Journal of Biomedical Sciences | ISSN: 2960-9356
Research Article Peer-review, Open Access

Serum D-Serine Enantiomeric Imbalance and Clinical Progression in Neurodegenerative Disease: A Multi-Diagnosis Cohort Analysis

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Abstract

Background: Predicting which patients with neurodegenerative disease will decline quickly still rests largely on clinical judgement. Serum D-serine, a co-agonist at the N-methyl-D-aspartate (NMDA) receptor whose balance with L-serine reflects glutamatergic and glial activity, is an attractive blood-based candidate, but its prognostic behaviour across neurodegenerative syndromes has not been defined. We prospectively followed 50 consecutive patients with Alzheimer's disease, Parkinson's disease, mild cognitive impairment, amyotrophic lateral sclerosis, Huntington's disease, frontotemporal dementia, or other neurodegenerative disorders for a mean of 18.7 months (range 12–24). Serum D- and L-serine were measured by chiral chromatography (HPLC-DAD, n = 29; LC-MS/MS, n = 21). Associations were tested with Spearman correlation under Benjamini–Hochberg false-discovery-rate (FDR) control, logistic and linear regression, receiver-operating-characteristic (ROC) analysis, and Cox modelling. At baseline, serum D-serine was 2.82 ± 0.70 µmol/L and the D-serine percentage of total serine 2.35 ± 0.47%. The D-serine percentage correlated with activities-of-daily-living impairment ($\rho = -0.70$), Clinical Dementia Rating ($\rho = +0.70$) and TNF-α ($\rho = +0.66$), all surviving FDR control. Serum D-serine rose in 39 of 50 patients during follow-up (median +0.31 µmol/L; $P < 0.001$) while L-serine was unchanged. Twenty-six patients (52%) progressed. The baseline D-serine percentage did not significantly discriminate progressors from stable patients (AUC 0.612, 95% CI 0.448–0.770; Cox hazard ratio 1.75 per percentage point, 95% CI 0.77–3.96; $P = 0.179$), whereas neurofilament light chain (NfL) discriminated modestly (AUC 0.724, 95% CI 0.575–0.857). An association that appeared only after covariate adjustment (OR 7.34, 95% CI 1.21–44.55) is unstable with 26 events, and the change in D-serine is coupled to the outcome definition and cannot be interpreted as a predictor. Serum D-serine enantiomeric metrics therefore mirror current disease severity and neuroinflammation; they remain a research-stage candidate rather than a validated prognostic marker.

Keywords: D-serine; enantiomeric imbalance; serine racemase; NMDA receptor; neurodegeneration; prognostic biomarker; disease progression; chiral amino acid analysis.

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Introduction

The prognostic problem in neurodegenerative disease

Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and frontotemporal dementia (FTD) run long and heterogeneous courses: two patients who look alike at diagnosis can diverge markedly within a year. This heterogeneity complicates trial design, the timing of disease-modifying therapy and prognostic counselling. Clinical rating scales remain the basis of staging, but they record damage already accrued rather than the activity of the underlying process [1].

The best-supported fluid biomarkers report structural injury rather than mechanism. Neurofilament light chain (NfL) rises with axonal damage in ALS, HD, FTD and atypical parkinsonism, and its trajectory predicts survival and phenoconversion. NfL, however, is a downstream integrator: it reflects how much tissue has been lost, not which excitotoxic, inflammatory or proteinopathic process is responsible. A marker of an upstream, druggable mechanism would add information that NfL cannot provide [2].

D-serine and the NMDA receptor co-agonist site

D-serine is the principal endogenous ligand of the glycine modulatory site of the forebrain NMDA receptor. It was localised to astrocytes and shown to be released in response to glutamate; serine racemase, the pyridoxal-5′-phosphate-dependent enzyme converting L-serine to D-serine, was then purified, and D-serine was confirmed as an endogenous glycine-site ligand. Because the receptor opens only when this site and the glutamate site are both occupied, D-serine availability acts as a gain control on excitatory transmission [3].

The biologically relevant quantity is therefore D-serine relative to L-serine rather than its absolute concentration. Peripheral L-serine is roughly two orders of magnitude more abundant than D-serine, so small shifts in racemisation, or in clearance by D-amino acid oxidase (DAAO), change the D/L ratio appreciably while total serine barely changes. DAAO, expressed mainly in cerebellum and kidney, catalyses the oxidative deamination of D-serine and sets its regional steady-state concentration. DAAO inhibition raises D-serine and has been explored therapeutically, and serine racemase variation has been linked to neuropsychiatric phenotypes [4].

Why enantiomeric imbalance might signal progression

At physiological occupancy, NMDA receptor activity supports plasticity and neuronal survival; sustained excess co-agonist supply, particularly with glial glutamate release and impaired astrocytic uptake, favours extrasynaptic receptor activation, calcium overload and mitochondrial stress. D-serine has accordingly been described as a co-agonist that can switch from a plasticity signal to a toxic one [5]. A rising D-serine fraction might therefore mark a process that contributes to damage (Figure 1). Disease-specific data support this axis: D-serine is a determinant of glutamate toxicity in ALS and DAAO activity modifies motoneuron degeneration, and in AD the astrocytic L-serine synthetic pathway is impaired, altering the substrate available for racemisation.

World of Medicine: Journal of Biomedical Sciences | ISSN 2960-9356

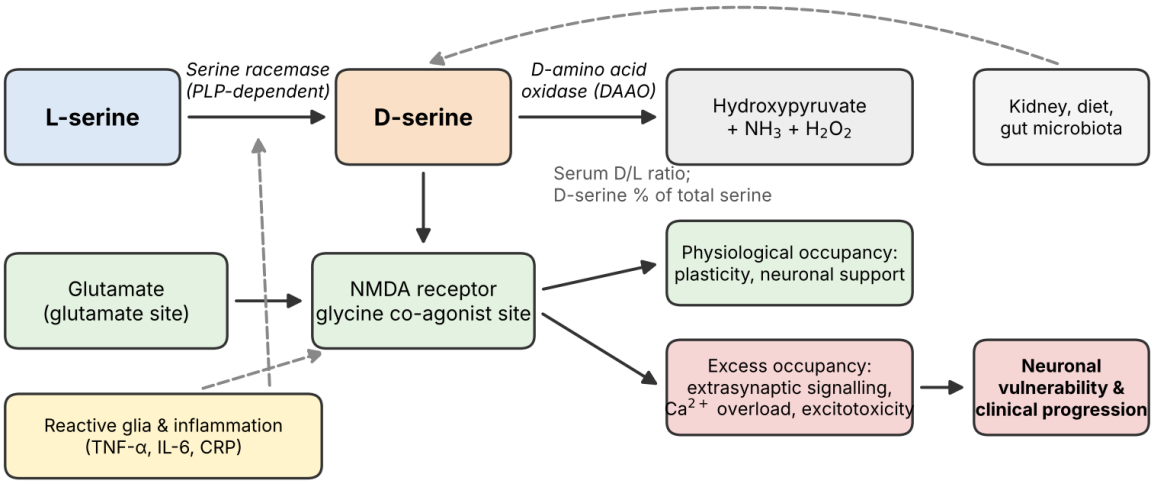


Figure 1. Mechanistic framework linking peripheral serine metabolism to neurodegeneration. L-serine is converted to D-serine by serine racemase and degraded by D-amino acid oxidase; the resulting enantiomeric balance sets occupancy of the NMDA receptor glycine site and is hypothesised to influence neuronal vulnerability and clinical progression.

Current evidence and gaps

Reduced serum D-serine and a reduced cerebrospinal fluid (CSF) D-/total-serine ratio were reported in schizophrenia. In neurodegeneration the evidence is thinner and inconsistent: altered CSF D-serine was reported in AD, but a larger study across the AD spectrum found no change in CSF or serum. A serum study reported higher D-serine and D-/total-serine ratio with advancing AD stage, whereas chiral profiling pointed to D-proline, D-aspartate and D-phenylalanine instead [6]. Blood DAAO has been proposed as a more stable readout of cognitive ageing, and reviews conclude that the biomarker status of D-serine remains unsettled.

Three gaps follow. Most studies ask a diagnostic question (cases versus controls) rather than a prognostic one; enantiomeric metrics are rarely compared across syndromes; and longitudinal measurements are scarce. This study addresses all three.

Hypothesis and objectives

The primary hypothesis was that baseline serum D-serine enantiomeric imbalance, quantified as the D/L-serine ratio and the D-serine percentage of total serine, is higher in patients who subsequently progress and discriminates progression better than chance [7]. A secondary hypothesis was that the longitudinal increase in D-serine is larger in progressors. The objectives were to: (i) describe serum D-serine, L-serine, total serine, the D/L ratio and the D-serine percentage in a multi-diagnosis cohort; (ii) relate these metrics to disease severity, cognition, function and inflammatory and neurodegenerative biomarkers; (iii) quantify their association with progression using logistic, linear and time-to-event models; (iv) estimate discrimination with confidence intervals and internally validate a combined model; and (v) state explicitly which questions the data cannot answer [8].

Methods

Study design

This was a prospective, single-centre observational cohort study of consecutive patients evaluated at Al-Najaf Teaching Hospital (Najaf, Iraq). Each patient contributed baseline serum serine enantiomer measurements, a clinical, cognitive, functional, biochemical and neuroinflammatory panel, and a structured follow-up assessment 12–24 months later (mean 18.7 months). Statistical analysis was performed on the

de-identified dataset after follow-up was complete. The primary exposure (baseline D-serine percentage of total serine) and the primary outcome (binary progression status) were defined before inferential analysis. Reporting follows the STROBE and TRIPOD recommendations.

Participants and diagnostic classification

Inclusion criteria were age ≥ 39 years, a clinically established diagnosis of a neurodegenerative disease, a valid baseline serum sample and a completed follow-up assessment. Exclusion criteria were an invalid serum sample, Wilson's disease and viral hepatitis. Fifty patients met these criteria and none was excluded at the analysis stage. Diagnoses were assigned by the treating neurologist using the National Institute on Aging–Alzheimer's Association criteria for AD dementia and mild cognitive impairment (MCI), the revised El Escorial criteria for ALS, and standard clinical criteria for PD and HD. The groups were AD (n = 12), PD (n = 10), MCI (n = 7), ALS (n = 7), HD (n = 5), FTD (n = 5) and other neurodegenerative disease (n = 4; multiple system atrophy and progressive supranuclear palsy). Disease stage at baseline was early in 13, middle in 28 and late in 9 patients. Disease duration was the interval between reported age at onset and the baseline visit.

Specimen collection, processing and routine laboratory analyses

Fasting venous blood was drawn at the phlebotomy station (Figure 2a) with single-use tourniquets after skin antisepsis with 70% ethanol. Serum was collected into gel-separator clot-activator tubes (gold top) for serine enantiomers, biochemistry and immunoassays, and whole blood into K_2/K_3-EDTA tubes (purple top) for HbA1c and blood counts. Each tube was labelled with the study code at the bedside, and sharps were discarded into puncture-resistant safety boxes. Serum tubes were allowed to clot and were centrifuged on a benchtop centrifuge (Figure 2c); serum was then aliquoted and stored frozen until batch analysis.

Routine and inflammatory analytes were measured in the hospital laboratory (Figure 3). Fasting glucose, creatinine and lipids were measured on a FUJIFILM DRI-CHEM NX600 dry-chemistry analyser, HbA1c on a CLOVER A1c Plus analyser, C-reactive protein on a Zybio Z3 CRP analyser, and IL-6, TNF-α, NfL, tau, amyloid-β and GFAP on a Snibe MAGLUMI 800 chemiluminescence immunoassay analyser, according to the manufacturers' instructions with daily internal quality control.

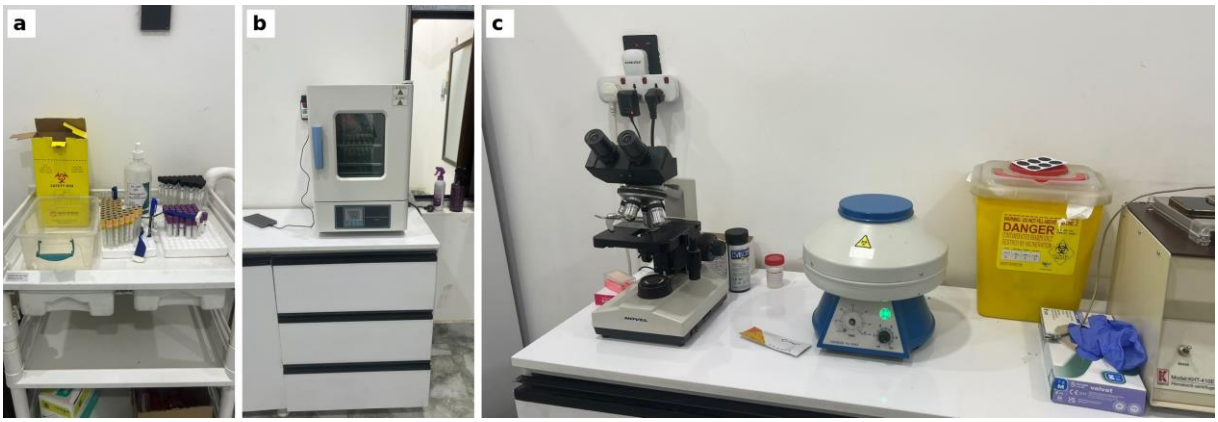


Figure 2. Pre-analytical equipment in the study laboratory. (a) Phlebotomy trolley with racks of gold-top gel-separator serum tubes, purple-top EDTA tubes and black-top tubes, a 70% alcohol antiseptic spray, a tourniquet, a marker pen for tube labelling, a clear specimen tray and a yellow biohazard sharps safety box. (b) Desktop constant-temperature incubator with a glass-windowed door, internal wire shelves and a digital temperature controller. (c) Laboratory bench with a binocular light microscope (NOVEL), a benchtop centrifuge (PLC series) with timer and speed controls used to separate serum, a yellow sharps container for incineration, and a microhaematocrit centrifuge (KHT-410E).

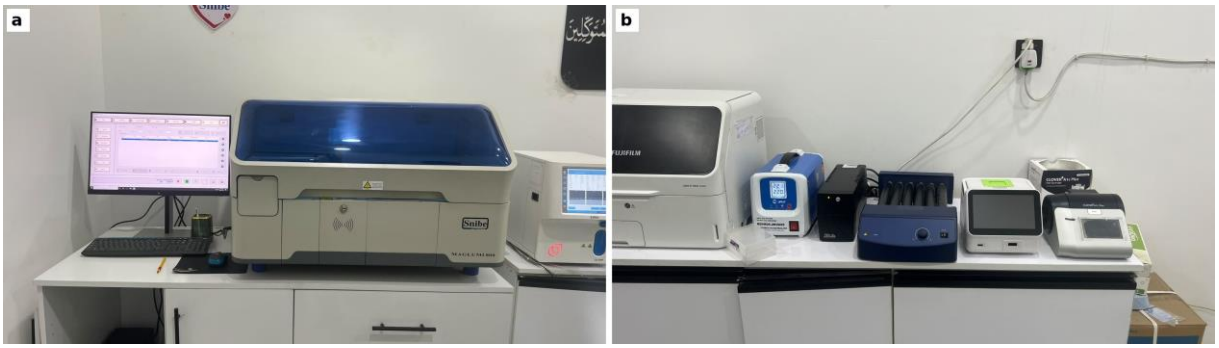


Figure 3. Clinical analysers used for routine and immunoassay measurements. (a) Snibe MAGLUMI 800 fully automated chemiluminescence immunoassay (CLIA) analyser, with a hinged transparent blue cover over the reagent and sample area and a dedicated computer workstation; a Zybio Z3 CRP haematology analyser stands on the right. (b) FUJIFILM DRI-CHEM NX600 dry-chemistry analyser (left), an automatic voltage regulator and an uninterruptible power supply protecting the instruments, a tube roller mixer for EDTA blood, a touchscreen point-of-care analyser, and a CLOVER A1c Plus HbA1c analyser with its test-cartridge box.

Serum serine enantiomer measurement

D- and L-serine were quantified in µmol/L by one of two chiral methods, recorded for each patient.

Chiral HPLC-DAD (n = 29, 58%). Analyses were performed on an Agilent 1260 Infinity II HPLC system (G7111B quaternary pump, G7129A vialsampler, G7116A multicolumn thermostat, G7115A diode-array detector; Agilent Technologies, Santa Clara, CA, USA; Figure 4). Serum D- and L-serine were derivatised with Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (L-FDAA; Marfey's reagent) to form diastereomers, separated on a reversed-phase C18 column and detected at 340 nm.



Figure 4. Agilent 1260 Infinity II HPLC system used for chiral HPLC-DAD analysis (n = 29). The modular stack comprises, from bottom to top, the G7111B quaternary pump, which mixes up to four mobile-phase solvents and delivers them at constant flow; the G7129A vialsampler, which holds the derivatised serum extracts and injects a fixed volume; the G7116A multicolumn thermostat, which keeps the C18 column at constant temperature; and the G7115A diode-array detector, which records absorbance of the Marfey derivatives at 340 nm. Solvent bottles sit in the tray on top of the stack, and the instrument is controlled from a workstation running the chromatography software.

Chiral LC-MS/MS (n = 21, 42%). Analyses were performed on an Agilent 6470 triple quadrupole mass spectrometer (G6470A; Agilent Technologies) coupled to a Nexera X3 UHPLC system (Shimadzu Corporation, Kyoto, Japan; Figure 5). Underivatised enantiomers were resolved on two CROWNPAK CR-I(+) chiral columns connected in tandem and detected in positive electrospray multiple-reaction-monitoring

mode. Because D- and L-serine are isobaric and share the same transition (m/z 106.1 → 60.1), they were identified by retention time; DL-serine-d3 was the internal standard (m/z 109.1 → 63.1).

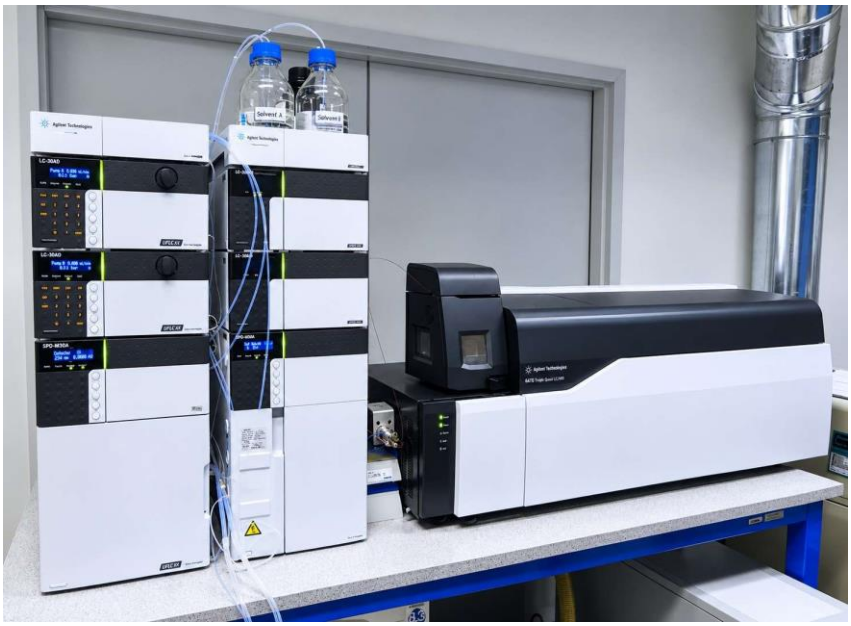


Figure 5. Agilent 6470 triple quadrupole LC/MS/MS system (G6470A) coupled to a Shimadzu Nexera X3 UHPLC system, used for chiral LC-MS/MS analysis (n = 21). The UHPLC stack (solvent delivery pumps, autosampler and column oven housing the tandem CROWNPAK CR-I(+) columns) separates D- and L-serine chromatographically. The column effluent enters the electrospray ion source on the front of the benchtop mass spectrometer, where ions are formed and passed through the first quadrupole (precursor selection, m/z 106.1), the collision cell (fragmentation) and the third quadrupole (product ion, m/z 60.1) before detection.

Assay performance characteristics (limit of quantification, linearity, precision, accuracy) were not available for either platform (see Limitations). Because two platforms were used, a method effect was tested: median serum D-serine was 2.848 µmol/L by HPLC-DAD and 2.921 µmol/L by LC-MS/MS (Mann–Whitney P = 0.555), and the median D-serine percentage 2.31% and 2.41% (P = 0.724). Data were therefore pooled; platform was recorded but not entered as a model covariate.

Enantiomeric metrics

With C_D and C_L denoting serum D- and L-serine concentrations, five exposures were derived:

Total serine (µmol/L) = C_D + C_L (1)

D/L-serine ratio = C_D / C_L (2)

D-serine percentage (%) = 100 × C_D / (C_D + C_L) (3)

ΔD-serine (µmol/L) = C_D,follow-up − C_D,baseline (4)

ΔD-serine (%) = 100 × (C_D,follow-up − C_D,baseline) / C_D,baseline (5)

The D/L ratio and the D-serine percentage are monotone transformations of each other (Spearman ρ = 1.000 in this dataset) and were never entered together in a model; the percentage, being closer to normally distributed, was the primary exposure. For future standardisation we also define an enantiomeric imbalance index, EII = $\log_2$[(C_D/C_L)_patient / (C_D/C_L)_reference] (6); because no validated reference interval exists, it was not used for inference. A categorical imbalance annotation in the dataset (increased, normal, decreased) had an undocumented reference range and was used descriptively only.

Clinical, cognitive and functional assessment

All patients completed the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA), domain scores for memory, executive function, attention and language, an activities-of-daily-living (ADL) score, and ordinal motor and cognitive categories. Disease-specific instruments were the Clinical Dementia Rating (CDR; n = 28), the Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog; n = 19), the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr stage (n = 10), the revised ALS Functional Rating Scale (ALSFRS-R; n = 7) and the Unified Huntington's Disease Rating Scale (UHDRS; n = 5). The dataset's composite clinical and cognitive scores draw on different instruments in different diagnoses; they were therefore used only for within-patient change, and cross-sectional cognitive inference used MMSE and MoCA.

Outcomes

The primary outcome was progression (progressing versus stable), based on the treating clinician's overall judgement of change between baseline and follow-up. Secondary outcomes were a three-level phenotype (stable, slow, rapid progressor), a continuous progression rate (score units per year) and time to progression. Time to progression was the event time for progressors and total follow-up for non-progressors; we verified that all 24 non-progressors were censored at the end of follow-up and that all 26 progressors had their event before it.

Biochemical and biomarker covariates

The panel comprised fasting glucose, HbA1c, creatinine, estimated glomerular filtration rate, lipids, C-reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), together with NfL, total and phosphorylated tau, amyloid-β42 and -β40, the Aβ42/Aβ40 ratio and glial fibrillary acidic protein (GFAP). Renal function was retained as a candidate covariate because serine is renally handled and DAAO is expressed in kidney.

Data quality control

Before inference, derived variables were recomputed from their constituents (agreement to floating-point precision), baseline fields were checked against the longitudinal block, baseline plus change was confirmed to equal follow-up for D- and L-serine in every patient, identifiers and units were checked, and all continuous variables were screened for implausible values. No value was edited, imputed or removed. The audit identified one structural problem: the change in D-serine separated progressors from non-progressors completely (largest increase in a non-progressor 0.280 µmol/L; smallest in a progressor 0.306 µmol/L). A variable that splits the outcome perfectly behaves as an outcome proxy, so change-based discrimination statistics are reported only to document this coupling and are excluded from prognostic claims.

Statistical analysis

Continuous variables are summarised as mean ± SD and median (IQR), and categorical variables as n (%). Normality was assessed with the Shapiro–Wilk test. Group comparisons used Mann–Whitney U or Kruskal–Wallis tests, paired comparisons the Wilcoxon signed-rank test, and effect sizes are given as Cliff's δ and Cohen's dz. Associations between the five enantiomeric exposures and 41 clinical and biomarker variables (205 tests) were assessed by Spearman correlation with Benjamini–Hochberg FDR control at 5% within each exposure family.

Binary progression was modelled by logistic regression, first univariably and then in two adjusted models: (a) D-serine percentage with age, sex and disease duration; and (b) absolute serum D-serine with NfL, age and sex. Progression rate was modelled by ordinary least squares (D-serine percentage alone; serum D-serine with disease duration; D/L ratio with age and sex). Coefficients affected by complete or quasi-complete separation are flagged as non-identifiable. Discrimination was assessed by the ROC area under the curve (AUC) computed as the Mann–Whitney statistic, with 95% CIs from 2,000 bootstrap resamples, and the Youden index for the optimal threshold. Time-to-event analyses used Kaplan–Meier estimation

with a log-rank test (median split of the D-serine percentage), Cox proportional hazards models and Harrell's concordance index. A combined logistic model (D/L ratio, NfL, age) was internally validated by leave-one-out cross-validation. Pre-specified sensitivity analyses compared the two platforms, examined diagnoses with ≥ 6 patients and ≥ 2 in each outcome state, and compared the three progression phenotypes. Tests were two-sided; analyses used Python 3.12 (pandas, NumPy, SciPy, statsmodels) and Matplotlib. There were no missing data for the primary analyses, and no imputation was performed.

Ethical considerations

The study was approved by the ethics committee of Al-Najaf Teaching Hospital (Chair: Dr. Karrar Al-Hilo, Hospital Director; Member: Dr. Ammar Al-Ziyadi, Director of the Technical Department; letter No. 5589, 1 May 2024). Written informed consent was obtained from all patients before sampling. Baseline specimens were collected from 1 June 2024 and follow-up specimens from 10 July 2025. Data were de-identified before analysis.

Results

Cohort characteristics

The cohort comprised 29 men and 21 women (mean age 64.9 ± 9.6 years, range 39–82). Most patients had middle-stage, moderate disease (56%); mean disease duration was 4.9 ± 3.0 years, 28% reported a family history of neurodegeneration, and mean follow-up was 18.7 ± 6.0 months (Table 1). All quality-control checks were passed [9].

Table 1. Baseline characteristics of the study cohort (N = 50).

Characteristic	Value
Age, years — mean ± SD (range)	64.9 ± 9.6 (39–82)
Age, years — median (IQR)	66.0 (59–72)
Sex — male / female, n (%)	29 (58) / 21 (42)
Body mass index, kg/m² — mean ± SD	25.6 ± 2.8
Smoking — never / former / current, n (%)	35 (70) / 12 (24) / 3 (6)
Family history of neurodegeneration, n (%)	14 (28)
Disease duration, years — mean ± SD	4.9 ± 3.0
Diagnosis — AD / PD / MCI / ALS, n	12 / 10 / 7 / 7
Diagnosis — HD / FTD / other, n	5 / 5 / 4
Stage (early / middle / late) = severity (mild / moderate / severe), n (%)	13 (26) / 28 (56) / 9 (18)
Follow-up, months — mean ± SD (range)	18.7 ± 6.0 (12–24)
Progressing / stable, n (%)	26 (52) / 24 (48)
Phenotype — stable / slow / rapid, n (%)	24 (48) / 11 (22) / 15 (30)
Platform — HPLC-DAD / LC-MS/MS, n (%)	29 (58) / 21 (42)

Baseline serine enantiomers

Baseline serum D-serine was 2.82 ± 0.70 µmol/L, L-serine 117.2 ± 17.5 µmol/L, the D/L ratio 0.0241 ± 0.0050 and the D-serine percentage 2.35 ± 0.47%; all were compatible with normality (Shapiro–Wilk P =

0.16–0.46). Between-patient variability was larger for the enantiomeric metrics (coefficients of variation 20–25%) than for L-serine (14.9%) (Table 2, Figure 6). D-serine correlated with L-serine (ρ = +0.54) and total serine (ρ = +0.56; both P < 0.001). By the dataset annotation, imbalance was increased in 21 patients (42%), normal in 28 (56%) and decreased in 1 (2%) [10].

Table 2. Serum serine enantiomer metrics at baseline and follow-up (N = 50).

Metric	Mean ± SD	Median (IQR)	Range	CV (%)
D-serine, μmol/L (baseline)	2.82 ± 0.70	2.89 (2.29–3.33)	1.46–4.18	24.8
L-serine, μmol/L (baseline)	117.2 ± 17.5	116.0 (106.5–128.6)	80.0–148.6	14.9
Total serine, μmol/L (baseline)	120.1 ± 17.9	119.2 (108.9–131.3)	81.5–151.3	14.9
D/L-serine ratio (baseline)	0.0241 ± 0.0050	0.0242 (0.0203–0.0279)	0.0138–0.0344	20.6
D-serine percentage, % (baseline)	2.35 ± 0.47	2.36 (1.99–2.72)	1.36–3.33	20.1
D-serine, μmol/L (follow-up)	3.17 ± 0.89	3.28 (2.47–3.74)	1.56–5.56	28.3
D-serine change, μmol/L	0.35 ± 0.38	0.31 (0.03–0.58)	−0.15 to 1.37	—
D-serine change, %	12.0 ± 12.5	10.5 (1.6–22.8)	−4.8 to 32.8	—
L-serine change, μmol/L	0.04 ± 4.78	−0.82 (−4.19 to 3.30)	−7.5 to 13.0	—

CV, coefficient of variation; not reported for change variables, whose means are close to zero.

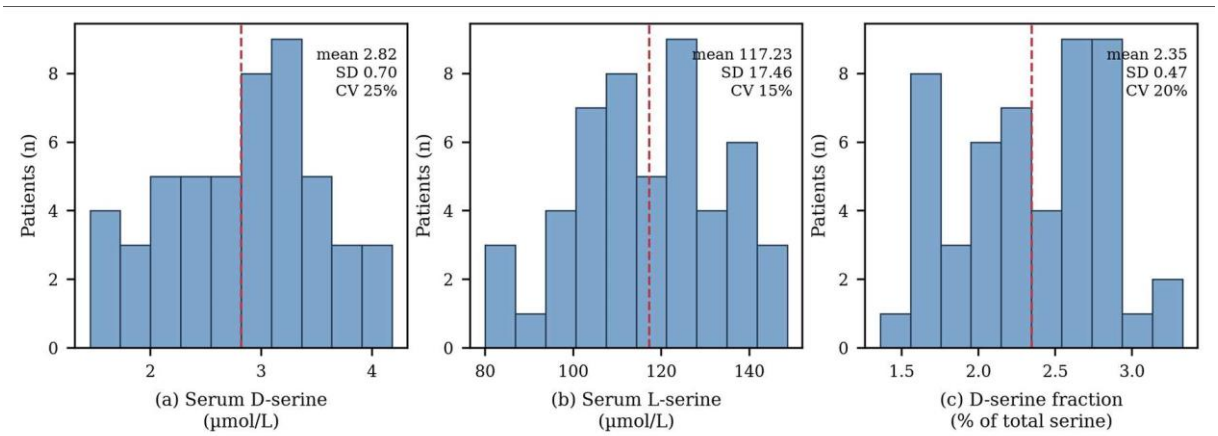


Figure 6. Distribution of baseline (a) serum D-serine, (b) serum L-serine and (c) D-serine percentage of total serine in the 50 patients. Dashed lines indicate the mean.

Longitudinal change in serum D-serine

Serum D-serine increased from 2.82 ± 0.70 to 3.17 ± 0.89 μmol/L (mean change +0.35 ± 0.38 μmol/L; median +0.31; mean relative change +12.0%, median +10.5%). The increase occurred in 39 of 50 patients (78%; Wilcoxon P < 0.001; Cohen's dz = 0.90). L-serine did not change (117.2 to 117.3 μmol/L; Wilcoxon P = 0.74; dz = 0.008; Figure 7), so the enantiomeric shift was driven by D-serine rather than by the L-serine pool [11].

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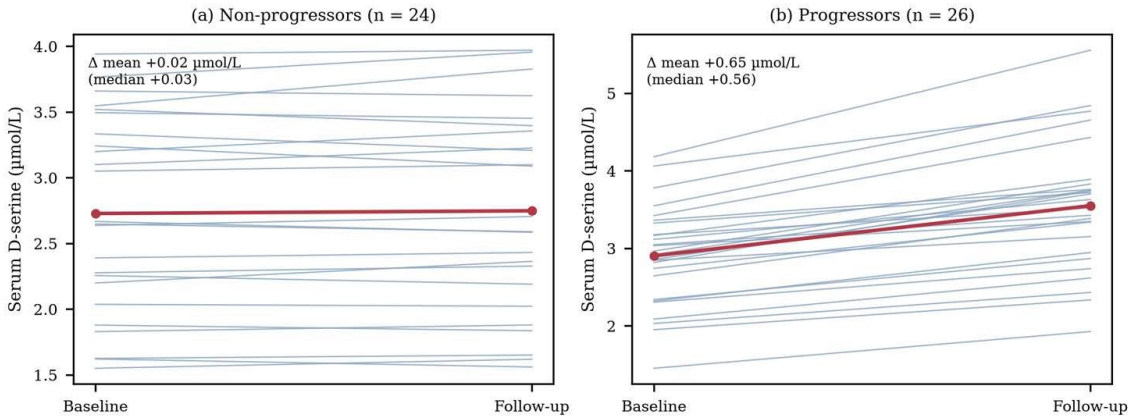


Figure 7. Individual (grey) and mean (red) trajectories of serum D-serine between baseline and follow-up in (a) non-progressors and (b) progressors.

Enantiomeric metrics and cross-sectional disease severity

Of 205 Spearman correlations, 63 were nominally significant and 40 survived FDR control. A higher D-serine percentage was consistently associated with greater severity: lower ADL performance (ρ = −0.70), higher CDR (ρ = +0.70) and ADAS-Cog (ρ = +0.69), worse motor category (ρ = +0.49), and lower MoCA, MMSE and executive scores (Table 3). In the 10 PD patients it correlated with Hoehn and Yahr stage (ρ = +0.74, q = 0.048), and serum D-serine correlated nominally with UPDRS total score (ρ = +0.73, P = 0.018). The D-serine percentage also correlated with TNF-α (ρ = +0.66), IL-6 (ρ = +0.47) and CRP (ρ = +0.44), and with depression and anxiety scores [12]. By contrast, associations with NfL (serum D-serine ρ = +0.24, P = 0.089; percentage ρ = +0.26, P = 0.074) and GFAP (serum D-serine ρ = +0.34, q = 0.053) did not survive FDR control. The enantiomeric metrics therefore track inflammatory and functional burden, but not the principal marker of axonal injury [13].

Table 3. Spearman correlations of the serum D-serine percentage that survived FDR control (q < 0.05).

Variable	n	ρ	P	q
Activities of daily living	50	−0.704	<0.001	<0.001
TNF-α, pg/mL	50	+0.658	<0.001	<0.001
Clinical Dementia Rating	28	+0.695	<0.001	0.001
ADAS-Cog	19	+0.694	0.001	0.005
Depression score	50	+0.505	<0.001	0.002
Global motor function category	50	+0.489	<0.001	0.002
Interleukin-6, pg/mL	50	+0.473	<0.001	0.003
C-reactive protein, mg/L	50	+0.440	0.001	0.007
Hoehn and Yahr stage	10	+0.744	0.014	0.048
Anxiety score	50	+0.388	0.005	0.023
MoCA score	50	−0.365	0.009	0.035
Executive function score	50	−0.341	0.015	0.049
MMSE score	50	−0.337	0.017	0.049
Progression rate, units/year	50	+0.332	0.019	0.049
Neurofilament light, pg/mL (reference; NS)	50	+0.255	0.074	0.18

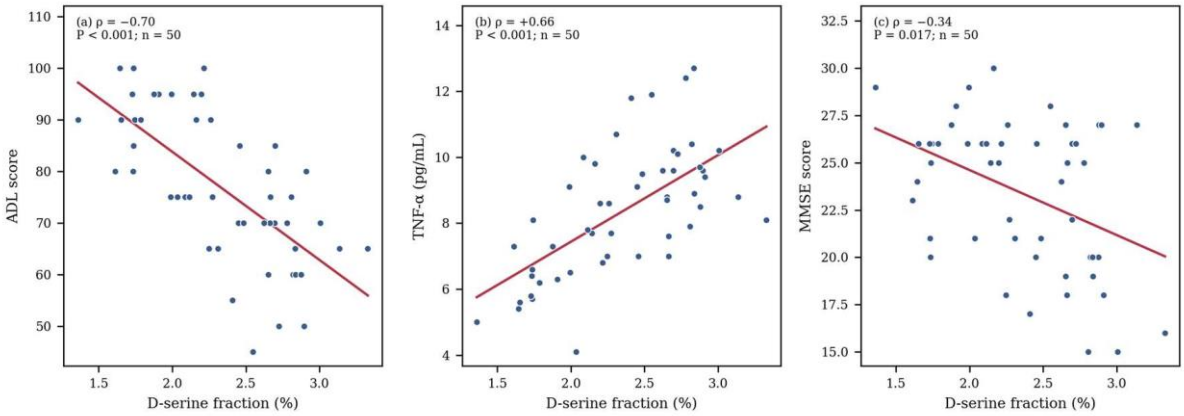


Figure 8. Serum D-serine percentage versus (a) ADL score, (b) TNF-α and (c) MMSE score. Spearman coefficients and P values are shown; least-squares lines are for orientation only.

Baseline metrics and subsequent progression

Twenty-six patients (52%) progressed (11 slow, 15 rapid) and 24 (48%) remained stable. Baseline enantiomeric metrics were slightly higher in progressors but did not differ significantly: median D-serine percentage 2.52% versus 2.22% (P = 0.177; Cliff's δ = 0.224); serum D-serine 2.94 versus 2.66 µmol/L (P = 0.49) [14]. NfL differed clearly (43.1 versus 24.1 pg/mL; P = 0.007; δ = 0.449), whereas GFAP, total tau and phosphorylated tau did not (Table 4). The categorical imbalance annotation was not associated with progression (13/21 [62%] increased versus 13/28 [46%] normal; $\chi^2 = 0.62$, P = 0.43) [15].

The change in D-serine was much larger in progressors (median +0.563 versus +0.028 µmol/L; +22.7% versus +1.2%; both P < 0.0001; δ = 1.000). Because the distributions do not overlap, the corresponding AUC of 1.000 (cut-point 0.31 µmol/L) is a mathematical consequence of the coupling between this variable and the outcome, not a performance estimate, and the variable was excluded from all prognostic inference [16]. Change in the composite clinical score did not correlate with change in D-serine (ρ = +0.08, P = 0.58); change in the composite cognitive score did (ρ = −0.70, P < 0.001), but this contrast is affected by the same coupling and by mixing of instruments. Change in L-serine was unrelated to progression (P = 0.93) [17].

Table 4. Baseline biomarkers in progressors and stable patients, median (IQR).

Biomarker	Progressors (n = 26)	Stable (n = 24)	P	Cliff's δ
Serum D-serine, µmol/L	2.94 (2.42–3.29)	2.66 (2.16–3.38)	0.491	+0.115
Serum L-serine, µmol/L	116.0 (107.3–127.1)	118.2 (105.9–129.8)	0.763	−0.051
Total serine, µmol/L	119.2 (109.8–130.5)	120.4 (108.5–132.4)	0.749	−0.054
D/L-serine ratio	0.0258 (0.0220–0.0287)	0.0227 (0.0177–0.0275)	0.177	+0.224
D-serine percentage, %	2.52 (2.15–2.79)	2.22 (1.74–2.67)	0.177	+0.224
Neurofilament light, pg/mL	43.1 (25.3–72.4)	24.1 (19.1–36.5)	0.007	+0.449
GFAP, pg/mL	182.8 (126.5–221.4)	144.7 (130.8–233.1)	0.734	+0.058
Total tau, pg/mL	2.47 (2.30–2.91)	2.40 (2.10–3.24)	0.554	+0.099
Phosphorylated tau, pg/mL	1.38 (1.14–1.60)	1.41 (1.07–2.73)	0.884	−0.026

P values from the Mann–Whitney U test.

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Regression models

In univariable logistic regression the D-serine percentage had an OR of 2.51 per percentage point (95% CI 0.73–8.66; P = 0.146). After adjustment for age, sex and disease duration, the OR rose to 7.34 (95% CI 1.21–44.55; P = 0.030), with shorter disease duration independently associated with progression (OR 0.66 per year; P = 0.006). NfL was associated with progression univariably (OR 1.038 per pg/mL; P = 0.013) and after adjustment for serum D-serine, age and sex (OR 1.060; P = 0.005), whereas serum D-serine was not (OR 0.91; P = 0.84) (Table 5) [18]. For progression rate, the D-serine percentage was not significant (β = +0.178 units/year per percentage point; P = 0.100), whereas serum D-serine was in a model adjusted for disease duration (β = +0.220 per μmol/L, 95% CI 0.078–0.361; P = 0.003; R^2 = 0.189) (Table 6). Baseline D-serine percentage showed no gradient across phenotypes (2.22% stable, 2.45% slow, 2.55% rapid; Kruskal–Wallis P = 0.38).

Table 5. Logistic regression for progression.

Model and predictor	**β**	**OR (95% CI)**	**P**
Univariable: D-serine percentage (per pp)	+0.919	2.51 (0.73–8.66)	0.146
Univariable: D/L-serine ratio (per unit)	+87.24	not identifiable	0.148
Univariable: serum D-serine (per μmol/L)	+0.372	1.45 (0.64–3.27)	0.371
Univariable: NfL (per pg/mL)	+0.037	1.038 (1.008–1.069)	0.013
Univariable: GFAP (per pg/mL)	−0.001	0.999 (0.992–1.005)	0.693
Model A (pseudo-R^2 = 0.196): D-serine percentage	+1.993	7.34 (1.21–44.55)	0.030
Age (per year)	−0.017	0.98 (0.92–1.05)	0.627
Male sex	+0.980	2.66 (0.67–10.62)	0.165
Disease duration (per year)	−0.409	0.66 (0.50–0.89)	0.006
Model B (pseudo-R^2 = 0.201): serum D-serine	−0.099	0.91 (0.35–2.36)	0.840
NfL (per pg/mL)	+0.058	1.060 (1.018–1.104)	0.005
Age (per year)	+0.082	1.09 (0.99–1.19)	0.078
Male sex	+0.367	1.44 (0.38–5.53)	0.592

Table 6. Linear regression for progression rate (score units per year).

Model and predictor	**β (95% CI)**	**P**	**R^2**
Model 1: D-serine percentage (per pp)	+0.178 (−0.035 to 0.391)	0.100	0.056
Model 2: serum D-serine (per μmol/L)	+0.220 (0.078 to 0.361)	0.003	0.189
Disease duration (per year)	−0.032 (−0.065 to 0.001)	0.054	

Model and predictor	β (95% CI)	P	R^2
Model 3: D/L-serine ratio (per unit)	+14.91 (−5.61 to 35.42)	0.150	0.112
Age (per year)	−0.006 (−0.016 to 0.005)	0.299	
Male sex	+0.158 (−0.050 to 0.365)	0.133	

Discrimination and time-to-event analysis

The D-serine percentage and D/L ratio each gave an AUC of 0.612 (95% CI 0.448–0.770), not significantly better than chance; at the Youden threshold of 2.12% sensitivity was 80.8% and specificity 45.8% (13 of 24 stable patients misclassified; positive predictive value 62%). Serum D-serine (AUC 0.558) and GFAP (0.529) were weaker, whereas NfL reached 0.724 (95% CI 0.575–0.857) (Table 7, Figure 9) [19]. For rapid versus non-rapid progression the D-serine percentage gave an AUC of 0.554. The combined model (D/L ratio, NfL, age) reached a cross-validated AUC of 0.692 (95% CI 0.535–0.832); because the NfL estimate was not cross-validated, the two values are not directly comparable, and their CIs overlap widely.

Median observed time to progression among progressors was 10.5 months. A median split of the D-serine percentage (2.36%) gave 16 events above versus 10 below the median (log-rank $\chi^2 = 2.75$, P = 0.098; Figure 10). In Cox models neither the D-serine percentage (HR 1.75; P = 0.179) nor serum D-serine (HR 1.12; P = 0.686) was associated with time to progression, whereas NfL was (HR 1.010 per pg/mL; P = 0.047). After mutual adjustment with age and sex, neither the D-serine percentage (HR 1.46; P = 0.396) nor NfL (HR 1.009; P = 0.125) remained significant, as expected with 26 events. Harrell's C was 0.576 for the D-serine percentage and 0.603 for NfL (Table 8) [20].

Table 7. Discriminative performance for progression.

Predictor	AUC (95% CI)	Threshold	Sensitivity	Specificity
D-serine percentage	0.612 (0.448–0.770)	2.12%	80.8%	45.8%
D/L-serine ratio	0.612 (0.448–0.770)	0.0216	80.8%	45.8%
Serum D-serine	0.558 (0.390–0.721)	2.74 µmol/L	69.2%	54.2%
Neurofilament light	0.724 (0.575–0.857)	29 pg/mL	69.2%	66.7%
GFAP	0.529 (0.362–0.699)	171 pg/mL	57.7%	66.7%
Combined model, leave-one-out	0.692 (0.535–0.832)	0.563	61.5%	75.0%
D-serine change (outcome-coupled; not interpretable)	1.000	0.31 µmol/L	100%	100%

World of Medicine: Journal of Biomedical Sciences | ISSN 2960-9356

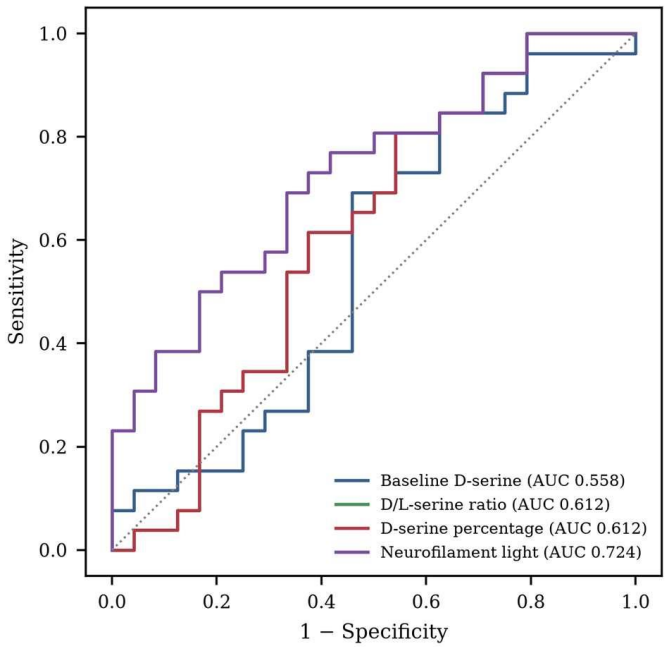


Figure 9. ROC curves for prediction of progression. Baseline enantiomeric metrics did not discriminate significantly better than chance, whereas NfL discriminated modestly; the outcome-coupled change variable is not shown.

Table 8. Cox proportional hazards and Kaplan–Meier analysis of time to progression.

Analysis	**Estimate**	**95% CI**	**P**
D-serine percentage (per pp), univariable	HR 1.75	0.77–3.96	0.179
Serum D-serine (per µmol/L), univariable	HR 1.12	0.65–1.94	0.686
NfL (per pg/mL), univariable	HR 1.010	1.000–1.020	0.047
Age (per year), univariable	HR 1.001	0.956–1.048	0.972
GFAP (per pg/mL), univariable	HR 0.999	0.995–1.004	0.719
D-serine percentage, adjusted (age, sex, NfL)	HR 1.46	0.61–3.46	0.396
NfL, adjusted	HR 1.009	0.998–1.020	0.125
Log-rank, median split of D-serine percentage	$\chi^2 = 2.75$	—	0.098
Harrell's C: D-serine percentage / NfL	0.576 / 0.603	—	—

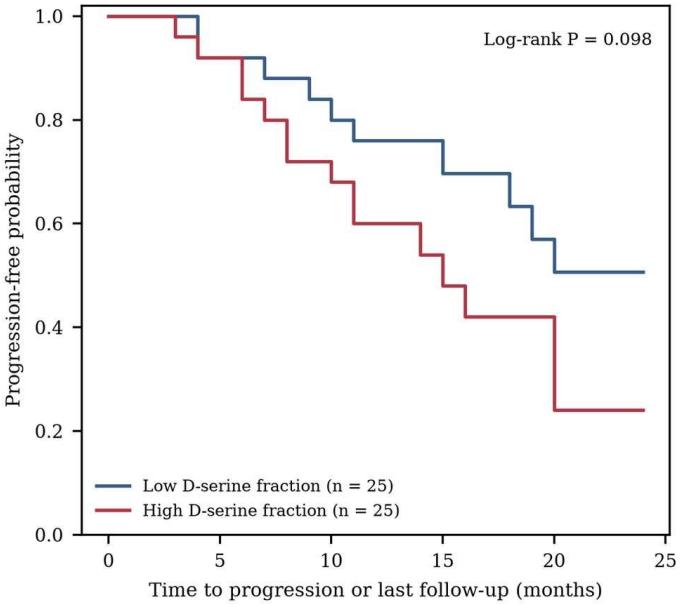


Figure 10. Kaplan–Meier progression-free probability by median baseline D-serine percentage (2.36%); log-rank P = 0.098.

Subgroup analyses

Within-diagnosis comparisons were possible for MCI (n = 7; median D-serine percentage 2.11% in 4 progressors versus 1.74% in 3 stable; P = 0.229), AD (n = 12; 2.41% versus 2.66%; P = 0.530) and PD (n = 10; 2.88% versus 2.56%; P = 0.400); none was significant, and the direction differed between AD and the other groups [21]. ALS, HD, FTD and other diagnoses lacked enough patients in both outcome states. The four MCI patients who converted to dementia (the four MCI progressors) had a mean relative D-serine change of +24.0% versus −1.6% in the three who did not, which reflects the same outcome coupling and is not independent evidence [22].

Discussion

Three findings emerge, with very different robustness. First, a higher serum D-serine fraction was consistently associated with greater functional dependence, dementia stage, cognitive and motor impairment and higher TNF-α, IL-6 and CRP, and these associations survived FDR control. Second, serum D-serine rose over 12–24 months while L-serine stayed constant, indicating a genuine shift in enantiomeric balance [23]. Third, the prognostic performance of baseline metrics was poor: null in univariable and time-to-event models, significant only after covariate adjustment, and with discrimination CIs that included chance. Serum enantiomeric imbalance thus appears to be a readout of current disease severity rather than a prognostic test, a distinction that matters because only prognostic markers can justify a change in management [24].

Comparison with previous work

Previous results in neurodegeneration conflict: altered D-serine in AD was not replicated in CSF or serum across the AD spectrum, while serum D-serine and the D-/total-serine ratio were reported to increase with AD stage and other chiral profiles implicated different D-amino acids [25]. One explanation is dilution: the brain contributes only a small fraction of circulating D-serine, and renal clearance, hepatic metabolism, gut microbiota and diet all shape serum concentrations. Our finding that serum D-serine tracked inflammation but not NfL is consistent with this: the peripheral measure seems to reflect the systemic inflammatory context of neurodegeneration more than the rate of axonal loss [26]. Our cross-sectional association with severity is in line with the reports of serum increases with disease stage, while the absence of prognostic value agrees with the generally negative or inconsistent literature.

The longitudinal rise of about 12%, with static L-serine, has little precedent. It could reflect increased production by reactive astrocytes expressing serine racemase or reduced DAAO-mediated clearance, a mechanism that modifies motoneuron survival in ALS models. Distinguishing these requires measurement of enzyme activity, not of the amino acid alone [27].

Mechanistic interpretation

Within a physiological window, co-agonist occupancy supports plasticity; beyond it, sustained availability favours calcium overload and excitotoxicity. Our data are compatible with occupancy of the upper part of this window in more advanced disease [28]. The correlation with inflammatory mediators is biologically plausible, because activated glia release glutamate and cytokines and a pro-inflammatory environment may favour toxic extrasynaptic NMDA receptor signalling; however, cross-sectional data cannot establish direction, and shared dependence on disease severity is an equally likely explanation [29]. The lack of correlation with NfL, a cumulative marker of axonal destruction, suggests that the two markers capture different aspects of disease, which could give the D-serine axis incremental value that this sample is too small to demonstrate.

Why the prognostic signal was weak

Four explanations are likely. First, power: with 26 events only large effects are detectable, and the observed effect was small to moderate ($\delta = 0.22$; AUC CI 0.45–0.77). Second, diagnostic heterogeneity: pooling diseases in which the relationship may differ in sign or slope, as the inconsistent subgroup directions suggest, yields an average that understates disease-specific performance [30]. Third, a single baseline value cannot capture a dynamic process; the change variable could not be tested because it was coupled to the outcome. Fourth, measurement noise from two platforms without documented validation would attenuate any true association [31]. The adjusted OR of 7.34 (upper bound 44.6) should not be over-interpreted: it is unstable with 26 events, and its distance from the unadjusted OR of 2.51 shows that adjustment, not the exposure, is doing much of the work. Likewise, the association of serum D-serine with progression rate is nominal and the outcome is partly derived from clinical change [32].

Relation to established fluid biomarkers

NfL behaved as expected, separating progressors from stable patients and predicting time to progression in univariable analysis, although only modestly ($C = 0.603$) and not after adjustment. That an established marker performs modestly in a heterogeneous cohort of 50 sets a realistic ceiling for any new candidate. The combined model did not show incremental value over NfL, but three predictors estimated on 26 events cannot be reliably separated; a pre-registered comparison with several hundred events would be needed [33].

The D-serine fraction is inexpensive to measure by chiral chromatography, changes over clinically meaningful intervals and correlates with function and neuroinflammation. It is therefore a defensible pharmacodynamic endpoint in early-phase trials of agents targeting serine racemase, DAAO or NMDA receptor co-agonism. It should not, on current evidence, be used to counsel individual patients about prognosis, to select trial participants or to guide therapy; this would require a pre-specified threshold in a defined population, demonstrated calibration and discrimination, and independent replication [34].

A formal data audit preceded inference and identified the complete separation of the change variable, which would otherwise have produced a spurious prognostic claim. Multiple testing was controlled, effect sizes and CIs accompany P values, platforms were compared before pooling, the longitudinal design permitted paired and time-to-event analyses, and cross-sectional cognitive inference was restricted to instruments given to the whole cohort.

With 50 patients and 26 events the study is adequate for descriptive and correlational analysis but underpowered for prognostic modelling, and all discrimination estimates should be considered hypothesis-generating. The study was conducted at a single centre, which limits generalizability [35]. Assay validation

data (limit of quantification, precision, accuracy, recovery and stability) were unavailable for both platforms, and individual-level cross-platform equivalence was not established against a certified reference material. No validated reference interval exists for serum D-serine enantiomers, so threshold-based classification could not be evaluated fairly.

Although samples were drawn fasting, time of venepuncture, dietary serine intake and gut microbial metabolism were not recorded, and residual confounding cannot be excluded; renal function was measured but was not modelled as a covariate [36]. Pooling five syndromes and two additional categories assumes a common relationship that the small subgroups could not test. Composite clinical and cognitive scores mix instruments, and progression was a clinician's integrated judgement rather than an algorithmic criterion. Most importantly, the change in D-serine partitioned the outcome perfectly, so this dataset cannot show whether D-serine change precedes and predicts progression; this requires serial pre-outcome sampling with blinded, independent outcome adjudication. Finally, there was no external validation or calibration analysis.

Conclusion

In this multi-diagnosis cohort, serum D-serine enantiomeric imbalance behaved as a marker of current disease burden rather than a predictor of future decline. The D-serine fraction was higher in patients with greater functional dependence, worse staging, cognitive and motor impairment and higher TNF-α, IL-6 and CRP, and serum D-serine rose by a median of 10.5% over 12–24 months while L-serine remained stable. Baseline metrics did not discriminate progressors significantly, a nominal association appeared only after adjustment in an unstable model, and the perfect discrimination of D-serine change reflects coupling with the outcome definition. Serum D-serine enantiomeric imbalance should be regarded as a research-stage candidate and possible pharmacodynamic marker. Prospective, diagnosis-specific studies with harmonised and validated chiral assays, reference intervals, serial pre-outcome sampling, independent outcome adjudication and external validation are needed.

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