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# Reduced superoxide dismutase-1 (SOD1) in cerebrospinal fluid of patients with early psychosis in association with clinical features

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ABSTRACT

Oxidative stress is implicated in the underlying pathophysiology of psychosis from studies of animal models and of tissues obtained from patients. Superoxide dismutase 1 (SOD1) is an antioxidant responsible for reducing free radicals. SOD1 levels in cerebrospinal fluid (CSF) reportedly correlate with those in brain. We hypothesized that patients in early-stages of psychotic disease may have altered SOD1 in CSF compared to healthy controls. We previously reported in a pilot study that SOD1 levels in CSF of patients with recent onset schizophrenia (SZ) were lower compared to healthy controls. Building on that work, in the present study we examined SOD1 levels in CSF acquired from two additional cohorts. Specifically, we studied SOD1 levels in CSF from a cohort of 15 patients with recent-onset psychosis and 18 healthy controls, as well as the second cohort of 18 antipsychotic-naïve patients with SZ and 20 healthy controls. In the first cohort, recent onset of illness was defined as within five years of onset of psychotic symptoms, and performance on neuropsychological testing as well as symptom severity were assessed. We observed 26.5% lower SOD1 in CSF from patients across both cohorts compared to controls ($P = 0.045$) that was consistent with our previous report (30%). Among the cohort of patients with recent onset of SZ, SOD1 in CSF was positively correlated with composite performance on neuropsychological testing. Our results support further study of the relationship between cognitive deficits and oxidative stress in the central nervous system of patients with psychosis, including through study of SOD1.



## 1. Introduction

Several lines of evidence suggest that oxidative stress contributes to the pathophysiology of psychosis and associated cognitive impairment (Emiliani et al., 2014; Fraguas et al., 2012; Hardingham and Do, 2016; Johnson et al., 2013; Landek-Salgado et al., 2016; Owen et al., 2016). Nevertheless, among studies of patients with psychosis, including schizophrenia (SZ), the reported alterations in markers of oxidative stress vary between tissue types, methodology used, and marker of interest (Emiliani et al., 2014; Flatow et al., 2013). Despite the role that oxidative stress is believed to play in the pathophysiology of psychosis, key mediators involved in the process remain elusive.

Among studies on the antioxidant defense system in the central nervous system (CNS) of SZ patients, an important antioxidant, glutathione (GSH) has been estimated in the brain using proton magnetic resonance spectroscopy (Matsuzawa and Hashimoto, 2011). Brain GSH has shown negative correlation with the severity of negative symptoms in patients (Matsuzawa et al., 2008). Another study reported a reduction of GSH in cerebrospinal fluid (CSF) (Do et al., 2000). Further molecular studies are expected to elucidate whether GSH levels in the CSF reflect those in the brains of patients. Complementary studies on oxidative stress markers in animal models for psychosis link altered reduction-oxidation (redox) balance to relevant cognitive and behavioral changes (Cabungcal et al., 2007; Jacobsen et al., 2005; Johnson et al., 2013; Kulak et al., 2012; Steullet et al., 2010).

Superoxide dismutase 1 (SOD1) is a homodimeric metalloenzyme that catalyzes the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen (McCord and Fridovich, 1969; Rosen et al., 1993). Among several antioxidant proteins and markers of oxidative damage, multiple studies have reported altered levels of SOD1 in the blood (e.g., serum, plasma, erythrocyte) from patients with psychosis relative to healthy controls (Akyol et al., 2002; Flatow et al., 2013; Gama et al., 2006; Kunz et al., 2008; Martinez-Cengotitabengoa et al., 2012; Wu et al., 2012, 2014; Zhang et al., 2006, 2007). However, across the data included in the meta-analysis of oxidative stress in SZ, there

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were no replicated correlations between peripheral markers of oxidative stress, including SOD1, and clinical characteristics (Flatow et al., 2013). This finding is in contrast to the strong preclinical evidence that oxidative stress is linked to neurocognitive deficits relevant to psychotic disease (Hardingham and Do, 2016; Johnson et al., 2013).

In contrast, SOD1 levels measured in tissues of the CNS (e.g., brain and CSF) may more directly reflect the pathophysiological processes underlying neuropsychiatric symptoms in SZ than those measured in peripheral tissues. First, SOD1 has a relatively long half-life in the CSF, which is advantageous (Crisp et al., 2015; Winer et al., 2013). Second, levels of SOD1 in CSF have been shown to correlate with those in brain tissue in mouse models of disease (Winer et al., 2013). Therefore, we recently conducted a pilot study of CSF from patients with recent onset of SZ by having a particular focus on SOD1, and reported lower SOD1 in CSF of patients compared to healthy controls (Coughlin et al., 2013). However, studies with independent cohorts are needed to validate further this preliminary observation.

In the present study, we assessed CSF SOD1 levels of patients with psychosis and healthy controls from two distinct cohorts: patients with recent onset of psychosis and antipsychotic-naïve patients with SZ. We also tested the relationship between CSF SOD1 and performance on neuropsychological testing.

## 2. Materials and methods

### 2.1. Participants

#### 2.1.1. Johns Hopkins cohort

All participants at the Johns Hopkins site (referred to as the Hopkins cohort) provided written informed consent. A Johns Hopkins University Institutional Review Board approved this study. Fifteen patients with recent onset of psychosis (defined as within five years of first psychotic symptoms) were recruited from outpatient clinics, inpatient services, and psychiatric day hospitals affiliated with the Johns Hopkins Medical Institutions and from hospitals in the surrounding, Greater Baltimore area. Eighteen healthy controls without history of psychiatric diagnosis were recruited from the Greater Baltimore area. Each participant was evaluated for history of psychotic symptoms and psychiatric diagnosis by a board-certified psychiatrist using the Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (First MB, 2002). All patients completed Scales for the Assessment of Positive and Negative Symptoms (SAPS and SANS) (Andreasen and Olsen, 1982), except for one patient who refused the SAPS and SANS assessment. Reported antipsychotic medication adherence was converted to chlorpromazine (CP) equivalent dose (Andreasen et al., 2010). Participants were excluded for: (a) age below 18 years old or above 35 years old, (b) history of structural brain injury and/or history of traumatic brain injury, (c) any unstable medical condition, (d) history of recent viral infection, HIV or hepatitis, (e) contraindication to lumbar puncture (clotting deficiency, impaired wound healing), and (f) abuse of alcohol or any other drugs of abuse in the past two months with the exception of cannabis or nicotine.

All lumbar punctures were performed at Johns Hopkins Hospital Outpatient Lumbar Puncture Clinic. CSF samples were collected, aliquoted, and stored at −80 °C. Samples were not thawed more than twice before measurement of SOD1.

#### 2.1.2. German cohort

All participants who were recruited from the University of Cologne and the Central Institute of Mental Health in Mannheim (referred to as the German cohort) provided written informed consent. The study protocol was approved by the ethical committees at University of Cologne and the Central Institute of Mental Health in Mannheim. Eighteen anti-psychotic naïve patients with SZ provided CSF through non-traumatic lumbar puncture before initiation of antipsychotic treatment using previously published methods (Leweke et al., 2004). Diagnosis of SZ was defined by DSM-IV criteria. Twenty healthy controls, without history of psychiatric illness, were recruited within the same geographic area (Cologne and Mannheim, Germany). Participants were excluded for contraindication to lumbar puncture (clotting deficiency, impaired wound healing).

All lumbar punctures were performed in accordance with the code of ethics of the world medical association for experiments involving humans (Declaration of Helsinki). CSF samples were collected and stored at −80 °C or lower. Samples were not thawed more than twice before measurement of SOD1.

### 2.2. Western blot for SOD1 levels in CSF

SOD1 levels in CSF were assessed by Western blot analysis, using the published methods previously shown to be in agreement with that of a commercial immune-multiplex assay (Coughlin et al., 2013). Briefly, 20 ul of CSF was mixed with SDS sample buffer (2x: 125 mM Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.005% bromophenol blue) and heated at 37 °C for 30 min. CSF was run on a 4–12% Bis-Tris gel at 125 V and transferred to a polyvinylidene fluoride membrane. The membrane was washed and blocked in 5% milk before incubating overnight with sheep anti-human SOD1 antibody (1:1500 in 5% milk, 574597, Millipore) at 4 °C. The next day the membranes were washed and incubated with donkey anti-sheep IgG-HRP secondary (1:100,000 in 5% milk, sc-2473, Santa Cruz) for 1 h at room temperature. Immunoreactive bands were visualized by the enhanced chemiluminescent substrate (ECL, Pierce) on X-ray film and quantified using the image software TINA (Manchester University). The 17 kDa SOD1 bands were subtracted from background and normalized to standard control CSF samples in order to compare results across several blots. Final results from each individual were reported relative to the mean of the SOD1 measurements from the healthy controls, which was set to 100%.

### 2.3. Neuropsychological assessment

All participants in the Hopkins cohort completed a two-hour battery of neuropsychological tests to assess cognitive function in six domains, namely processing speed, attention/working memory, verbal memory, visual memory, ideational fluency, and executive function, as previously described (Coughlin et al., 2015; Ojeda et al., 2012; Schretlen et al., 2013). A list of the specific tests included in this battery are provided in Supplementary Table 1 and were administered and scored according to standard instructions by the same study team neuropsychologist who was blind to the clinical data of all participants.

Factor scores were calculated for each domain after controlling for age, sex, race and premorbid intelligence (Parmenter et al., 2010; Testa et al., 2009), using the Calibrated Neuropsychological Normative System (CNNS) (Schretlen and Vannorsdall, 2010). Premorbid intelligence was estimated using the Hopkins Adult Reading Test (Schretlen et al., 2009). A composite cognitive score was calculated for each participant by calculating the average value of the six domain scores.

### 2.4. Statistical analysis

Statistical analyses were performed using STATA 13.1 for Macintosh (STATA, College Station, Texas, USA). Group comparisons of demographic and clinical data were calculated using *t*-tests for continuous variables and chi-square or Fisher's exact test for categorical variables. In the Hopkins cohort, we tested for correlation between CSF SOD1 and neurocognitive performance or symptom severity (SAPS, SANS scores) using Pearsons's or Spearman's rank correlation analysis, depending on the normality of the data. Normality of the data was judged based on Shapiro-Wilk test for normality. Data were expressed as mean ± standard deviation. Statistical significance was defined as $P < 0.05$ except for results across the six subdomains of neurocognitive

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