Plasma dopa decarboxylase activity in treatment-resistant recent-onset psychosis patients

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Abstract: Treatment resistance (TR) in psychosis is a major clinical problem. A biomarker predicting TR against conventional antipsychotic drugs would be relevant, potentially reducing unnecessary delay to adequate treatment with clozapine. Dopa decarboxylase (DDC) activity in the striatum, measured with positron emission tomography, is elevated in responders, but not in treatment-resistant patients. Plasma DDC activity could be a surrogate marker for DDC brain activity, and thus a potential biomarker that could be used in daily clinical practice. Therefore, we determined plasma DDC activity in 40 male patients with recent-onset psychosis, of whom the majority had started treatment, whereby 21 turned out to be treatment responders and 19 treatment resistant during follow up. We observed no significant group differences. Furthermore, symptom severity was not associated with plasma DCC activity. We did observe a trend level difference in the distribution of plasma DDC activity across categories of medication, with subsequent post hoc analysis showing lower DDC activity in risperidone-using patients. This may suggest that risperidone could influence plasma DDC activity. Based on these results, plasma DDC activity does not appear to be a promising biomarker for TR in recent-onset psychosis patients who are already receiving antipsychotic treatment.

Keywords: antipsychotics, dopa decarboxylase, psychosis, treatment resistance

Received: 13 February 2019; revised manuscript accepted: 2 August 2019.

Introduction

Treatment resistance (TR) in psychosis is a major clinical problem, with 20–35% of psychotic patients showing nonresponse to conventional antipsychotic treatment.1 This leads to months or years of delay in effective treatment, resulting in hospitalization and the unnecessary side effects of ineffective antipsychotics. Therefore, a biomarker is needed that could be used to guide treatment decisions, for example to switch TR patients at an early stage to clozapine, the only antipsychotic with recognized superior effectiveness in TR.2 Recent findings indicate that a longer duration of TR before instalment of clozapine could diminish the potential for clozapine to still render a therapeutic response.3–5 This further underlines the necessity of pursuing a biomarker that can be used in clinical practice.

A well-established finding in psychosis is increased dopamine synthesis capacity in the striatum, which has not only been replicated in patients with schizophrenia,6,7 but also in other psychiatric disorders such as bipolar disorder.8 Increased dopamine concentrations are related to positive symptoms (i.e. hallucinations, delusions) of the illness.9 However, TR patients seem to have lower striatal dopamine synthesis capacity than the responders, actually being comparable to healthy controls.10,11 This was demonstrated with positron emission tomography (PET) imaging using [18F]F-DOPA, which is processed by dopa decarboxylase (DDC), an enzyme required for dopamine synthesis. Moreover, a genome-wide association study also identified a locus next to the gene encoding DDC that was associated with TR.12 DDC activity would therefore be
a good candidate biomarker for TR. However, the gold standard for assessing dopamine synthesis, \(^{[18]F}\)F-DOPA PET imaging, is not feasible for routine screening to identify TR patients, since it is costly, invasive, time-consuming, and leads to radiation exposure.

Apart from being required for the synthesis of dopamine, DDC is also the rate-limiting enzyme for the production of the neuromodulator 2-phenylethylamine. 2-Phenylethylamine concentration is demonstrated to be higher in plasma in patients with schizophrenia and also in cerebrospinal fluid.\(^{13,14}\) This suggests that plasma DDC activity might be an accurate reflection of DDC activity in the brain.

Therefore, the current study aims to explore plasma DDC activity as a potential biomarker by testing the hypothesis that plasma DDC activity is lower in TR patients than in responders. In addition, we will explore whether there is an effect of different antipsychotics on plasma DDC activity.

**Experimental procedures**

Data originate from a longitudinal study in recent-onset psychosis patients, investigating the influence of immune disturbances on the clinical profile and course of psychosis, with follow up at 1, 2, and 3 years after baseline. The research protocol of this study was reviewed and approved by the Medical Ethics Committee of the Academic Medical Center (AMC) of Amsterdam (10.141 # 11.17.0174). Patients with recent-onset psychosis, that is, within 4 years of symptom onset, were recruited during diagnostic intake at the specialized Early Psychosis Clinic of the Academic Medical Center (AMC) of Amsterdam, the Netherlands. Detailed medical and psychiatric histories, as well as baseline demographic data, symptom severity, medication status and blood samples were obtained during the diagnostic assessment at the clinic for all participants and defined as baseline measurement. Diagnosis was defined by the standardized criteria of the Comprehensive Assessment of Symptoms and History (CASH). Symptom severity was scored by the clinician at intake with the Positive and Negative Syndrome Scale for Schizophrenia (PANSS).\(^{15}\) The total score of the PANSS (PANSS\(t\)) and the score of the positive subscale (PANSS\(p\)) were chosen as outcome measurements.

Plasma was quickly separated after blood withdrawal and stored at \(-80^\circ\)C. DDC activity and the dopamine metabolites vanilglycolic acid (VGA), 3-methoxy-4-hydroxyphenylethylene glycol (MPHG), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were measured as described by Leuzzi and colleagues.\(^{16}\)

TR was determined based on information in the medical records during follow up, defined as showing no adequate response to a minimum of two sufficiently dosed conventional antipsychotics for a minimum of 6 weeks. Adequate response is defined as a PANSS score lower than 4 on the positive symptoms. Data were available for a minimum follow-up time of 1 year and a maximum of 3 years after baseline measurements.

Plasma DDC activity was compared between TR patients and responders, and between different categories of medication (including medication free) to assess the possible confounding effect of medication status. In addition, the relationship between plasma DDC activity and the PANSS total and positive subscale score was assessed. Appropriate parametric and nonparametric tests were used, and a probability value of 0.05 was selected as level of significance for all analyses.

**Results**

Plasma samples of 24 responders and 20 TR recent-onset psychosis patients were analyzed. We excluded three outliers (mean ± 2SD), two responding females and one TR male, as outliers might indicate bad data or an experimental error. In addition, we excluded the only female (responder) that was left from the analyses, to exclude gender as a confounding factor, leaving 21 male treatment responders and 19 male TR patients. Demographics of the subjects are reported in Table 1.

DDC activity (mean ± SD) did not differ significantly between TR patients [23.0 ± 8.7 nmol/(l/min)] and treatment responders [23.7 ± 8.2 nmol/(l/min)] (Table 2 and Figure 1; \(t=0.28, p=0.78\)). Adding the excluded patients in analyses increased DDC activity (mean ± SD) for TR patients [24.4 ± 10.5 nmol/(l/min)] and treatment responders [26.8 ± 12.8 nmol/(l/min)]; however, the results remained nonsignificant [\(t(42)=0.68, p=0.50\)]. Furthermore, excluding the bipolar and psychotic disorder not otherwise specified patients showed similar results [\(t(30)=0.20, p=0.85\)], with similar DDC activity (mean ± SD) for the responders...
In addition, DDC activity (mean ± SD) between schizophrenia TR patients [23.1 ± 9.4 nmol/(l/min)] and schizophrenia treatment responding patients [23.3 ± 8.2 nmol/(l/min)], yielded no significant results [t(25) = 0.056, p = 0.96]. Explorative nonparametric analyses demonstrated that the dopamine metabolites also did not differ significantly between TR patients and treatment responders (Table 1). As expected, positive symptom severity (mean ± SD) did differ between TR (20.1 ± 7.9) patients and treatment responders (13.6 ± 6.4), whereas total symptom severity did not differ significantly between TR patients and treatment responders (20.1 ± 7.9 vs. 13.6 ± 6.4).
Severity did not differ between TR (76.5 ± 23.7) and treatment responders (61.9 ± 22.0) from baseline (Table 1). Symptom severity was not related to plasma DCC activity (PANSSp $r = 0.00$, $p = 1.00$; PANSStr $r = -0.045$, $p = 0.71$). Controlling for nicotine and cannabis use, by adding the variables as covariate in a general linear model, yielded similar results as the original analyses. No significant relationship was found between antipsychotic medication levels at chlorpromazine-equivalent doses and plasma DCC activity ($r = -0.032$, $p = 0.79$), nor between symptom onset in weeks and plasma DCC activity ($r = -0.090$, $p = 0.42$). Correlation between medication use in weeks and plasma DCC activity showed a trend towards significance ($r = -0.195$, $p = 0.081$). In addition, the distribution of plasma DCC activity across the categories of antipsychotic medication approached trend level significance [$H(9) = 15.41$, $p = 0.080$]. Post hoc analyses revealed significantly lower DCC activity in risperidone [$n = 6$, $Mdn = 14.10$ nmol/(l/min)] compared with the other antipsychotic medication categories [$n = 34$, $Mdn = 25.25$ nmol/(l/min), $U = 12.0$, $p < 0.001$]. Excluding risperidone from analyses resulted in no significant difference in the distribution of plasma DCC activity across the categories of medication [$H(8) = 4.78$, $p = 0.78$]. Repeating the other analyses with exclusion of risperidone yielded similar results as the original analyses.

Discussion
The current study assessed plasma DCC activity as a candidate biomarker for TR in recent-onset psychosis. No significant group differences in plasma DCC activity or dopamine metabolites were observed between TR patients and responders, indicating that DCC plasma activity is an unlikely predictor of TR. We did, however, observe a trend level difference in the distribution of plasma DCC activity across categories of medication, based on lower plasma DCC activity in risperidone-using patients.

As expected, we found a greater severity of positive symptoms in the TR group compared with the responders. These results are in line with previous research, and can be expected by the fact that TR is defined mainly by severity of (positive) symptoms. However, although it is well known that higher striatal dopamine levels contribute to the positive symptoms in psychosis, we did not find an association between severity of positive symptoms and plasma DCC activity.

A possible explanation for the lack of difference in plasma DCC activity between TR patients and responders could be that peripheral plasma DCC activity insufficiently reflects the central dopamine synthesis capacity. Studies using [$^{18}$F]F-DOPA PET, in which the PET tracer is processed by DDC, previously showed elevated uptake in the striatum in psychosis, but not in TR patients as a subgroup, even while they were using antipsychotics. In line with the [$^{18}$F]F-DOPA PET studies, also amphetamine-induced dopamine release is elevated in the striatum in schizophrenia. In contrast, cortical dopamine release is lower in schizophrenia patients. This shows that, even within the brain, dopamine synthesis capacity, and, related to that, DCC activity, might be region specific. Thus, plasma DCC activity may not necessarily be related to striatal DCC activity. To verify this assumption, it would be necessary to correlate [$^{18}$F]F-DOPA PET with plasma DCC activity measurements in future studies.
The observed trend level effect of type of medication on the plasma DDC activity and trend level correlation between medication use in weeks and plasma DDC activity, could indicate that the use of antipsychotics might have influenced our results. This might also suggest that chronic schizophrenia patients with long-term medication use differ in plasma DDC activity compared with recent-onset patients, therefore our results are not applicable to chronic schizophrenia patients. Subsequent post hoc analysis showed lower DDC activity in risperidone-using patients. Risperidone is less lipophilic than most antipsychotics and has a lower brain-to-plasma ratio, resulting in a relatively higher peripheral concentration.23,24 Especially the active metabolite 9-hydroxy-risperidone of risperidone might influence the DDC activity. 9-Hydroxy-risperidone has strong dopamine D₂ receptor antagonistic properties, and, compared with risperidone, an even lower brain-to-plasma ratio and a longer half-life.24 In addition, this metabolite appears to play a role in plasma prolactin elevation,25 and elevated prolactin level increases dopamine synthesis in pituitary neurons by increasing tyrosine hydroxylase activity. Although the mechanism is not exactly clear, this may suggest that risperidone could influence plasma DDC activity as well. Exclusion of these patients from the analyses yielded no relevant change of results though. Other antipsychotic medications were not significantly associated with DDC activity. Also nonuse of medication did not result in significantly different DDC activity (n=7, mean ±SD=23.1 ± 5.4). However, the current study included a modest sample size and there was a wide variety in antipsychotic use. Ideally, plasma DDC activity should be assessed in medication-naïve patients, or at least in patients with short period of treatment with antipsychotic medication and who are medication-free at time of plasma sampling. Evaluating plasma DDC activity in these patients may still be of interest before ruling out plasma DDC activity measure as potential biomarker.

This study has several limitations, including modest sample size, heterogeneity in diagnosis, heterogeneity in antipsychotic use, and inclusion of patients with nicotine and cannabis use (which may affect presynaptic striatal dopamine).26 Furthermore, results are only applicable to male recent onset psychotic patients, because women were not included in the analyses. Taken together, it should be regarded as a pilot study to assess the potential of plasma DDC activity as biomarker for TR. The current findings point out that a future study that would investigate plasma DCC activity as biomarker for TR should include a larger sample size, ideally in medication-naïve schizophrenia male and female patients without substance abuse. A longitudinal study design would preferably include structured follow up with PANSS assessment and blood sampling. Plasma DDC activity measurements at follow up could provide information about changes in plasma DDC activity in TR and responders and the effect of medication use on plasma DDC activity. Furthermore, correlation of plasma DDC activity with [¹⁸F]{F-DOPA} PET could validate the plasma measure as surrogate for striatal DDC activity. In spite of the limitations of the current study, the results show very similar plasma DDC activity between responders and TR. We cannot exclude the possibility of a false negative finding with this study, but it does suggest that a potential effect size would be too small for clinical use. Taken together, plasma DDC activity does not appear to be a promising biomarker for TR in patients currently using antipsychotic medication. Future research on biomarkers for TR could focus on other potential derivatives of dopamine synthesis in the brain or on other neurotransmitter systems (e.g. glutamate).27,28

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and publication of this article: The study was funded by a Veni grant (91618075) from the Netherlands Organisation for Health Research and Development (ZonMw). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest statement
The authors declare that there is no conflict of interest.

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