A Multicenter, Add-On Randomized Controlled Trial of Low-Dose D-Serine for Negative and Cognitive Symptoms of Schizophrenia

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ABSTRACT

Background: Observations that antagonists of the *N*-methyl-D-aspartate (NMDA) receptor of glutamatergic neurons can mimic symptoms of schizophrenia have raised the hope that NMDA agonists can improve symptoms. On the basis of encouraging results of trials in which NMDA agonists were added to antipsychotics, we conducted an adequately powered randomized controlled trial adding D-serine, an NMDA modulator, to antipsychotics.

Method: This study was a 195-patient, multicenter, double-blind, randomized, placebo-controlled, 16-week trial of D-serine 2 g/d as an add-on treatment to antipsychotics. Subjects had *DSM-IV* schizophrenia or schizoaffective disorder and were inpatients or outpatients stabilized on antipsychotics, with persistent negative symptoms. The primary outcome measures were changes in negative symptoms and cognition as measured by the Scale for the Assessment of Negative Symptoms (SANS) and the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) battery, respectively. The study was performed between 2003 and 2007.

Results: Mean total Positive and Negative Syndrome Scale scores at baseline were 75.5. Subjects receiving D-serine and placebo improved in scores on the SANS and MATRICS, but no significant differences were observed between groups: improvement on SANS was 11.4% for D-serine vs 14.8% for placebo, $F_{1,147} = 1.18$, $P = .32$; and improvement on MATRICS was 6.8% for D-serine vs 6.1% for placebo, $F_{1,125} = 0.96$, $P = .39$, respectively. D-Serine was well tolerated.

Discussion: This study did not find a significant difference between drug and placebo. However, the results are limited by a relatively large placebo response and somewhat lower-achieved doses than in prior studies. Future studies will administer higher doses and will attempt to affect the NMDA receptor using other mechanisms, such as agonists of the presynaptic metabotropic glutamate 2/3 receptor or glycine reuptake inhibitors.

Trial Registration: ClinicalTrials.gov identifier: NCT00138775

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Several observations have suggested that impaired *N*-methyl-D-aspartate (NMDA) neurotransmission might underlie some of the symptoms of schizophrenia. First, administration of NMDA antagonists, such as phencyclidine (PCP [a street drug]) or the anesthetic ketamine, have been shown to mimic positive and negative symptoms and cause cognitive deficits similar to those seen in schizophrenia.[1] In laboratory settings, administering ketamine produces schizophrenia-like disturbances in set shifting,[2] working memory,[3] attention,[4] and auditory and visual processing.[5] Postmortem studies have shown decreased NMDA receptor responsiveness in the prefrontal cortices of schizophrenia patients.[6] Together, these findings have lead to the hypothesis that positive and negative symptoms and cognitive deficits in schizophrenia might arise from impairment in NMDA neurotransmission.[7] Hence, enhancing NMDA activity might benefit patients with schizophrenia.

Trials have reported positive results for glycine,[8–10] D-alanine,[11] D-serine,[12,13] the partial agonist D-cycloserine,[14–18] and the glycine reuptake inhibitor sarcosine[19,20] when added to antipsychotic medications, but studies have also reported results in which NMDA agonists did not differ from placebo.[21–24] A National Institute of Mental Health–funded multicenter trial, the Cognitive and Negative Symptoms in Schizophrenia Trial,[24] which compared glycine and D-cycloserine to placebo as add-on treatment to antipsychotics, found no advantage for these putative drugs.

The goal of the present study was to evaluate the effects of D-serine, a naturally occurring allosteric modulator of the NMDA receptor complex and thought to be preferentially transported into the brain.[25] D-Serine was added on to standard antipsychotic therapy in the treatment of patients with schizophrenia with cognitive impairment and negative symptoms. The primary outcome measures were the Scale for the Assessment of Negative Symptoms (SANS)[26] and a Hebrew translation of the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) cognitive battery.[27]

METHOD

Subjects

Patients were recruited from 10 community mental health centers or hospitals throughout Israel between 2003 and 2007. The trial was approved by local institutional review boards and conducted according to Good Clinical Practice. Each participant received oral and written explanations of the study, and then written informed consent was obtained.

Participants were inpatients and outpatients aged 18–64 years meeting *DSM-IV* criteria for schizophrenia or schizoaffective disorder diagnosed using the Structured Clinical Interview for

DSM-IV Axis I Disorders.[28] Subjects had a score of at least 18 on the 3-factor on negative symptoms subscale of the Positive and Negative Syndrome Scale (PANSS),[29,30] with no baseline severity requirement for cognitive impairment or positive symptoms. Patients had to be on stable doses of antipsychotic medications for a month before randomization. Adjunctive treatment with anticholinergic agents, β-blockers, mood stabilizers, antidepressants, and anxiolytics was allowed provided that patients had been on their current dose for at least 4 weeks prior to entry into the screening phase of the study. Patients receiving clozapine were excluded from the trial.

Study medication and treatment assignment: D-serine was purchased from REXIM SA (Paris, France), and purity (> 98%) was verified by Bactochem Laboratories (Ness-Ziona, Israel). Capsules containing 400 mg of D-serine or identical placebo capsules containing cellulose were prepared by Shor Tabachnik Pharmacies Inc (Tel Aviv, Israel). Assignment to D-serine or placebo was randomly allocated on a stratified basis by using a computer-generated list that was not available to investigators. Compliance was monitored through weekly capsule and bottle counts and interviews.

Study Design

After a 4-week lead-in phase to assure clinical stability, patients were randomized to a 16-week double-blind treatment. Because of potential differences between first- and second-generation antipsychotics and between inpatients and outpatients, treatment was stratified based on antipsychotic treatment (first- vs second-generation antipsychotics) and inpatient versus outpatient status. Therapy was initiated at 1 capsule per day and increased every 3 days to a maximum dose of 5 capsules by day 14, equaling 2 g/d of D-serine. When patients complained of any side effect, the blinded psychiatrist was allowed to omit the next dose and then continue the patient on 2 g/d, or to drop the dose to 1.6 g per day. Patients who relapsed during the treatment phase were removed from the study; criteria for relapse were based on Csernansky et al.[31]

We planned to recruit 200 subjects. To this end, 259 patients were screened, of whom 195 were randomized. Forty-six dropped out during the study, leaving 149 completers. The study was registered on ClinicalTrials.gov (identifier: NCT00138775).

Clinical Assessments

The PANSS,[29,30] SANS,[26] and Clinical Global Impressions scale (CGI)[32] were administered. Because the detailed MATRICS cognitive battery was administered, we used a modified version of the SANS that did not include the attention item. Scale ratings were administered at the beginning and end of the lead-in phase, and at weeks 4, 8, and 16 of the study. In order to monitor for adverse effects of antipsychotic medications, the Simpson-Angus Scale for Extrapyramidal Side Effects,[33] Abnormal Involuntary Movement Scale (AIMS),[34] and Udvalg for Kliniske Undersøgelser (UKU) Side Effect Rating Scale[35] were administered, and safety assessments included monthly assessment of clinical laboratory parameters (complete blood counts and serum metabolites and chemistry, including kidney and liver function tests).

Clinical Points

- Recent findings suggest that positive and negative symptoms and cognitive deficits in schizophrenia might arise from impairment in *N*-methyl-D-aspartate (NMDA) neurotransmission.
- D-Serine is a naturally occurring allosteric modulator of the NMDA receptor complex.
- This large, double-blind, randomized controlled trial of add-on D-serine showed no improvement in negative or cognitive symptoms of schizophrenia.

Neurocognitive Assessments

Neurocognitive assessment was conducted using a Hebrew translation of the MATRICS.[36,37] The battery covers 10 subtests: category fluency,[38] Brief Assessment of Cognition in Schizophrenia-symbol coding,[39] Trail Making A,[40] Continuous Performance Test—identical pairs,[41] University of Maryland—letter-number span,[42] Wechsler Memory Scale-III (WMS-III)—spatial span,[43] Rey Auditory Verbal Learning Test,[44] Brief Visuospatial Memory Test-Revised,[45] Neuropsychological Assessment Battery-mazes,[46] Mayer-Salovey-Caruso Emotional Intelligence Test—managing emotions (D and H).[47] The Mayer-Salovey-Caruso Emotional Intelligence Test has yet to be analyzed and will not be presented in the scope of the current article.

The neurocognitive assessments were carried out by trained, MA-level research assistants. The battery was performed at weeks 0 (baseline), 8, and 16. In order to prevent practice effects, the neurocognitive assessments were counter balanced.

Plasma D-Serine Levels

In order to examine correlations between plasma D-serine levels and response to treatment, plasma D-serine levels were measured at week 8 using liquid chromatography, with O-methylserine as the internal standard. Plasma (0.25 mL) was deproteinized and extracted prior to derivatization with phenylisothiocyanate reagent (Thermo Fisher Scientific Inc, Waltham, Massachusetts) to enhance separation and spectrographic detection at 254 nm.[48] Calibration standards that included the expected concentration range for this study preceded each batch of samples. In addition, a set of quality controls was assayed to validate each day's analyses.[13]

Statistical Methods and Data Management

Changes in scores between baseline, week 4, week 8, and week 16 were assessed using repeated-measures multivariate analyses of variance (MANOVAs), with treatment (D-serine vs placebo) and time (week 0, 8, 16) as within-subject factors. Separate analyses were conducted for the SANS total score, PANSS total score, PANSS positive subscale, PANSS negative subscale, and the PANSS general psychopathology

Table 1. Baseline Characteristics of D-Serine and Placebo Groups[a]

Variable	D-Serine n = 97	Placebo n = 98	*t* or χ^2 Statistic	*P*[b]
Age, mean (SD), y	39.39 (12.0)	39.75 (12.3)	0.2	.84
Education, mean (SD), y	11.15 (2.5)	10.53 (2.8)	−1.62	.11
Male gender, n (%)	74 (76.3)	70 (71.4)	0.6	.44
Immigrant status, n (%)	31 (32.0)	38 (38.8)	0.99	.32
Inpatient status, n (%)	42 (43.3)	42 (42.9)	0.004	.95
Duration of illness, mean (SD), y	17.1 (11.73)	15.3 (10.3)	−1.068	.29
Treatment with second-generation antipsychotics, n (%)	58 (59.8)	60 (61.2)	0.042	.88
PANSS score, mean (SD)	75.5 (13.4)	75.5 (14.6)	0.03	.97
PANSS negative subscale score, mean (SD)	26.7 (4.78)	26.2 (5.04)	−0.648	.52
SANS score, mean (SD)	60.6 (14.2)	59.0 (16.5)	−0.744	.46

[a]Values shown in parentheses are either standard deviations or percentages of the total groups as indicated by (SD) or (%), respectively.
[b]No statistical difference between the D-serine and placebo groups was found on any measure.

subscale scores. Secondary analyses evaluated percent change from baseline at each visit by using Student *t* tests to compare D-serine and placebo.

The normality of the distribution of the MATRICS test scores for each outcome variable at baseline, week 8, and week 16 was examined. All tests met the criteria for normality, excluding the Trail Making A outcome measures, which was therefore transformed by logarithmic conversion. Where high scores indicated impairment, scores were transformed (direction reversed) so that high scores always indicated better cognitive functioning. All raw or transformed raw scores were standardized to *Z* scores based on the sample of 195 participants. A factor analysis (principal components analysis, with varimax rotation) revealed that all tests belonged to 1 factor. Thus, domain scores were not calculated. For those who completed at least 6 of the 9 cognitive tests at baseline and at the end of follow-up, an overall composite score for global cognition was also calculated: a mean of the valid test *t* scores was calculated and standardized to a *t* score of its own.

In order to assess changes in cognitive test performance between baseline, week 8, and week 16, MANOVAs, 1 for each neuropsychological test and 1 for the cognitive composite score, were conducted with treatment (D-serine vs placebo) and time (week 0, 8, 16) as within-subject factors.

Pearson correlations were computed to determine the association between serum D-serine levels and the effect size of cognitive improvement at week 8. In an attempt to examine treatment response in patients with higher plasma D-serine levels, the D-serine group was split according to the median plasma level, and correlations between change in SANS and cognition were examined in the group with the above-median plasma D-serine levels. Data analysis was done by using SPSS 15.0 for Windows (International Business Machines, Armonk, New York).

General growth mixture modeling[49,50] was applied to test whether there were distinct subgroups that have different patterns of response to treatment (as seen in SANS, PANSS, and global cognition composite scores). Models were fitted in Mplus Version 4.0 (Muthén & Muthén, Los Angeles, California) by using maximum likelihood estimation. Following the steps outlined in Muthén,[49] a series of models were fitted beginning with a 1-class model and progressing sequentially to a 4-class model.

RESULTS

Of 259 patients screened, 195 entered the randomization phase. Among these, 97 received D-serine and 98 received placebo. The 2 groups did not differ significantly in their age, years of education, gender, immigrant status, duration of illness, baseline PANSS total and negative subscale scores, or SANS score (Table 1). At week 8, 84.5% of the D-serine group and 83.7% of the placebo group remained in the study. Completion rates after 16 weeks for the D-serine and placebo groups were 76.3% and 76.5%, respectively (see Table 2). χ^2 Analysis showed no significant difference in the proportion of noncompleters between the 2 groups ($\chi^2 = 0.002$, $P = .968$).

Clinical Symptoms

On the SANS scores, there was a significant effect of time ($F_{3,441} = 46.49$, $P < .001$), with both groups showing improvement; there was no effect of treatment group or group-by-time interaction (Table 2). When adding type of medication to the model (first- vs second-generation antipsychotics), there was no significant group × treatment × medication interaction ($F_{3,435} = 0.76$, $P = .52$).

Similar findings were observed in the total PANSS scores, as well as the positive, negative, and general psychopathology subscales; there was a significant effect of time ($F_{3,441} = 94.51$, $P < .001$; $F_{3,441} = 30.33$, $P < .001$; $F_{(3,441)} = 92.56$, $P < .001$; $F_{3,441} = 55.26$, $P < .001$, respectively), with both groups showing improvement; there was no significant effect of treatment group or group-by-time interaction (Table 2). When adding type of medication to the model (first- vs second-generation antipsychotics), neither the PANSS total nor any of the subscales showed significant group × treatment × medication interactions.

The pattern of results did not change when week-16 scores were removed from the model, leaving baseline, week-4, and week-8 data (SANS and PANSS scores: placebo, n = 82; D-serine, n = 82; data not shown). However, when examining percent change from baseline at week 4, there was a significantly greater improvement in the PANSS negative symptom in D-serine (n = 94, 9.9% change) than in placebo (n = 88, 6% change) patients ($t = 1.98$, $P < .05$; Figure 1).

An additional last-observation-carried-forward analysis showed that D-serine and placebo did not differ in the proportion of subjects who had at least 20% reduction on the SANS (n = 25 [28.4%] vs n = 26 [27.7%], respectively; $\chi^2_1 = 0.01$, $P = .91$) or the PANSS total score (n = 46 [52.3%] vs n = 48 [51.1%], respectively; $\chi^2_1 = 0.03$, $P = .8$).

Neurocognitive Assessment

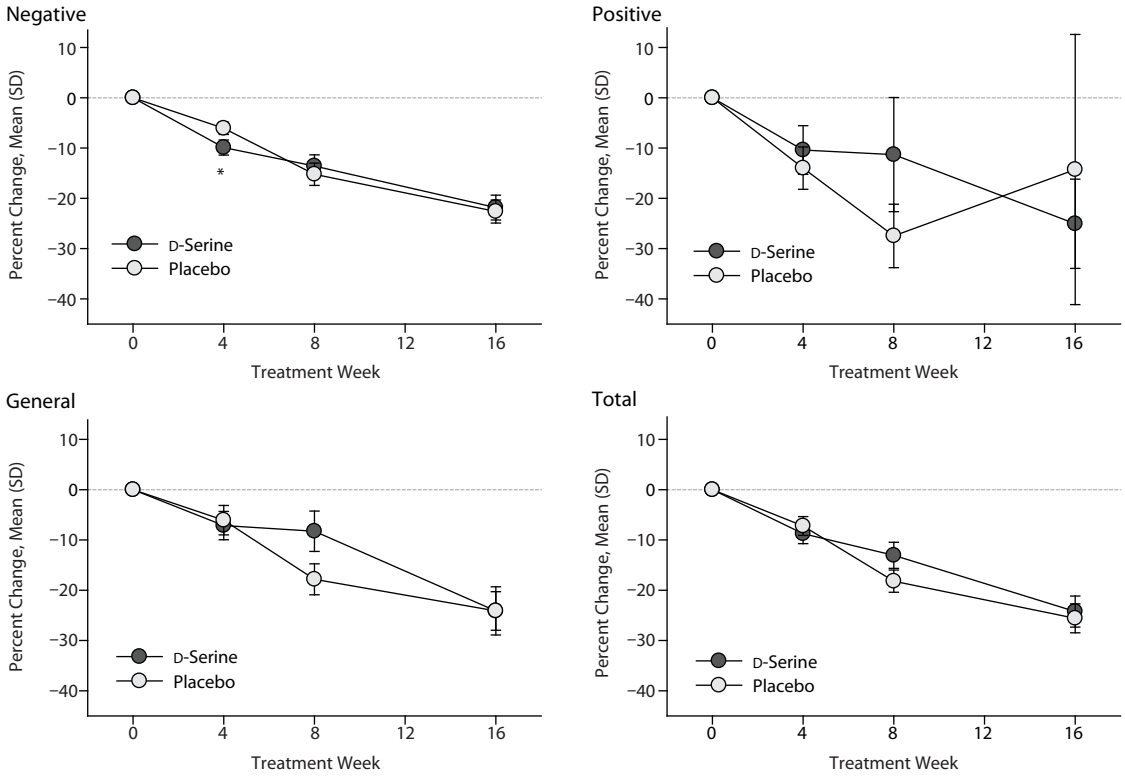
Among both treatment groups, the neuropsychological composite score showed improvement over time

Table 2. Clinical Outcome Measures

Measure	Baseline		Week 4		Week 8		Week 16		Time × Group Interaction	
	D-Serine, Mean (SD)	Placebo, Mean (SD)	D-Serine, Mean (SD)	Placebo, Mean (SD)	D-Serine, Mean (SD)	Placebo, Mean (SD)	D-Serine, Mean (SD)	Placebo, Mean (SD)	*F*	*P*
SANS score	60.68 (14.07)	58.85 (16.68)	58.76 (15.00)	57.00 (16.44)	57.03 (15.60)	53.31 (18.23)	53.76 (15.31)	50.15 (18.72)	1.18	.32
PANSS Total	74.82 (13.53)	75.41 (14.32)	71.38 (14.76)	71.88 (15.06)	68.05 (15.05)	67.03 (15.62)	64.24 (16.40)	63.55 (14.78)	0.68	.57
Positive symptom subscale	13.01 (4.56)	13.12 (4.19)	12.57 (4.43)	12.41 (4.03)	11.58 (4.50)	11.55 (3.90)	11.30 (4.37)	11.08 (4.00)	0.24	.87
Negative symptom subscale	26.43 (5.02)	26.24 (5.22)	24.73 (5.50)	25.01 (5.61)	23.76 (5.37)	23.21 (6.05)	22.28 (5.97)	21.95 (5.67)	1.26	.29
General psychopathology subscale	35.38 (8.09)	36.05 (8.44)	34.08 (8.09)	34.45 (8.15)	32.72 (8.29)	32.27 (8.29)	30.66 (8.56)	30.52 (8.30)	0.65	.59

Abbreviations: PANSS = Positive and Negative Syndrome Scale, SANS = Scale for the Assessment of Negative Symptoms.

Figure 1. Change in Scores on Positive and Negative Syndrome Scale Total and Subscales Over Time



*$P < .05$.

($F_{2,250} = 78.91$, $P < .001$); there was no effect of treatment group or group-by-time interaction. The same was true for each of the 9 cognitive tests (all *P* values for time < .01; all *P* values for interaction ≥ .18; Table 3).

The pattern of results did not change when week-16 scores were removed from the model, leaving baseline and week-8 data (composite score: placebo, n=69; D-serine, n=73; n for the subtests ranged from 58 to 74 for placebo and 63 to 76 for D-serine 82; data not shown).

Side Effects

D-Serine was generally well tolerated, differing from placebo significantly in only 3 adverse effects: mouth sores (4.1% [D-serine] vs 0% [placebo], $\chi^2 = 4.13$, $P = .04$), dizziness (11.3% [D-serine] vs 22.4% [placebo], $\chi^2 = 4.28$, $P = .04$), and headache (13.4% [D-serine] vs 29.6% [placebo], $\chi^2 = 4.28$, $P = .01$). Only mouth sores were more common in the treatment group, whereas others were less frequent.

Plasma D-Serine Levels

Mean ± SD plasma D- and L-serine levels in treated patients (week 8) were 77.3 ± 28.1 μmol/L and 88.8 ± 62.0 μmol/L, respectively. No correlation was found between plasma D-serine level and change in cognition ($r = -0.055$, $P = .65$) or total SANS scores ($r = 0.08$, $P = .51$).

In a further analysis, the D-serine group was split according to the median plasma level. Among subjects with plasma levels above the group's median (suggesting adherence to treatment), the mean ± SD plasma D-serine level was 144.3 ± 43.7 μmol/L. In this group, there was no correlation

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Table 3. Cognitive Measures as Assessed by the MATRICS Battery

Test	n		Baseline		Week 8		Week 16		Time × Group Interaction	
	D-Serine	Placebo	D-Serine, Mean (SD)	Placebo, Mean (SD)	D-Serine, Mean (SD)	Placebo, Mean (SD)	D-Serine, Mean (SD)	Placebo, Mean (SD)	*F*	*P*
Composite score	66	61	50.52 (9.09)	49.44 (10.95)	53.39 (9.64)	51.57 (11.17)	54.04 (10.26)	52.46 (12.11)	0.96	.39
Trail Making A	68	64	50.10 (10.80)	49.24 (11.03)	53.20 (11.14)	51.30 (11.02)	53.48 (10.53)	52.67 (11.56)	0.48	.62
Letter Number Span	54	46	51.09 (9.58)	50.22 (10.39)	53.79 (8.55)	51.28 (11.50)	53.64 (9.38)	53.11 (11.62)	1.73	.18
NAB-mazes	62	58	51.62 (10.49)	50.17 (10.69)	53.20 (11.18)	50.72 (10.43)	54.05 (12.09)	51.92 (11.32)	0.34	.71
Category fluency	69	60	50.24 (9.73)	49.72 (10.81)	52.56 (11.04)	51.22 (10.60)	52.18 (11.22)	51.74 (11.96)	0.39	.68
BACS-symbol coding	66	59	51.35 (9.24)	50.39 (11.15)	53.55 (10.28)	51.57 (12.58)	53.42 (10.15)	52.70 (12.71)	0.86	.43
RAVLT	65	58	51.62 (10.60)	49.88 (9.56)	54.87 (10.57)	53.98 (10.01)	57.15 (12.74)	54.34 (10.58)	1.22	.30
WMS-III—spatial span	65	61	49.06 (8.89)	50.26 (10.74)	51.02 (9.79)	50.73 (10.44)	51.86 (10.60)	51.42 (11.24)	1.32	.27
BMVT- R	69	62	50.52 (10.42)	49.35 (10.29)	52.43 (11.00)	50.66 (10.22)	53.69 (11.44)	51.45 (11.00)	0.56	.57
CPT-IP	56	50	50.51 (9.38)	50.33 (11.25)	52.55 (9.34)	53.45 (11.43)	53.26 (10.23)	53.57 (12.30)	0.61	.55

Abbreviations: BACS = Brief Assessment of Cognition in Schizophrenia, BMVT-R = Brief Visuospatial Memory Test-Revised, CPT = Continuous Performance Test—identical pairs, MATRICS = Measurement and Treatment Research to Improve Cognition in Schizophrenia, NAB = Neuropsychological Assessment Battery, RAVLT = Rey Auditory Verbal Learning Test, WMS-III = Wechsler Memory Scale-III.

between plasma D-serine level and change in cognition ($r = 0.03$, $P = .85$) or total SANS scores ($r = -0.03$, $P = .86$).

Patterns of Response to Treatment

Growth mixture modeling was applied to explore whether subgroups showing distinct treatment-response trajectories could be identified. No evidence for differential response trajectories was revealed for patients receiving D-serine.

A discriminant analysis showed that the groups could be significantly differentiated based upon negative symptom response at 4 weeks ($P < .05$).

DISCUSSION

D-Serine is a naturally occurring, endogenous allosteric modulator of the NMDA receptor complex. Prior single-site studies[12,13] found significant, large effect-size improvements across symptom dimensions when used in chronic patients in combination with typical or atypical antipsychotics other than clozapine. Its effectiveness when applied to larger scale clinical populations, however, remains unknown. This is the first study to evaluate D-serine in a multicenter randomized controlled trial and the first to incorporate objective measures of neurocognitive function in addition to symptom ratings.

In the primary analysis of the present study, no differences were shown between drug and placebo. However, both showed highly significant (pretreatment-posttreatement $P < .0001$), large effect-size improvement in negative symptoms after 16 weeks, as measured by the SANS (Cohen $d = 0.8$). A significant between-group difference in negative-symptom response was also observed at week 4 in favor of D-serine but disappeared later in the study. No significant effect of D-serine on neurocognitive measures versus placebo was observed, with neither group showing significant change in cognitive performance during the course of the study.

Given the lack of placebo effect on cognitive measures, the present study must be considered a negative result with regard to neurocognition. While some beneficial effect of D-serine on neurocognition has been reported previously,[12] the majority of prior D-serine studies have not included neurocognitive outcome measures, and this present study does not find a beneficial response of D-serine, at the present dose, on neurocognition. Results are consistent with a recent open-label, dose-escalation study of D-serine, which also found no significant improvement of neurocognition at a dose of 30 mg/kg, but did find significant improvement at higher doses.[51]

However, interpretation of the negative finding with regard to negative symptoms is more complicated, since a substantial improvement in negative symptoms was observed in both groups. A limitation of negative symptom studies in schizophrenia, in general, is the absence of a "gold standard" treatment that can be used as an active comparator. Thus, in the presence of a substantial placebo response, there is no way to determine whether a trial should be considered a negative trial or a failed trial.

If the present study is interpreted as a negative study, then it argues against further development of D-serine as a treatment for persistent negative symptoms of schizophrenia and calls into question prior positive results with this compound. Conversely, if the study is interpreted as failed, it needs to be repeated with greater safeguards against placebo response. Schizophrenia symptoms were once considered to be relatively stable measures that were relatively immune from placebo effects. Over recent years, however, there has been increasing realization that such effects may occur and may lead to failure of even large, phase-3 clinical trials.[52] Strategies suggested to potentially reduce random symptom change over time include use of video-taped and audio-taping assessment, placebo lead-in phases at the beginning of the study, staggered start times, blinded and/or off-site raters, and randomized discontinuation studies. Such strategies should be considered in future studies of D-serine or other add-on treatments for persistent negative symptoms in schizophrenia.

Other considerations may also account for the differential findings of the present study versus previous studies. First, in the present study, fixed 2 g/d dosing was employed instead of 30 mg/kg dosing, as in prior studies. Since many schizophrenic patients weigh more than 70 kg, this dose led to an achieved dose and plasma levels that were approximately 30% lower than in Tsai et al.[12] However, our analysis of patients

with above-median plasma D-serine levels showed no correlation between plasma levels and either cognition or total SANS scores, despite levels approximately 35% higher than those reported by Tsai et al.[12] As we do not have baseline levels, we cannot know to what extent plasma D-serine increased from baseline. Our week-8 mean plasma D-serine levels were 77 μmol/L. If the plasma D-serine levels in our sample were similar to those reported by Tsai et al,[12] with a mean of 2.3 μmol/L, that would suggest that plasma D-serine levels increased roughly 30-fold from baseline in the present study. At present, dosing of D-serine is limited by concerns about nephrotoxicity rather than dose-limiting central nervous system side effects. In the absence of a target engagement biomarker, the dose-response relationship for D-serine versus central NMDA receptors remains unknown. Thus, higher doses could produce greater clinical response, as suggested by a recent open-label pilot study.[51]

Second, because of the multicenter design and greater inclusion of outpatients, patient characteristics were somewhat different than in prior studies.[12,13] In those studies, patients were selected for either treatment resistance[13] or deficit syndrome.[12] Accordingly, the baseline mean ± SD PANSS positive symptom subscale scores in these studies (19.5 ± 6.3 and 22.1 ± 4.3) were ~ 50% higher than in the present investigation (13.0 ± 4.6). Recently, Lane et al[53] have also reported a relatively lesser effect of D-serine than of the glycine transport inhibitor sarcosine in a nondeficit, persistently symptomatic patient population similar to that recruited here. Nevertheless, across all studies with D-serine, including the present study, a 15%–20% improvement in negative symptoms is consistently observed. The difference in statistical outcome across studies thus might be driven by sample size and magnitude of the placebo response rather than factors intrinsic to the D-serine arm itself.

Use of D-serine and other similar agents is based, in part, on NMDA models of schizophrenia. These, in turn, are based primarily upon the observation that PCP and other NMDA receptor antagonists induce a clinical syndrome closely resembling schizophrenia. Furthermore, schizophrenia may be associated with reduced plasma D-serine levels and with altered function of serine racemase, which is the primary synthetic enzyme for D-serine, and D-amino acid oxidase, which is the primary degratory enzyme. In animal models, effects of NMDA antagonists are reversed by compounds such as D-serine, supporting the potential therapeutic effectiveness of NMDA-stimulating agents. However, the majority of animal studies utilize only acute treatment, so it remains to be determined whether such compounds are effective during sustained clinical interventions. Until treatments are developed that are known to significantly stimulate NMDA function in vivo, it will not be possible to adequately test the underlying hypotheses.

In addition to natural compounds such as glycine and D-serine, other approaches for increasing central glycine/D-serine levels include use of glycine transport inhibitors to prevent glycine removal from the presynaptic terminal, or use of D-amino acid oxidase antagonists to prevent D-serine degradation. Several studies have reported significant improvements[19,54,55] with sarcosine, a naturally occurring glycine transport inhibitor. Eli Lilly is developing an agonist for metabotropic glutamate 2/3 receptors, in which initial studies[56] showed encouraging results, and a series of monotherapy and add-on studies on this compound that are in progress. Roche recently presented the results of a phase-2 add-on study comparing a glycine reuptake inhibitor to placebo, which showed improvement in negative symptoms,[57] although definitive phase-3 studies remain ongoing.

In summary, the present study does not support prior results showing effectiveness of 2 g/d D-serine in treating schizophrenia but is limited by a substantial placebo symptom effect and somewhat lower achieved doses than in prior studies. D-Serine treatment was safe and well tolerated, potentially opening the way to higher dose studies. Ongoing studies with higher dose D-serine and with other glutamatergic treatments may provide additional opportunities to test predictions of NMDA models of schizophrenia.

Drug names: clozapine (Clozaril, FazaClo, and others), ketamine (Ketalar and others).

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Author contributions: Drs Weiser and Heresco-Levy were co-principal investigators of this study.

Potential conflicts of interest: **Dr Javitt** has intellectual property rights for use of glycine, D-serine, and glycine transport inhibitors in schizophrenia and has an equity interest in Glytech. **Drs Weiser**, **Heresco-Levy**, **Davidson**, **Werbeloff**, **Gershon**, **Abramovich**, **Amital**, **Doron**, **Konas**, **Levkovitz**, **Liba**, **Teitelbaum**, **Mashiach**, and **Zimmerman** report no financial or other relationships relevant to the subject of this article.

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