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An observational study investigating cytokine levels in the cerebrospinal fluid of patients with schizophrenia spectrum disorders

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ABSTRACT

Introduction: The role of immunological mechanisms in the pathophysiology of mental disorders has been discussed with increasing frequency. In this context, especially schizophrenia has become the focus of attention after the discovery of autoimmune encephalitis, which might present with psychotic symptoms. Furthermore, multiple studies have identified associations between infections or autoimmune diseases and schizophreniform disorders. Cerebrospinal fluid (CSF) analysis plays a central role in identifying potential inflammatory processes in the central nervous system. Therefore, the rationale of this retrospective study was the analysis of different cytokines, including interleukin-8 (IL-8) levels, in the CSF of patients with schizophrenia spectrum disorders.
Methods: The authors examined the CSF of 40 patients with schizophrenia spectrum disorders, in comparison to the CSF of a mentally healthy control group of 39 patients with idiopathic intracranial hypertension (IIH). Magnetic bead multiplexing immunoassay was used to retrospectively determine different cytokines in the participants' CSF.
Results: Participants with schizophrenia spectrum disorders had significantly higher IL-8 levels in their CSF than controls (mean ± SD: 41.83 ± 17.50 pg/ml versus 21.40 ± 7.96 pg/ml; $p < 0.001$).
Conclusion: The main finding of this study is the presence of significantly higher IL-8 concentrations in the CSF of patients with schizophrenia spectrum disorders when compared to the control group. This supports the hypothesis that immunological processes may be involved in the pathophysiology of a subgroup of patients with schizophrenia spectrum disorders. However, the study's results are limited by the retrospective design, methodological aspects, and the control group with IIH.

1. Introduction

Schizophrenia is a severe mental disorder that presents psychopathologically with delusions, hallucinations, catatonic movement disorders, or negative symptoms, such as impaired motivation and social withdrawal (Owen et al., 2016). The pathophysiology of the disorder remains largely obscure despite intensive research. A distinction can be made between primary forms, in which high heritability plays an important role (Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2014), and secondary forms, in which the symptoms can be attributed to organic disease or substance abuse (Tebartz van Elst, 2017). Due to the discovery that autoantibody-induced autoimmune encephalitis can mimic schizophrenia, immunological mechanisms in the pathogenesis of secondary forms have been discussed with increasing frequency over the past decade (Dalmau and Graus, 2018; Pollak et al., 2020; Endres et al., 2020a). In this context, several studies have reported associations between infections or autoimmune diseases and schizophrenia spectrum disorders (SSDs) (Eaton et al., 2010; Arias et al., 2012; Hjorthøj et al., 2020). A genome-wide association study identified several gene loci of immune regulation to be most significantly associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., 2014).

In the detection and characterization of immunological processes in mental disorders, the examination of cerebrospinal fluid (CSF) is essential. Due to its proximity to the brain, inflammation can be detected in the CSF by assessing various parameters that may not be linked to

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abnormalities in cerebral magnetic resonance imaging (cMRI) or electroencephalography (EEG). In a recently published study in which the authors evaluated the CSF of 992 patients with schizophreniform and affective psychoses, signs of neuroinflammation with increased white blood cell (WBC) counts, elevated immunoglobulin (Ig)G indices, or CSF-specific oligoclonal bands (OCBs) were detected in 8% of the patients (Endres et al., 2020b). Pro-inflammatory cytokines, which are secreted by activated leukocytes, play an important role in mediating neuroinflammatory processes in the central nervous system (CNS). The overwhelming majority of studies on cytokines in patients with mental disorders have been conducted on serum. For schizophrenia, multiple studies have reported elevated serum levels in IL-6 and IL-8 (e.g., Zhang et al., 2002; Zhang et al., 2004; Boerrigter et al., 2017; Maxeiner et al., 2014). Furthermore, for both cytokines, elevated mRNA expression has been found in postmortem brain tissue (Fillman et al., 2013). IL-8 seems to play a CSF-specific role in brain diseases and has been identified as a cytokine marker for intrathecal inflammatory processes in several neurological diseases (Bielekova et al., 2012). Only a few studies in patients with SSDs have used CSF. Some of them found elevated IL-8 concentrations compared with control groups (Hayes et al., 2014; Gallego et al., 2018), while others did not (Söderlund et al., 2009; Schwieler et al., 2015) (cf. Table 1). Previous meta-analyses have described increased CSF concentrations of interleukin-6 (IL-6) and interleukin-8 (IL-8) in patients with schizophrenia (Wang and Miller, 2018; Gallego et al., 2018; Orlovska-Waast et al., 2019), but the number of studies using CSF material and their comparability are limited.

Selected case-control studies of pro-inflammatory cytokines in CSF are summarized in Table 1.

1.1. Rationale of the current study

The primary objective of this study was to investigate pro-inflammatory cytokines in the CSF of patients with SSDs. A recent meta-analysis of previous studies revealed abnormalities in the form of elevated IL-6 and IL-8 concentrations in patients with schizophrenia, but the studies varied considerably in CSF findings and often contained some kind of bias, such as the representativeness of cases or their comparability to the controls (Orlovska-Waast et al., 2019). Moreover, there is a lack of cytokine studies with a broad determination of other CSF parameters, such as the albumin quotient (AQ), to gain a better understanding of possible pathomechanisms (Gallego et al., 2018). Therefore, the rationale of the current study was to compare a well-characterized group of patients with SSDs and a mentally healthy control group to investigate possible inflammatory processes in the CNS of patients with schizophrenia.

2. Materials and methods

The local ethics committee approved this study as part of a larger research project (Medical Faculty of the University of Freiburg, EK-Fr 609/14). All study participants gave written informed consent before undergoing lumbar puncture.

2.1. Study sample

The patient group consisted of 40 patients with SSDs who underwent lumbar puncture during their acute inpatient stay to exclude secondary organic causes of the disorder. They had to have received prior MRI imaging and be able to give consent. Experienced senior physicians determined the diagnosis and comorbidities according to ICD-10 criteria on admission. Patients with schizophrenia (ICD-10: F20), delusional disorders (ICD-10: F22), brief psychotic disorder (ICD-10: F23) and schizoaffective disorders (ICD-10: F25) were included. The information about diagnosis and comorbidity came from chart review. Patients with neurodegenerative diseases, acute clinical infections, or current substance dependence disorders, as well as neurological or immunological diseases with known brain involvement were excluded from the study. A detailed list is provided in Supplementary Box 1.

As a control group, 39 mentally healthy patients with idiopathic intracranial hypertension (IIH), a non-inflammatory neurological disorder, were included. IIH was diagnosed by expert neurologists primarily according to the modified Dandy criteria (Smith, 1985). Patients with a secondary form of intracranial hypertension, with a psychiatric or other neurological disease (except headaches), or patients who were treated with psychotropic medication were excluded. The control group was described in recently published papers (Kuzior et al., 2020; Runge et al., 2020).

Table 1
Selected CSF cytokine studies comparing patients with schizophrenia spectrum disorders and healthy controls (meta-analyzed in Orlovska-Waast et al., 2019 and Gallego et al., 2018). Not all studies on IL-1 alpha/beta, IL-2 and TGF, neopterin and MIP-1 alpha were listed. *Only 11 cases and 12 controls underwent lumbar puncture. Abbreviations: IL = Interleukin, TNF = Tumor necrosis factor, ELISA = Enzyme linked immunosorbent assay.

	Cohorts	Average age in years (% of males)	Cytokine(s) measured (Method)	Results (Schizophrenia patients vs controls)
Van Kammen et al., 1999	N = 61 schizophrenia or schizoaffective disorder N = 25 controls	38.1 (100) 35.0 (100)	IL-6 (ELISA)	No difference in IL-6
Garver et al., 2003	N = 31 schizophrenia N = 14 controls	34.1 (90) 32.9 (71)	IL-6 (Sandwich ELISA)	Non-significant trend for IL-6
Söderlund et al., 2009	N = 26 schizophrenia N = 30 controls	27.5 (100) 25.4 (100)	IL-1β, IL-6, IL-8. IL-2, IL-4, GM-CSF, IFNγ, TNF-α, IL-5, IL-10 (Sandwich-immunoassay-based protein-array system)	Higher IL-1β, no difference in IL-6 and IL-8, IL-2, IL-4, IL-5, IL-10, GM-CSF, IFNγ, TNF-α not detectable or only in low concentration
Sasayama et al., 2013	N = 32 schizophrenia N = 35 controls	40.8 (63) 41.3 (60)	IL-6 (ELISA)	High IL-6
Hayes et al., 2014	N = 46 schizophrenia N = 35 controls	25.8 (78) 26.4 (60)	IL-6, IL-6R, IL-8, C3, MCP-2, and TNFR2 (ELISA)	Higher IL-8 and MCP-2, no difference in IL-6, IL-6R, C3, and TNFR2
Schwieler et al., 2015	N = 23 schizophrenia or schizoaffective disorder N = 37 controls	36.3 (65) 23.6 (62)	IL-6, IL-8, IFN-gamma, IL-1beta, IL-4: undetectable IL-2, IL-10, IL-18, TNF-alpha, IFN-alpha-2a (Electrochemiluminescence detection, liquid chromatography/mass spectrometry)	Higher IL-6, no difference in IL-8, other cyto-/chemokines not detectable or only in low concentration.
Coughlin et al., 2016	N = 14 schizophrenia N = 16 controls	24.1 (79) 24.9 (56)	IL-6 (V-Plex Custom Human Biomarkers kit)	Higher IL-6
Gallego et al., 2018	N = 10 schizophrenia N = 10 controls	43 (90) 40 (90)	IL-1 β, IL-6, IL-8 (Q-plex Human Cytokine Screen array - ELISA)	Higher IL-1 β and IL-8, no difference in IL-6

Table 2
Clinical and demographic data of patients and controls. Abbreviations: IIH = Idiopathic intracranial hypertension, F = Female, M = Male, SD = Standard deviation, OCD = Obsessive-compulsive disorder, PTSD = Posttraumatic stress disorder, CSF = Cerebrospinal fluid, cMRI = Cerebral magnetic resonance imaging, EEG = Electroencephalography, SSRI = Selective serotonin reuptake inhibitor, SSNRI = Selective serotonin/noradrenaline reuptake inhibitor. Values in bold indicate statistically significant findings ($p < 0.05$).

	Patients (N = 40)	IIH controls (N = 39)	Statistics
Sex	16 M: 24 F	6 M: 33 F	**p = 0.015**, φ = 0.275
Average age ± SD at the time of sampling (age range)	33.6 ± 13.4 (18–65 years)	34.6 ± 12.0 (18–61 years)	p = 0.731, d = −0.078
Diagnoses[a]			
Paranoid schizophrenia [F20.0]	25 (63%)		
Hebephrenic schizophrenia [F20.1]	1 (3%)		
Catatonic schizophrenia [F20.2]	1 (3%)		
Delusional disorder [F22.0]	1 (3%)		
Acute polymorphic psychotic [F23.1]	1 (3%)		
Schizoaffective disorder [F25.X]	11 (28%)		
Course of disease			
First episode	13 (33%)		
Recurrent/chronic	27 (68%)		
Psychiatric comorbidity			
Depression	2 (5%)		
ADHD	5[b] (13%)		
Autism spectrum disorder	1 (3%)		
Other developmental disorders	2 (5%)		
- Mixed developmental disorder	1 (3%)		
- Constitutional dwarfism	1 (3%)		
OCD	1 (3%)		
Borderline personality disorder	1 (3%)		
PTSD	1 (3%)		
Anorexia nervosa in youth	1 (3%)		
Substance dependence in history	3 (8%)		
- Benzodiazepines	2 (5%)		
- Cannabis	1 (3%)		
Neurologic comorbidity			
Seizures	2[c] (5%)		
Concussion	3[d] (8%)		
Migraine	1 (3%)		
Overall	6 (15%)		
Psychotropic drugs at the time of sampling			
SSRI	4 (10%)		
SSNRI	1 (3%)		
Mirtazapine	1 (3%)		
"Typical neuroleptics" with high-potency	3 (8%)		
- Haloperidol	3 (8%)		
"Typical neuroleptics" with low-potency	6 (15%)		
- Pipamperone	3 (8%)		
- Promethazine	1 (3%)		
- Prothipendyl	1 (3%)		
- Levomepromazine	1 (3%)		
"Atypical neuroleptics"	40 (100%)		
- Risperidone	14 (35%)		
- Olanzapine	11 (28%)		
- Amisulpride	10 (25%)		
- Clozapine	9 (23%)		
- Aripiprazole	6 (15%)		
Lithium	7 (18%)		
Benzodiazepine	9 (23%)		
Anticonvulsant	7 (18%)		
Unmedicated	0 (0%)		
Civil status			
Single	30 (75%)		
Married	6 (15%)		
Divorced	1 (3%)		
Widowed	1 (3%)		
Unknown	2 (5%)		
Educational level			
Low	11 (28%)		
Intermediate	7 (18%)		
High	19 (48%)		
Unknown	3 (8%)		
Employment			
Unemployed	7 (18%)		
Others not working	0 (0%)		
Working	13 (33%)		
In training	11 (28%)		
Retired	6 (15%)		
Housewife/-man	2 (5%)		
Unknown	1 (3%)		
Living situation			
Alone	13 (33%)		
With partner/family	11 (28%)		
With parents/custodian	12 (30%)		
Others	4 (10%)		
Family history for any psychiatric disease[e]			
Positive	22 (55%)		
Negative	16 (40%)		
Unknown	2 (3%)		
Number of previous inpatient stays			
None	12 (30%)		
1	6 (15%)		
2	3 (8%)		
3	3 (8%)		
More than 3	14 (35%)		
Unknown	2 (5%)		
Number of suicide attempts			
None	28 (70%)		
1	2 (5%)		
2	4 (10%)		
More than 2	3 (8%)		
Unknown	3 (8%)		

[a] In four cases, an organic genesis of the schizophrenia spectrum disorders was discussed differential-diagnostically (in one patient with questionable anti-NMDA receptor antibodies, in another patient in connection with constitutional dwarfism, and for two patients with elevated anti-thyroid antibodies). One patient with autism since early childhood developed psychotic symptoms with visual hallucinations, ego disturbances, and thought insertion at age 20, so the unusual comorbidity of a schizophrenia spectrum disorder and autism was diagnosed.
[b] Of which one only in childhood.
[c] One patient with drug-induced (>10 years ago) and one with two unclear generalized seizures.
[d] One 7 years before lumbar puncture and two in childhood.
[e] In first-degree relatives.

During the inpatient treatment of patients with SSDs, psychometric scales are routinely administered as part of the clinical diagnostic process. This comprises psychopathological scores based on guidelines published by the German Association for Methodology and Documentation in Psychiatry (AMDP; Arbeitsgemeinschaft für Methodik und Dokumentation, 2018), as well as the Global Assessment of Functioning (GAF; American Psychiatric Association, 2009) and the Clinical Global Impression (CGI) scores (Rush, 2000). The number of suicide attempts and previous inpatient stays, family history and all demographic data were also recorded.

2.2. Cerebrospinal fluid and instrument-based diagnostics

All routine CSF analyses and antineuronal autoantibody testing were performed in the CSF laboratory of the Department of Neurology, University of Freiburg, as described previously (Stich et al., 2015; Endres et al., 2020b). Fixed cell-based assays (Euroimmun®) were used to

detect antineuronal autoantibodies against cell surface antigens (NMDA receptor [R], LGI1, CASPR2, AMPA1/2-R, GABA-B-R) in CSF, while immunoblot (Ravo line assay®) was used for autoantibodies in serum against paraneoplastic intracellular antigens (Yo, Hu, CV2/CRMP5, Ri, Ma1/2, SOX1, Tr, Zic4, GAD65, amphiphysin). After routine diagnostics, residual material was deep-frozen at −80 °C for possible follow-up examinations.

Cytokine levels in the participants' CSF were measured as described in a previous paper reporting cytokine findings of patients with unipolar depression (Kuzior et al., 2020). We used the magnetic bead-based multiplex immunoassay *Human Cytokine Magnetic 30-Plex Panel* (ThermoFisher, Waltham, MA; https://www.thermofisher.com/order/catalog/product/LHC6003M#/LHC6003M, last accessed 21.02.2021) and the corresponding detection system *MAGPIX®* (ThermoFisher, Waltham, MA) to detect cytokine/chemokine levels ("Cytokine 30-Plex Human Panel"). For cytokine measurement, the manufacturer's instructions were followed and CSF cytokines were measured once (not duplicated). The panel was designed for cytokine detection in serum but was used with undiluted CSF in this and previous studies. All measurements with a net median fluorescence intensity lower than the lowest standard of the standard curve of the respective cytokine and a bead count below 20 were set to zero. Only cytokines for which more than half of the samples could be measured above the detection limit were analyzed. All patients with an SSD underwent cerebral MRI, which was assessed by experienced neuroradiologists, as well as EEG, assessed by the attending physicians. Intermittent generalized rhythmic delta- and theta-activity were automatically analyzed using a previously published method (Endres et al., 2017).

2.3. Statistical analyses

All collected data were processed and statistically analyzed with Statistical Package for the Social Sciences (SPSS) software, version 25 (IBM Corp., Armonk, NY). To investigate statistical differences between the patient and control groups, Pearson's Chi-square tests were conducted for categorical variables (e.g., sex and the number of patients with abnormal CSF diagnostics), and two-sided independent-sample *t*-tests were conducted for continuous variables (e.g., age and total protein concentration). All *p*-values below 0.05 were considered statistically significant. The measured cytokine concentrations were tested for normal distribution using the Kolmogorov-Smirnov test. For interferon gamma-induced protein 10 (IP-10), normal distributions could not be assumed, so the nonparametric Mann-Whitney *U* test for independent samples was performed for all group comparisons of cytokines (also in the following sub-analyses). To account for potential influences of sex, ANCOVAs were performed with sex as covariate. Effect sizes were calculated with the use of Cohen's d for unpaired t-tests, φ for chi-squared tests, η^2 for ANCOVAs, and Pearson's correlation coefficient r for Mann-Whitney *U* (Cohen, 1988; Fritz et al., 2012; Lakens, 2013). A post-hoc power analysis for the cytokine parameters using G*Power 3.1.9.7 (Faul et al., 2007) was conducted, and results are noted in the Supplementary Tables 1, 2 and 3. Correlations between cytokines/chemokines, CSF basic parameters (WBC count, total protein, AQ, and IgG index), the above-mentioned psychometric data (AMDP/GAF/CGI scores, number of suicide attempts, and previous hospitalizations) and automatically analyzed IRDA/IRTA rates (before/after hyperventilation, overall) were investigated with Spearman's rank correlation coefficient. The significance level for these correlation analyses was set to $p < 0.05$ due to the exploratory nature of these analyses. The profiles of patients with the most extreme cytokine values of the measurable pro-inflammatory cytokines were described in detail.

3. Results

3.1. Demographic data

The clinical and demographic data of patients and controls are summarized in Table 2. Both groups did not differ in age ($p = 0.731$, d = −0.078), but there was a significant sex difference ($p = 0.015$, φ = 0.275). Most patients in the SSD group were diagnosed with paranoid schizophrenia (63%) or schizoaffective disorder (28%). For psychiatric comorbidities ADHD was most common (13%).

Of the 40 patients, 13 (33%) were experiencing a schizophrenic episode for the first time. All patients were treated with neuroleptic medication at the time of the lumbar puncture procedure. Most patients were single (75%), had a high level of education (48%), and either had a regular job (33%) or were in training (28%). Fifty-five percent of patients had a family history of psychiatric illness, and at least nine (23%) had attempted suicide.

3.2. Cytokine findings

In the CSF samples, the cytokine levels of monocyte chemoattractant protein 1 (MCP-1), IP-10, and IL-8 could be determined. In the control group, one sample for IP-10 and one for MCP-1 were below the detection threshold. The readings of the other cytokines measured were considered unreliable as less than half of the samples were below the detection limit. A significant difference between patients and the control group was only found for IL-8 ($p < 0.001$, r = 0.648; Table 3 and Supplementary Table 1, Fig. 1).

Subsequent analysis was adjusted for the influence of sex, the group difference for IL-8 persisted ($F(1,76) = 48.508$, $p < 0.001$, $\eta^2 = 0.390$), while for MCP-1 and IP-10 no significant group difference was observed again when adjusting for sex (MCP-1: $F(1,76) = 0.109$, $p = 0.742$, $\eta^2 = 0.001$; IP-10: $F(1,76) = 2.420$, $p = 0.124$, $\eta^2 = 0.031$). As for the influence of sex, no significant effect was detected on IL-8 ($F(1,76) = 3.097$, $p = 0.082$, $\eta^2 = 0.039$), MCP-1 ($F(1,76) = 0.679$, $p = 0.173$, $\eta^2 = 0.002$), or IP-10 ($F(1,76) = 1.046$, $p = 0.310$, $\eta^2 = 0.014$) levels.

Even in the sub-sample of only female patients (patients with SSD $n = 24$, IIH controls $n = 33$), similar results were achieved with a significant difference in IL-8 levels ($p < 0.001$, $r = 0.723$) and no significant difference in MCP-1 ($p = 0.783$, $r = 0.035$) or IP-10 ($p = 0.457$, $r = 0.098$) levels (see Supplementary Table 2). When excluding the patient with acute polymorphic psychotic disease, the difference in IP-10 levels is significant ($p = 0.010$, $r = 0.293$; Supplementary Table 3) but does not persist when adjusting for sex ($F(1,75) = 3.241$, $p = 0.076$, $\eta^2 =$

Table 3
Results of the cytokine multiplex assay. Abbreviations: IIH = Idiopathic intracranial hypertension, SD = Standard deviation, MCP = Monocyte chemoattractant protein, IP = Interferon-γ-induced protein, IL = Interleukin. Values in bold indicate statistically significant findings ($p < 0.05$).

	Patients with schizophrenia spectrum disorders (N = 40)	IIH controls (N = 39)	Statistics
MCP-1 (Mean ± SD)	192.99 ± 115.91 pg/ml	182.27 ± 86.71 pg/ml	p = 0.845, r = 0.022
IP-10 (Mean ± SD)	7.41 ± 7.09 pg/ml	9.94 ± 9.69 pg/ml	p = 0.181, r = 0.266
IL-8 (Mean ± SD)	41.83 ± 17.50 pg/ml	21.40 ± 7.96 pg/ml	**p < 0.001**, r = 0.648

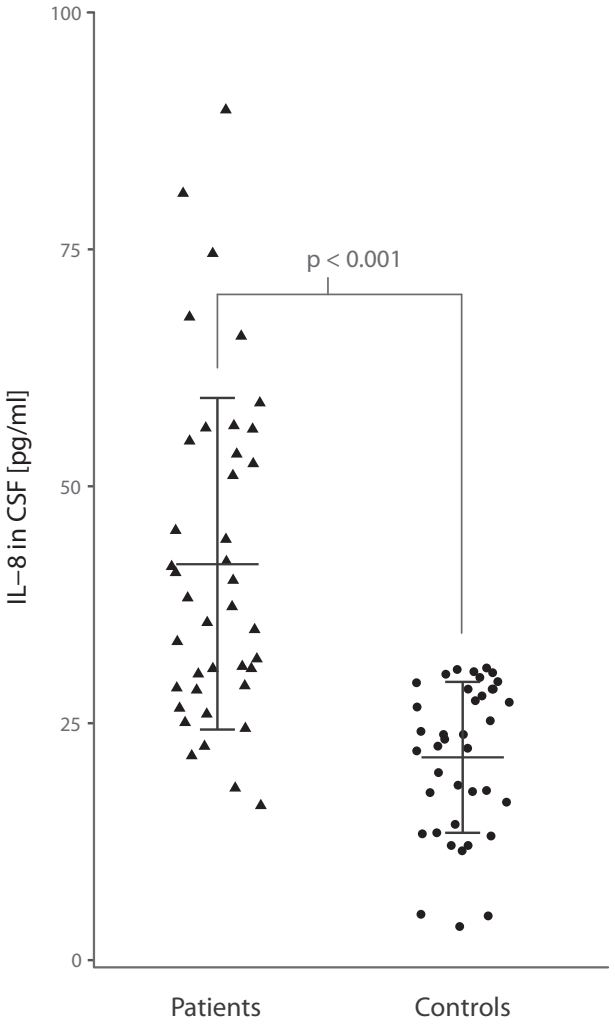

Fig. 1. Cerebrospinal fluid interleukin-8 concentrations in patients with schizophrenia spectrum disorder and controls. Error bars indicate mean value ± one standard deviation. Abbreviations: IL-8 = Interleukin-8, CSF = Cerebrospinal fluid.

0.041). Unchanged, there is a significant effect for IL-8 ($p < 0.001$, $r = 0.642$) and none for MCP-1 ($p = 0.693$, $r = 0.045$).

3.3. Basic cerebrospinal fluid diagnostics and antineuronal autoantibody findings

In the patient group, significantly higher total protein levels ($p = 0.014$, d = 0.565) and AQs ($p = 0.022$, d = 0.525) were detected compared to the control group. Tables 4 and 5 display all results of the basic CSF measurements. Antineuronal autoantibodies were only investigated in the SSD patients. One patient had questionable anti-NMDA-R autoantibodies; therefore, an organic psychosis was discussed differential-diagnostically. All other analyses for antineuronal autoantibodies against cell-surface antigens in CSF (and/or serum) and autoantibodies against intracellular antigens in serum were negative.

3.4. Instrumental diagnostics

Of the patients with SSDs, alterations were identified on more than half of the MRI scans and in one-quarter of the EEG readings. On the MRI scans, mostly non-specific white matter lesions and pineal cysts were noticeable, while most EEG abnormalities were characterized by intermittent delta/theta slow waves. All findings are listed in Supplementary Table 4.

3.5. Correlation analyses

In the correlation analysis, CSF IL-8 concentrations correlated significantly with IP-10 ($r = 0.879$, $p < 0.001$; $N = 40$), MCP-1 ($r = 0.702$, $p < 0.001$; N = 40) and AQ ($r = 0.332$, $p = 0.037$; N = 40). Furthermore, IP-10 concentrations correlated with MCP-1 ($r = 0.733$, $p < 0.001$; N = 40), total protein ($r = 0.346$, $p = 0.029$; N = 40) and AQ ($r = 0.25$, $p = 0.041$; N = 40). MCP-1 correlated with AMDP memory and attention deficits ($r = 0.385$, $p = 0.027$; $N = 33$) as well as fears and obsessive-compulsive behavior ($r = 0.522$, $p = 0.002$; N = 33). Cytokine concentrations and intermittent generalized slow EEG activity did not correlate. All correlations can be seen in Supplementary Tables 5 and 6.

3.6. Characteristics of Interleukin-8 outliers and subanalyses

Since IL-8 concentrations were normally distributed, no clear outliers were identified. Nevertheless, five patients with the highest IL-8 values are presented in detail (Table 6). The two patients with the highest IL-8 concentrations suffered from a chronic course and additional EEG (Case 1) or MRI (Case 2) abnormalities. Case 2 suffered from immunological comorbidity (i.e., psoriasis) and questionable positive anti-NMDA-R autoantibodies. Both patients were treated with clozapine. In a subsequent sub-analysis of patients receiving clozapine at the time of the lumbar puncture procedure ($n = 9$) and patients treated with other neuroleptics ($n = 31$), there were no statistical differences in IL-8 concentrations ($p = 0.332$). IL-8 concentrations in CSF also did not differ significantly ($p = 0.94$) between patients with an initial diagnosis of SSD ($n = 13$) and patients with longer standing SSD ($n = 27$).

When patients with the third- to fifth-highest IL-8 levels were analyzed, no relationships between high IL-8 and sex, age, or medication were apparent. The diagnostic findings were mainly inconspicuous except for slightly elevated total protein in one patient and an unspecific OCB in another. In general, when compared to patients with inconspicuous CSF ($N = 26$), patients with alterations in CSF basic diagnostics (N

Table 4
Cerebrospinal fluid basis diagnostics. Abbreviations: IIH = Idiopathic intracranial hypertension, SD = Standard deviation, IgG = Immunoglobulin G, N = number. Values in bold indicate statistically significant findings ($p < 0.05$).

	Reference (Hufschmidt et al., 2017)	Patients (N = 40)	IIH controls (N = 39)	Statistics
White blood cell count (Mean ± SD)	<5/μl	1.85 ± 1.46	2.60 ± 7.59[a]	p = 0.542, d = −0.138
Total protein (Mean ± SD)	≤450 mg/l	406.45 ± 196.15	309.33 ± 142.5	p = **0.014**, d = 0.565
Albumin quotient (Mean ± SD)	<40y.: 6.5×10^{-3}; 40–60y.: 8×10^{-3}; >60y.: 9.3×10^{-3}	5.02 ± 2.29	3.93 ± 1.81	p = **0.022**, d = 0.525
IgG index (Mean ± SD)	≤0.7 mg/l	0.49 ± 0.04	0.50 ± 0.04	p = 0.196, d = −0.293

[a] Data of only 35 controls are available. One of them suffered from reactive pleocytosis (46 cells/μl), which regressed independently to normal white blood cell counts. The magnetic resonance imaging was normal in this patient. No other cause for the transient, self-limiting pleocytosis was detected in this patient.

Table 5
Number of participants with abnormal cerebrospinal fluid diagnostics. IIH = Idiopathic intracranial hypertension, WBC = White blood cell, SD = Standard deviation, IgG = Immunoglobulin G, OCBs = Oligoclonal bands, CSF = Cerebrospinal fluid, N = Number.

	Patients (N = 40)	IIH controls (N = 39)	Statistics
Increased white blood cell counts (≥5/μl)	3 (8%)	1 (2.9%)[a]	p = 0.372, φ = 0.103
Increased total protein (>450 mg/l)	12 (30%)	6 (15.4%)	p = 0.122, φ = 0.174
Increased age-dependent albumin quotient (<40y.: 6.5×10^{-3}; 40–60y.: 8×10^{-3}; >60y.: 9.3×10^{-3})	6 (15%)	2 (5.1%)	p = 0.146, φ = 0.164
Increased IgG index (>0.7 mg/l)	0 (0%)	0 (0%)	–
CSF specific OCBs	1[b] (0%)	0[c] (0%)	–

[a] Only data of 35 controls are available. One of them suffered from reactive pleocytosis (46 cells/μl) due to repetitive lumbar punctures, which regressed independently to normal WBC counts in the follow-up lumbar puncture. The magnetic resonance imaging was normal in this patient. No other cause for the transient, self-limiting pleocytosis was detected in this patient.
[b] Two findings were not considered positive: One patient had some weak identical bands in CSF and serum as well as another patient had an isolated OCB in the CSF.
[c] Only data of 38 controls was available. Three patients had an isolated OCB which were not considered positive.

= 14) showed a trend toward higher IL-8 concentrations ($p = 0.071$). Concerning blood-CSF barrier (BCSFB) functioning, no significant differences were found in patients with elevated ($N = 6$) and normal ($N = 34$) AQ regarding IL-8 concentrations ($p = 0.234$).

4. Discussion

The main finding of this study is a statistically significantly elevated level of IL-8 in the CSF of patients with SSDs compared to controls.

4.1. Elevated interleukin-8 levels in cerebrospinal fluid

Recent meta-analyses of these studies have suggested an increase in IL-6 and IL-8 levels in the CSF of schizophrenic patients compared to controls (Wang and Miller, 2018; Gallego et al., 2018; Orlovska-Waast et al., 2019). The current study substantiates the finding of elevated IL-8 in CSF in a well-characterized patient cohort. Compared with the previous studies listed in Table 1, this cohort has a more balanced sex ratio (40% males compared with 63–100% males) with an age average similar to those of the other studies. In the past, similar findings, but with less elevated IL-8 values, were reported for patients with unipolar depressive disorders (Kuzior et al., 2020). Therefore, increased IL-8 levels do not seem to reflect a disease-specific change in schizophrenia. No correlations between IL-8 concentrations and the collected psychometric data were observed, which is consistent with the results of previous studies that found no correlation between IL-8 and disease severity (Coelho et al., 2008; Schwieler et al., 2015; Dahan et al., 2018). Interestingly, IL-8 was correlated with AQ. The age-adapted AQ is considered the gold standard for the assessment of BCSFB functioning and may indicate a dysfunction at elevated levels (Reiber and Peter, 2001).

4.2. Interleukin-8 and blood-CSF barrier functioning

Increased AQs in patients with schizophrenia were described previously (Kirch et al., 1992; Endres et al., 2015, 2020b; Orlovska-Waast et al., 2019), and significant differences were also found between the presently investigated patient and control groups. This finding raises the question of whether increased IL-8 concentrations are caused by a passive transfer of IL-8 from the blood into CSF due to BCSFB dysfunction or whether IL-8 is also produced in the CNS. Evidence against the assumption of pure flow effects of IL-8 via a damaged BCSFB is that only one of the five patients with highest IL-8 concentrations displayed a manifest BCSFB dysfunction (Table 6), and no significant differences in IL-8 concentrations were found between patients with and without increased AQs. Furthermore, other studies have shown that in patients with SSDs, IL-8 reaches even greater concentrations in CSF than in serum (Maier et al., 2005; Maxeiner et al., 2014; Boerrigter et al., 2017), and in some studies, it was only detectable in CSF (Schwieler et al., 2015). Therefore, it is also conceivable that vice versa, intrathecally-produced IL-8 passes from the CNS into the bloodstream due to BCSFB dysfunction, resulting in higher IL-8 serum concentrations. Moreover, there are cytokine-specific transporters at the BCSFB, influenced by daily rhythm or brain disease, making it even more difficult to reach reliable conclusions about cytokine correlations (Banks et al., 1995; Banks, 2009). Notably, even a purely traumatic brain injury could lead to an inflammatory process with intrathecal IL-8 production (Kossmann et al., 1997). In SSDs, such mild, low-level neuroinflammation would be compatible with the discussed theory of mild encephalitis in the pathogenesis of schizophrenia (Bechter, 2013, 2020). It is possible that intrathecal inflammation in a subgroup of SSDs leads to increased IL-8 levels and increased AQs. This would explain the correlation between AQ and IL-8, not as a causal relationship, but by a common etiology. Furthermore, it is still discussed whether an increased AQ, which indicates dysfunction of the BCSFB, is caused by leakage of the endothelium or is unrelated to a CSF flow disturbance with reduced CSF drainage (Asgari et al., 2017; Reiber, 1994). Interestingly, fetal exposure to IL-8 due to high maternal IL-8 serum levels was previously associated with increased ventricular CSF in adult patients with SSD (Ellman et al., 2010). Elevated ventricular CSF is, in turn, not only associated with schizophrenia itself (Patterson, 2009) but might also be caused by a disturbance in CSF flow, which could arguably lead to an increased AQ. Nevertheless, the exact pathomechanisms underlying elevated IL-8 levels and increased AQs remain elusive.

4.3. Interleukin-8 in the central nervous system and schizophrenia

IL-8 is an inflammatory mediator in the chemotactic recruitment of leukocytes, especially neutrophil granulocytes, as well as in other processes, such as in the genesis of new blood vessels as a proliferative factor (Brat et al., 2005). In many neurological diseases, it seems to be produced intrathecally with higher concentrations in CSF than in serum (Maxeiner et al., 2014), indicating inflammatory processes of the CNS (Bielekova et al., 2012). In this context, resident immune cells, endothelial cells, astrocytes, and especially microglia might play a key role in intrathecal IL-8 secretion. Interestingly, in the brains of patients with schizophrenia, increased microglial activity has been observed in positron emission tomography studies (Doorduin et al., 2009; van Berckel et al., 2008; Leza et al., 2015), and a higher density of microglia cells has been measured in post-mortem immunohistochemistry (Radewicz et al., 2000; Fillman et al., 2013). In addition, IL-8 may exert more specialized functions in the CNS beyond its classical inflammatory role, such as directly influencing synaptic activities. For instance, it has been found that increased expression of IL-8 in the anterior cingulate cortex stimulates synaptic transmission in this area (Cui et al., 2012), and it was suggested that IL-8 can directly modify ion channels in the CNS by binding to the receptors CXCR1 and CXCR2 (Puma et al., 2001).

Regarding the development of SSDs, increased fetal exposure to IL-8 in the second trimester of pregnancy has been identified as a risk factor

Table 6
Characteristics of patients with schizophrenia spectrum disorder and the highest CSF IL-8 concentrations. Abbreviations: F = Female, M = Male, OCB = Oligoclonal bands, CSF = Cerebrospinal fluid, Il-8 = Interleukin-8, AQ = Albumin quotient, MCP-1 = Monocyte Chemoattractant Protein 1, IP-10 = Interferon-γ-induced Protein.

Pat.	Sex	Age	Diagnoses	Somatic diseases	IL-8 in CSF	Other cytokines	Medication	CSF	Other immuno-logical findings	MRI	EEG	Previous inpatient stays	Course of disease
Pat. (1)	F	Mid 20	Paranoid schizophrenia	Premature birth, craniocerebral trauma in childhood	89.7 pg/ml	Increased MCP-1 (323.4 pg/ml) and high IP-10 levels (10.3 pg/ml)	Amisulpride, clozapine, risperidone	Incon-spicuous	None	Inconspicuous	Fronto-central theta slow waves	5	Chronic
Pat. (2)	M	Mid 30	Schizo-affective disorder	Psoriasis capitis, hypothyroidism, erythema migrans in childhood	80.9 pg/ml	Increased MCP-1 (615.6 pg/ml) and below average IP-10 (8.9 pg/ml)	Clozapine, lithium, escitalopram	Increased total protein (724 mg/l) and increased age-dependent AQ (8.7×10^{-3})1	Question-able positive anti-NMDA-R anti-bodies	Two T2 lesions on the right frontal lobe and right posterior horn	Inconspicuous	9	Chronic
Pat. (3)	M	Mid 30	Paranoid schizophrenia, comorbid ADHD	None	74.6 pg/ml	Increased MCP-1 (290.1 pg/ml) and above average IP-10 (9.9 pg/ml)	Amisulpride	Incon-spicuous	None	Inconspicuous	Inconspicuous	1	Chronic
Pat. (4)	F	≈60	Schizo-affective disorder	Hysterectomy due to myoma, macular degeneration	67.9 pg/ml	Below average MCP-1 (177.0 pg/ml) and average IP-10 (8.7 pg/ml)	Lorazepam, risperidone, lithium, mirtazapine, sertraline	Slightly increased total protein (501 mg/l)	None	Inconspicuous	Inconspicuous	2	First diagnosis
Pat. (5)	F	≈50	Paranoid schizophrenia	None	65.8 pg/ml	Increased MCP-1 (305.1 pg/ml) and increased IP-10 (12.4 pg/ml)	Risperidone	Non-specific OCB in the CSF	None	Slightly enlarged pituitary gland	Inconspicuous	0	Chronic

(Brown et al., 2004). Furthermore, elevated maternal serum IL-8 in the last two trimesters of pregnancy is associated with neuroanatomical abnormalities often linked to schizophrenia (Ellman et al., 2010).

In summary, IL-8 is an important mediator in inflammatory processes and in schizophrenia and other neurological diseases presumably produced intrathecally by activated microglia.

4.4. Other cytokines/chemokines

For MCP-1 and IP-10, no significant differences were found between SSD patients and controls. Interestingly, low concentrations of both cytokines were associated with suicide attempts in a previous study (Janelidze et al., 2013). A negative correlation between psychotic symptoms and MCP-1 concentrations in CSF has also been found (Janelidze et al., 2013). In the current study, these associations were not confirmed; however, we observed a correlation between MCP-1 and memory and attention deficits, as well as fears and obsessive-compulsive behavior, whereas no clinical correlations were found for IP-10. In the current study, IFN-α and IL-6 concentrations could not be measured reliably. Several studies have reported the same problem of undetectable cytokines in CSF material (Söderlund et al., 2009; Schwieler et al., 2015). For the measurement of cytokines, multiple methods are available, but they differ in sensitivity (Keustermans et al., 2013). However, it can be assumed that IFN-α and IL-6 concentrations are likely to be low in the current samples.

5. Limitations

Compared to previous studies of CSF cytokines in SSD, the current study included a relatively large and well-characterized cohort. Nevertheless, important limiting factors must be mentioned, starting with its open and retrospective design. The patients were not examined for study purposes, but as a routine part of the clinical diagnostic work-up to exclude secondary organic causes. Therefore, the diagnosis was determined by experienced physicians according to ICD-10 criteria without structured interviews upon admission. The findings came from a chart review, and subsequent determination according to DSM-5 criteria was not possible retrospectively. Most of the diagnostic criteria of SSD are similar in both classifications, but for certain conditions, such as schizoaffective disorders, there are still discrepancies, which could complicate comparability with other studies. However, patients in our hospital are routinely offered a lumbar puncture as part of the *Freiburg Diagnostic Protocol in Psychosis* (FDPP; Endres et al., 2020c). CSF routine diagnostics—except for severe infectious encephalitis—may have a relatively low sensitivity for neuroinflammation; therefore, cytokine measurement was performed. Following routine diagnostics, residual material was frozen for follow-up examinations. Since the samples were frozen only after routine diagnostics and not directly after lumbar puncture, some cytokines (such as IL-6) may have degraded. However, as discussed above, several other working groups have also reported similar problems with low detection thresholds for cytokines in CSF, depending on the detection technique. Additionally, with regard to multiplex platforms (Luminex-xMAP or Meso Scale Discovery), problems with cytokine samples, especially with low concentrations in blood and CSF, have been reported in the literature (Malekzadeh et al., 2012). The sex difference between patients and controls is noteworthy. In one study, no effects of sex on the measured IL-8 concentration in serum could be detected for IL-8 under regular circumstances, but after cortisol stimulation, higher IL-8 levels were found in men (Da Pozzo et al., 2018). Some sex differences in the production of certain cytokines seem to be due to different stimulation responses of, for example, monocytes (ter Horst et al., 2016). Therefore, the sex ratio difference in the present samples may have led to errors, although it must be mentioned that for several cytokines, including IL-6, no differences in cytokine levels between sexes could be found (Schwieler et al., 2015) and a subsequent analysis of our data showed no influence of sex on IL-8, IP-10 or MCP-1. Other important factors, such as body mass index or smoking, did not seem to cause group differences in cytokine

concentrations in previous studies (Schwieler et al., 2015; ter Horst et al., 2016). Medication may also be a confounding factor, as the SSD group received neuroleptics, while the control group received no psychotropic medication at the time of lumbar puncture. Several studies have found no associations between antipsychotic medication and IL-6 and IL-8 levels in CSF and serum (Zhang et al., 2004; Miller et al., 2011; Tourjman et al., 2013; Schwieler et al., 2015; Gallego et al., 2018), while others reported reduced concentrations of inflammatory cytokines due to neuroleptic treatment (Erbağci et al., 2001; Fillman et al., 2013; Goldsmith et al., 2016). Finally, the use of a control group with non-inflammatory neurological disorder IIH patients must be mentioned. For ethical reasons it is hardly feasible to perform lumbar punctures on healthy individuals. However, the pathophysiology of IIH may have influenced the group differences and may even have led to an over- or underestimation of the pathology in the SSD group. In IIH, some cytokine levels like IL-1β, TNF and IP-10 seem consistent with those observed in healthy controls (Dhungana et al., 2009), while for other cytokines, such as IL-2 and IL-17 in CSF, as well as IL-4, IL-10, IL-12 and IL-17 in serum, elevated levels were reported (Altıokka-Uzun et al., 2015; Edwards et al., 2013). For IL-8 no significant differences in CSF and serum levels in IIH patients compared with patients with other neurological conditions could be found in an earlier study (Edwards et al., 2013). Furthermore, it must be noted that although no IIH patients with a psychiatric diagnosis were included, no structured interview on mental illness was conducted in IIH controls.

6. Conclusions

The main finding of this study is the presence of significantly higher IL-8 concentrations in the CSF of patients with SSDs compared to the control group. This supports the hypothesis that inflammatory processes may be involved in the pathophysiology of a subgroup of patients with SSDs. Further research, including the determination of IL-8 concentrations over the course of the disorder, is necessary to gain a better understanding of this subgroup of SSDs. The additional measurement of IL-8 might be helpful in the future in a multimodal diagnostic work-up for the detection of secondary SSDs.

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Authors' contributions

KR wrote the paper. DE critically revised the first draft. KR, BLF, LTvE and DE organized the study and created the study design. BLF, HK, NMY and SWS performed the cytokine measurements. MAS and KD supervised the laboratory work and critically revised the manuscript. BB and RD performed the CSF basic analyses. KR performed the statistical analyses. SJM and SM supported the statistical analyses. KN and DD revised the manuscript critically focusing on clinical and statistical aspects. All authors were critically involved in the theoretical discussion and composition of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of competing interest

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Appendix A. Supplementary data

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