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Increased level of serum cytokines, chemokines and adipokines in patients with schizophrenia is associated with disease and metabolic syndrome

Wouter Beumer [a,*], Roosmarijn C. Drexhage [a], Harm De Wit [a], Marjan A. Versnel [a], Hemmo A. Drexhage [a,1], Dan Cohen [b,1]

[a] Department of Immunology, Erasmus MC, Rotterdam, The Netherlands
[b] Department of Severe Mental Illness, Mental health Organization North-Holland North, Heerhugowaard, The Netherlands

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Monocytes;
Macrophages

Summary At present there are strong indications of a shared vulnerability factor for schizophrenia (SZ), diabetes and the metabolic syndrome (metS). In this study we focus on an aberrantly activated monocyte/macrophage system as the shared factor.

We measured in SZ patients ($n = 144$), the serum levels of monocyte/macrophage cytokines/chemokines/adipokines CCL2, CCL4, IL-1β, TNF-α, IL-6, PTX3, leptin, adiponectin, PAI-1, OPG and ICAM-1 and compared these levels to healthy controls (HC) ($n = 138$). Using multivariate analysis, we studied the effect of the presence of the disease SZ, the components of the metS including BMI, the levels of lipids (HDL cholesterol and triglycerides (TG)), diabetes (hyperglycemia) and the use of antipsychotic medication, on the serum levels of these immune compounds.

We found all measured immune compounds with the exception of PAI-1 and OPG to be elevated in the SZ patient population. Multivariate analysis showed that elevations were linked to gender (ICAM-1, leptin, TNF-α and adiponectin), an increased BMI (leptin, adiponectin), hyperglycemia/diabetes (CCL4, and OPG), reduced HDL-cholesterol or increased levels of TG (adiponectin and PTX3) or the metS (CCL2, leptin and adiponectin). IL-1β and IL-6 were the only immune compounds raised in the serum of patients not affected by any of the included factors.

Although many of the immune compounds were found linked to (components of) the metS, the most dominant linkage was found with the disease schizophrenia, confirming earlier reports on increased monocyte/macrophage activation as a key component for understanding the pathogenesis of schizophrenia.

* Corresponding author at: Dept. of Immunology, Erasmus MC, Dr. Molenwaterplein 50, 3015CE Rotterdam, The Netherlands. Tel.: +31 0107044091; fax: +31 10704 4731.
E-mail address: w.beumer@erasmusmc.nl (W. Beumer).
[1] These authors contributed equally to this work.

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1. Introduction

Increased prevalence of both components and the metS itself has been a systematic finding in metabolic research in SZ (De Hert et al., 2006). It is well known that 1 year of antipsychotic drug treatment in the first episode of schizophrenia induces clinical relevant weight gain and rise of fasting plasma glucose levels (Kahn et al., 2008). In a cross-sectional study of 200 Caucasian patients with SZ or schizoaffective disorder (mean age 41 years) the prevalence of hyperglycemia was 7% and that of type 2 DM 14.5%, being significantly increased compared to the general population (Cohen et al., 2006b). However, the development of diabetes was unrelated to duration of antipsychotic treatment or to a specific antipsychotic drug (Cohen et al., 2006a), suggesting that disease-related instead of treatment-related effects determined the higher risk for diabetes.

Studies in drug-naïve SZ patients suggests that diabetes and the metS are linked to the to the disease state schizophrenia itself (Ryan et al., 2003). This direct linkage was already suggested in 1879 by Sir Maudsley in his paper ''Diabetes and Insanity'' far before the era of antipsychotic medication, where he noted that diabetes was more prevalent in psychotic patients and their family members (Maudsley, 1879). A more recent paper found increased diabetes prevalence and increased IL-6 blood concentrations in drug-naïve recent onset SZ patients, irrespective of BMI, gender, age and other confounding factors (Fernandez-Egea et al., 2009).

Presently there are strong indications that several vulnerability factors for schizophrenia, diabetes and the metS are shared (Mitchell and Malone, 2006; De Hert et al., 2011) and possibly interlinked. We focus in this report on the contribution of an aberrantly activated monocyte/macrophage system.

Evidence is accumulating that an aberrantly activated monocyte/macrophage system found in SZ patients is a key to the understanding of the disease. We previously reported on monocytosis and an up regulated inflammatory gene expression profile in circulating monocytes of SZ patients involving genes such as

IL-1β, TNF-α, IL-6, PTX3, CCL2 and CCL4 (Drexhage et al., 2010b). Also increases in the serum concentrations of these cytokines and chemokines have been reported in SZ patients (data reviewed in Drexhage et al., 2010a). It is thought that these excessively produced immune compounds destabilize the brain in such a way that other genetic and environmental influences are capable of precipitating the signs and symptoms of SZ (Smith, 1992; Smith and Maes, 1995). Indeed, receptors for inflammatory cytokines are expressed in various brain nuclei (Silberstein et al., 2009) and via their triggering, deregulations of important neurotransmitter and neuro-developmental systems are introduced, facilitating the development of psychiatric signs and symptoms. Moreover, infusion with pro-inflammatory cytokines (such as IFN-α and TNF-α) is capable of inducing psychiatric symptoms (Keefe, 2007; Khairova et al., 2009). Anti-inflammatory therapies, such as anti-TNF (Soczynska et al., 2009), COX-2 inhibitors (Muller et al., 2002, 2010; Akhondzadeh et al., 2007), aspirin (Laan et al., 2010) and strong macrophage-dampening anti-oxidants, such as n-acetyl cysteine (NAC) (Dodd et al., 2008; Dean et al., 2011), are capable of alleviating symptoms of depression and SZ. Furthermore, increased levels of the osteoclastogenesis factor OPG have been described in SZ patients (Hope et al., 2010). OPG is a member of the tumor necrosis factor receptor family and known to act as a competitive decoy receptor for RANKL preventing the genesis of the macrophages of the bone, the osteoclasts. Interestingly SZ patients also have a higher frequency of osteoporosis (Hummer et al., 2005; Meyer and Lehman, 2006)

An aberrantly activated monocyte/macrophage system is also a key player in T2D and the metS. In T2D monocytes/macrophages are involved by excessively secreting inflammatory cytokines (such as CCL2 and TNF-α), which are capable of inducing insulin-resistance in muscle and liver cells (Kamei et al., 2006; Drexhage et al., 2008).

In patients with obesity, macrophages in the white adipose tissue are in a chronic inflamed state and produce an array of (pro-inflammatory) cytokines including ICAM-1, CCL2, CCL4, IL-1β, TNF-α, IL-6, leptin, adiponectin, PAI-1 (Weisberg et al., 2003; Xu et al., 2003). PAI-1 is considered to be an anti-fibrinolytic adipokine synthesized by macrophages in adipose tissue and the level of PAI-1 in serum is known to be increased in individuals with obesity (De Taeye et al., 2005). Increased PAI-1 expression that accompanies abdominal obesity is the most well documented abnormality associated with the metS. Interestingly, we have found the PAI gene overexpressed in the monocytes of SZ patients (Drexhage et al., 2010b). With regard to dyslipidemia, reduced HDL levels are correlated to a high inflammatory set point of monocytes and macrophages (Sarov-Blat et al., 2007).

The aim of this study was to investigate, using an array-based system, whether monocyte/macrophage related ''inflammatory'' cytokines/chemokines/adipokines were raised in the serum of SZ patients as compared to HC. We focused in particular on those compounds which have been described in the literature as abnormal or which we found previously as abnormally expressed in the monocytes of SZ and diabetes patients (Padmos et al., 2008a,b; Drexhage et al., 2010b). We therefore tested for the chemokines CCL2, CCL4, the cytokines IL-1β, TNF-α, IL-6, PTX3 OPG, the adipokines leptin, adiponectin and PAI-1 and the adhesion factor ICAM-1 and studied whether putative elevations in the serum of SZ patients were related to the disease, to the BMI, the levels of lipids (HDL, TG), diabetes (hyperglycemia) or the use of antipsychotic medication.

2. Materials and methods

The study protocols for the inclusion of both the study patients' samples and HC were reviewed and approved by two independent medical ethical review committees METIGG (Utrecht, The Netherlands) and Utrecht University, respectively.

2.1. Patients with schizophrenia

Chronic stable SZ patients between the ages of 19 and 65 years, with minimum disease duration of 5 years, were recruited from the mental health care organizations Rijngeestgroep (Oegstgeest, Voorhout, and Noordwijkerhout), De Grote Rivieren (Dordrecht) and Anoiksis, the Dutch organization for patients with schizophrenia. Most participants of

the study were recruited from the semirural part of the Dutch province Zuid-Holland.

After providing written informed consent, the M.I.N.I. Plus (Sheehan et al., 1998), a structured diagnostic interview for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) diagnosis (American Psychiatric Association, 2000), and the available medical records were used for a DSM-IV diagnosis of schizophrenia or schizoaffective disorder. The study design as well as the demographic data of the study population has been described before (Cohen et al., 2006b). Patients were classified as on typical (n = 39/144) and atypical (n = 94/144) medication. The two treatment groups did not differ in age, BMI, or waist-to-hip ratio.

Although the group of SZ patients share a long history of antipsychotic medication use, a small subgroup (n = 11) was *currently* drug-free. This subgroup did not use anti-psychotic medication for at least 3 months. The drug-free population had significantly lower BMI and higher HDL (Table S3)

The demographic data for the SZ patients are listed in Table 1.

2.2. Healthy controls

HC (n = 138) were laboratory staff, medical staff and medical students at the Erasmus MC. The inclusion criteria for HC were an absence of any major psychiatric disorder including schizophrenia, bipolar and major depressive disorder and an absent history of these disorders in first-degree family members. The HC had to be in good health and free of any diagnosed medical disorder or illness for at least 2 weeks before blood withdrawal. The demographic data for the HC are listed in Table 1.

2.3. Clinical variables

Clinical variables including TG, HDL, mean fasting glucose levels were only determined in the SZ patient group. BMI was determined only in 43 of 144 HC. Overall, the HC were considered lean.

The metabolic syndrome (metS) was defined according to a modified definition of the NCEP-ATP III (National Cholesterol Education Program (U.S.). Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2002).

Hyperglycemia was diagnosed when the mean fasting glucose plasma level was between 6.1 and 7.0 mmol/l, or when the oral glucose tolerance test (OGTT) plasma glucose levels at T = 120 min were between 7.8 and <11.1 mmol/l. The glucose measurements were performed in the morning between 09:00 h and 11:00 h. In addition, we also determined the metS according to International Diabetes Federation guidelines (Alberti et al., 2005), but this did not result in a change of the total number of SZ patients with metS.

2.4. Laboratory methods

2.4.1. Serum collection

After a 12 h fasting period a blood sample was taken between 09:00 h and 11:00 h in both populations. Blood was collected in clotting tubes for serum preparation and stored at −20 °C.

2.4.2. Cytokine and adipokine measurements

The serum cytokine/chemokine/adipokine concentrations were measured using the Cytometric Bead Array kits (BD Biosciences, San Diego, USA) according to the manufacturer's protocol. Samples were analyzed with a Calibur flow cytometer (BD Biosciences, San Diego, USA) using BD FCAP Array Software (BD Biosciences). Results are expressed as picograms per milliliter.

Undetectable cytokine levels were considered as 0 pg/ml and included in the statistical analysis. Subjects with missing serum cytokine values as a result of limited amount of serum were excluded from the specific test.

Table 1 Patient characteristics. Characteristics of HC, SZ patients and SZ patients with the metS.

	HC	SZ	p-Value HC vs. SZ	SZ-metS	p-Value SZ vs. SZ-metS	p-Value HC vs. SZ-metS
Group size n	138	98		46		
Age (mean)	18–62 (33)	19–65 (40)	**<0.001**	22–65 (41)	0.857	**<0.001**
Gender						
Female n (%)	84 (61%)	24 (24%)		19 (41%)		
Male n (%)	54 (39%)	74 (76%)		27 (49%)		
Medication use						
None	138	8		3		
Typical	·	26		13		
Atypical	·	64		30		
Clozapine	·	14		11		
Risperidone	·	22		9		
Olanzapine	·	25		10		
Quetiapine	·	3		0		
BMI mean (range)	24(18–36)[a]	26 (18–35)	**<0.001**	32 (19–47)	**<0.001**	**<0.001**
TG mean (range) mmol/l	NA	1.4 (0.3–9.0)	NA	2.8 (0.6–9.4)	**<0.001**	NA
HDL mean (range) mmol/l	NA	1.1 (0–5.6)[a]	NA	0.9 (0.0–1.8)	**<0.001**	NA
Diabetes mellitus (%)	0	5 (5%)	.	7 (15%)	.	.

Values in bold denote a significant difference between two groups.
[a] BMI was measured in 42 HC.

Table 2 Cytokines/chemokines/adipokines in HC, SZ and SZ-metS patients. Group size, median with IQR, *p*-values obtained by Mann–Whitney *U*-test and Kruskal–Wallis *H*-test. *p*-Values in bold denote significant differences between groups after Bonferroni correction for 3 groups. Similar analysis excluding the 11 current drug-free patients is shown in Table S4.

	HC		SZ			SZ-metS				Kruskal Wallis H	
	N	Median (IQR)	N	Median (IQR)	*p*-Value (HC vs. SZ)	N	Median (IQR)	*p*-Value (HC vs. SZ-metS)	*p*-Value (SZ vs. SZ-MetS)	H	*p*-Value
CCL2	89	149.5 (83.2)	98	179.0 (110.8)	**<0.001**	46	221.9 (228.8)	**<0.001**	0.017	28.3	**<0.001**
CCL4	93	55.6 (33.2)	87	79.7 (38.0)	**<0.001**	36	93.7 (49.0)	**<0.001**	0.07	42.0	**<0.001**
IL-1β	89	0.0 (11)	94	9.3 (14.8)	**<0.001**	44	11.3 (12.8)	**<0.001**	0.824	24.8	**<0.001**
TNF-α	83	0.0 (22.2)	89	24.0 (65.4)	**<0.001**	41	22.2 (31.8)	**0.005**	0.323	18.6	**<0.001**
IL-6	83	0.0 (38.9)	89	31.9 (64.2)	**0.001**	38	40.8 (56.3)	**0.002**	0.862	14.9	**0.001**
PTX3	96	213.6 (524.0)	98	430.4 (523.0)	**<0.001**	45	388.2 (504.1)	**0.001**	0.99	20.0	**<0.001**
Leptin											
Female	48	0.0 (23.2)	20	80.5 (95.2)	**<0.001**	13	98.7 (120.2)	**<0.001**	0.169	40.0	**<0.001**
Male	36	0.0 (0.0)	67	0.0 (0.0)	0.129	23	0.0 (66.9)	**<0.001**	**<0.001**	21.7	**<0.001**
Adiponectin	93	75.4 (32.4)	87	90.6 (26.4)	**<0.001**	36	109.7 (37.1)	**<0.001**	**<0.001**	45.9	**0.001**
PAI-1	93	9.7 (2.6)	87	9.8 (2.2)	0.783	36	9.8 (2.4)	0.85	0.962	0.9	0.063
OPG	93	79.4 (54.66)	87	87.0 (39.2)	0.334	36	89.0 (32.5)	**0.017**	0.125	5.5	0.958
ICAM-1											
Female	48	372.7 (486.4)	20	592.2 (699.5)	0.026	13	545.9 (726.7)	0.149	0.624	5.8	0.054
Male	36	501.9 (360.6)	67	656.8 (469.3)	0.136	23	661.0 (546.3)	0.036	0.456	4.3	0.118

2.4.3. Statistics

Statistical analysis was performed using the SPSS 20 (IBM, Inc.) package for Windows. Data were tested for normal distribution using the Kolmogorov–Smirnov test. Depending on the distribution pattern and the total number of subjects, parametric (normal distribution) or nonparametric group comparison (Mann–Whitney *U* and Kruskal–Wallis *H*-tests) were applied. Correlations were determined by Spearman correlation. The effect of components of the metS on the serum cytokine/chemokine/adipokine concentrations were determined by generalized linear models with bootstrapping to improve model fit. Levels of significance were set at $p = 0.05$ (two-tailed). The specific tests and group size are mentioned in tables, footnotes and in figure legends. Graphs were designed with Graphpad Prism 5.04 (Graphpad Software, Inc) for windows.

3. Results

We compared the serum cytokines/chemokines/adipokines concentrations between the study groups, i.e. HC, SZ patients with and without the metS. The studied serum proteins appeared not normally distributed according to the Kolmogorov–Smirnov test; therefore we used non-parametric tests for group comparison and correlation. According to the Kruskal–Wallis *H*-test (Table 2) there are significant differences in serum levels between the study groups for CCL2, CCL4, IL-1β, TNF-α, IL-6, PTX3, leptin and adiponectin. There was a significant difference in age between the HC and SZ patient groups (Table 1, $p < 0.001$). Generalized linear models applied to our data set demonstrated that age had a significant, but small effect on adiponectin serum concentrations in HC and SZ patients (Table S1, $\beta = 3.9$). However, the regression coefficient of age was smaller than the beta of the disease presence ($\beta = 13.2$), indicating that the effect of age on the adiponectin concentrations was smaller than the effect of disease. In addition, no significant correlation between age and the studied clinical variables was found by Spearman correlation (Table S2).

Similarly, we studied the effect of gender on the serum protein concentrations with generalized linear models. A negative beta value corresponding to gender means an increase in cytokine/chemokine/adipokine concentration in males, whereas a positive beta corresponds to an increase of the specific immune compound in females. There was a significant effect of gender on ICAM-1, TNF-α, leptin and adiponectin (Table S1). Our finding of higher leptin concentration in the serum of females compared to males is supported by literature (Rosenbaum et al., 1996; Saad et al., 1997). For ICAM-1 such literature data is not available. Only in the case of ICAM-1 and Leptin the effect of gender was larger than the disease presence and we therefore stratified ICAM-1 and leptin into male and female groups.

For each of the immune compounds the individual study groups were thereafter compared using Mann–Whitney *U*-tests. Group size, median, interquartile range (IQR) and *p*-values are summarized in Table 2.

3.1. CCL2 and CCL4

We found a significant increase of CCL2 (Fig. 1A) and CCL4 (Fig. 1B) in the serum of SZ patients without the metS as

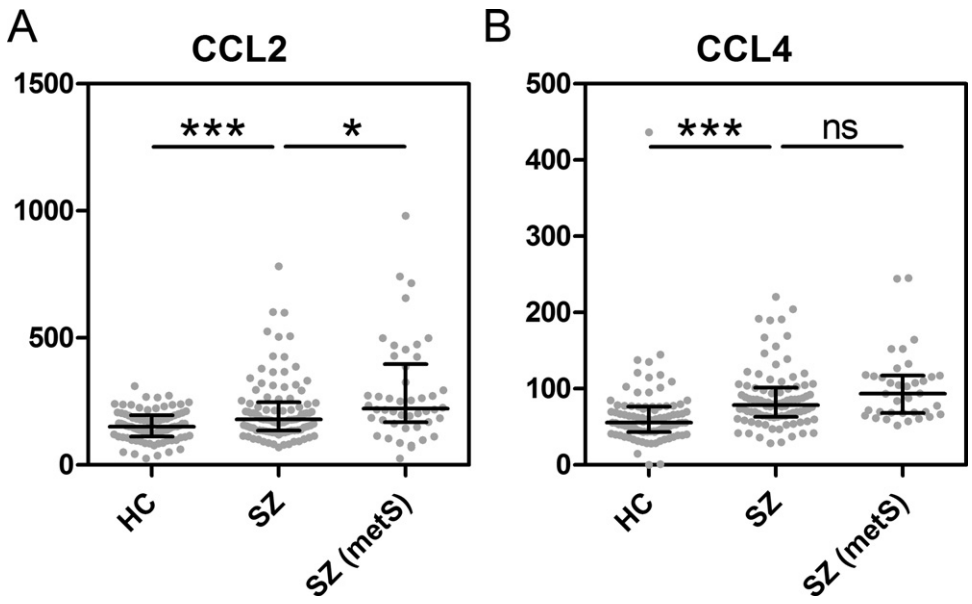

Figure 1 (A) CCL2 and (B) CCL4 serum concentrations. Dots depict individual study subjects, the line represents the median and the whiskers depict the interquartile range (summarized in Table 2). *p*-Values were obtained by Mann—Whitney *U*-tests $^{*}p < 0.05$, $^{***}p < 0.001$.

compared to HC ($p < 0.001$ for both chemokines), indicating a significant effect of disease on these chemokines. SZ patients with the metS had an even more and significantly raised serum level ($p = 0.017$) of CCL2 in comparison to the serum levels found in patients without the metS. For CCL4 there was also such increase, but this did not reach statistical significance in comparison to the patients without the metS. Outcomes of the generalized linear model showed that none of the components of the metS did have a significant effect on the CCL2 levels. There was a significant effect of a disturbed OGTT on the CCL4 serum concentration in the SZ patient group (Table 3; $\beta_{OGTT} = 28.4$, $p = 0.035$) and we therefore checked whether there was a correlation between the CCL4 serum concentrations and fasting glucose serum levels (Spearman correlation). There was no such correlation ($R_s = 0.215$, $p = 0.017$).

3.2. IL-1β, TNF-α, IL-6 and PTX3

We measured significant rises in the serum levels of IL-1β (Fig. 2A: $p < 0.001$), TNF-α (Fig. 2B; $p < 0.001$), IL-6 (Fig. 2C; $p = 0.001$) and PTX3 (Fig. 2D; $p < 0.001$) in SZ patients without the metS in comparison to the HC. The presence of the metS and its components in SZ patients had no further effect on the raised serum levels of IL-1β, TNF-α and IL-6. Decreased HDL had a significant effect on the serum concentration of PTX3 (Table 3: $\beta_{HDL} = -78.6$, $p = 0.05$).

3.3. Leptin

Females with SZ (without the metS) had a significantly higher level of leptin as compared to female HC (Fig. 3A: $p < 0.001$). This increase was not present in male patients, indicating that a disease effect was only noticeable in the female gender. For both genders the additional presence of the metS resulted in higher leptin levels, but values only reached statistical significance for males ($p < 0.001$). Gender and BMI appeared to be components of the metS showing the strongest effect on the serum leptin levels (Table 3: $\beta_{gender} = 73.4$, $p = 0.001$; $\beta_{BMI} = 31.1$, $p = 0.003$).

3.4. Adiponectin

We found a significant increase in adiponectin levels in the serum of SZ patients without the metS as compared to HC (Fig. 3B; $p < 0.001$), indicating an effect of presence of SZ on the expression of this adipokine in serum. SZ patients with the metS had an even more and statistically raised serum level of adiponectin in comparison to patients without the metS. Gender, BMI and TG had the strongest effect on adiponectin serum levels (Table 3: $\beta_{gender} = -9.4$, $p = 0.049$; $\beta_{BMI} = 8.7$, $p = 0.001$; $\beta_{TG} = 6.3$, $p = 0.020$).

3.5. PAI-1

There was no increase of PAI-1 in the serum of SZ patients compared to HC irrespective of the absence or presence of the metS (Fig. 3C). In addition, we did not find any effect of the components of the metS on serum PAI-1 levels (Table 3).

3.6. OPG

We did not find differences in OPG serum levels between HC and SZ patients. However, we did find a significant difference between HC and SZ patients with the metS (Fig. 4A: $p = 0.017$) There was an effect noticeable of a disturbed OGTT on OPG levels (Table 3: $\beta_{OGTT} = 46.9$, $p = 0.001$).

3.7. ICAM-1

Females with SZ and without the metS had a higher level of serum ICAM-1 as compared to female HC ($p = 0.026$). There

Table 3 Effect of components of the metS on serum cytokines/chemokines/adipokines in SZ patients. Generalized linear model to identify the effect of gender, age and components of the metS on the serum cytokine/chemokine/adipokine concentrations within the SZ patient group. A negative beta value corresponding to gender means an increase in cytokine/chemokine/adipokine concentration in males, whereas a positive beta corresponds to an increase of the specific immune compound in females. Similar analysis excluding the 11 current drug-free patients is shown in Table S5.

	Gender Beta (95% CI)	Age[a] Beta (95% CI)	BMI[a] Beta (95% CI)	HDL[a] Beta (95% CI)	TG[a] Beta (95% CI)	OGTT Beta (95% CI)
CCL2 (n = 143)	−3.8 (−53.5, 56.4)	−13.4 (−35.6,9.9)	15.1 (−18.2,44.8)	−15.2 (−70.4,−1.2)	−18.5 (−40.3,7.8)	48.9 (−30.8,142.1)
CCL4 (n = 122)	−14.9 (−30.0, 2.4)	−1.6 (−10.6,8.9)	2.7 (−7.0,12.3)	−5.3 (−22.1,3.7)	0.5 (−8.6,11.9)	**28.4 (−4.1, 52.4)***
IL-1β (n = 137)	−3.3 (−7.5,1.5)	0.8 (−1.3, 3.0)	2.0 (−0.9, 4.8)	−0.3 (−6.9, 1.1)	−1.4 (−4.4, 0.2)	0.7 (−4.9, 6.7)
TNF-α (n = 129)	**−23.4 (−39.6, −5.5)***	2.3 (−6.4, 11.7)	7.9 (−3.9, 19.8)	1.5 (−19.5, 6.8)	−6.9 (−19.6, 0.6)	−1.7 (−26.2, 21.1)
IL-6 (n = 126)	−15.7 (−48.9, 11.4)	2.9 (−6.5, 12.8)	13.2 (−2.1, 35.1)	0.3 (−17.0, 9.3)	−7.3 (−26.1, 3.3)	−12.7 (−43.5, 11.0)
PTX3 (n = 142)	73.3 (−104.8, 292.0)	−8.1 (−79.8, 75.8)	−9.8 (−112.9, 95.3)	**−78.4 (−266.1, −38.8)***	−17.9 (−103.6, 45.0)	−38.9 (−240.4, 146.6)
Leptin (n = 122)	**74.3 (47.1, 103.2)****	−7.1 (−19.1, 3.5)	**31.3 (12.5, 47.6)***	−0.9 (−18.1, 17.2)	−8.3 (−18.8, 2.1)	35.4 (6.6, 75.4)
Adiponectin (n = 122)	**−9.4 (−18.4, −0.5)***	−1.9 (−5.7, 2.6)	**8.7 (2.9, 13.4)****	−1.9 (−15.3, 0.4)	**6.3 (0.9, 12.8)***	7.8 (−19.6, 3.4)
PAI-1 (n = 122)	−0.5 (−1.3, 0.4)	−0.0 (−0.4, 0.4)	0.4 (0.1, 0.8)	0.1 (−0.7, 0.3)	0.2 (−0.1, 0.5)	−0.3 (−1.3, 0.7)
OPG (n = 122)	13.6 (−8.6, 36)	−0.1 (−10.5, 10.5)	7.7 (−6.7, 21.9)	−1.2 (−25.4, 8.9)	2.8 (−8.4, 13.3)	**46.9 (21.6, 76.3)****
ICAM-1 (n = 122)	**−234.7 (−434.4, −13.6)***	−41.4 (−150.3, 68.6)	−65.7 (−195.0, 59.9)	−74.4 (−355.0, −20.2)	−25.7 (−143.5, 41.6)	221.5 (530.9, 101.7)

Values in bold denote a significant effect.
p-Values are depicted as:
* $p < 0.05$.
** $p < 0.01$.
[a] Age, BMI, HDL and TG were standardized as z-scores to correct for the effect size of each cofactor.

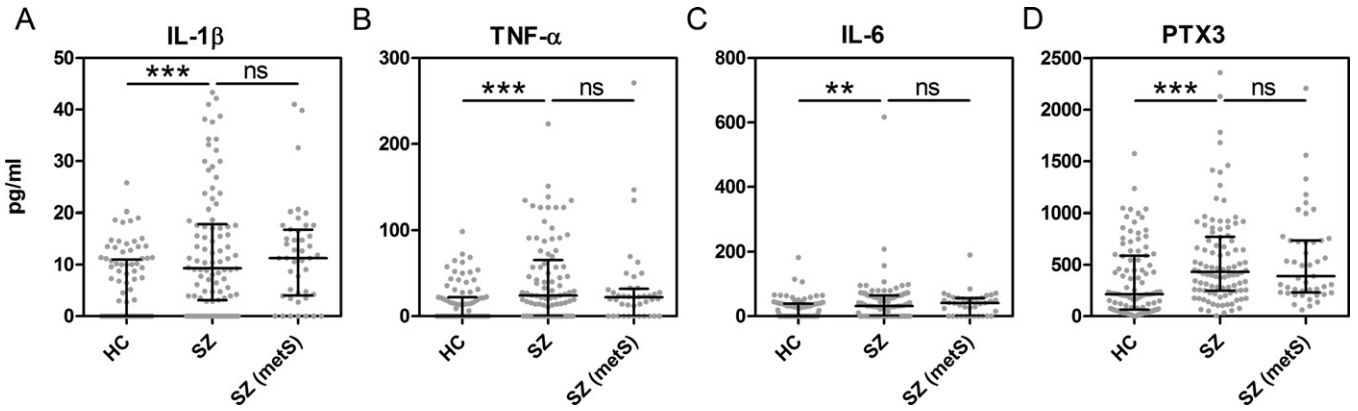

Figure 2 (A) IL-1β, (B) TNF-α, (C) IL-6 and (D) PTX3 serum concentrations. Dots depict individual study subjects, the line represents the median and the whiskers depict the interquartile range (summarized in Table 2). p-Values were obtained by Mann−Whitney U-tests $^{**}p < 0.01$, $^{***}p < 0.001$.

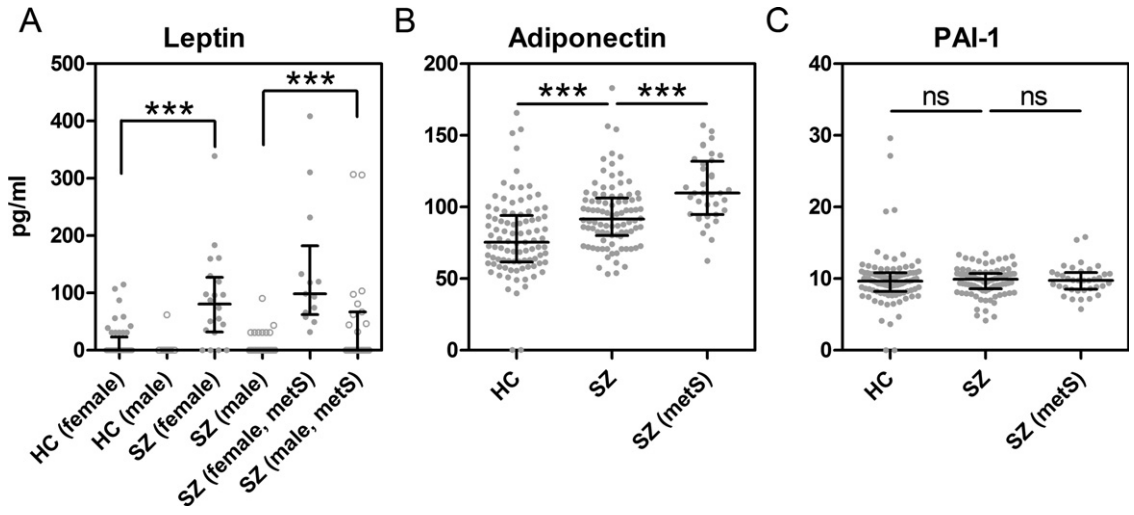

Figure 3 (A) Leptin, (B) adiponectin and (C) PAI-1 serum concentrations. Dots depict individual study subjects, the line represents the median and the whiskers depict the interquartile range (summarized in Table 2). *p*-Values were obtained by Mann—Whitney *U*-tests ***$p < 0.001$.

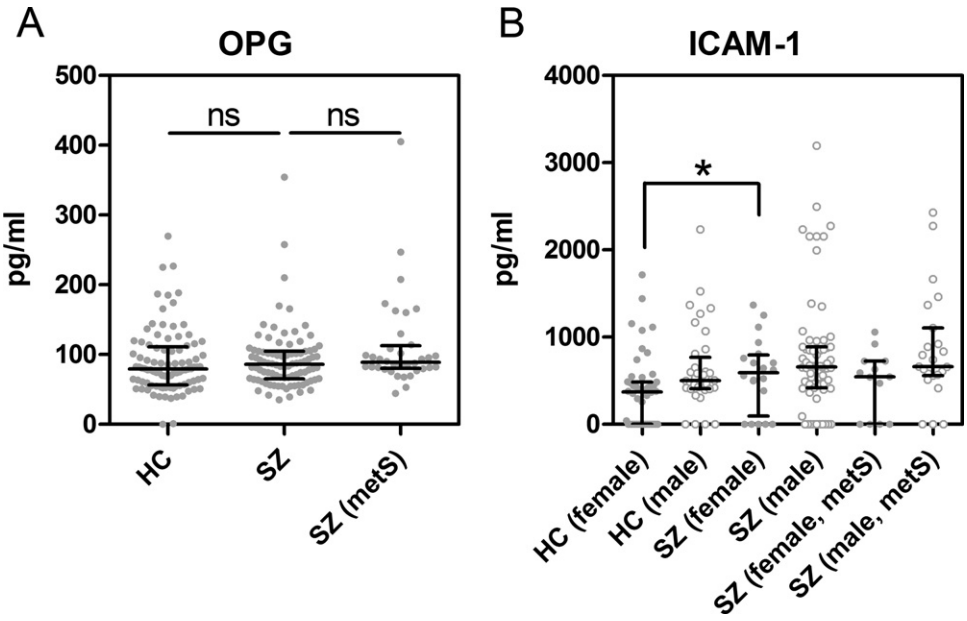

Figure 4 (A) OPG, (B) ICAM-1 serum concentrations. Dots depict individual study subjects, the line represents the median and the whiskers depict the interquartile range (summarized in Table 2). *p*-Values were obtained by Mann—Whitney *U*-tests *$p < 0.05$.

was no difference between the ICAM-1 levels in males, indicating that a small disease effect was only noticeable in the female gender (Fig. 4B). For both genders the additional presence of the metS and its components (Table 3) did not have any effect on ICAM-1 levels.

3.8. Effect of antipsychotic medication

We studied the effect of antipsychotic medication use (users vs. currently 11 non-users) as well as the type of antipsychotic medication (as summarized in Table 1) on the serum cytokine/chemokine/adipokine levels and did not find a correlation with the measured levels (Table S3). The use of antipsychotic medication did correlate weakly, but significantly with HDL ($R_s = -0.174$, $p = 0.03$) and the BMI ($R_s = 0.209$, $p = 0.012$).

Omission of the 11 drug-free cases (Table S4) from the statistical analysis did not change the outcome of the study group comparison shown in Table 3. Furthermore, generalized linear models to study the effect of the components of the metS on the serum cytokine/chemokine/adipokine levels did not reveal any major changes when removing the 11 drug-free patients from the analysis (Table S5). However, the effect of HDL levels on PTX3 serum levels, the effect of TG on serum adiponectin levels and the effect of a positive outcome of the OGTT on CCL4 lost significance.

Table 4 Results synopsis. Synopsis of results obtained from the statistical models.

	Disease	Age	Gender	metS	BMI	TG	HDL	OGTT
Chemokines								
CCL2	+ + +	·	·	+	·	·	·	·
CCL4	+ + +	·	·	·	·	·	·	+ +
Pro-inflammatory cytokines								
IL-1β	+ + +	·	·	·	·	·	·	·
TNF-α	+ + +	·	+ (♂)	·	·	·	·	·
IL-6	+ +	·	·	·	·	·	·	·
PTX3	+ + +	·	·	·	·	·	+	·
Adipokines								
Leptin	+ + + (♀)	·	+ + + (♀)	+ + (♂)	+	·	·	·
Adiponectin	+ + +	·	+ (♂)	+ + +	+ +	+	·	·
PAI-1	·	·	·	·	·	·	·	·
OPG	(+)[b]	·	·	·	·	·	·	+ +
Adhesion factor								
ICAM-1	(+)[a] (♀)	·	+ + + (♂)	·	·	·	·	·

+: Represent the effect of each component on the serum cytokine/chemokine/adipokine concentration. ♀: effect in females, ♂ effect in males.

[a] Not significant after Bonferroni correction.

[b] Significant difference in OPG serum concentration between HC and SZ patients suffering from the metS.

Since the use of antipsychotic medication resulted in a higher BMI (Table S3, $p = 0.012$) and a reduced HDL (Table S3, $p = 0.039$) in our series, the omission of the drug-free patients (and thus of the leanest patients with the most favorable lipid spectrum) might have influenced indirectly the loss of correlation between the lipid levels/OGTT on PTX3, adiponectin and CCL4 levels.

Since it has been found that clozapine treatment increases serum IL-6 concentrations (Maes et al., 1997) and since clozapine mobilized CD34 progenitor cells (Loffler et al., 2010), we tested for clozapine correlation separately in our group of SZ patients. A considerable proportion of SZ patients in this study were taking clozapine ($n = 25$). Spearman correlation indicated that the use of clozapine did not correlate with any of the serum cytokine/chemokine/adipokine levels (Table S6). In addition no differences in serum cytokine/chemokine/adipokine levels between clozapine users and users of other anti-psychotic medication were detected (Table S7).

4. Discussion

Although many studies report increased serum levels of monocyte/macrophage related cytokines IL-1β, IL-6, TNF-α in SZ patients (Drexhage et al., 2010b), the majority of studies did not take confounders such as age, gender, BMI, medication and presence of the metS into account.

This study shows an elevation of the monocyte/macrophage cytokines IL-1β, TNF-α, IL-6, PTX3, the chemokines CCL2 and CCL4 and the adipokines leptin and adiponectin in the serum of patients with chronic schizophrenia (for synopsis of findings see Table 4). Multivariate analysis showed that these elevations were linked to both the disease state itself as well as to confounders such as gender (ICAM-1, leptin, TNF-α and adiponectin), a high BMI (leptin, adiponectin), hyperglycemia (CCL4 and to some extent OPG), reduced levels of HDL/high levels of TG (adiponectin and PTX3) and/or the presence of metS in general (CCL2, leptin and adiponectin). IL-1β and IL-6 were the only cytokines raised in the serum of SZ patients not affected by any of the here studied confounding factors.

PAI-1 was not raised in the serum of chronic schizophrenia patients. Carrizo et al. (2008) did not find significant differences in serum PAI-1 levels between SZ patients and their healthy relatives; however, they did find a strong correlation of PAI-1 levels and BMI and antipsychotic medication use, something we did not find.

OPG was slightly elevated in SZ patients, but only the difference between HC and SZ-metS was significant and a prime role for hyperglycemia in this subgroup of patients is likely (Tables 3 and 4). Hope et al. (2010) did find increased OPG levels in SZ patients, but these authors did not take the presence of the metS into account.

Monocyte migration into the tissues is — besides on the action of chemokines — also dependent on the action of integrins and the ICAM-1 system is important in this. The concentration of the adhesion molecule ICAM-1 was elevated in SZ patients, but with only a p-value of 0.026 and linked to gender. It is questionable whether we should apply Bonferroni testing on this limited array of cytokines/chemokines/adipokines/adhesion factors studied here (in particular since we focused on monocyte/macrophage compounds from a hypothesis-driven approach), but in doing so the significance for ICAM-1 levels was lost (see legend Table 2, not for the other studied compounds). Despite the questionable significance for the rise in ICAM-1, it is in line with results from a previous study (Schwarz et al., 2000), where also increased ICAM-1 levels were detected in SZ patients. However elevations in ICAM-1 were found in that study in medicated patients, while drug-naïve patients did not show such increase. Therefore the authors suggested that the anti-psychotic medication was responsible for the rise in serum ICAM-1 levels. Only a small group of our SZ patients ($n = 11/144$) did not use antipsychotic medication at the time of serum collection, but they had used such medication in the past.

Although we did not find any difference in serum levels of the immune compounds in this study between medicated vs. currently non-medicated chronic SZ patients (including clozapine), it is possible that long term medication in the presently 11 drug-free patients may have had a long lasting stimulating influence on the immune system being responsible for a long-lasting rise in the level of the immune compounds. It must be noted however that antipsychotic medication, has in general an immune suppressive effect (Janssen et al., 2010), confirmed by a meta-analysis of (Miller et al., 2011), who found significantly decreased IL-1β and IL-6 levels after initiation of antipsychotic treatment. Suffice to say that more in depth studies on the effects of anti-psychotic medication on the levels of the here reported cytokines/chemokines/adhesion molecules is indicated.

Our study has several limitations. First, the drug-free group of patients is of insufficient size and insufficient length of — drug-free — time. Secondly, the HC group composed of laboratory and hospital staff was not only younger than the SZ patients and had a lower BMI than the SZ patients, but maybe even more important, they had a higher socioeconomic status (SES) than the SZ patients. Because of the normal BMI, we assume that these healthy hospital and medical faculty staff were not or hardly suffering from the metS, though exact data are not available on the HDL, TG and OGTT values in the healthy control group. In comparison to the levels found in this HC group, the levels CCL4, IL-1β, TNF-α, IL-6, PTX3, leptin (females) and adiponectin were significantly raised in the serum of SZ patients without the metS. If the prevalence of the metS in our healthy controls would have been considerable and would have had an impact, it would have blurred the difference between the two groups (since the metS has an increasing effect on the cytokine/chemokine/adipokine levels). We are therefore confident that our data strongly suggest that the higher expression of CCL4, IL-1β, TNF-α, IL-6, PTX3, leptin and adiponectin are linked to the disease state of SZ itself and not to the presence of the metS.

A low SES was found to be correlated to higher levels of circulating inflammatory markers including IL-6 and TNF-α (O'Connor et al., 2009). However, strong association of smoking, drinking, less exercise and obesity with low SES might explain this increase in inflammatory makers. Schizophrenia is associated with a greater probability of ever daily smoking compared to other mood disorders and the general population (de Leon et al., 2002). Tobacco smoking leads to increased IL-6 levels in the serum; decreased TNF-α however, was found to be associated with smoking in females only (Haddy et al., 2005).

Suffice to say, that our data should be verified in new cohorts with healthy controls matched for age, gender, signs and symptoms of the metabolic syndrome, smoking habits and particularly socioeconomic status.

In a previous study of serum cytokine levels, using the same assay, but in younger (mean age 24) psychotic recent-onset SZ patients with a treatment duration under 3 months, the levels of CCL2, IL-1β, TNF-α, IL-6 and PTX3 were found to be normal (Drexhage et al., 2011). However, we *did* find in this young group of SZ patients an up regulation in the circulating monocytes for CCL2, IL-1β, TNF-α, IL-6 and PTX3 mRNA and we then argued that there must be a control over the excessively up regulated monocyte genes not to result in excessive protein production. The present data suggest that during the development of the disease to the chronic phase, these control factors are lost. However our data did not clearly indicate which conditions were responsible for breaking control, since the presence of the metS did not influence the expression of the studied cytokines apart from that of CCL2, leptin (in males) and adiponectin. It might be that the cumulative treatment with antipsychotic medication resulting in obesity (this report confirmed the correlation of antipsychotic medication with the BMI) has an indirect increasing effect on serum cytokine levels; macrophages in fat are producers of (pro-inflammatory) cytokines including ICAM-1, CCL2, CCL4, IL-1β, TNF-α, IL-6 and adipokines leptin, adiponectin and PAI-1 (Weisberg et al., 2003; Xu et al., 2003). However, we were unable to find an effect of increased BMI on the serum levels of the mentioned cytokines (Table 3). Also in a separately small series study of morbid obese non-SZ patients, we could not find higher serum levels of the above mentioned inflammatory cytokines in comparison to lean controls (van der Weerd et al., 2012).

The diabetes found in antipsychotic treated SZ patients has three outstanding characteristics. First it occurs at a younger age (Cohen et al., 2006a; De Hert et al., 2006). Second, it might develop into a diabetic form of ketoacidosis (DKA) or a hyperglycemic hyperosmolar state (HHS) (Koller et al., 2001, 2003, 2004; Koller and Doraiswamy, 2002). Third, the increased incidence of DKA/HHS notwithstanding, no indications of autoimmune origin were found: antibodies and antibodies to GAD65 are not present in SZ patients with diabetes (Cohen et al., 2005). Although we did not find a direct correlation between CCL4 serum levels and fasting glucose or glucose levels after the OGTT, we did find a small, but significant effect of CCL4 levels on the outcome of the OGTT, indicating that the increase of CCL4 is linked to the presence of impaired glucose tolerance or (pre)-diabetes in SZ patients. In addition, CCL4 was encountered as a predominant T2D-expressed monocyte gene of T2D patients in a preliminary set of gene-finding experiments in monocytes of T2D patients (unpublished data). Collectively these data support a view that CCL4 is an important gene in both SZ and T2D.

In conclusion, chronic schizophrenia patients clearly show an activated monocyte/macrophage system as evidenced by raised serum levels of the chemokines CCL2 and CCL4, the cytokines IL-1β, TNF-α, IL-6 and PTX3, the adipokines leptin and adiponectin. Although many of these immune compounds were found linked to gender, the metS and hyperglycemia/diabetes, the most dominant linkage was found with the disease state of schizophrenia itself supporting our earlier expressed view (studying psychotic patients with recent onset schizophrenia) that immune system activation is a key to understand the pathogenesis of schizophrenia (Drexhage et al., 2011). These findings support the rational for an (add-on) anti-inflammatory treatment in patients with chronic SZ.

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Conflict of interest statement

D. Cohen:

- speakers fee: AstraZeneca, Bristol-Myers Squibb
- Advisory board: AstraZeneca, Bristol-Myers Squibb.
- contribution to congress costs: Bristol-Myers Squibb, Eli-Lilly
- unconditional grant: Eli-Lilly, Janssen-Cilag.

H. A. Drexhage:

- speakers fee: AstraZeneca.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2012.04.001.

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