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Diminished treatment response in relapsed versus first-episode schizophrenia as revealed by a panel of blood-based biomarkers: A combined cross-sectional and longitudinal study

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ABSTRACT

There is a paucity of biomarkers for the prediction of treatment response in schizophrenia. In this study, we aimed to investigate whether diminished antipsychotic treatment response in relapsed versus first-episode schizophrenia can be revealed and predicted by a panel of blood-based biomarkers. A cross-sectional cohort consisting of 655 schizophrenia patients at different episodes and 606 healthy controls, and a longitudinal cohort including 52 first-episode antipsychotic-naïve schizophrenia patients treated with the same antipsychotic drugs during the 5-year follow-up of their first three episodes were enrolled. Plasma biomarker changes and symptom improvement were compared between the drug-free phase of psychosis onset and after 4 weeks of atypical antipsychotic drug (AAPD) treatment. In response to treatment, the extent of changes in the biomarkers of bioenergetic, purinergic, phospholipid and neurosteroid metabolisms dwindled down as number of episode and illness duration increased in relapsed schizophrenia. The changes of creatine, inosine, progesterone, allopregnanolone, cortisol and PE(16:0/22:6) were significantly correlated with the improvement of symptomatology. Inosine and progesterone at baseline were shown to be strong predictive biomarkers of treatment response. The results suggest that AAPD treatment response is diminished in the context of relapse, and our findings open new avenues for understanding the pathophysiology of treatment-resistance schizophrenia.

1. Introduction

Many patients with schizophrenia usually relapse multiple times, which not only causes considerable psychological stress to the patient and his or her family, but also leads to an increased social and economic burden (Pennington and McCrone, 2017). Factors that were associated with an increased or decreased risk of relapse included adherence to medication, stress, psychosocial therapies, previous hospitalization/recurrence, and patient insight (Olivares José et al., 2013). Although it is acknowledged that continuous antipsychotic treatment plays a key role in preventing relapse in schizophrenia (Leucht et al., 2012), non-adherence to antipsychotic medication remains the most

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prominent factor in relapse, especially early in the course of the illness (Alvarez-Jimenez et al., 2012).

The clinical significance and outcome of relapse is partially clear. Response to antipsychotic treatment tends to be more pronounced and rapid in the first episode and its optimal dose of antipsychotics is generally lower than in the later stages of schizophrenia (Zhu et al., 2017). This has led to the speculation that subsequent psychotic episodes and active psychosis may be involved in the process of diminished antipsychotic response (i.e., treatment resistance) (Takeuchi et al., 2019). As such, it is noteworthy that a proportion of relapsing schizophrenia patients may not return to their previous level of functioning and may develop treatment resistance (Lieberman, 1996). On the other hand, more questions remain unanswered regarding the potential biological risk of relapse. Active psychotic phases may be neurotoxic and represent a pathological process leading to disease progression and treatment impairment (Emsley et al., 2013). Likewise, relapses representing active psychotic episodes may be associated with disease progression. In a 7-year follow-up study, it was found that 80% of patients with schizophrenia were judged to deteriorate over time and that the degree of deterioration was significantly associated with the number of relapses the patient had (Curson et al., 1985). In another long-term follow-up study, the natural course of the first four episodes of schizophrenia was investigated. Notably, about one sixth (17%) of patients failed to remit after each episode, regardless of which one it was (Wiersma et al., 1998). More direct evidence was obtained in a cohort of 130 patients who took the same antipsychotic medication during their first and second episodes. The results of the study showed a reduced or delayed response to antipsychotic medication during the second episode, despite the effectiveness of the treatment (50% response rate: 48.7 vs. 10.4% at week 7; 88.2 vs. 27.8% at week 27) compared to the first episode of schizophrenia (Takeuchi et al., 2019), suggesting that relapse may be associated with treatment resistance in schizophrenia. To date, the pathogenesis of diminished antipsychotic response in schizophrenia is far from clear. Current hypotheses on its biological basis have focused on alterations in the function of the dopaminergic system (i.e., dopamine receptor hypersensitivity), or changes in the glutamatergic or other neurotransmitter systems (Howes et al., 2012; Schwartz et al., 2012; Galling et al., 2018). However, these hypotheses are not universally accepted and clinical data do not consistently support them (Potkin et al., 2020). Further elucidation of the pathophysiology of relapse and the underlying mechanisms of attenuated antipsychotic drug response may help to better select treatments and inform the development of future treatments. Researches on biomarkers of attenuated antipsychotic drug response and treatment resistance are urgently needed.

Previously, there were several studies done on cytokines and neural biomarkers involved in the pathophysiology of schizophrenia. For example, it has been reported that pro-inflammatory cytokines including interleukin-6 (IL-6), IL-12, tumor necrosis factor-α (TNF-α), IL-1β, and interferon-γ (IFN-γ) were increased in the blood and cerebrospinal fluid (CSF) in first-onset and acute relapse patients with schizophrenia (Na et al., 2014), supporting the role of inflammatory abnormalities in the pathogenesis of schizophrenia. However, evidence from another angle indicates that increased levels of inflammatory biomarkers do not lead to substantially higher schizophrenia risk (Hartwig et al., 2017), and atypical antipsychotic treatment can even be associated with enhanced inflammatory response and comorbid somatic diseases (Na et al., 2014; Scorza et al., 2020). In addition, a recent system review and meta-analysis has suggested that certain antipsychotic drugs might have a lowering effect on serum IL-6, but this effect is not significant despite marked treatment response (Zhou et al., 2021). Together with cytokines, the selectin family of adhesion molecules also plays a role in immune/inflammatory responses in schizophrenia (Meyer et al., 2009). Elevated levels of serum soluble selectins in unmedicated schizophrenia patients as compared with either healthy subjects or depressive patients have been found (Iwata et al., 2007). However, it seems that this abnormality would be worsened by atypical antipsychotic drugs (AAPDs) such as risperidone (Aboul-Fotouh and Elgayar, 2013), and is considered as a biomarker for cardiovascular disease in overweight/obese adults with schizophrenia (Bourassa et al., 2020). As such, AAPD-induced changes in these inflammatory markers may be more related to the metabolic side effects rather than therapeutic effects. S100B is a calcium binding protein mainly produced by glial cells and has been found increased in serum/plasma of patients with schizophrenia, though there were no significant correlations between S100B and psychotic symptoms or cognition (Deng et al., 2018; Kozłowska et al., 2021). It is noteworthy that higher plasma S100B concentrations are independently associated with Inflammatory markers such as IL-17, IL-23 and IL-10, which implicates a pivotal role of inflammatory response and balance to glial dysfunction in patients with schizophrenia (Hong et al., 2016). By contrast, studies investigating the treatment with antipsychotic agents have produced conflicting results, which suggest that antipsychotics might even increase or have no significant effects on circulating S100B levels (Yelmo-Cruz et al., 2013; Aleksovska et al., 2014). Meanwhile, multiple lines of evidence also support a role of the renin-angiotensin system (RAS) in schizophrenia and were suggestive of a therapeutic potential of RAS modulation (Oh and Fan, 2019). Angiotensin-converting enzyme (ACE) converts angiotensin I to the hypertensive peptide angiotensin II, and is considered as the key element of RAS. Most of the studies suggested higher ACE levels in plasma and CSF of schizophrenia patients as compared with healthy subjects, though it is not always the case (Mohite et al., 2018). The increased activity of ACE may be positively associated with cytokines in schizophrenia (Gadelha et al., 2015), but antipsychotic treatment did not normalize ACE levels in CSF/plasma, or represent a relationship between ACE changes and psychotic symptom improvement (Nani et al., 2020; Wahlbeck et al., 1997). Taken together, these abovementioned inflammatory and neural damage biomarkers may be trait-like and not sensitive enough to reflect the improvement of schizophrenic symptomatology after AAPD treatment.

Recently, using an ultraperformance liquid chromatography-mass spectrometry (UPLC-MS/MS)-based metabolomics approach and a multicriteria-assessment strategy (Cai et al., 2017), we identified a panel of biomarkers revealing impaired metabolic pathways in the pathophysiology of schizophrenia shown in Fig. S1. The results suggest that aberrant metabolic underpinnings in schizophrenia can induce oxidative damage by disrupting energy metabolism (creatine-phosphocreatine circuit), purinergic and neurosteroid pathways, leading to increased membrane lipid peroxidation, which can be specifically restored by atypical antipsychotic drug (AAPD) treatment (Cai et al., 2012, 2017). To further investigate the underlying biological mechanisms of diminished antipsychotic drug response in relapse, we developed a quantitative method that allows sensitive and precise monitoring of their changes in circulating blood (Cai et al., 2019). Therefore, we conducted a multicenter pilot study that recruited two independent cohorts: (1) a cross-sectional cohort of patients with schizophrenia of different episodes, together with age- and sex-matched healthy controls, and (2) a longitudinal cohort of patients with first episode and antipsychotic-naïve schizophrenia who were followed up with the same antipsychotic medication for their first three episodes. For patients in both cohorts, biomarker changes in plasma and symptom improvement were compared between the drug-free phase of psychosis onset and after 4 weeks of AAPD treatment.

Our working hypotheses were as follows. (1) diminished response to antipsychotic treatment in patients with relapsed versus first-episode schizophrenia can be revealed by this panel of blood-based biomarkers, and (2) changes and baseline levels of certain biomarkers can predict treatment responsiveness.

2. Materials and methods

2.1. Participants

This project was approved by ethics committees of all the participating sites. Written informed consent was obtained from the participants or their legal guardians. The participants' confidentiality was well protected, and the contents of the recruitment and follow-up forms were not disclosed to any third party.

2.2. Cross-sectional cohort

The flow-chart of the clinical trial design is shown in Fig. 1. Patients aged 15–60 years were recruited from the Second Xiangya Hospital of Central South University, Hunan Provincial Brain Hospital, and Changsha Psychiatric Hospital (Changsha, Hunan, China) between 2016 and 2021. All patients were Han Chinese and met the following inclusion criteria: (1) DSM-5 criteria for schizophrenia, schizoaffective disorder, or schizophreniform disorder (American Psychiatric Association, 2009); and (2) newly hospitalized patients with first-onset/recurrent psychosis and without taking antipsychotic medication for more than one month prior to admission. We excluded patients complicated with Alzheimer's disease, epilepsy, alcohol or drug abuse, or other severe physical or mental illness. Patients who were taking medications for hyperlipidemia or diabetes mellitus were also excluded. In addition, because nicotine is used significantly in schizophrenia population and unfortunately can substantially impact cytochrome 1A2 medication like olanzapine, and can drastically increase inflammatory markers (Sagud et al., 2019), the patients we enrolled were taken care of in the no-smoking wards, and nicotine use /cigarette smoking was prohibited during the antipsychotic treatment period in the hospital. Antipsychotic treatment strategies and doses were determined by experienced psychiatrists who performed initial screening and adhered to a standardized antipsychotic regimen until recovery at week 4. At the end of recruitment, patients received either monotherapy with atypical antipsychotics or combination therapy, with the combination including all the ways in which one drug may be added to another (Goodwin et al., 2009). We recruited healthy subjects who matched the patients in age and gender from the same regions. The healthy subjects had no history of mental disorders and no first- or second-degree relatives with psychotic illnesses.

2.3. Longitudinal cohort

Patients with repeat admissions during the same time period and at the same site were included in the longitudinal cohort without any overlap with participants in the cross-sectional cohort. For the majority of patients, risperidone or olanzapine was administered at the first episode. Therefore, a treatment algorithm was implemented to standardize medication management during their hospitalization. Patients were administered monotherapy (olanzapine/ risperidone) in two phases, as needed and tolerated, for a 4-week observation period: Phase I, low-dose therapy (olanzapine 5–10 mg/day and risperidone 2–3 mg/day), and Phase II, full-dose therapy (olanzapine 12.5–20 mg/day and risperidone 4–6 mg/day). To be included in this cohort, patients must meet the following criteria. (1) a diagnosis of schizophrenia by DSM-5 criteria and a first episode of psychosis successfully treated with olanzapine/risperidone resulting in minimal response criteria [see "Clinical Assessment" section below]; (2) second and third episodes (i.e., relapses [see "Clinical Assessment" section below]) due to nonadherence to treatment after discharge; and (3) in the judgment of the psychiatrist, no modification of the treatment algorithm and dosage for the second and third episodes. Blood samples and Positive and Negative Syndrome Scale (PANSS) symptom presentation were obtained before and after a 4-week treatment period.

2.4. Clinical assessments

In addition to collecting general demographic, clinical and pharmacological information, patients were scored on the PANSS at admission and after 4 weeks of treatment by three senior and experienced psychiatrists (Kay et al., 1987). They were all trained to administer the PANSS to establish good inter-rater reliability (kappa coefficient >0.81). The PANSS total score, three subscales (positive syndrome, negative syndrome, and general psychopathology) and five cluster scores (anergia, thought disturbance, activation, paranoid/belligerence, and depression) were used to assess psychopathological symptoms (Kay et al., 1991). The rate of change (RC) in PANSS score was calculated to assess the symptom improvement with the formula [(PANSS score at baseline-PANSS score at 4 weeks)/ PANSS score at baseline] × 100%. The RCs of 20 and 50% were chosen as the thresholds of response because they correspond to "minimal response" and "good response", respectively (Leucht et al., 2017).

To date, the variation in relapse definitions may be a main source of heterogeneity in comparative effectiveness studies of schizophrenia

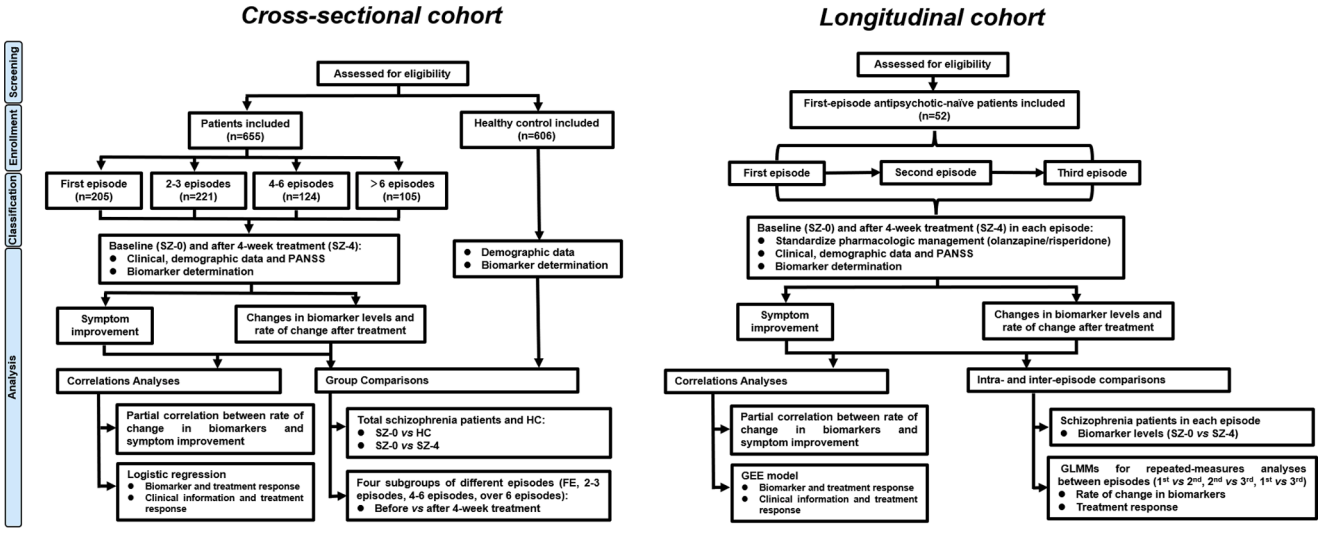

Fig. 1. Flow diagram of the clinical trial. SZ, schizophrenia; HC, healthy control; FE, first episode; PANSS, Positive and Negative Syndrome Scale; GEE, generalized estimating equation; GLMM, generalized linear mixed model.

treatment (Moncrieff et al., 2020; Cristarella et al., 2022). Therefore, in this study, relapse was defined under the prerequisite of discontinuing antipsychotic medication for more than one month prior to psychiatric re-hospitalization, and included the functional manifestation as any of the following: increased level of psychiatric care and exacerbation/re-emergence of psychiatric symptoms with a 25% increase in PANSS total score; deliberate self-harm, clinically significant suicidal or homicidal ideation as judged by the investigator; resulting in clinically significant violence against another person or property (Csernansky et al., 2002).

2.5. Sample collection and biomarker analysis

The blood collection protocol for human subjects was in accordance with the principles outlined in the Declaration of Helsinki (The World Medical Association, 2013). Patients' samples were collected in the morning (approximately 7:00 am) at baseline and 4 weeks after treatment, whereas the samples of healthy controls were collected only once. In addition, because steroid hormones change dramatically throughout the menstrual cycle, for female subjects, we asked for the date of their last menstrual bleeding and restricted sampling to the early follicular period (days 1–7). Also, to avoid potential effects of food on biomarker levels, all patients were provided with a standard diet from the hospital cafeteria each day, and the meal composition is shown in Table S1.

Whole blood in the fasted state was collected in 5 ml of EDTA K_2 vacuum blood collection tubes and then centrifuged at 1200 g for 10 min at 4 °C. The upper plasma layer was transferred to Eppendorf tubes and stored at −80 °C until analysis. As published elsewhere (Cai et al., 2019), we developed an LC-MS/MS method for the simultaneous determination of this panel of blood-based biomarkers, including choline, creatine, hypoxanthine, uric acid, allantoic acid, inosine, allopregnanolone, progesterone, cortisol, corticosterone, lysophosphatidylcholines [LysoPC (16:0), LysoPC (18:1), and LysoPC (18:0)], and phosphatidylethanolamines [PE (16:0/22:6) and PE (18:0/22:6)] in human plasma. Briefly, these biomarkers in plasma were extracted with methyl tert-butyl ether/methanol (1:1, v/v) and separated on a reversed-phase C8 column under gradient elution with a combination of aqueous phase of 5 mM ammonium acetate buffer solution containing 0.1% formic acid and organic phase of acetonitrile/2-propanol (3:7, v/v). Four consecutive time periods were set up to measure each biomarker in either positive or negative ion mode, with specific multiple reaction monitoring transitions set up as appropriate. Calibration curves and quality control samples were inserted at regular intervals and analyzed in batches of 20 samples.

2.6. Analyses

2.6.1. Setting 1: cross-sectional cohort

All data analyses were performed with SPSS (version 23, IBM, Armonk, NY, USA). Chi-square tests were used for comparisons between healthy control (HC) and schizophrenia patient (SZ) groups with respect to gender composition, and between different subgroups by episodes (first episode, 2–3 episodes, 4–6 episodes and more than 6 episodes). To account for the dose of antipsychotic drugs, the defined daily dose (DDD) method was used to calculate the chlorpromazine equivalent dose (Leucht et al., 2016). For all variables of biomarkers, the Kolmogorov-Smirnov test for the assumption of normality in one or more groups was rejected when $p<0.05$, and this finding did not change after data transformation. Therefore, the data were non-normally distributed and the Mann-Whitney U test was used to analyze changes between the HC and SZ groups. The Kruskal-Wallis test was used for subgroup analysis. Group-paired differences between the SZ groups before and after treatment were determined by the Wilcoxon matched-pairs signed rank test. Two-tailed partial correlation tests controlling for age, sex, and chlorpromazine equivalent dose were used to explore possible associations between RCs in biomarkers and PANSS scores [(SZ-baseline - SZ-4 weeks)/SZ-baseline] × 100%]. An ordinal logistic regression model was developed to identify the best predictors of diminished antipsychotic response using RCs of biomarkers as explanatory variables, controlling for age, sex, body mass index (BMI), number of episodes, duration of illness, and equivalent dose of chlorpromazine. Relative risk was estimated by the odd ratio (OR) and its 95% confidence interval (CI). Treatment response as a primary outcome was divided into three categories: poor response (PANSS total score <20% reduction from baseline), minimal response (PANSS total score ≥20 and <50% reduction from baseline), and good response (PANSS total score ≥50% reduction from baseline). Statistical tests were considered significant at $p < 0.05$.

2.6.2. Setting 2: longitudinal cohort

In each episode, levels of biomarkers between baseline and 4 weeks were compared by Wilcoxon matched pairs signed rank test. Associations between RCs of biomarkers and PANSS scores were analyzed by two-tailed partial correlation tests controlling for age, sex, and chlorpromazine equivalent dose. Generalized linear mixed models (GLMMs) for repeated measures analysis were used to compare the mean rates of change in PANSS and biomarker parameters for the first, second, and third episodes of schizophrenia, which included fixed effects for episodes, AAPD type, gender, chlorpromazine equivalent dose, and episode-by-AAPD type interaction terms. In the analysis of response rates for the three consecutive episodes, generalized estimating equation (GEE) models were used for the longitudinal data, including gender and AAPD type as factors, the RCs of biomarkers with significant OR in the cross-sectional cohort, age, BMI, and chlorpromazine equivalent dose as covariates, and number of episodes as within-subjects variables. Unstructured covariance matrices were used to study within-subject correlations. Because no patient in this cohort had a reduction in PANSS total score of less than 20% from baseline, the dependent variable (response rate) was divided into only two categories: a reduction in PANSS total score <50% from baseline was considered poor response, and a reduction in PANSS total score ≥50% from baseline was considered a good response. Estimates of the exponential parameters including OR and Cl were calculated, and the significance level was set at $p < 0.05$.

2.6.3. Setting 3: total cohorts at baseline

Significant metabolites were analyzed at baseline between good responders (≥50% reduction from baseline in PANSS total score) and poor responders (<50% reduction from baseline in PANSS total score) to identify potential predictive biomarkers of treatment responsiveness. Chi-square or Mann-Whitney *U* tests were then used to compare the differences in demographic and clinical characteristics and baseline biomarker concentrations between good and poor responders. A receiver-operating characteristic curve (ROC) analysis was performed to quantify the diagnostic performance of individual biomarker/clinical factor. The values of area under the ROC curve (AUC), cut-off and Youden index were calculated.

3. Results

3.1. Demographic and clinical characteristics of patients

A cross-sectional cohort consisting of 655 (male/female: 298/357) schizophrenia patients (numbers of patients: first-episode antipsychotic-naïve 205; 2–3 episodes 221; 4–6 episodes 124; over 6 episodes 105) and 606 (male/female: 250/356) healthy subjects, and a longitudinal cohort comprising 52 (male/female: 28/24) first-episode antipsychotic-naïve SZ patients were recruited (Fig. 1). The clinical and demographic characteristics of the two cohorts are shown in Tables S2 and S3, respectively.

3.2. Metabolic biomarkers in plasma

3.2.1. Aberrant creatine-phosphocreatine shuttling, purinergic, hormonal and phospholipid metabolism in schizophrenia at onset

As illustrated in Fig. 2, the panel of blood-based biomarkers except uric acid showed significant differences between the SZ and HC groups. Creatine (Cohen's $d = 0.591$, $U = 133{,}481$, $p < 0.001$), inosine (Cohen's $d = 1.802$, $U = 44{,}879$, $p < 0.001$), hypoxanthine (Cohen's $d = 0.822$, $U = 111{,}229$, $p < 0.001$), allopregnanolone (Cohen's $d = 1.05$, $U = 91{,}779$, $p < 0.001$), choline (Cohen's $d = 0.445$, $U = 148{,}627$, $p < 0.001$), PE(16:0/22:6) (Cohen's $d = 0.726$, $U = 120{,}226$, $p < 0.001$) and PE(18:0/22:6) (Cohen's $d = 0.613$, $U = 131{,}260$, $p < 0.001$) were significantly decreased in the SZ group at onset stage. In contrast, allantoic acid (Cohen's $d = 3.226$, $U = 3472$, $p < 0.001$), progesterone (Cohen's $d = 0.511$, $U = 141{,}717$, $p < 0.001$), corticosterone (Cohen's $d = 0.894$, $U = 104{,}868$, $p < 0.001$), cortisol (Cohen's $d = 0.272$, $U = 167{,}593$, $p < 0.001$), and LysoPC(16:0) (Cohen's $d = 0.328$, $U = 161{,}280$, $p < 0.001$), LysoPC(18:1) (Cohen's $d = 0.823$, $U = 111{,}195$, $p < 0.001$) and LysoPC(18:0) (Cohen's $d = 0.612$, $U = 131{,}358$, $p < 0.001$) were significantly increased at baseline.

3.2.2. Variations of the AAPD effects on biomarkers among different episodes

The therapeutic effects of 4-week AAPD treatment on this panel of biomarkers in plasma were confirmed in both the cross-sectional (Fig. 2) and longitudinal cohorts (Fig. 3). Overall, in the cross-sectional cohort, creatine (η^2=8.627, $W = 65{,}501$, $p < 0.001$), inosine (η^2=7.600, $W = 87{,}851$, $p < 0.001$), hypoxanthine (η^2=8.994, $W = 57{,}841$, $p < 0.001$), uric acid (η^2=11.036, $W = 17{,}771$, $p = 0.022$), allopregnanolone (η^2=9.907, $W = 39{,}412$, $p < 0.001$), PE(16:0/22:6) (η^2=9.764, $W = 42{,}235$, $p < 0.001$) and PE(18:0/22:6) (η^2=9.272, $W = 52{,}128$, $p < 0.001$) were significantly increased after treatment. Meanwhile, progesterone (η^2=15.359, W=−56,240, $p < 0.001$), corticosterone (η^2=15.995, W=−66,209, $p < 0.001$), and cortisol (η^2=16.097, W=−67,780, $p < 0.001$) were significantly reduced at week 4. During the treatment period, we did not find significant changes in allantoic acid, choline, LysoPC(16:0), LysoPC(18:1) and LysoPC(18:0). Fig. S2 shows the results of the subgroup analyses of the cross-sectional cohort. The effect of AAPD medications on creatine was persistent in all four subgroups (>6 episodes), the effect on inosine and hypoxanthine was significant in the first three subgroups (≤6 episodes), and that the effect on PE(16:0/22:6) and PE(18:0/22:6) occurred mainly in the first two subgroups (≤3 episodes). Elevations in uric acid and allopregnanolone only appeared in the first-episode subgroup. In addition, decreases in progesterone, corticosterone, and cortisol after treatment also occurred only in the first-episode subgroup.

Accordingly, as indicated in Fig. 3, the biomarkers that significantly increased in response to 4-week AAPD treatment in the longitudinal cohort were as follows: (1) creatine (1st, η^2=3.207, $W = 1338$, $p < 0.001$; 2nd, η^2=3.117, $W = 1358$, $p < 0.001$; 3rd, η^2=8.146, $W = 496$, $p < 0.001$), inosine (1st, η^2=3.126, $W = 1356$, $p < 0.001$; 2nd, η^2=4.416, $W = 1092$, $p < 0.001$; 3rd, η^2=8.218, $W = 486$, $p < 0.001$), hypoxanthine (1st, η^2=3.090, $W = 1364$, $p < 0.001$; 2nd, η^2=3.627, $W = 1248$, $p < 0.001$; 3rd, η^2=8.146, $W = 496$, $p < 0.001$), PE(16:0/22:6) (1st, η^2=4.052, $W = 1162$, $p < 0.001$; 2nd, η^2=5.008, $W = 984$, $p < 0.001$; 3rd, η^2=8.808, $W = 406$, $p < 0.001$) and PE(18:0/22:6) (1st, η^2=3.428, $W = 1290$, $p < 0.001$; 2nd, η^2=4.119, $W = 1149$, $p < 0.001$; 3rd, η^2=9.952, $W = 258$, $p = 0.010$) in all the three episodes; (2) allopregnanolone (1st, η^2=3.068, $W = 1369$, $p < 0.001$) and uric acid (1st, η^2=3.037, $W = 1376$, $p < 0.001$) in the first episode. The biomarkers with reduction after AAPD treatment included corticosterone (1st, η^2=25.621, W=−1252, $p < 0.001$; 2nd, η^2=26.006, W=−1282, $p < 0.001$; 3rd, η^2=14.585, W=−268, $p = 0.008$), choline (1st, η^2=27.257, W=−1378, $p < 0.001$; 2nd, η^2=17.739, W=−579, $p = 0.008$), allantoic acid (1st, η^2=27.178, W=−1372, $p < 0.001$), progesterone (1st, η^2=26.006, W=−1282, $p < 0.001$), and cortisol (1st, η^2=25.036, W=−1206, $p < 0.001$) in various episodes. LysoPCs remained at high levels and were not reduced by AAPD in any of the three episodes. In the repeated-measures analysis of GLMM, the RCs of biomarkers in response to AAPD showed significant differences across episodes. Compared to

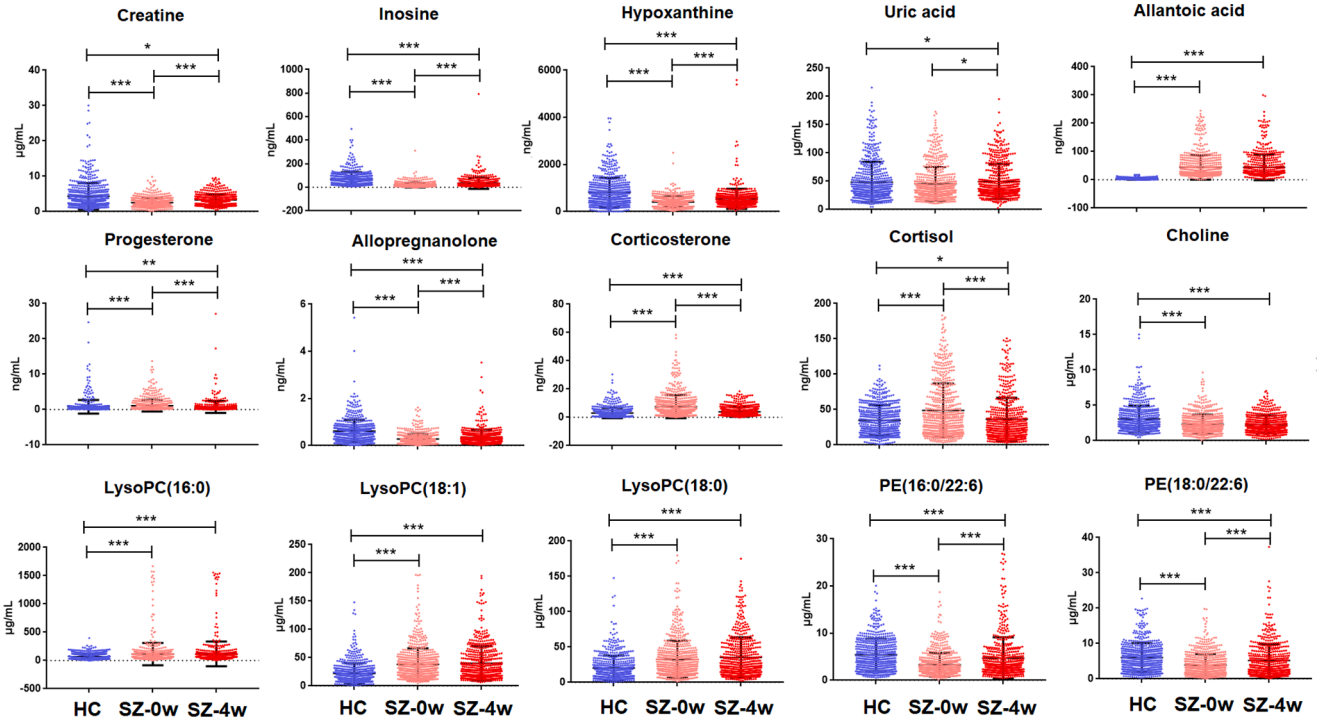

Fig. 2. Differences in the levels of a targeted panel of blood-based biomarkers between patients with schizophrenia at baseline (SZ-0w) and healthy controls (HC) as well as changes after 4 weeks of antipsychotic treatment (SZ-4w) of the cross-sectional cohort. Scatter dot plots are shown with error bars of the means and standard deviations; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

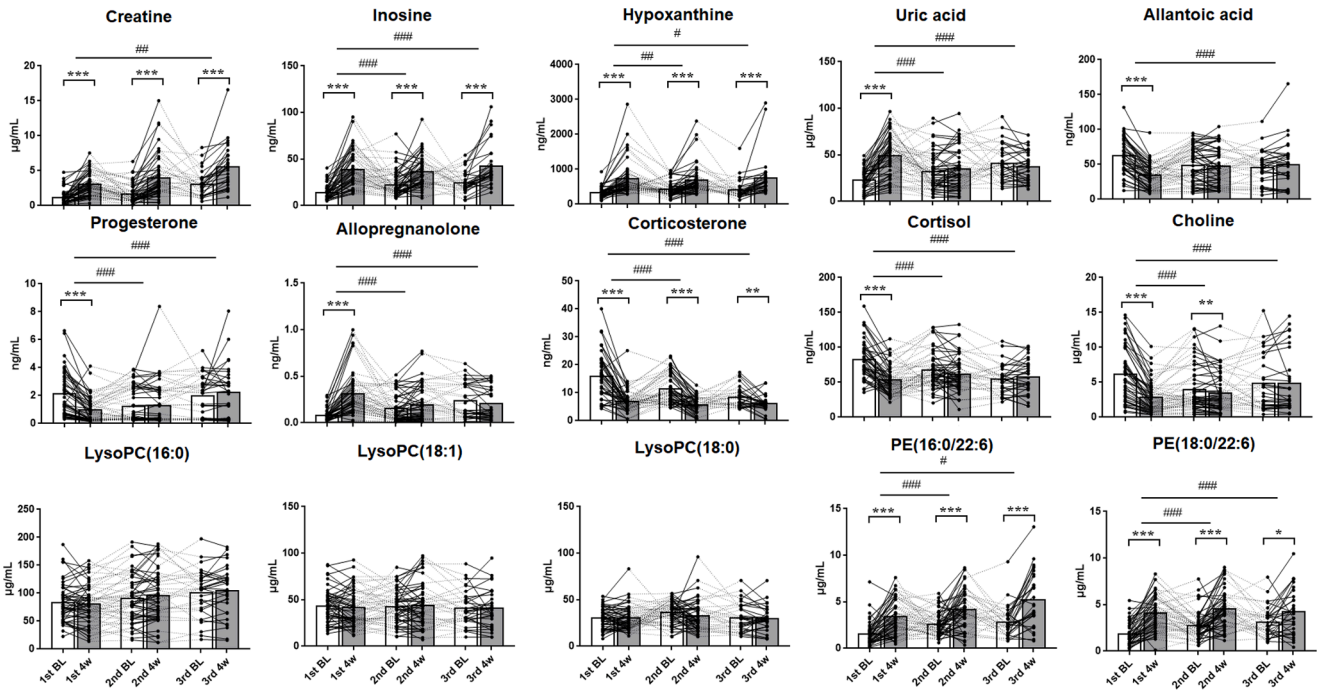

Fig. 3. Effects of atypical antipsychotic monotherapy on the panel of blood-based biomarkers in the longitudinal cohort of first-episode antipsychotic-naïve schizophrenia patients during the follow-up of their first three episodes. Before-after comparisons within each episode using Wilcoxon matched-pairs signed rank test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; between episode comparisons by generalized linear mixed models for repeated-measures analysis, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$.

the first episode, the third episode showed a significant decrease in creatine (contrast estimate=0.933, $t = 3.012$, $p = 0.003$), inosine (contrast estimate=1.537, $t = 4.870$, $p < 0.001$), hypoxanthine (contrast estimate=0.122, $t = 2.373$, $p = 0.019$), uric acid (contrast estimate=1.455, $t = 8.421$, $p < 0.001$), allantoic acid (contrast estimate=−0.533, t=−7.696, $p < 0.001$), progesterone (contrast estimate=−0.667, t=−7.026, $p < 0.001$), allopregnanolone (contrast estimate=4.541, $t = 5.343$, $p < 0.001$), corticosterone (contrast estimate=−0.312, t=−4.723, $p < 0.001$), cortisol (contrast estimate=−0.391, t=−5.426, $p < 0.001$), PE(16:0/22:6) (contrast estimate=0.948, $t = 2.593$, $p = 0.011$) and PE(18:0/22:6) (contrast estimate=1.659, $t = 5.415$, $p < 0.001$). The responsiveness of biomarkers was also significantly reduced in the second versus the first episode, including inosine (contrast estimate=1.406, $t = 5.324$, $p < 0.001$), hypoxanthine (contrast estimate=0.658, $t = 3.133$, $p = 0.002$), uric acid (contrast estimate=1.243, $t = 8.470$, $p < 0.001$), progesterone (contrast estimate=−0.485, t=−5.676, $p < 0.001$), allopregnanolone (contrast estimate=3.865, $t = 5.875$, $p < 0.001$), corticosterone (contrast estimate=−0.288, t=−4.358, $p < 0.001$), cortisol (contrast estimate=−0.274, t=−4.452, $p < 0.001$), PE(16:0/22:6) (contrast estimate=1.058, $t = 3.695$, $p < 0.001$) and PE(18:0/22:6) (contrast estimate=1.085, $t = 4.386$, $p < 0.001$). The differences in biomarker responses between the third and second episodes did not approach significance.

3.3. Symptomatology among different episodes

3.3.1. Cross-sectional cohort

The number of episodes was negatively correlated with the decrease in PANSS total score (r=−0.2041, $p < 0.001$, Fig. S3a). The proportion of good responses was highest in the first-episode subgroup (Fig. S3b). In addition, first-episode patients showed the greatest improvement in positive, negative, general psychopathology, anergia, paranoia/belligerence, depression, and PANSS total scores compared to other episode subgroups (Fig. S3c).

3.3.2. Longitudinal cohort

As with the findings in the cross-sectional cohort, the number of episodes was also negatively associated with a reduction in PANSS total score in the longitudinal cohort (r=−0.6131, $p < 0.001$, Fig. S4a). The proportion of good responses was highest at their first episode (good responses/total: 46/52) and lowest at their third episode (good responses/total: 12/52) (Fig. S4b). The same schizophrenia patients showed the greatest improvement in positive, negative, general psychopathology, thought disturbance, paranoid/belligerence, depression and PANSS total scores across episodes (Fig. S4c).

3.4. Correlations between biomarker changes and symptom improvement

3.4.1. Partial correlation analysis

Fig. S5a shows the results of the 2-tailed partial correlation analysis for the cross-sectional cohort controlling for sex, age, and chlorpromazine equivalent dose. The heatmap indicates that creatine (r=−0.093, $p = 0.013$), inosine (r=−0.090, $p = 0.006$), progesterone ($r = 0.083$, $p = 0.011$), allopregnanolone (r=−0.099, $p = 0.002$), cortisol ($r = 0.075$, $p = 0.022$), PE(16:0/22:6) (r=−0.098, $p = 0.003$) and PE(18:0/22:6) (r=−0.127, $p < 0.001$) were significantly associated with the RC in PANSS total score, as well as with the RCs in several other PANSS subscale scores.

These correlation results were partly replicated in the longitudinal cohort, as depicted in Fig. S5b. The RCs in creatine (r=−0.488, $p < 0.001$), inosine (r=−0.318, $p < 0.001$), uric acid (r=−0.469, $p < 0.001$), allantoic acid ($r = 0.391$, $p < 0.001$), progesterone ($r = 0.437$, $p < 0.001$), allopregnanolone (r=−0.527, $p < 0.001$), cortisol ($r = 0.410$, $p < 0.001$) and PE(16:0/22:6) (r=−0.244, $p = 0.004$) were significantly correlated with the RC in PANSS total score.

3.4.2. Regression modeling analysis

For the cross-sectional cohort, the ordinal logistic regression model (Fig. 4a) revealed correlations between the biomarkers and treatment response in schizophrenia patients after controlling for age, gender,

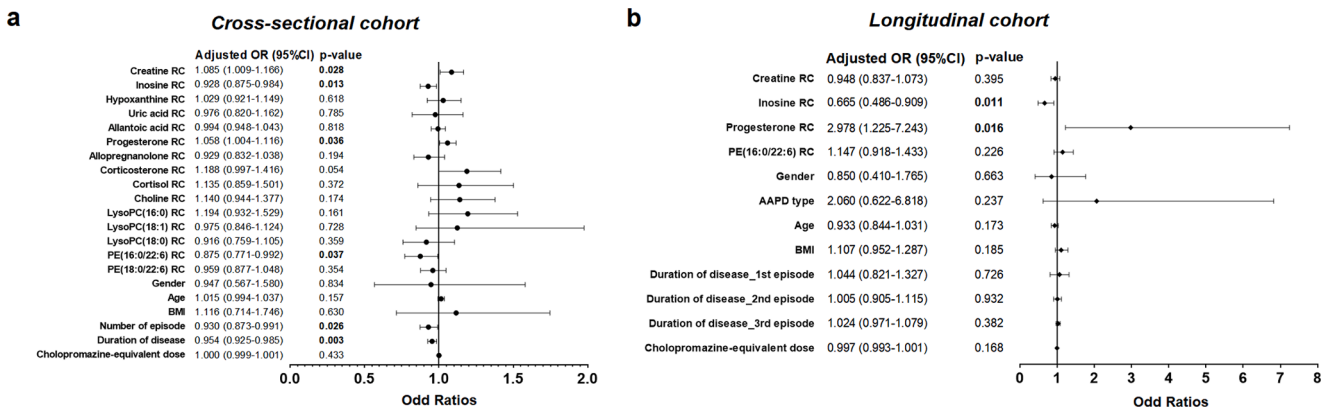

Fig. 4. Forest plots illustrating the relevance between rate of change in biomarker and treatment responsiveness in schizophrenia patients, which were analyzed by an ordinal logistic regression model for the cross-sectional cohort (a) and by a generalized estimating equation model for the longitudinal cohort (b). Clinical characteristics such as gender, age, BMI, AAPD type, chlorpromazine-equivalent dose, number of episodes and disease course were added to the regression models to control the influence of these confounding factors on drug response.

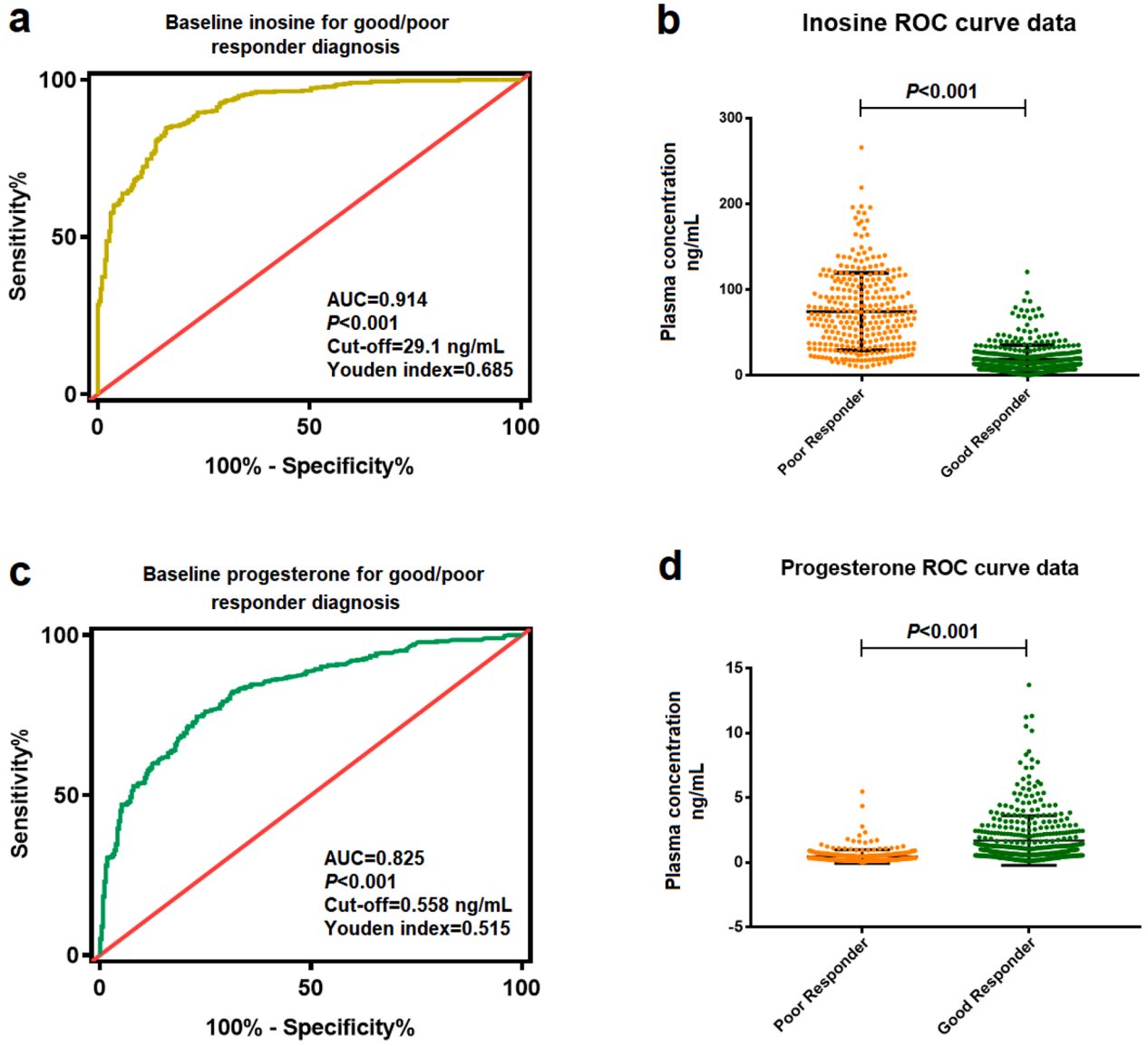

Fig. 5. Receiver-operating characteristic (ROC) curves and related curve data of inosine (a and b) and progesterone (c and d) for good/poor responder diagnosis. AUC, area under the ROC curve.

BMI, number of episodes, duration of disease and chlorpromazine-equivalent dose. The Omnibus test (likelihood ratio Chi-Square=48.4, $p < 0.001$) and the test of parallel lines (likelihood ratio Chi-Square=20.6, $p = 0.150$) indicated that the model was a good fit and valid. The RCs in creatine (adjusted OR 1.085, 95% CI 1.009–1.166, $p = 0.028$), inosine (adjusted OR 0.928, 95% CI 0.875–0.984, $p = 0.013$), progesterone (adjusted OR 1.058, 95% CI 1.004–1.116, $p = 0.036$) and PE(16:0/22:6) (adjusted OR 0.875, 95% CI 0.775–0.992, $p = 0.037$) were associated with treatment response. In addition, number of episode (adjusted OR 0.930, 95% CI 0.873–0.991, $p = 0.026$) and duration of disease (adjusted OR 0.954, 95% CI 0.925–0.985, $p = 0.003$) were risk factors negatively correlated with treatment response.

The biomarkers significantly associated with treatment response in the cross-sectional cohort [creatine, inosine, progesterone and PE(16:0/22:6)] were further identified in the longitudinal cohort with GEE analysis. The goodness-of-fit of the model shown as quasi-likelihood under the independence model criterion (QIC) value was 171.5. As shown in Fig. 4b, only the correlations of inosine (adjusted OR 0.665, 95% CI 0.486–0.909, $p = 0.011$) and progesterone (adjusted OR 2.978, 95% CI 1.225–7.243, $p = 0.016$) with treatment response remained significant. The duration of disease no longer appeared to be a risk factor for treatment response in the first three episodes of the longitudinal cohort.

3.5. Treatment response prediction

The cross-sectional and longitudinal cohorts were combined and categorized into good and poor responder groups, using a 50% reduction in total PANSS score as the threshold. The demographic and clinical characteristics of schizophrenia patients with good ($N = 415$) /poor ($N = 292$) responses are presented in Table S4. As compared with the poor responders, the good responders were younger, and had lower BMI, smaller number of episodes, shorter duration of disease, as well as higher values of PANSS positive syndrome, thought disturbance, activation, and paranoid/belligerence scores. ROC analysis was performed to assess the diagnostic performance of each biomarker at the baseline level. Inosine and progesterone were identified as the two most effective diagnostic biomarkers in distinguishing the good and poor responders (Fig. 5a and 5c), with AUCs of 0.914 (95% CI 0.894–0.935, $p < 0.001$, cut-off=29.1 ng/mL, Youden index=0.685, sensitivity: 84.6%, specificity: 83.9%) and 0.825 (95% CI 0.795–0.856, $p < 0.001$, cut-off=0.558 ng/mL, Youden index=0.515, sensitivity: 74.5%, specificity: 77.1%), respectively. Before the treatment, poor responders showed higher levels of inosine (Cohen's $d = 1.993$, $U = 10{,}406$, $p < 0.001$, Fig. 5b) and lower concentrations of progesterone (Cohen's $d = 1.332$, $U = 21{,}176$, $p < 0.001$, Fig. 5d) in plasma as compared with good responders. The ROC curves of other biomarkers are presented in Fig. S6. The ROC curves of significant clinical factors are depicted in Fig. S7.

4. Discussion

To our knowledge, this is the first study to investigate a panel of blood-based biomarkers revealing attenuated antipsychotic treatment response in patients with relapsed versus first-episode schizophrenia, from the cross-sectional and longitudinal cohorts simultaneously. By utilization of these biomarkers, we observed significant differences in the metabolic signatures between schizophrenia patients and healthy subjects, as well as in the antipsychotic effects between multi-episode and first-episode schizophrenia across several metabolic pathways.

Creatine can cross the blood-brain barrier (BBB), leading to a significant increase in the concentrations of creatine and phosphocreatine across the brain regions (Dechent et al., 1999; Lyoo et al., 2003). Using ^{1}H MRS technologies, a significant decrease in creatine levels in the anterior cingulate cortex and parieto-occipital cortex has been found in patients with schizophrenia compared to controls (Ongür et al., 2009). In line with this report, we confirmed that plasma creatine levels in schizophrenia patients were low at baseline, suggesting that a sustained reduction in creatine-phosphate signaling is associated with disruption of brain energy production and deprivation of adenosine triphosphate (ATP) at the onset of schizophrenia (Roberts, 2017). This explanation is consistent with previous findings of a dramatic decrease in creatine kinase (CK) enzyme activity in the frontal cortex, anterior and posterior cingulate cortex, hippocampus and cerebellum of schizophrenia patients (Burbaeva et al., 2003). A direct byproduct of this ATP production is reactive oxygen species (ROS), which is highly detrimental to neurons. However, the dysregulated antioxidant defense system in schizophrenia is known to be closely related to the dynamics of the purinergic pathway, particularly in relation to the end product uric acid (Yao et al., 2010, 2012). Uric acid acts as a pivotal free radical scavenger in the body, scavenging oxygen radicals and exerting a non-enzymatic antioxidant effect. There is growing evidence that uric acid levels do not differ significantly between subjects with schizophrenia as a whole and healthy controls, but instead are only decreased in patients with first-episode psychosis (He et al., 2020). More interestingly, our data showed that allantoic acid, the oxidized catabolite of uric acid, was significantly increased in the schizophrenia patients. It is noteworthy that allantoic acid is not a normal purine end product in humans, although trace amounts have been found in human plasma (Sorensen, 1965; Seymour et al., 2013). In our study, the surge of allantoic acid may result from the increased oxidative stress in schizophrenia patients (Fraguas et al., 2019), leading to excessive oxygen free radicals, which further oxidize uric acid into allantoic acid (Seymour et al., 2013). In response to AAPD treatment, the increase in creatine levels in schizophrenia patients may indicate an increased brain energy supply due to stimulation of CK activity by AAPD (Laoutidis and Kioulos, 2014; Ignácio et al., 2015). Interestingly, our findings suggest that this regulation of creatine is sustained and unaffected by relapse, while downstream inosine, hypoxanthine and uric acid are regulated to a lesser extent. Together with the fact that allantoic acid remained at high levels and largely unresponsive to AAPD, the results suggest that the pathophysiology of oxidative stress appears to be partially restored and that the therapeutic effect on antioxidant purine metabolism diminishes with increasing number of episodes. We believe this is one of the leading causes for the development of treatment-resistant schizophrenia (TRS). Interestingly, using an iridium-reducing capacity assay as a global measure of oxidative stress, it was found that a subgroup of TRS patients treated with clozapine showed greater levels of oxidative stress and more severe psychiatric symptoms (Kim et al., 2019). In addition, the elevations of creatine and inosine induced by AAPD treatment were both positively associated with the PANSS total score improvement in schizophrenia patients of the two cohorts, supporting the notion that AAPD can exert the therapeutic effects via upregulation of purinergic signaling. Meanwhile, the changes of inosine were specifically correlated with improvements in general psychopathology, thought disturbance and paranoid/belligerence subscale scores, suggesting that inosine may be a more indicative treatment biomarker in purinergic pathway.

In the pathophysiology and etiology of schizophrenia, shortage of ATP and increased ROS are coupled (Prabakaran et al., 2004). Phospholipase A_2 (PLA_2) is a key player activated by elevated ROS levels, resulting in the hydrolysis of various products of the plasma membrane such as peroxidized fatty acids (Beaulieu et al., 2014). Recent evidence increasingly links disturbances in membrane phospholipid metabolism with excessive activation of PLA_2 as a cause of dopaminergic dysfunction and associated cognitive impairment in schizophrenia (Schaeffer et al., 2012; Tessier et al., 2016). Here, prior to treatment, an increasing production of LysoPCs was found in schizophrenia patients, suggesting that PLA_2 activity upregulated by ROS accelerates the breakdown of membrane phospholipids in this disorder (Gattaz and Brunner, 1996). Notably, high levels of LysoPCs are cytotoxic and can in turn drive demyelination, leading to apoptosis in the central nervous system (CNS) (Sun et al., 2009; Plemel et al., 2018). In support, our data suggest that

PEs, a major component of myelin, together with choline, are reduced at baseline in schizophrenia patients, which is also consistent with previous findings (Schmitt et al., 2001). In addition, phospholipid PEs were significantly reduced in the caudate region of dissected, medicated schizophrenia patients, which is also consistent with our findings in plasma (Yao et al., 2000). After treatment, the increase of plasma PE (16:0/22:6) was also associated with the improvement in PANSS total score of both cohorts, which may be suggestive of the therapeutic effects of AAPD on promoting myelination in schizophrenia (Wang et al., 2021). The three LysoPCs (16:0, 18:1, 18:0) appear to be relatively "blunted" to AAPD treatment, as reflected in both cross-sectional and longitudinal cohorts. Interestingly, a study reported that LysoPC levels were decreased in the first three weeks during antipsychotic treatment of neuroleptic-free schizophrenia patients, but increased again in the following six months, reaching significantly higher levels than controls (Schmitt et al., 2001). These results imply instead that the increase in phospholipid catabolism may be reversed only by the initial antipsychotic treatment, while the final trend of change is determined by the development of schizophrenia pathology. Moreover, the upregulation of PEs and downregulation of choline induced by AAPD appear to be manifested only in the first three episodes as a compensatory mechanism for the loss of phosphatidylcholine.

It has been suggested that the dysregulation of neurosteroids is associated with schizophrenia (Cai et al., 2018a). Due to their highly lipophilic nature, those neurosteroids and their precursors produced by peripheral endocrine glands can also subsequently exert their biological functions by crossing the BBB. In the cross-sectional cohort, we found increased baseline levels of corticosterone and cortisol. These abnormalities may suggest hypothalamic-pituitary-adrenal (HPA) axis hyperactivity in patients with schizophrenia (Cullen et al., 2015). It has been reported that activation of the HPA axis and the consequent excessive release of corticosterone can induce glial cell activation and increased neuroinflammation, leading to neuronal apoptosis and structural damage in the hippocampal region of rats (Xu et al., 2019). As demonstrated in patients with first-episode schizophrenia, an association between higher pre-treatment afternoon cortisol levels and the manifestation of impaired memory function has also been reported (Havelka et al., 2016). In addition, we found increased plasma progesterone and decreased allopregnanolone, its active metabolite, in patients with schizophrenia, which may reflect a parallel downregulation of 5α-reductase, the rate-limiting enzyme that catalyzes the conversion from progesterone to allopregnanolone (Cai et al., 2018a). Thus, previous data suggest that levels of allopregnanolone and 5α-reductase may be altered in neural tissue of patients with schizophrenia (Paba et al., 2011). In response to the AAPD treatment, plasma levels of corticosterone and cortisol in both cohorts of schizophrenia patients were markedly decreased, suggesting an alleviation of HPA axis hyperactivity. The observed data are consistent with the previous literature (Bradley and Dinan, 2010). In the present study, the AAPD-induced reduction of cortisol was correlated with PANSS total score improvement. However, this effect may be non-specific, as AAPD has been shown to significantly reduce cortisol levels even in healthy individuals (Cohrs et al., 2006). Of note, the AAPD exerted therapeutic effects via normalizing the progesterone-allopregnanolone conversion, which was demonstrated by its significant associations with the improvement of PANSS total score in this study. The CNS is a highly steroidogenic environment synthesizing steroids *de novo*, as well as metabolizing steroids derived from the circulation. Allopregnanolone has been shown to reduce neuroinflammatory responses and promote remyelination via binding to γ-aminobutyric acid (GABA) A receptor (Yilmaz et al., 2019). Surprisingly, the therapeutic effect of AAPD on progesterone and allopregnanolone was no longer prominent from the second episode onwards, as demonstrated in both cohorts. Although exploratory, this phenomenon seems to be inevitable, even in the absence of relapse. Previously, in a small cohort of first-episode antipsychotic-naïve schizophrenia patients, we found that the normalization of plasma allopregnanolone levels seen after 1 month of treatment, waned with continued treatment over the next 11 months when the patients were stable (Cai et al., 2018b).

Neither in the cross-sectional nor in the longitudinal cohorts, we observed significant odds ratios indicating the associations between chlorpromazine-equivalent dose and treatment response, which are in accordance with the previous findings (Zhu et al., al.,2017). Interestingly, the chlorpromazine-equivalent dose in the cross-sectional cohort showed that the patients at 2–3 episodes received relatively higher dosage than the first-episode. Even though the dose difference was not significant, the fact suggests that the psychiatrists followed the International Consensus Study on Antipsychotic dose (Gardner et al., 2010), which recommends 25–30% lower doses for first-episode patients than for chronic patients. Another key finding is that we found a strong association between specific plasma biomarkers and treatment responsiveness, which may have profound impact on the clinical practice. How to optimize antipsychotic medication in the treatment of schizophrenia largely remains a trail-and-error process, with no specific biomarkers or methods to lend decision support in prediction of treatment response (Lally and MacCabe, 2015). Therefore, there would be great value in using these blood-based biomarkers to help establish a diagnosis and personalize predictions of future treatment response to antipsychotic medications. By performing partial correlation, regression modeling, and ROC analysis on a combined cohort of all 707 patients with schizophrenia, we gradually narrowed the range of biomarkers to only inosine and progesterone having the strongest predictive power, suggesting that lower inosine and higher progesterone levels at baseline were associated with better medication response. Since both inosine and progesterone penetrate the BBB, their changes in plasma may also indicate alterations in the central nervous system. Inosine is the first metabolite of adenosine catabolism, but has a longer half-life (adenosine: <10 s, inosine: 15 h) and may act as a functional agonist at the adenosine A_{2A} receptor (Nascimento et al., 2021). Indeed, the adenosine hypothesis of schizophrenia suggests that the dysregulation of dopaminergic signaling is secondary to a hypoadenosinergic state, possibly due to reduced adenosine A_{2A}-dopamine D_2 receptor heteromerization in schizophrenia (Valle-León et al., 2021). Meanwhile, multiple lines of evidence also support the notion that progesterone can modulate core regions involved in the central dopaminergic pathways by stimulating the release of dopamine, suggesting that progesterone is a key modulator implicated in schizophrenia (Sun et al., 2016). To date, dopamine supersensitivity and hypofunctional dopamine uptake have been proposed to explain the pathophysiology of TRS (Potkin et al., 2020). In this context, it is highly interesting to note that inosine and progesterone may act as predictors of treatment responsiveness in schizophrenia. It would be meaningful to compare these biomarkers with conventional clinimetric evaluations for individualized prediction of treatment response (Guidi et al., 2021). Several other clinical factors known to predict good response or favorable course in schizophrenia were also taken into account in our study. As indicated in Table S4, these factors showing significant differences between the two outcome groups include: age, BMI, number of episodes, duration of disease, preponderance of symptoms such as positive syndrome, thought disturbance, activation, and paranoid/belligerence. In our study, the poor responders did not show significantly earlier age at onset, and prominent negative symptoms, which are usually considered as predictors of poor response in schizophrenia (Takeuchi et al., 2019; Potkin et al., 2020). Nevertheless, the AUC of these clinical factors ranged from 0.551 to 0.646 (Fig. S7), suggesting that the biomarkers inosine and progesterone perform better at identifying responders than these conventional predictors of poor course/outcome.

We note several limitations of our study. First, as compared with the cross-sectional cohort, the relatively small sample size of the longitudinal cohort may restrict the generalization of the findings. This is because it is very difficult to recruit the same patients without changing the treatment algorithm during the follow-up to the third episode. However, this is by far the first study investigating plasma biomarkers

associated with treatment response in the face of relapse with a combined cross-sectional and longitudinal design. Second, the biomarker changes and treatment response were assessed after 4 weeks of treatment, which is a relatively brief treatment timeframe. There are two main reasons for that: (1) one month is a common standard hospitalization period for the schizophrenia patients in China, so we can enroll as many patients as possible within this timeframe; (2) outpatients with longer treatment timeframe were not considered since their dietary status and treatment compliance may become uncontrollable without supervision, which can influence the biomarker profiles. Third, no correction was made for multiple comparisons thereby increasing the potential risk for Type I errors. While multiple testing correction reduces Type I error, it increases Type II error. We decided not to adjust for multiple comparisons to avoid missing important findings as this was an exploratory analysis and should be considered hypothesis-generating. Meanwhile, the p-values are impressive (mostly $p < 0.001$) and will remain significant after Bonferroni correction ($\alpha<0.05/15=0.003$), which can also partly be related to the high number of subjects. Fourth, different presentations of TRS exist including early TRS from illness onset and late TRS during subsequent development (Potkin et al., 2020). Due to the lack of accurate diagnostic information for TRS, we cannot analyze the differential biomarker features between the two types. The message to be conveyed by our findings is that good response in biomarkers and symptomatology cannot be guaranteed in the face of relapse, even if the same treatment is restarted (Takeuchi et al., 2019). Last, we did not collect the data about patient previously/later being on any other psychotropic medications. Although the lack of national network for medical tracking system and migrations of patients after they leave the hospital prevent us from doing so nowadays, the focus on this aspect would be valuable to look into those biomarker correlations later in future.

5. Conclusion

In summary, as revealed by the panel of blood-based biomarkers, we firstly observed metabolic changes associated with reduced treatment responsiveness in the face of relapse, from both the cross-sectional and longitudinal cohorts of schizophrenia patients. Among the perturbed metabolic pathways, the sort order of the reduced treatment response from fast to slow is neurosteroid metabolism, phospholipid metabolism, and purinergic metabolism. We further identified inosine and progesterone as potential predictive biomarkers for treatment response in schizophrenia. Our findings of biomarkers not only provide a step toward individualized identification and treatment response prediction of schizophrenia, but also open new avenues for understanding the pathophysiology of TRS. Further mechanism studies focusing on the inosine- and progesterone-related systems in TRS would advance these findings towards the goals of clinical and research utility.

Author contribution statement

H.C., P.Y., and B.Z. designed the study and drafted the manuscript. P. Y., H.W., X.G., X.L., J.W., and Y.Y. performed or coordinated patient recruitment, psychometric assessment, and blood sample collection. R. W., W.G., P.Y., and X.G. supervised patient recruitment and sample collection, and ascertained the psychometric data. X.Z., Y.L., R.W., W. G., and H.T. planned and performed all of the statistical analyses, and/or commenting on/editing the drafts of this work. C.Z., S.Z., T.C. and N.L. recorded the data and performed biomarker analysis. H.C. supervised the whole work and revised the manuscript according to the comments from the other authors. All authors have approved the final manuscript. The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

Declaration of Competing Interest

The authors have no conflict of interests to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2022.114762.

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