, the head of the caudate nuclei is bilaterally hyperactive (Menzies et al. 2008; Baxter et al. 1988; Abramovitch et al. 2012; Whiteside, Port, and Abramowitz 2004).

Animal studies with optogenetics show that excitation of a pathway that connects the OFC and the striatum produces excessive grooming, in other words, repetitive behavior (Ahmari et al. 2013). Another study shows that optogenetic excitation of the lateral OFC can suppress repetitive behavior in a genetic mouse model of OCD (Burguère et al. 2013). Therefore, these animal studies provide evidence that the CSTC model is in fact possible.

From the studies and proposed hypotheses, the CSTC model is a suitable explanation for the neurobiological substrate that produces OCD, and although some of the studies have small sample sizes, it is a step in understanding how the symptoms and biological findings are associated with OCD. Therefore, further comprehensive research and larger sample sizes will bring new evidence to the understanding of the etiology of OCD.

### 2.3 Autoimmunity in obsessive-compulsive disorder

There are several neuropsychiatric disorders that secondary to infection or medical disease, manifest OCD and movement disorders. Several of these neuropsychiatric disorders have shown that an autoimmune response occurs by diverse mechanisms that usually involve auto-antibodies against several catecholamine receptors.

Research of autoimmunity in patients with OCD began in the 1980s, and by the year 1998 the first case series of an autoimmune response that causes OCD was documented in a pediatric group that acutely developed severe OCD after an infection by a group A beta-hemolytic streptococcus (GABHS) and showed presence of motor symptoms, termed Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS), that have a recurring course and exacerbation of symptoms when infection is present (S. E. Swedo et al. 1998). However, a recent evaluation of the symptom criteria for PANDAS excluded cases in post pubertal ages and it was therefore agreed that a broader inclusion criteria be set (mainly changing the acute onset by adding chronic cases and
patients up to 18 years of age) and thus the term Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) was created (E. Swedo 2012).

Rheumatic fever occurs secondary to streptococcus pyogenes infection, which belongs to the group A streptococcus, and causes diverse symptoms, among them, is Sydenham’s Chorea (SC), which is the neuropsychiatric sequelae of the acute phase of rheumatic fever and can last six months after the infection (Duckett Jones 1944). It is characterized by emotional lability, acute onset of OCD, hyperactivity and involuntary movements (Marques-Dias et al. 1997). Sydenham's Chorea is linked to a cross-reactive immune response between the GABHS cell wall and gangliosides of the basal ganglia neurons and is implicated in the motor symptoms that SC produces (Kirvan et al. 2003). Cross-reactivity produces anti-streptococcal antibodies that target antigens of the human basal ganglia and titer elevation of the antibodies correlate with the duration and severity of the symptoms (Bronze and Dale 1993; Husby et al. 1976). Animal studies show that group A streptococcus (GAS) antigens produce anti-neuronal antibodies which affect behavior and cause motor dysfunction and shows that such IgG reacts to dopamine 1 (DRD1) and dopamine 2 (DRD2) receptors as well as serotonin receptors 5HT-2A and 5HT-2C (Lotan et al. 2014). Symptom severity is associated with neuronal antibodies elevation in children with SC and exacerbations with acute or chronic GABHS infections (Harvey S. Singer et al. 2015).

Signal transduction is involved in the brain by the function of gangliosides (Kotani and Tai 1997), and the surface antigen N-acetyl-β-D-glucosamine (GlcNAc) which is a carbohydrate epitope of the cell wall of the group A streptococcus (Shikhman, Greenspan, and Cunningham 1994) cross-reacts with gangliosides, specifically with lysoganglioside GM1 (LGN-GM1) which promotes signal transduction (Kirvan et al. 2003). Antibody titers against LGN-GM1 are often elevated in acute SC, thus showing that immune activation in the brain takes place during the acute phase of SC (Kirvan et al. 2006).

Calcium/calmodulin-dependent protein kinase II (CAMKII) can modulate neurotransmitter synthesis and behavior, and in SC it can be activated by antibody interactions (Chen et al. 1994). Elevation of CAMKII indicates an active phase of SC which ultimately triggers a signal transduction cascade (Kirvan et al. 2006). Dopamine exocytosis by phosphorylation of synapsins, which are vesicle proteins that cause dopamine release, can be regulated by CAMKII (Consogno et al. 2001; Iwata et al. 1997) and exocytosis causes the release of dopamine by activation of tyrosine hydroxylase, which may cause the neuropsychiatric and movement symptoms of SC (Kirvan et al. 2006).

Basal ganglia dysfunction is involved in several neuropsychiatric disorders that have an autoimmune origin such as PANS, SC, (Brimberg et al. 2012; S. E. Swedo et al. 1998; Susan E. Swedo, Leonard, and Rapoport 2004) and Tourette syndrome (Kansy et al. 2006; H. S. Singer et al. 1998). The dysfunction is believed to occur by molecular mimicry when cross-reaction of antibodies produced by infection from a GABHS react with the surface epitopes of the neurons in the basal ganglia, which could alter cell signaling and hence produce the different symptoms of OCD (Kirvan et al. 2006; Dale et al. 2004; Dale and Brilot
2012). Experimental studies show that a neuropsychiatric phenotype can be induced by exposing rats to group A streptococcus or serum of subjects with PANS or SC, and also produce anti-basal ganglia antibodies (Brimberg et al. 2012; Yaddanapudi et al. 2009). Positivity of anti-basal ganglia antibodies (ABGA) is present in CSF and sera of patients with primary OCD as well as other autoimmune neuropsychiatric disorders (Pearlman et al. 2014).

Limbic encephalitis (LE) also causes an autoimmune response in the absence of a concomitant medical disease (Dalmau et al. 2008) that is accompanied by severe neuropsychiatric symptoms. Antineuronal antibodies target neuronal synaptic proteins and cause aberrant synaptic transmission that causes acute and chronic neuropsychiatric symptoms (Kayser, Kohler, and Dalmau 2010; Moscato et al. 2010). Anti-NMDA receptor encephalitis is a type of LE that often has a viral prodrome and is characterized by severe neuropsychiatric symptoms such as paranoia, hallucinations, delusions and motor symptoms and occurs when the NMDA-type glutamate receptor is affected by an autoimmune response (Dalmau et al. 2008; Kayser and Dalmau 2011). IgG antibodies that bind to NMDA receptor (NMDAR) induce an internalization in the neuronal synapse and the loss of NMDARs reflects the elevation of antibody titers (Hughes et al. 2010). In vitro studies in neurons show that continuous exposure reduces the density of NMDARs (Moscato et al. 2014). However, the symptoms are reversible once immunotherapy is started (Dalmau et al. 2008; Hughes et al. 2010).

Autoimmunity has a strong link to the production of diverse neuropsychiatric symptoms and there is a definite link between autoimmune reactions and triggering of OCD, therefore, the underlying autoimmune response could be associated with humoral immune reactions that may produce symptoms of OCD. Understanding the autoimmune basis for OCD could provide new clues to the etiology of OCD and ultimately change the diagnosis and treatment of the disorder.

2.5 Cytokines in obsessive-compulsive disorder

Inflammation has been thoroughly studied in diverse psychiatric disorders but the etiological pathway(s) that associates inflammation to symptoms remains unclear, although 3 major pathways have been suggested that associate the exposure to stress and an inflammatory response. First, humoral routes where peripheral cytokines leak through the blood brain barrier in areas that involve the circumventricular organs and the activation of cytokine specific saturable transporters that transport cytokines to the brain parenchyma (Quan and Banks 2007). Second, a neural route that activates cytokine receptors of afferent nerve fibers which elicit cytokine signaling to the brain (Luheshi et al. 2000; Watkins et al. 1995). Third, chemokines that are released by the activation of microglia and adhesion molecules expressed throughout the central nervous system (CNS) that could attract peripheral activated cells such as monocytes and T cells to the meninges and parenchyma (D’Mello, Le and Swain 2009; Lewitus, Cohen and Schwartz 2008).

Several hypotheses state that diverse neurotransmitters (serotonin, dopamine, and glutamate) are involved in the etiology of OCD (Zohar, Greenberg, and Denys 2012; Klanker, Feenstra, and Denys 2013; Maina et al. 2008; Rodriguez et al. 2015), and cytokines may have an effect on their release, reuptake and synthesis, and therefore influence the clinical manifestation of symptoms (Miller et al. 2013).
Diverse cytokines influence the increase or decrease of diverse neurotransmitters. One study shows that increased levels of interleukin-6 (IL-6) in the brain decrease levels of serotonin and dopamine when the production of tetrahydrobiopterin, which is a naturally occurring necessary cofactor for the synthesis of serotonin and dopamine, is decreased by IL-6 (Miller et al. 2013). There is also another study where depletion of brain serotonin secondary to the consumption of tryptophan by the production of several neuroactive metabolites and kynurenine, is caused by interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) via the activation of indoleamine 2,3-dioxygenase (Dantzer et al. 2008). There is also evidence that glutamate can also be released from astrocytes by the presence of cytokines (Ida et al. 2008), which is another neurotransmitter involved in the CSTC pathway in the etiology of OCD. To this day, however, the results are still contradictory due to factors such as medication, medical and psychiatric comorbidities, age, and sample size. A meta-analysis of cytokine profiles in adult OCD populations shows that IL-β is decreased in OCD patients, and no changes are seen in IL-6 and TNF-α titers (Gray and Bloch 2012). However, in a subgroup analysis, IL-6 levels are increased in drug-naive OCD adults, and IL-6 levels are lower in OCD children with pharmacological treatment (Gray and Bloch 2012).

Another study shows that adults with OCD have increased plasma levels of chemokines CXCL8/IL-8 and CCL3/MIP-1α and soluble receptors of TNF-α type 1 (sTNFR1) and type 2 (sTNFR2) (Fontenelle et al. 2012). Interestingly, a study shows that the soluble receptors of TNF-α (sTNFR1 and sTNFR2) could be appropriate putative markers for the overall activity of TNF-α (Scalzo et al. 2009). A recent study in drug-naive adult OCD patients shows higher levels of IL 2, 4, 6, 10 and TNF-α compared to healthy controls (Rao et al. 2015), supporting the findings that cytokines have a possible role in the pathogenesis of OCD and that comorbidities and medication affect the levels of cytokines in OCD. A study of drug-naive children with OCD without PANDAS or tics, shows that serum TNF-α is increased, but IL-12 levels are lower which could be a self-regulating cytokine mechanism (Çolak Sivri, Bilgiç, and Kılınç 2018), however, TNF-α appears to be a consistent finding in both adult and pediatric OCD populations.

Although the findings are contradictory, there is evidence that cytokines are altered in patients with OCD, suggesting that an underlying inflammatory response could be secondary to an autoimmune reaction, since the association of autoimmune disease and OCD symptoms is highly significant. Thus, cytokines merit further study since the implications of an inflammatory response in OCD could be a new path for diagnosis and treatment of adult and pediatric patients with OCD, a condition where exacerbation of symptoms and relapses are a commonly occurring.
3. Aims

- To validate earlier findings of elevated inflammatory markers in a cohort of young adults with OCD compared to age matched controls.
- To identify novel markers in a cohort of young adults with OCD using proximity extension assay (PEA).
- To test our hypothesis that the inflammatory changes described earlier will be present even in young patients with OCD and that a combination of several inflammatory markers will increase the power of these biomarkers in separating the OCD group from the control group.
- To test our hypothesis that pharmacological treatment influences the level of inflammation markers in patients with OCD.
- To evaluate whether clinical data such as obesity or high BMI related to underlying inflammation is associated with inflammation markers.
4. Material and Methods

4.1 Study design
A cross-sectional study was performed with OCD patients from the UPP cohort. Two groups were created, OCD+inflammation markers and a control group+inflammation markers.

4.2 Inflammation marker selection
Uppsala University library and PubMed were searched for relevant bibliography using the search terms “cytokines” AND “obsessive-compulsive disorder” AND “adult.” Based on a meta analysis by Gray and Bloch in 2012, further studies of OCD in adult or pediatric cohorts that measured the same or other cytokines that were not measured previously were chosen for the validation in this cohort.

4.3 Participants
The data for this study was obtained from the patient cohort Uppsala Psychiatric Patient Samples (UPP). The UPP cohort consists of ambulatory patients within 18-25 years old with affective and anxiety disorders from the “Young Adults” section of the Department of General Psychiatry at Uppsala University Hospital. This cohort is described in detail in the paper by Cunningham et al. 2017. The time range of the available data was from August 2012 to Feb 2016. From the UPP sample, 43 patients with DSM IV (American Psychiatric Association 2013) diagnosis of OCD were selected for this study. All patients agreed and consented to the study. A control group of 139 subjects was used, subjects were excluded if values for BMI and cytokine levels were missing, resulting in a cohort of 45 healthy controls without any psychiatric disorders, which were recruited from the university students and hospital personnel to UPP between 2013 and 2015.

4.4 Clinical assessment
The diagnosis of OCD of the patients from the UPP sample was made using the Structured Clinical Interview for DSM-IV Axis I Disorders- Clinical Version (First M, et al., 1996) or Mini-International Neuropsychiatric Interview (Hergueta et al. 1998; Sheehan et al. 2010). Clinical examination data was also obtained which consisted of height, weight, waist-hip measurement, blood pressure, and pulse. Body mass index (BMI) was also calculated by the body weight (kg) divided by the square of the patient's height (m²). Blood sample collection was performed in conjunction with the clinical examination.
The OCD patient sample was screened for depressive symptoms using the Montgomery-Asberg depression rating scale (MADRS) which consists of a 10-item questionnaire to measure the severity of depressive episodes. Higher scores indicate higher depression severity, the overall score ranges from 0 to 54 (Svanborg and Åsberg 1994; 2001) Based on MADRS-S scores, participants were arbitrarily grouped as suffering from “None or mild” (0-19 points), “moderate” (20-29 points) or “severe” (30+ points) depressive symptoms. The Sheehan disability scale (SDS) was also used to measure functional impairment in the sample, it is a self-report that measures impairment in three items: work/school, social life, and home life or family responsibility. The scoring is from 0 to 10 for each individual item of impairment. There is no cut-off score but scores of 5 or higher in any of the three items indicate significant functional disability (Sheehan 1983; Leon et al.
1992). The control samples were obtained from students and staff at the Department of General Psychiatry at Uppsala University Hospital. The control group was matched with the patient population according to age, exclusion of controls included subjects younger than 18 years and older than 26 years, subjects who scored $>13$ in the MADRS-S score or AUDIT score $>13$ for women or $>15$ for men; subjects who had current or past psychiatric disease, and ongoing medical treatment with SSRI, rituximab, zopiclone and methylphenidate.

4.5 Blood sample collection and analysis

Plasma was isolated and stored at $-70^\circ$C. The samples were thawed on ice and the samples were transferred to 96-well plates with 90 samples and 6 controls (three inter-plate controls and three negative controls (buffer). After incubation, mixes (3 μL) containing 92 pairs of unique DNA oligonucleotides each labeled to a corresponding antibody were combined with plasma samples (1 μL) and incubated at 4°C overnight. PEA enzyme and PCR reagents were added in a 96 μL extension mix and incubated at room temperature for 5 minutes. Initial DNA extension was performed for 20 minutes at 50°C and subsequent DNA amplification. According to the manufacturer's instructions (Fluidigm, South San Francisco, CA, USA), a 96.96 Dynamic Array IFC was prepared and primed, while sample mixture (2.8 μL) was mixed with 7.2 μL detection mix in a new plate. The right side of the primed 96.96 Dynamic Array IFC was loaded with 5 μL of this mixture and the left side was loaded with the unique primer pairs for each protein. Using Proseek instructions, the protein expression program was run in Fluidigm Biomark reader. Each sample was spiked with one detection control, one extension control and two incubation controls. Data normalization was performed using GenEx software in Olink Wizard. The normalized protein expression (NPX) was obtained with the quantification cycle (Cq) values that were generated in the real-time PCR in three steps. First, technical variations were corrected by subtracting the extension control from the Cq-value of every sample (CqSample = CqSample − CqExtension control). Second, the interplate control was subtracted to compensate for potential variations between runs (Cq = CqSample − CqInterplate control). Third, the NPX was calculated by normalization against a calculation correction factor (NPX = Correction factor − CqSample). The normalized protein expression (NPX) data was presented on a Log2-scale. The 92 samples were analyzed in tandem with 569 other plasma samples. Normalization of plate differences was done using a median normalization for each protein. The normalization produced 72 proteins with detectable values in 80% of the plasma samples.

4.6 Statistics

Statistics were performed using SPSS program, software version 21. A description of the cohort can be seen in on table 1. The comparisons of the categorical variables in table 1 were calculated with Fisher's exact test or Chi-square when appropriate. Normal distribution was tested for the continuous variables with the Shapiro-Wilk test and were non normally distributed. The continuous variables, which are the levels of IL-6, IL-8, MIP-1α, and IL-10, were compared between patients with OCD and age matched controls with the Mann-Whitney U test. Z-scores for each cytokine level were performed and afterwards a factor analysis was used. To determine the sample adequacy the Kaiser-Meyer-Olkin (KMO) test was used and the Bartlett test of Sphericity was used to determine if the data was to homogenous. After the solution Varimax rotation was used and produced a new variable which was assessed with T-test for significance. A logistic regression analysis was performed to test for the effects of BMI, sex, comorbid anxiety, comorbid depression, smoking and
current medication with selective serotonin reuptake inhibitors (SSRI) or serotonin-norepeniphrine reuptake inhibitors (SNRI), antipsychotics or antihistamines, MADRS scale, Sheehan scale and GAF scale.
5. Results

The literature search produced several matches of studies that measured cytokine levels, cytokine production or chemokine levels in OCD patients compared with controls and the studies included measurements for IL-6, IL-8, MIP-1α, and IL-10. These cytokine measurements were chosen for this study since they have consistently been reported elevated in adult and young adult patients with OCD (Fontenelle et al. 2012; Gray and Bloch 2012; Rao et al. 2015).

There were 43 subjects with OCD (M/F, 11/32) and 45 healthy controls (M/F, 15/30). Samples did not differ in gender (p=0.487). Clinical characteristics of the OCD patients are shown in table 1.

Table 1. Characteristics for controls and patients

<table>
<thead>
<tr>
<th>General</th>
<th>Controls (n=45)</th>
<th>Patients (n=43)</th>
<th>P^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>15/30</td>
<td>11/32</td>
<td>0.4879</td>
</tr>
<tr>
<td>BMI kg/m² (mean)</td>
<td>21.45</td>
<td>22.95</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>28.09</td>
<td>37.36</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>16.92</td>
<td>17.21</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>1</td>
<td>10</td>
<td>0.0029*</td>
</tr>
<tr>
<td>Pharmacological Treatment</td>
<td>9</td>
<td>18</td>
<td>0.0372*</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Asthma treatment</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Contraceptives</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI/SNRI</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zolpidem</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other^c</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (mean)</td>
<td>63.7</td>
<td>65.8</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>92</td>
<td>104.2</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>50</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>
Pharmacological treatment in some of the patients was concurrent. Nine out of the 18 patients had treatment with only one drug. The rest had a combination of the treatments mentioned in table 1. The non-psychopharmacological drugs included one patient taking mesalazine and azathioprine, one patient taking thyroxine, and one patient taking omeprazole.

Cytokine levels for the OCD patients and controls are shown in table 2. The mean± SD serum levels of IL-8 were 5.35 ±0.34 and 5.13 ±0.43 in the OCD and control groups, respectively. OCD patients had significantly higher serum levels of IL-8 (P=0.007) than controls. The mean± SD serum levels of MIP-1α were 1.63 ±0.44 and 1.53± 0.29 in the OCD and control groups, respectively. There was no significant difference between groups (P=0.158). The mean ±SD serum levels of IL-6 were 2.13 ±0.87 and 1.92 ±0.78 in the OCD and control groups, respectively. There was no significant difference between groups (P=0.114). The mean ±SD serum levels of IL-10 were 3.91 ±1.38 and 3.74 ±0.47 in the OCD and control groups, respectively. There was no significant difference between groups (P=0.203).

Table 2. Proximity extension assay plasma cytokine values between OCD patients and controls.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Controls (n=45)</th>
<th>Patients (n=43)</th>
<th>U⁺</th>
<th>P⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>5.13 (0.43)</td>
<td>5.35 (0.34)</td>
<td>645.0</td>
<td>0.007*</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>1.53 (0.29)</td>
<td>1.65 (0.44)</td>
<td>798.5</td>
<td>0.158</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.92 (0.78)</td>
<td>2.13 (0.87)</td>
<td>778.0</td>
<td>0.114</td>
</tr>
<tr>
<td>IL-10</td>
<td>3.74 (0.47)</td>
<td>3.91 (1.38)</td>
<td>815.0</td>
<td>0.203</td>
</tr>
</tbody>
</table>

aProximity extension assay; bStandard deviation
*P<0.05

Some patients had two or more concurrent treatments; Chi square test; Pharmacological treatment for hypothyroidism, rheumatoid arthritis, irritable bowel syndrome, antihistamines; obsessive-compulsive disorder; generalized anxiety disorder

P<0.05 level
Figure 1. Proximity extension assay total values between OCD patients and controls

Mann-Whitney U test; *P<0.05

Obsessive-compulsive disorder patients with a major depressive episode (MDE) had significantly higher serum levels of IL-8 (P=0.027) compared to OCD patients without a major depressive episode. Generalized anxiety disorder and major depressive disorder (MDD) did not reach significance for OCD patients.

There were no significant differences of OCD patients with generalized anxiety disorder (GAD) or MDD and the serum levels of IL-8, MIP-1α, IL-6 or IL-10.

A logistic regression analysis with the Z-scores for each individual cytokine showed that separately they do not reach significance, therefore, a factor analysis between IL-8, MIP-1α, IL-6 or IL-10 produced the variable FAC1 which was significant (P=0.006).

The logistic regression analysis results are in table 3. The analysis showed that the cytokines as a group are significant in OCD patients vs. controls (P=0.031; OR=2.22). Gender (P=0.507) and BMI (P=0.271) did not reach significance in the model.
Table 3. Logistic regression analysis results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance</th>
<th>aO.R.</th>
<th>95% bC.I. Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine Factor</td>
<td>0.031</td>
<td>2.22</td>
<td>1.07</td>
<td>4.59</td>
</tr>
<tr>
<td>Gender</td>
<td>0.507</td>
<td>1.44</td>
<td>0.48</td>
<td>4.31</td>
</tr>
<tr>
<td>BMI</td>
<td>0.271</td>
<td>1.10</td>
<td>0.92</td>
<td>1.31</td>
</tr>
</tbody>
</table>

*Odds ratio

bConfidence interval

A logistic regression analysis of smoking vs non-smoking OCD patients showed that there is no significant association with the cytokine factor (P=0.220). Treatment with SSRI or SNRI (P=0.720), antihistamine treatment (P=0.559) or antipsychotic treatment (P=0.659) showed no significant association with the cytokine factor. Analysis of the MADRS, Sheehan and GAF scales showed no significant association with the cytokine factor.

Individually the cytokine factor showed the highest significance in the logistic regression model (P=0.008) and BMI (P=0.027). Major depressive episode, major depressive disorder, and generalized anxiety disorder did not reach significance in the logistic regression model.
6. Discussion

The present study sought to investigate whether there is a difference in the cytokine levels of young adult patients with OCD compared to healthy controls and validate previous findings of cytokine and chemokine elevation. This study used a new technique for measuring cytokine and chemokine levels in patients with OCD, which had not been previously used in OCD studies, which is the proximity extension assay (PEA) developed by Olink in Uppsala, Sweden. Besides using a new method to measure levels of circulating peripheral cytokines and chemokines, this study also evaluated a combination of cytokines and chemokines that have consistently been found in other studies of adult and pediatric cohorts with OCD (Fontenelle et al. 2012; Gray and Bloch 2012; Rao et al. 2015) and in this study, the cohort was robust and composed of young adults with OCD and healthy controls, which is larger than other studies that measure cytokine levels in plasma.

This study showed that increased levels of the chemokine IL-8 were present in patients with OCD and, interestingly, IL-8 was the only marker that showed to have a higher significance for OCD patients. Chemokines function by chemotaxis and control migration and positioning of different cells in different situations such as inflammatory states, homeostasis, and development. One of the key roles of chemokines in the immune system is to handle the coordination of the migration of cells since the immune system depends on the activation of chemokines to successfully produce an immune response. Thus, they guide effector cells to areas where there is ongoing inflammation or infection and they also coordinate diverse interactions between different immune cell types. Hence, chemokines function by promoting interactions between the innate and adaptive immune systems and therefore provide the necessary setting for an optimal immune response (Sokol and Luster 2015), therefore this finding agrees with previous studies and strengthens the evidence of an immune response in the etiology of OCD.

To this day, chemokines have been studied in other psychiatric disorders such as bipolar disorder (Tokac et al. 2016), autism spectrum disorders (Han et al. 2017) and schizophrenia (Hong et al. 2017) with results that associate clinical data with the presence of elevated chemokine levels. In accordance to another study where chemokines have been studied in a cohort of adult patients with OCD, IL-8 and MIP-1α are both elevated in patients with OCD (Fontenelle et al. 2012), and interestingly a subgroup analysis in our cohort of OCD patients with an MDE had higher levels of IL-8 (P=0.027), suggesting that comorbid depression might influence the elevation of cytokines in OCD patients and that both disorders share a common inflammation pathway that results in clinical symptoms. IL-8 (CXCL8) is associated with the chemokine receptor CXCR2 that is expressed in the neurons of diverse areas of the brain such as the striatum, cortex, and the hypothalamic, thalamic, pontine and mesencephalic nuclei. Once astrocytes and microglia become activated by proinflammatory cytokines, they also express the CXCR2 receptor (Semple, Kossmann and Morganti-Kossmann 2009) which would be interesting to study in specific areas of the CSTC pathway to observe if astrocytes and microglia have a role in the production of OCD. When diverse neurons express the CXCR2 receptor, it can produce a response that can modulate synaptic transmission by altering neurotransmitter release through the alteration of calcium channel excitability (Cartier et al. 2005; Semple, Kossmann and Morganti-Kossmann 2009). In accordance to Fontenelle et al., this study shows that IL-8 is significant for patients with OCD, therefore the
presence of IL-8 in OCD patients suggests that an inflammatory response is underway during the disease and that neurotransmitter release could be influenced by the presence of IL-8 and the presence of OCD symptoms.

An in-vitro study shows that hippocampal neurons chronically exposed to MIP-1α (CCL3) alters neuronal-specific and glia proteins which in turn increase intracellular calcium levels in the hippocampal neurons and also increase the NMDA receptors. Therefore MIP-1α can, in fact, induce adaptive changes in neurons which could ultimately have a key role in diverse central nervous system disorders that have a neuroinflammatory etiology (Kuijpers et al. 2010). In contrast to Fontenelle’s study, MIP-1α did not reach significance in our study, which could be due to different methods and a selective cohort which included OCD patients with an MDE with treatment with an antipsychotic and antidepressant, and could also therefore influence the levels of cytokines. Our study used PEA which has never been used in a cohort of OCD young adults, however, further studies with our method need to be performed to observe if this has a greater sensitivity and reliability than ELISA. The advantage of using this technique in OCD patients is that it is highly sensitive and specific for the detection of low-level abundant proteins such as cytokines and chemokines which can be detected and quantified by qPCR (Lundberg et al. 2011). Another study of a pediatric cohort with OCD drug-naive patients and healthy controls shows that the chemokine levels of IL-8 and MIP-1α did not differ (Çolak Sivri, Bilgiç, and Kılıç 2018), and in our study, however, we did find higher levels of IL-8 in adult OCD patients. As seen with other studies that measure chemokines, a contributing factor for this contradicting finding could be the differences in age of the cohorts, pharmacological treatment and methods for analyzing the samples.

A meta-analysis of the levels of proinflammatory cytokines in different studies of OCD cohorts, carried out by Gray and Bloch, found that IL-6 levels in OCD patients did not significantly differ from that of controls, and in a subgroup analysis, age apparently has a moderating effect, since children with any type of pharmacological treatment had significantly lower IL-6 levels compared to adults. In our study, the cohort included young adult patients that were on current pharmacological treatment, therefore, age and treatment could have influenced the levels of IL-6 and the significance. The levels of IL-6 still remains contradictory in different studies, where elevation and lowering of IL-6 varies greatly and can be due to the already mentioned factors that influence cytokine levels. From this study we show that a selected cohort of young adult OCD patients with pharmacological treatment do not have higher levels of IL-6 or lower levels as mentioned by Gray and Bloch. To this day only one study (Rao et al., 2015) shows that levels of IL-10 are elevated in patients with OCD and have a relation to the symptom dimensions, however, the cohort was composed of drug-naive OCD patients without any comorbidities. Our study did not find any association with elevated IL-10 levels and OCD patients, however, this study included patients with ongoing pharmacological treatment and psychiatric comorbidities which could ultimately influence the fluctuation of cytokine levels in plasma.

Although individually the cytokines and chemokines we analyzed show a low significance for OCD patients, when a factor analysis score between IL-8, MIP-1α, IL-6 and IL-10 was used in a logistic regression model, the combination of these proinflammatory cytokines and chemokines proved to be a significantly associated with OCD regardless of the other factors that influence the presence of inflammation. Since cytokines have direct and indirect
interactions among themselves, and ultimately auto-regulate each other to form a complex feedback system (Cavaillon 2002; van der Poll 1994) it is still difficult to analyze how the fluctuation of cytokine/chemokine levels occur since most studies are performed in a cross-sectional manner. Therefore, it is often difficult to elucidate when an increase of a particular cytokine is a consequence or a response to another cytokine elevation or decrease. In previous studies, the elevation of IL-4 and IL-10, which are anti-inflammatory cytokines, suggests that it could be a secondary response to a proinflammatory state that is meant to be controlled through a feedback mechanism, which is the proinflammatory response (Ng et al. 2003). Therefore, it would be tempting to say that in our study, the absence of elevated levels of the different cytokines could be due to a feedback mechanism and the next step would be to perform a longitudinal study which examines cytokine levels in OCD patients, since there could be a proinflammatory response in OCD patients that causes an elevation of anti-inflammatory cytokines such as IL-10, and that through feedback mechanisms, fluctuations in the levels of other cytokines can also occur, and since this is a cross-sectional study, it would be difficult to infer which cytokine influences the latter.

Obsessive compulsive-disorder is a multidimensional disorder and different symptom dimensions could account for the presence of different cytokine elevations, however, in the present study, the different symptom dimensions were not assessed for correlation with cytokine levels. Only the study by Fontenelle et al. has correlated different symptom dimensions with cytokine and chemokine levels, and found that levels of serum TNF receptor 1 is correlated to the severity of cleanliness symptoms and CCL24, which is a chemokine, is negatively correlated with the severity of hoarding. Although our study used scales to measure dysfunction, the cytokine factor showed no association to the severity of dysfunction in OCD patients. Future studies with cytokines and chemokines in cohorts of OCD patients should include symptomatic dimensions correlations.

Studies that analyze correlations between other factors that influence inflammation markers in patients with psychiatric disorders, such as obesity, have shown no difference in the levels of cytokines. A study shows that a cohort of depressed patients have no significant correlation between being overweight and the levels of TNF-α, which can suggest that obesity and inflammation alterations might not be as straightforward in a cohort of patients with psychiatric disease (Himmerich et al. 2008). In accordance with the previously mentioned study, this study shows that there is no significant associations in the BMI measurements and the presence of OCD.

Strengths in this study include that diagnosis of was performed using the same instrument for patients and controls, and both groups had a large number of participants compared to other studies. This study also introduces a new way to obtain the cytokine and chemokine levels which is through the proximity extension assay. There are limitations in this study and some include that several of the OCD patients had a comorbid major depressive episode or disorder which is known for producing a proinflammatory state (Capuron and Miller 2011), and could, therefore, raise the question whether the comorbidity produces the elevation of cytokines and chemokines. The patients were also on current pharmacological treatment, which could ultimately influence the levels of circulating cytokines.
7. Conclusion

In conclusion, this study indicates that OCD young adults have higher levels of cytokines and chemokines compared to healthy controls, which supports the findings of previous studies done in adult and pediatric populations and that the use of PEA could be a new method for the detection of diverse cytokines and chemokines in OCD cohorts. Even though the immunological responses might differ in different stages of life, from this study we can observe that OCD patients do in fact, have a proinflammatory state which can affect the immune system. Elevation of the selected proteins in OCD patients supports the hypothesis that cytokines and chemokines have a role in the immunologic response of OCD and therefore could indicate a pathophysiological mechanism that is intertwined with the innate immune system. There are still, however, questions that should be handled in future studies. For example, elucidating which cell types synthesize the proteins that are relevant for OCD and how do these cells influence the symptoms that are present in OCD, and future studies could also focus on targeting cytokines and chemokines as a novel line of treatment.
8. Acknowledgments

I would like to thank Janet Cunningham for providing me with the data and samples from the UPP cohort and as well for organizing the database and providing feedback for the whole project. I would also like to thank SciLife lab for the analysis of the samples. Special thanks to Annica Rasmusson for the analysis and management of the samples, organizing and providing me with the database of the cytokine levels from the Olink dataset. I would also like to thank Mikaela Syk for the collection of clinical data and providing me with the dataset from the UPP patient cohort. I would also like to thank Hans Arinell who contributed to the statistical analysis and guided me through the process of obtaining my results.
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