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# Pronounced immunological abnormalities in unmedicated first episode as compared to chronic schizophrenia patients

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ABSTRACT

There is major evidence for the involvement of immunological processes in the pathophysiology of schizophrenia. Especially alterations of T-cell function and activation of the inflammatory response system appear to be linked to schizophrenia. A mild chronic inflammation process has been proposed and repeated findings of altered serum cytokine levels led to the hypothesis of a TH2 shift or cytokine imbalance in schizophrenia. We investigated serum levels of TH1 and TH2 related cytokines and immune markers in 25 patients suffering an acute schizophrenic episode (all unmedicated, 22 neuroleptica-naïve) at different stages of disorder (18 first episode, FEP; 7 recurrent episode, REP) compared to 25 age and sex matched healthy controls.

In patients, we found an increase of the TH2 system cytokine IL-13 ($p = 0.039$) and a decrease of the TH1 system markers sICAM-1 ($p = 0.011$) and sIL-2R ($p = 0.063$, n. s.). Elevation of the pro-inflammatory cytokine IL-6 was not significant ($p = 0.052$). The effect of sIL-2R decrease was greater in the FEP subgroup ($p = 0.01$) of patients. We found no group differences in the other investigated immune markers: IL-4, IL-8, TNF-alpha, and Interferon-gamma, in which most readings were below the lower detection limit of the respective assay.

Our findings support the notion of a TH1/TH2 imbalance particularly in the acute manifestation phase of schizophrenia. In the long run, this may lead to the identification of cytokine patterns that are applicable as trait or state markers, may be helpful in making or ensuring diagnosis or in monitoring therapy.

## 1. Introduction

Schizophrenia is currently understood as a group of complex debilitating mental disorders with neurodevelopmental and neurodegenerative elements (Keshavan, Nasrallah, & Tandon, 2011; Tandon et al., 2013). Symptoms include disturbances in perception, thinking and emotional responses, but can also include vegetative symptoms and motor symptoms (Tandon, Keshavan, & Nasrallah, 2008; Walther & Strik, 2012). Though schizophrenia varies in course and outcome (Huber, 1997), a typical course of paranoid schizophrenia begins in early adulthood with a prodromal stage with unspecific symptoms, followed by a distinguishable first acute psychotic episode (FEP), a longer recovering period with different outcomes, mostly partial remission, and later relapses in form of recurrent psychotic episodes (REP).

While twin studies suggest a high amount of genetic determination (81%) and smaller but significant common environmental effects (11%) on the liability to develop schizophrenia (Sullivan, Kendler, & Neale, 2003), the exact causes and pathomechanisms of schizophrenia are still unknown. However, there is broad knowledge about the affected systems and structures (Keshavan et al., 2011; Tandon et al., 2008). Known alterations in schizophrenia (Tandon et al., 2008) include structural and functional gray and white brain matter abnormalities, alterations in dopaminergic and glutamatergic pathways and related neural networks, changes in antioxidative defense systems (Yao & Keshavan, 2011), and alterations of immune function (Goldsmith, Rapaport, & Miller, 2016; Khandaker et al., 2015; Müller, Weidinger, Leitner, & Schwarz, 2015).

Indeed, interactions between symptoms of mental disorders and immunological processes have been described for centuries

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(Himmerich, Sorge, Kirkby, & Steinberg, 2012), also causing the first biological approaches in terms of therapy in the early 20th century (Wagner-Jauregg, 1936). Therapeutical approaches in modern times include application of immunomodulatory agents such as COX-2 inhibitors (Müller et al., 2004) or interferon-gamma (Gruber, Bunse, Weidinger, Reichard, & Muller, 2014). A variety of observations in schizophrenia could be integrated once alterations of immune function have been considered as part of the illness, e.g. the observation that the risk to develop schizophrenia increases after a maternal infection during pregnancy (Brown, 2006; Meyer & Feldon, 2009). More recent studies base the assumption of deregulated immune function in schizophrenia on the finding of altered cytokines, the key signaling molecules of the immune system (Nawa & Takei, 2006). Meanwhile, several alterations of cytokines and other immunological markers in the blood could be replicated in the affected individuals (Goldsmith et al., 2016; Müller et al., 2015; Potvin et al., 2008). These findings raised expectations in the use of cytokines or patterns thereof as potential biomarkers for the disease or its course (Cox, Chan, & Bahn, 2015; Lai et al., 2016). However, findings in terms of cytokine alterations in schizophrenia are still heterogeneous, in part even contradictory, and thus the idea of using cytokines as biomarkers for schizophrenia also has been a subject to criticism (Koola, 2016; Potvin et al., 2008). One important factor in this discussion is the potential effect of antipsychotic medication on cytokine patterns (Baumeister, Ciufolini, & Mondelli, 2016; Bresee et al., 2006; Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011). While the earliest studies have been conducted in chronic patients on stable antipsychotic medication (for an overview see Potvin et al. (2008), more recent studies were performed in unmedicated patients (Goldsmith et al., 2016; Upthegrove, Manzanares-Teson, & Barnes, 2014).

In order to better understand the complex interrelation between immune function and the development and outcome of schizophrenia, i.e. its changeability during the course of illness, the investigation of cytokine patterns at different stages of illness seems to be a suitable approach (at-risk phase (Föcking et al., 2016; Smesny et al., 2017); first acute psychotic episode phase (FEP); recurrent episode phase (REP), partial/complete remission or before/after medication phase (Baumeister et al., 2016; Upthegrove et al., 2014) etc.). In the long run, this research aims to identify cytokine patterns that are applicable as trait or state markers and may be helpful in making/ensuring diagnosis or in monitoring therapy (Ritsner & Gottesman, 2009).

In a recently published study (Smesny et al., 2017) we have been investigating a selected number of cytokines and other immune-related soluble receptors in an ultra-high-risk (UHR) population for psychosis and found an influence of omega-3 dietary supplementation on sICAM-1. In the present study we investigated a number of cytokines and other immune-related soluble receptors and mediators in acutely ill schizophrenia patients (n = 25). The study population initially consisted of 99 patients and controls. After excluding all patients with assumed covariates that were thought to have a greater impact on the measured biological markers (see study population/participants in the next section), our remaining selected study population consisted of 18 first episode patients (FEP) and 7 recurrent episode patients (REP), all suffering an acute schizophrenic episode at time of investigation. All selected FEP were neuroleptic naïve, i.e. never treated with any antipsychotic agents. Three patients of the REP group had received prior neuroleptic treatment but were free of neuroleptic medication for at least 7 days at time of investigation. The schizophrenia patients were compared to 25 healthy individuals (HC), matched for age, gender and lifestyle characteristics. Selected cytokines included the well “established” IL-6 as well as uncommon but previously investigated immune markers like sICAM-1 (Schwarz, Riedel, Ackenheil, & Müller, 2000).

We deliberately selected markers of the TH1 and TH2 systems to investigate the repeatedly postulated TH1/TH2 imbalance in acutely ill schizophrenic patients (Avgustin, Wraber, & Tavcar, 2005; Schwarz, Müller, Riedel, & Ackenheil, 2001). TH1 cytokines promote the type 1 cell-mediated immune response especially against microbial pathogens by activating the differentiation of CD4 positive naïve T-cells to the subtype T-helper cells 1 (TH1). TH2 cytokines stimulate the B-lymphocyte maturation and the antigen production (e.g. IgE) as well as the differentiation of naïve T-lymphocytes to the subtype T-helper cells 2 (TH2) after being activated by IL-4 which is secreted by cells of the innate immune system (Potvin et al., 2008).

As representatives of the TH1 system we included sIL-2R, IFN-gamma, and sICAM-1. For the TH2 system we included IL-4, IL-8, and IL-13. These cytokines and immune-related markers can also be classified based on their pro-inflammatory (sIL-2R, IL-6, IL-8, TNF-alpha), respective anti-inflammatory (IL-4, IL-13) effects. IL-6 was included because of its importance in acute (Luo & Zheng, 2016) and chronic inflammatory processes (Chun et al., 2007; Doan & Massarotti, 2005) and because it is one of the most frequently studied cytokines in schizophrenia (Potvin et al., 2008). Experimental studies suggest that IL-6 might inhibit TH1 (Diehl & Rincon, 2002) and promote TH2 responses (Sofi, Li, Kaplan, & Chang, 2009). For further information on each selected cytokine respective immune-related receptor or mediator we compiled additional information in the Supplementary material.

Our hypotheses were (i) that there would be alterations in the signature of the measured immune-related markers in schizophrenia patients, independently from assumed effects of antipsychotic medication. According to previous findings, we expected (ii) a shift towards TH2. Further, (iii) we expected group differences between first and recurrent patient groups.

## 2. Subjects and methods

### 2.1. Study population/participants

The study population initially encompassed 99 persons (acute psychosis patients and healthy controls). Application of the below described strict exclusion criteria resulted in a remaining study population of 25 schizophrenia (SZ) patients (mean age ± SD [years]: 28.1 ± 7.5, gender ratio male/female: 12/13) and 25 healthy controls (HC), matched for age and gender (mean age ± SD [years]: 27.4 years ± 7.5, gender ratio male/female: 12/13). Regarding the stage of the disease, two subgroups of patients were distinguished. The first episode patient (FEP) group included 18 individuals, the recurrent episode group (REP) 7 individuals. At the time of investigation all FEP were drug-naïve (Table 1). In the REP group, 4 patients were drug-naïve and 3 patients had received sporadic neuroleptic medication but were medication-free for at least 7 days prior measurement.

*All patients* suffered an acute schizophrenic episode according to DSM-IV criteria (American Psychiatric Association, 2000). Diagnoses were made by two independent, board certified psychiatrists (SS, BM). Structured interviews were conducted to affirm diagnosis (Wittchen, Zaudig, & Fydrich, 1997). *Healthy volunteers* were recruited by paper advertisement from hospital stuff (therapists, nurses, trainees) or the general population. It was ensured that the control population was similar to patients in terms of education and lifestyle and therefore included students, low-level and mid-level-workers, academics, smokers, and non-smokers.

**Table 1**
Study population/participants, SD: standard deviation, HC: healthy controls, SZ: schizophrenia patients, FEP: first episode psychosis, REP: recurrent episode psychosis.

| | HC | SZ |
|---|---|---|
| Sex (male/female) | 12/13 | 12/13 |
| Age (in years, mean ± SD) | 27.29 ± 7.45 | 28.18 ± 7.63 |
| FEP/REP | | 18/7 |
| neuroleptica-free > 7d | | 25 |
| thereof neuroleptica-naive | | 22 |

**Table 2**
Results of laboratory analyses (ELISA). Lower detection limits, mean, median, and standard deviation per group in [pg/mL]. HC: healthy controls; SZ: patients; N: number of valid data points, SD: standard deviation, Below detection limit: number of data points below the detection limit of the respective assay (those values have been assigned the value 0.00 pg/mL), P: p-value of Wilcoxon-test (2-sided), * statistically significant; $^{T}$ trend.

| Assay | Lower detection limits (pg/mL) | Group | N | Below detection limit | Mean | Median | SD | P |
|---|---|---|---|---|---|---|---|---|
| IL-2R | 0.27 | HC | 25 | 0 | 3.66 | 3.43 | 1.15 | 0.063$^{T}$ |
| | | SZ | 25 | 0 | 3.00 | 2.66 | 1.43 | |
| IL-4 | 0.1 | HC | 21 | 18 | 7.18 | 0 | 20.06 | 0.398 |
| | | SZ | 17 | 10 | 24.86 | 0 | 55.56 | |
| IL-6 | 0.02 | HC | 25 | 6 | 0.52 | 0.09 | 1.31 | 0.052$^{T}$ |
| | | SZ | 24 | 7 | 2.32 | 0.495 | 5.88 | |
| IL-8 | 3.5 | HC | 21 | 15 | 1.15 | 0 | 3.00 | 0.779 |
| | | SZ | 17 | 12 | 0.97 | 0 | 1.90 | |
| IL-13 | 0.73 | HC | 21 | 11 | 8.02 | 0 | 13.69 | 0.039* |
| | | SZ | 18 | 5 | 81.10 | 16.8 | 142.81 | |
| sICAM-1 | 2.2 | HC | 25 | 0 | 414.71 | 416.3 | 82.13 | 0.011* |
| | | SZ | 25 | 0 | 358.31 | 345.4 | 70.64 | |
| IFN-gamma | 0.99 | HC | 21 | 20 | 0.17 | 0 | 0.77 | 0.317 |
| | | SZ | 17 | 17 | 0.00 | 0 | 0.00 | |
| TNF-alpha | 0.06 | HC | 21 | 19 | 0.57 | 0 | 1.98 | 0.273 |
| | | SZ | 17 | 14 | 2.96 | 0 | 9.75 | |

*Exclusion criteria: for patients*: acute suicidal ideation and involuntary hospitalization; *for all participants:* acute or chronic inflammatory or autoimmune disease (as screened for by standard laboratory tests (white blood cell count, C-reactive protein), measurement of body temperature, and obtaining the medical history of the participant); intake of nonsteroidal or steroidal anti-inflammatory drugs; current alcohol abuse; consumption of illegal drugs within the last 8 weeks; IQ below 70 or brain developmental abnormalities as obtained by standard cranial MRI scan; differential diagnoses of bipolar disorder, borderline personality disorder, antisocial personality disorder, psychotic major depression, delirium. It was ensured that none of the healthy controls had a personal or family history of any mental disorder. All participants gave written informed consent.

We excluded 26 patients from the initial sample. 19 patients were excluded because they did not receive the diagnosis of schizophrenia but another diagnosis (mood disorders including schizoaffective disorder and bipolar disorder: 9; other psychosis 3; personality disorders: 3; anxiety disorders: 3; anorexia nervosa: 1). Two patients were excluded because of neuroanatomical malformations obtained by cranial MRI scanning. Two patients were excluded because of heavy drinking. Five patients were excluded because of current drug abuse (all of them used cannabis). Five patients had prior or current neuroleptic medication (2 olanzapine, 1 clozapine, 2 haloperidole). There were some patients who met more than one exclusion criterion (like drug abuse and being on neuroleptic medication).

The study design was approved by the Research Ethics Committee of the University Hospital of Jena.

### 2.2. Blood collection

Fasting morning blood was drawn in sitting position from an antecubital vein into serum separator tubes using a standard Sarstedt® blood collecting system. After clotting, the blood samples were centrifuged (2500 × *g*, 10 min) to obtain serum, and aliquots (1 mL) were immediately frozen and stored at −72 °C until analysis. Biochemical analyses were performed blinded in the laboratory of the Department of Dermatology at the University Hospital Jena. Before analysis, aliquots were thawed at 4 °C overnight and centrifuged once more (2500 × g, 10 min). Repeated freeze-thaw cycles were avoided as recommended by the manufacturer.

### 2.3. Determination of cytokines, cytokine receptors and other immune mediating receptors

Serum levels of sIL-2R, IL-4, IL-6, IL-8, IL-13, sICAM-1, IFN-γ, TNF-α were quantified utilizing commercially available enzyme-linked immunosorbent assay (ELISA) kits (Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions with double determination of each parameter. To determine very low values of IL-4, IL-6, IFN-γ, and sICAM-1, high sensitivity ELISA (Bender MedSystems, Vienna, Austria) were carried out. Manufacturer catalog numbers, lower detection limits of the used test kits and %CV-values are given in the Supplementary material.

### 2.4. Statistical analysis

Statistical analyses were performed using the software package IBM SPSS Statistics 24. Additional graphics were created using the software package Prism 7 by GraphPad Software. Immune marker concentrations were represented as mean and standard deviation.

Tests for distribution (Kolmogorov-Smirnov; Shapiro-Wilk) and variance homogeneity (Levene) were performed for cytokine data and resulted in non-normal distribution and heterogeneous variance. Hence, non-parametric tests were chosen for all following analyses. We performed the nonparametric Wilcoxon-test for dependent samples (matched pairs) to evaluate group differences in cytokine levels. Values below the lower detection limit were treated as zero. Due to limited availability of serum samples we were not able to measure all cytokines in all patients and controls. Missing data have been treated as such and were excluded pairwise during the respective statistical analysis.

In the main initial analysis we compared patients (SZ) with healthy controls (HC). Subgroup analyses were performed between FEP or REP and their respective age and gender matched HC group. Group differences were considered significant at $p < 0.05$.

## 3. Results

### 3.1. Patients vs. healthy controls (SZ vs. HC)

Results of the initial group comparison of all patients and all HC are summarized in Table 2. Results of Wilcoxon-Tests are also provided in Table 2. An elevation of IL-13 ($p = 0.039$) and a reduction of sICAM-1 ($p = 0.011$) in the entire patients group reached significance level. Elevated IL-6 ($p = 0.052$) and diminished sIL-2R ($p = 0.63$) levels in patients did not exceed the chosen threshold for significance. When excluding extreme outliers (IL-13: 565.94 (FEP) pg/mL; IL-6: 23,03 (FEP) and 19.39 (REP) and pg/mL) and their matching healthy controls, the group difference in IL-6 would be significant with $p = 0.049$ and the group difference in IL-13 would remain significant at $p = 0.048$). For a graphical representation see also Fig. 1.

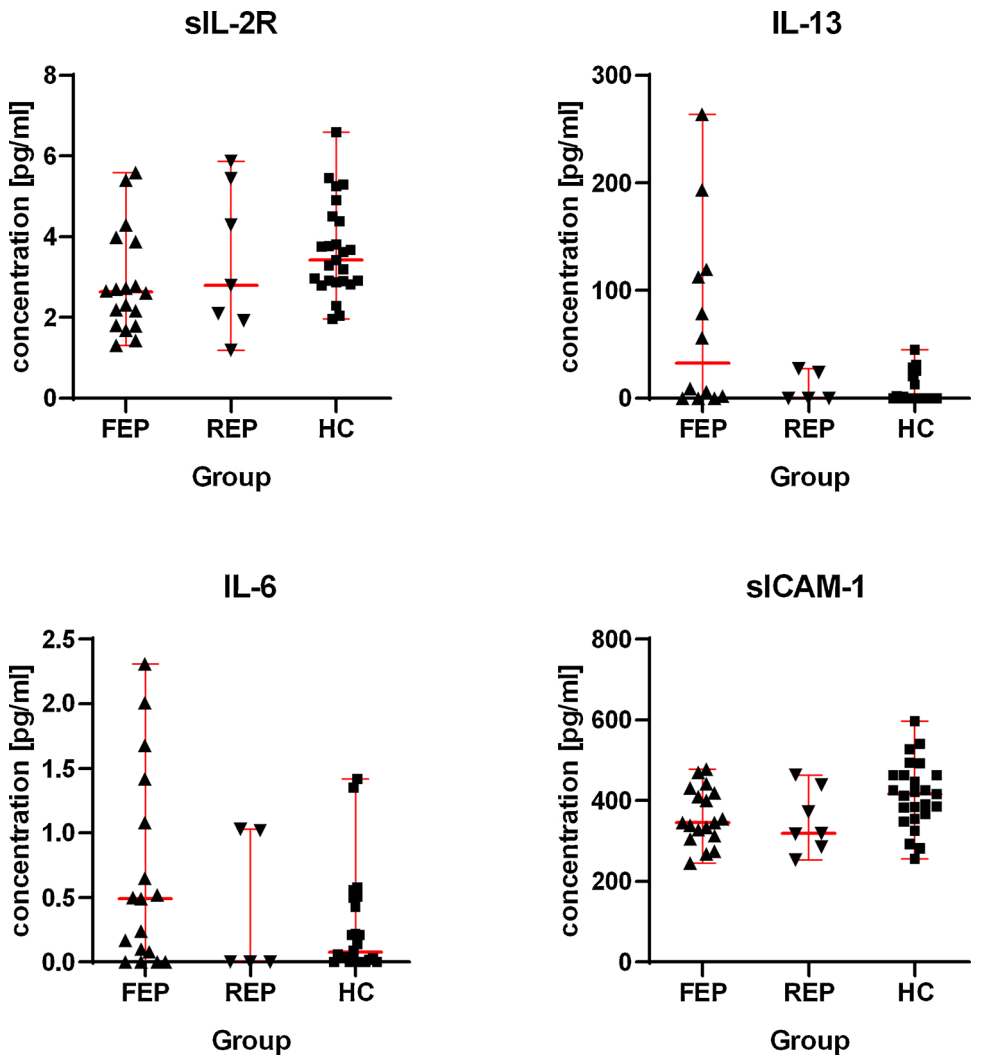


**Fig. 1.** Graphical representation of results of selected markers. Scatter plots; median and range. For better readability, extreme outliers and their matching counterparts in healthy controls have been omitted in this figure (IL-13: 565.94 pg/mL (FEP); IL-6: 23,03 pg/mL (FEP) and 19.39 pg/mL (REP).

In IL-4, IL-8, TNF-alpha, and IFN-gamma, most readings were below the lower detection limit of the respective assay. In IFN-gamma, there were no elevated levels in patients at all.

### 3.2. Subgroup analysis

The comparison of FEP (N = 18) with their respective matched controls (N = 18, Table 3) yielded the same result (increased IL-13, p = 0.026 and reduction of sICAM-1, p = 0.020) as for the overall patient group. Additionally, sIL-2R was significantly diminished (p = 0.010) in the FEP group.

When comparing REP (N = 7) against their respective matched controls (N = 7), we found no significant group differences.

### 3.3. Secondary and negative findings

We also performed a direct comparison (Mann-Whitney-*U* test) between FEP (N = 18) and REP (N = 7) and found no group differences at this sample size.

IFN-gamma: There was only one individual in the healthy control group who had an elevated IFN-gamma value (3.52 pg/mL). All other IFN-gamma values in patients and controls were below the detectable limit.

**Table 3**
Subgroup analysis FEP vs HC Mean, median and standard deviation per group in [pg/mL]. HC: healthy controls; FEP: first episode psychosis; N: number of valid data points; Below detection limit: number of data points below the detection limit of the respective assay (those values have been assigned the value of 0.00 pg/mL); P: p-value of Wilcoxon-test (two-sided); * statistically significant. pg/mL.

| Assay | Group | N | Below detection limit | Mean | Median | SD | P |
|---|---|---|---|---|---|---|---|
| sIL-2R | HC | 18 | 0 | 3.919 | 3.760 | 1.23 | 0.010* |
| | FEP | 18 | 0 | 2.853 | 2.850 | 1.277 | |
| IL-4 | HC | 15 | 13 | 7.925 | 0.000 | 22.63 | 0.249 |
| | FEP | 12 | 6 | 33.49 | 0.010 | 64.68 | |
| IL-6 | HC | 18 | 3 | 0.6606 | 0.175 | 1.522 | 0.171 |
| | FEP | 18 | 4 | 1.904 | 0.495 | 5.323 | |
| IL-8 | HC | 15 | 11 | 0.796 | 0.000 | 2.022 | 0.463 |
| | FEP | 12 | 8 | 1.274 | 0.000 | 2.186 | |
| IL-13 | HC | 15 | 9 | 8.257 | 0.000 | 14.47 | 0.026* |
| | FEP | 13 | 3 | 108.3 | 56.39 | 161.1 | |
| sICAM-1 | HC | 18 | 0 | 423.1 | 419.385 | 66.6 | 0.020* |
| | FEP | 18 | 0 | 361.4 | 345.53 | 69.51 | |
| IFN-gamma | HC | 15 | 14 | 0.2347 | 0.000 | 69.51 | 0.317 |
| | FEP | 12 | 12 | 0 | 0.000 | 0 | |
| TNF-alpha | HC | 15 | 13 | 0.8033 | 0.000 | 3.323 | 0.655 |
| | FEP | 12 | 11 | 2.319 | 0.000 | 11.51 | |

## 4. Discussion

We investigated immune markers in acutely ill schizophrenia patients at different phases of illness. We excluded as best as possible the influence of antipsychotic medication by including (in the FEP and REP group) mainly neuroleptic-naïve patients.

Our main results are a reduction of sICAM-1 and an elevation of IL-13 in the entire patient group. Subgroup analysis of FEP revealed a significant reduction of sIL-2R. We also found an elevation of IL-6 ($p = 0.052$) which was not significant at the chosen threshold for significance. We did not find significant group differences in the other investigated immune markers (IL-4, IL-8, TNF-alpha, IFN-gamma), in which most readings were below the lower detection limit of the respective assay.

Our first finding, the decrease of sICAM-1, is in line with previous studies (Kronig et al., 2005; Schwarz et al., 2000). As sICAM-1 represents a signaling molecule required for the activation of TH1 helper cells (see also Supplementary material) and is therefore representing a marker of the cellular immune system, this finding suggests a weakening of the cellular immune system in schizophrenia. This finding also supports the hypothesized relative shift towards the TH2 immune response in schizophrenia.

IL-13 is a TH2 associated anti-inflammatory cytokine. Therefore, the finding of an elevation of IL-13 in patients suggests TH2 activation. Considering the mild inflammation process presumed in schizophrenia (Bechter, 2013; Maxeiner et al., 2014), this finding could reflect a counter-reaction. This assumption is supported by the previous finding of elevated maternal IL-13 being associated with a higher risk of the offspring to develop schizophrenia (Allswede, Buka, Yolken, Torrey, & Cannon, 2016). Though one previous study found a trend towards higher IL-13 (Maxeiner et al., 2014), to our knowledge, serum IL-13 has not been frequently investigated in schizophrenia populations so far.

Also our third finding, reduced sIL-2R in the drug-naïve FEP, may refer to a relative weakening of the TH1 system (Hilkens et al., 1995). This result supports the pattern proposed by our hypotheses. However, many previous studies reported *increased* sIL-2R levels in schizophrenia patients (Akiyama, 1999; Rapaport & Lohr, 1994; Rapaport et al., 1994; Sirota, Meiman, Herschko, & Bessler, 2005) as described in detail in a recent meta-analysis, which refers to these studies (Goldsmith et al., 2016). As sIL-2R is very sensitive to drug treatment and other influences (Baumeister et al., 2016; Na, Jung, & Kim, 2014) this discrepancy could be caused by different characteristics of the investigated patient populations. Müller et al. found an increasement of sIL-2R after medication, too (N. Müller, Empl, Riedel, Schwarz, & Ackenheil, 1997). Interestingly, they reported no group differences *before* medication. Also, Bresee et al. found increased sIL-2R levels in schizophrenia on stable medication (Bresee & Rapaport, 2009). They refer to previous studies by Rapaport et al. who investigated sIL-2R in different schizophrenia populations, including unmedicated first episode patients and found consistently elevated levels (Rapaport & Lohr, 1994). We cannot plausibly explain the differences between our results and most previous findings. One difference is that Bresee and Rapaport used commercially available tests from R&D Systems, Inc., USA, while we used tests from Bender MedSystems GmbH, Austria. Other technical differences might be found in the preanalytic procedure. From our point of view and our results it is conceivable that reduced sIL-2R occurs only in the acute FEP phase of schizophrenic illness.

Consistent with previous studies (for an overview see Potvin et al. (2008)), we found an elevation of the pro-inflammatory cytokine IL-6, too. However, it was not significant. Since there is evidence that IL-6 is elevated by smoking (Di Nicola et al., 2013), this may be explained by the relatively small amount of smokers in our sample (see also Supplemental material).

Since in this study all subjects of both patient groups suffered an acute psychotic episode, we assume that the found alterations of immune markers are part of the pathology leading to acute exacerbations.

## 5. Limitations of the study

We measured drug-naïve and drug-free schizophrenia patients during a psychotic episode. Due to ethical reasons this naturally leads to a comparably small number of suitable patients during a limited study period. The sample size of 25 vs. 25 therefore has to be seen as a critical factor that affects the sensitivity to detect group differences, and also increases the risk of misinterpretation. This is due to the possibility of random effects which, at this sample size, could affect the results.

We were not able in this study to investigate associations between immune marker concentrations and measures of psychopathology, e.g. the severity of delusional ideas or hallucinations. However, all patients were acutely help-seeking in a routine hospital setting. Our interpretation therefore is limited by the fact that we cannot finally exclude that the reported findings reflect unspecific aspects of acute psychotic episodes, e.g. stress or tension. We need to state that this obviously is a limitation of this research in general, as only a small fraction of studies investigated or reported associations between immune marker findings and symptomatology so far (e.g. only 70 of 1202 or 0.6% of all studies identified by PUBMED search on 2018-02-07 with the keywords *schizophrenia* and *cytokine* also contained the keyword *psychopathology*).

## 6. Conclusion

We conclude that our results—significant reduction of TH1-associated sICAM-1 and elevation of TH2-associated IL-13—support the notion of a TH1/TH2 imbalance in schizophrenia (Schwarz et al., 2001) expressed by a relative cytokine shift in favor of the TH2 system. The result of reduced sIL-2R only in FEP is suggestive that this TH1/TH2 shift is more pronounced in the first acute manifestation phase of illness. We assume that these alterations of immune markers reflect processes inherent to the disease, and that the pathomechanisms underlying these alterations may be stronger during the first acute manifestation of the disease.

In healthy controls and in our schizophrenia patient groups, measurements of IL-4, IL-8, TNF-alpha, IFN-gamma were in general below the detection limit of standard industrial assays. Therefore, these markers may be less suited as candidates for a selection of biomarkers to detect or classify schizophrenia or monitoring therapy.

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The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Authors' contributions

BM had the original idea, selected immune markers based on literature research, screened patients, was responsible for pre-analytics, did statistical analysis and conceptualized and wrote the article. KR screened patients and performed pre-analytics. She also screened literature and prepared the literature database. JH and CM developed laboratory routines and SOPs. JH, KR, CM performed analyses in the laboratory. JH wrote the biochemical methods part and contributed significantly to the overall text and discussion. She also prepared all raw data for statistical analyses. CM did proof-reading and contributed to the text. KL contributed significantly in preparing and processing data and contributed importantly to the final text. UM improved the manuscript significantly and contributed major ideas due to her broad knowledge in this field. PE is the head of the dermatology department and provided the means and structures to perform the measurements. UCH is the director of the laboratory, provided working structures, material, and expertise. SS screened patients, contributed important ideas how to embed the results into a meaningful context, and improved the manuscript. UCH and SS are the senior authors.

## Declaration of Competing Interest

All authors declare that there were no possible conflicts of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.npbr.2019.10.002.

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