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Streptococcal Seroreactivity and Neuropsychiatric Symptom Patterns Relevant to PANS/PANDAS in Young Adults

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Abstract: PANDAS and PANS are paediatric neuropsychiatric constructs, with limited evidence for related patterns in adulthood. We examined whether current antistreptolysin O (ASO) titres covaried with PANS/PANDAS-relevant symptoms in 50 psychiatric patients aged 18–34 years selected using a pragmatic history consistent with previous streptococcal exposure. Participants completed an investigator-adapted Romanian PANS 31-item symptom rating scale; routine EEG and clinical covariates were recorded. Continuous ASO was analysed against the total score and ten study-defined domains using Spearman correlations, bootstrap confidence intervals and Benjamini–Hochberg correction; item-level and clinical-context analyses were exploratory. ASO showed a modest association with the total score ($\rho=0.305$, $p=0.031$), whereas the ≥ 200 -IU/mL contrast was nonsignificant and the association attenuated after adjustment for paediatric psychiatric history. The strongest multiplicity-robust associations involved tics ($\rho=0.496$, $q=0.0025$) and cognitive/motor symptoms ($\rho=0.421$, $q=0.0117$); vocal tics were the only item surviving correction across 31 items. Higher ASO was also associated with paediatric psychiatric history and recorded autoimmune pathology, while routine EEG did not independently converge with symptom findings. Total-scale internal consistency was low ($\alpha=0.535$). These cross-sectional findings support a narrow, hypothesis-generating association between streptococcal seroreactivity and selected PANS/PANDAS-relevant symptom dimensions in young adults, but do not establish adult PANDAS, active neuroinflammation, or ASO as a diagnostic biomarker.

Keywords: PANS; PANDAS; antistreptolysin O; streptococcal seroreactivity; neuropsychiatric symptoms; young adulthood; tics.

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1. Introduction

Can a paediatric post-infectious neuropsychiatric construct leave a measurable trace after the patient is no longer a child? This question is clinically important because paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) are defined by prepubertal onset, whereas the cortico-striato-thalamo-cortical systems implicated in obsessive-compulsive symptoms, tic expression, motor regulation and executive control continue to mature throughout adolescence. Reaching adulthood may therefore mark the end of the current diagnostic framework without necessarily marking the end of every symptom, vulnerability or circuit-level consequence. PANDAS was originally proposed to describe abrupt, episodic obsessive-compulsive symptoms and/or tics temporally associated with group A streptococcal infection in children (Swedo et al., 1998). Paediatric acute-onset neuropsychiatric syndrome (PANS) subsequently broadened the phenotype to unusually abrupt obsessive-compulsive symptoms or severe food restriction accompanied by concurrent symptoms in at least two additional neuropsychiatric or somatic domains, without requiring a streptococcal trigger (*Chang et al., 2015*). The American Academy of Pediatrics clinical report recognises the clinical challenge of sudden-onset paediatric neuropsychiatric presentations while emphasising that disease-specific biomarkers, pathogenic certainty and high-quality treatment evidence remain limited (*American Academy of Pediatrics Board of Directors, 2025*). These cautions are particularly relevant when considering adults, for whom validated PANS/PANDAS diagnostic criteria have not been established.

The adult evidence gap remains substantial. Adult-onset or persistent PANDAS/PANS-like presentations have been described only in isolated reports. These include an early report questioning the applicability of PANDAS beyond childhood, a young adult with sudden-onset obsessive-compulsive symptoms following a streptococcal illness and associated magnetic-resonance abnormalities, and a clinicopathological report of a 26-year-old woman whose neuropsychiatric symptoms began at age 19 following group A streptococcal infection. More recently, a 20-year-old woman with a five-year relapsing-remitting PANS course in whom REM sleep without atonia remained detectable in adulthood (Bodner et al., 2001; Jory & Handelman, 2024; Kulumani Mahadevan et al., 2023; Mulas et al., 2025). These reports establish clinical possibility, not prevalence, causal validity, or a reproducible adult phenotype. They cannot determine whether an adult presentation represents persistence of a childhood syndrome, recurrence in a previously vulnerable individual, a distinct young-adult-onset phenomenon, or coincidence between common infections and common psychiatric symptoms.

The present study addressed this gap dimensionally rather than diagnostically. In a naturalistic cohort of young adults selected using a pragmatic history consistent with previous streptococcal infection or exposure, we asked whether current antistreptolysin O (ASO) titres were associated with current symptom expression measured with an investigator-adapted Romanian version of the PANS 31-item symptom rating scale. We examined whether the largest associations were domain-specific, whether paediatric psychiatric history altered interpretation, and whether routine electroencephalographic features converged with the serological and symptom findings. As an explicitly exploratory extension, we also asked whether ASO variation was associated with developmental history, recorded autoimmune pathology, routine EEG findings, or current diagnostic heterogeneity.

2. Literature Review: Neuroimmune and Developmental Framework

2.1. Mechanistic Plausibility and Evidentiary Boundaries

The neuroimmune hypothesis draws partly on conceptual continuity with Sydenham chorea, a recognised post-streptococcal movement disorder involving basal ganglia-related systems (Cunningham & Cox, 2016; Vreeland et al., 2023). Proposed mechanisms in PANDAS/PANS include molecular mimicry, cross-reactive antineuronal antibodies, altered calcium/calmodulin-dependent protein kinase II signalling, and dopaminergic dysregulation

(Cunningham, 2019; Cunningham & Cox, 2016). Experimental work suggests a potential route for immune access to the central nervous system: repeated intranasal group A *Streptococcus* exposure in mice promoted migration of streptococcal-specific T helper 17 cells into the brain, accompanied by blood–brain barrier disruption, serum IgG deposition and microglial activation (*Dileepan et al., 2016*). Antibodies from children with PANDAS have been reported to bind striatal cholinergic interneurons and alter their activity (Xu et al., 2021); more recent work in relapsing-remitting PANS found increased IgG binding to striatal cholinergic interneurons during symptom flares, with reduced binding in recovery samples (Xu et al., 2024).

Recent mechanistic evidence adds specificity without resolving the clinical controversy. Menendez et al. (2024) reported different dopamine D1- and D2-receptor autoantibody signalling patterns across post-streptococcal neuropsychiatric and movement phenotypes, supporting a model in which functionally active cross-reactive antibodies could influence dopaminergic pathways. Contemporary reviews likewise describe accumulating neuroimmune evidence while emphasising the absence of clinically rigorous diagnostic biomarkers and major gaps in controlled clinical evidence (Vreeland et al., 2023). At the same time, paediatric clinical data caution against treating systemic autoimmunity as a uniform defining feature. Stagi et al. (2014) found that thyroid autoimmunity, coeliac disease and broader organ- or non-organ-specific autoantibodies were not more frequent in children with PANDAS than in healthy controls and these findings did not support routine screening for such abnormalities. Larger studies were nevertheless considered necessary to clarify autoimmune heterogeneity. These observations strengthen biological plausibility while preserving an essential boundary. They do not establish a single ASO measurement as a brain-specific biomarker, demonstrate that the same mechanism operates in every PANS/PANDAS presentation, or validate a diagnosis in adults. ASO is an exposure-related serological marker reflecting the host antibody response to streptolysin O. A single titre cannot reliably date a preceding infection, and serial ASO measurements together with anti-DNase B provide stronger evidence when antecedent group A streptococcal exposure must be established (Sen & Ramanan, 2014). ASO does not identify a pathogenic neuronal antibody, demonstrate neuroinflammation, or function as a disease-specific biomarker for PANS/PANDAS (Wilbur et al., 2019). It should therefore be treated as an imperfect and temporally ambiguous exposure correlate rather than as a diagnostic test. Population-level data have associated both streptococcal and non-streptococcal throat infections with later psychiatric outcomes (Orlovska et al., 2017), while prospective paediatric follow-up found that group A streptococcal infection was neither the only nor the most common antecedent to tic or obsessive-compulsive exacerbations (Kurlan et al., 2008).

This distinction is central to the present article. The study measured ASO, self-reported symptoms and routine EEG features; it did not measure cytokines, blood–brain barrier integrity, cerebrospinal fluid inflammation, neural autoantibodies, dopamine-receptor signalling, or microglial activation. Consequently, the data can identify a statistical association between ASO variation and particular symptom dimensions. They cannot establish autoimmune encephalitis, an inflammatory state, or a causal pathway from streptococcal infection to adult psychopathology.

Gut–brain interactions may represent an additional component of the broader neuroimmune context, although evidence specific to PANS/PANDAS remains limited. Quagliariello et al. (2018) reported altered gut microbial community structure in children with PANS/PANDAS compared with controls, while exploratory ASO–taxa relationships were not evident in the full cohort and emerged only in a small subgroup with high ASO measured near microbiome sampling. These findings remain hypothesis-generating and do not establish a microbiome-mediated mechanism. Functional gastrointestinal pathology was therefore included in the present study only as an exploratory clinical-context variable.

2.2. Why a Paediatric Phenotype May Attenuate or Reorganise

Young adulthood is not simply an older version of childhood. Executive function follows a prolonged developmental trajectory and approaches adult-level stability in late adolescence or early adulthood (Tervo-Clemmens et al., 2023). In Tourette syndrome, the severity of tics and

comorbidities often declines through adolescence, although residual symptoms and clinically relevant persistence remain common (Groth et al., 2017). Childhood caudate volume in Tourette syndrome predicted tic and obsessive-compulsive symptom severity at follow-up in early adulthood (Bloch et al., 2005). Separately, poorer childhood fine-motor and visuospatial performance was associated with persistence of paediatric-onset obsessive-compulsive disorder into adulthood (Bloch et al., 2011). Longitudinal clinical data from a specialised PANS cohort likewise demonstrated heterogeneous flare and disease-progression patterns, prompting proposed standardised terminology for disease state and clinical course (Masterson et al., 2025). That work did not establish adult continuity, but it reinforces the developmental premise that persistence, recurrence, partial remission and reorganisation should be considered separately.

Several non-exclusive adult trajectories are therefore plausible. Improved executive and social control may reduce visible behavioural dysregulation or permit suppression of tics. Childhood school decline may later be experienced as executive inefficiency, “brain fog” or occupational difficulty. Separation anxiety and overt regression may become less prominent, while sensory intolerance, autonomic symptoms or subtle motor phenomena could persist. Alternatively, the childhood trigger may have no continuing biological activity, and adult symptoms may instead reflect stable psychiatric vulnerability, current diagnoses, treatment history or unrelated exposures. A cross-sectional adult sample cannot adjudicate among these pathways. It can only determine whether the observed symptom architecture is compatible with a focused residual pattern and identify measured contextual factors that may alter that interpretation.

Paediatric psychiatric history is especially important. It may represent pre-existing vulnerability, prior symptom expression, greater contact with mental health services or partial continuity of the putative paediatric phenotype. It can also confound an ASO–symptom association if individuals with earlier psychiatric morbidity differ systematically in infection history, treatment, diagnostic mix or adult symptom reporting. For that reason, paediatric history was treated both as a developmental stratifier and as a covariate in sensitivity models, not as proof of childhood PANS/PANDAS.

2.3. Dimensional Phenotyping and Analytical Restraint

The PANS 31-item Symptom Rating Scale was developed to identify and quantify PANS symptom severity and has been psychometrically evaluated as a parent-completed paediatric instrument (Bernstein et al., 2024). In 135 children with PANS, the total score showed strong internal consistency (Cronbach’s $\alpha=0.89$) and preliminary convergent validity. Those properties cannot be assumed to transfer to an investigator-adapted adult self-report version, particularly when age, informant, language, and diagnostic composition change simultaneously. A total score may combine symptom dimensions with different developmental trajectories and different relevance to streptococcal exposure. Study-defined operational content domains are therefore useful for describing where an association lies, provided they are not mistaken for validated adult subscales. Item-level analyses can localise a statistical signal further, but 31 simultaneous tests are highly vulnerable to false-positive findings unless multiplicity is controlled.

Small clinical datasets also invite unstable high-dimensional results. With 50 participants, flexible predictive models or other highly data-driven representations would be particularly vulnerable to sampling variation and unstable performance estimates. Internal cross-validation does not create an independent validation cohort and prediction estimates can have substantial uncertainty in small samples (Poldrack et al., 2020; Varoquaux, 2018). We therefore used a deliberately restrained analytical hierarchy: a global continuous association, study-defined operational domains, multiplicity-controlled item-level exploration, and parsimonious sensitivity models. No predictive model is presented as an empirical result.

3. Methodology

3.1. Design, Setting and Participant Flow

This was a naturalistic, prospectively recruited, cross-sectional observational study of young adults evaluated in two psychiatric care settings in northeastern Romania. Hospital-based recruitment occurred from February 2024 to February 2025, and outpatient recruitment from October 2024 to October 2025. The planned age range was 18–35 years; observed ages were 18–34 years. The upper age limit was a protocol-defined recruitment boundary intended to focus the study on early adulthood and should not be interpreted as a universally accepted developmental cut-off. Participants were not classified as having adult PANS or adult PANDAS. Eligibility required a pragmatic history consistent with previous streptococcal infection or exposure, supported by at least one of the following: prior medical documentation or diagnosis, a previous positive pharyngeal swab/culture, a previous ASO result, or a clinically suggestive history recorded during assessment. This criterion was used for cohort assembly and did not constitute uniform, contemporaneous, microbiologically confirmed group A streptococcal infection.

The research pathway was standardised as follows: written informed consent; screening questionnaire; initial psychiatric consultation with preliminary inclusion/exclusion assessment; participant completion of the Romanian PANS-31 study adaptation; full anamnesis, including psychiatric history and associated organic pathology; final inclusion/exclusion decision; ASO testing; and routine EEG. Of 63 adults evaluated, nine did not complete the required ASO/EEG pathway or did not return for completion, and four were excluded because major organic or neurodevelopmental conditions provided substantial alternative explanations for the study outcomes. The final analytical cohort therefore comprised 50 participants. Recruitment records and the locked analytical dataset were reconciled before manuscript finalisation. No formal a priori power calculation determined the final sample size; the analytical cohort reflects eligible participants who completed the full pathway during the defined recruitment periods. This constraint motivated emphasis on effect estimates and confidence intervals and the avoidance of high-dimensional modelling.

3.2. Eligibility and Exclusion Criteria

Eligible participants were adults aged 18–35 years who met the pragmatic streptococcal-history criterion described above and completed the clinical, serological, and EEG pathway. Exclusion criteria were designed primarily to limit major alternative explanations for psychiatric symptoms or EEG findings. They included major organic disease, especially organic cerebral pathology, epilepsy, moderate-to-profound intellectual disability, clinically important uncontrolled organic disease, and absence or withdrawal of informed consent. Examples identified during screening included an intracranial tumour, moderate intellectual disability, and therapeutically uncontrolled thyroiditis.

The screening questionnaire supported preliminary eligibility and guided the consultation; it was not treated as an outcome instrument. Final inclusion depended on clinical evaluation and completion of objective study procedures. This separation was intended to avoid using the same questionnaire simultaneously to define the sample and to generate the principal research outcome.

3.3. Clinical and Neurophysiological Variables

Age, sex, urban/rural residence, and recruitment source (hospital or outpatient) were recorded. Current psychiatric diagnoses were established by a psychiatrist during routine clinical evaluation. Paediatric psychiatric history was obtained from the available hospital information system and/or prior medical documentation. The clinical dataset also included previous penicillin treatment, atopic background, recorded autoimmune conditions, and functional gastrointestinal pathology. In this dataset, previous penicillin treatment referred to prior benzathine benzylpenicillin

exposure; indication, dose, timing, and duration were not standardised. All participants were receiving psychotropic treatment at the time of assessment; individual agents, doses, combinations, and treatment duration were not systematically encoded and therefore could not be adjusted for analytically. Paediatric psychiatric history was analysed both as the recorded historical information and as a binary indicator of any previous paediatric psychiatric follow-up.

Routine EEG was recorded using a Neuron-Spectrum-4/P system (Neurosoft), with a standard 21-electrode montage based on the international 10–20 system and bilateral auricular references. The source database recorded two routine EEG modifications: low-voltage alpha activity and polymorphic theta activity. Low-voltage alpha was a qualitative clinical EEG descriptor of alpha rhythm in the 8–12-Hz range; no quantitative amplitude threshold was available in the source data. A derived binary variable represented the presence of either recorded modification. These routine features are nonspecific and were analysed as exploratory neurophysiological context, not as biomarkers of PANS/PANDAS or inflammation.

3.4. ASO Measurement

ASO determinations were obtained as part of routine clinical laboratory work-up rather than through a study-specific assay protocol. When testing was performed in the hospital laboratory, latex agglutination was used, whereas determinations performed in private clinical laboratories used immunoturbidimetric methods. The assay method was not encoded at participant level, and some hospital participants were referred externally when local reagents were unavailable. The research team did not repeat, reanalyse, or experimentally standardise the assays. Numerical ASO values in IU/mL were transcribed from the original laboratory documentation without recalculation. ASO was analysed primarily as a continuous variable. A secondary threshold comparison used ASO ≥ 200 IU/mL versus < 200 IU/mL, matching the locked database coding. The threshold was treated as an operational cut point, not as proof of active infection, neuroinflammation, or neuroimmune disease.

3.5. Symptom Measure and Scoring

Participants completed an investigator-prepared Romanian working adaptation of the PANS 31-item Symptom Rating Scale (Murphy & Bernstein, 2024), with investigator clarification available when wording required explanation.

Table 1. Study-defined operational domains and corresponding PANS-31 items

Operational domain	Items	Clinical content
OCD/anxiety	I01–I03, I07–I08	Obsessions; compulsions; hoarding; separation anxiety; other anxiety, phobias or panic
Affective	I09–I13	Rapid mood changes; inappropriate crying/laughter; suicidal ideation/self-harm; depressive symptoms/withdrawal; irritability
Behavioural	I14–I16	Oppositionality/demand avoidance; rage/aggression; agitation, hyperactivity or impulsivity
Cognitive/motor	I17, I20–I22	Attention/concentration difficulties; reduced work/study/daily performance; deterioration in handwriting/copying/typing/drawing; cognitive slowing, confusion, “brain fog” or memory problems
Functional regression	I18–I19	Regression/change in mode of expression; behavioural or functional regression with reduced autonomy
Somatic/autonomic	I23–I26	Pain; sleep disturbance; urinary incontinence/enuresis; urinary frequency
Sensory hypersensitivity	I27	Increased sensitivity to sounds, smells, textures, light, clothing or touch
Psychotic	I28–I29	Hallucinations; delusional or paranoid ideation
Tics	I30–I31	Motor tics; vocal tics
Eating	I04–I06	Food refusal/avoidance; increased appetite/food preoccupation; fluid refusal/avoidance

The original instrument was developed for paediatric caregiver-rated use and has published preliminary psychometric support in children with PANS (Bernstein et al., 2024); responses in the present study came directly from adult participants. The Romanian working adaptation had not undergone formal transcultural or adult psychometric validation and was treated as an exploratory research measure. Each item was scored from 0 to 4. The total was the sum of all 31 item scores, with a theoretical range of 0–124.

Ten study-defined operational content domains were calculated using the fixed item-to-domain mapping shown in Table 1. Domain scores were item sums, and the ten domain totals exactly reconstructed the PANS-31 total for every participant. Because domain lengths differed, descriptive mean-per-item values were also generated, whereas inferential domain analyses used summed scores. These groupings were operational analytical constructs rather than validated PANS subscales or adult dimensions. For adult use, wording referring to school or developmental functioning was adapted to work, studies, daily activities, and functional autonomy while preserving the original 31-item structure.

3.6. Data Integrity and Statistical Analysis

The source workbook contained a clinical sheet and a participant-level item sheet. The analytical pipeline retained rows with valid participant identifiers, checked for duplicate identifiers, required all 31 item scores to be numeric and within the 0–4 range, and recalculated each participant's total and ten operational domain scores directly from the item-level data. Recalculated totals were cross-checked against both source sheets, and the sum of the ten operational domains was verified to reproduce the total score for every participant. The categorical ASO field was also checked against the ≥ 200 IU/mL rule, and the binary EEG variable was verified against the recorded EEG modifications. No invalid item values, missing analytical values, duplicated identifiers, or scoring discrepancies remained in the locked 50-participant dataset.

Continuous variables are summarised using mean, standard deviation, median, interquartile range, and range; categorical variables are reported as counts and percentages. The primary analysis was the Spearman correlation between continuous ASO and the PANS-31-derived total score. Spearman correlation was selected because the outcome was derived from ordinal symptom items and bivariate normality was not assumed; Pearson correlation was reported as a parametric companion estimate.

The secondary inferential layer comprised Spearman correlations between continuous ASO and the ten study-defined operational symptom domains. Benjamini–Hochberg false-discovery-rate (BH-FDR) correction was applied across these ten tests (Benjamini & Hochberg, 1995), and nonparametric percentile 95% confidence intervals were estimated using 5,000 bootstrap replications. Item-level ASO correlations were exploratory, used 2,500-replication bootstrap confidence intervals, and were corrected as a separate family of 31 tests using BH-FDR. Internal consistency was estimated using Cronbach's alpha for the total scale and multi-item domains, with 5,000-replication bootstrap confidence intervals; alpha was not calculated for the single-item sensory domain.

The secondary ASO threshold comparison used the Mann–Whitney U test, rank-biserial correlation, Welch's t test, Hedges' g, and a bootstrap confidence interval for the mean difference. Associations between the PANS-31-derived total and nine binary clinical variables—sex, residence, recruitment source, previous benzathine benzylpenicillin exposure, paediatric psychiatric history, atopic background, recorded autoimmune pathology, functional gastrointestinal pathology, and any routine EEG modification—were exploratory and BH-FDR corrected as one family. Exploratory domain-level comparisons by paediatric psychiatric history and by routine EEG modification were each BH-FDR corrected across the ten operational domains. Developmental strata combined paediatric psychiatric history with ASO category (≥ 200 / <200 IU/mL); their distributions were described and an adjusted interaction model was used as a sensitivity analysis.

Ordinary least-squares models with HC3 heteroskedasticity-consistent standard errors estimated the PANS-31-derived total-score change per 50-IU/mL increase in ASO. Models were sequentially unadjusted, adjusted for age and sex, additionally adjusted for paediatric psychiatric history, and additionally adjusted for any routine EEG modification. Rank-based outcome/exposure models assessed sensitivity to distributional assumptions. Covariates were selected on clinical grounds rather than by automated procedures, and model dimensionality was deliberately restricted given the sample size.

Additional clinical-context analyses were exploratory. Continuous ASO was compared between participants with versus without paediatric psychiatric history, recorded autoimmune pathology, atopic background, functional gastrointestinal pathology, and any routine EEG modification using Mann–Whitney U tests and rank-biserial effect estimates with bootstrap 95% confidence intervals; BH-FDR correction was applied across this five-variable family. Previous benzathine benzylpenicillin exposure was analysed separately because it represented a therapeutic exposure and potential determinant of the serological marker rather than a comorbidity. ASO distributions across the nine current diagnostic categories were examined using the Kruskal–Wallis test with a 20,000-permutation sensitivity analysis. The diagnostic-category $\times$ ASO-threshold contingency table was evaluated using the exact Fisher–Freeman–Halton test by conditional enumeration. Diagnosis-specific post-hoc tests were not performed because several cells were small. Finally, an exploratory HC3 OLS model with ASO as the dependent variable included age, sex, paediatric psychiatric history, recorded autoimmune pathology, any EEG modification, and previous benzathine benzylpenicillin exposure to examine whether bivariate clinical-context signals persisted under simultaneous adjustment.

All tests were two-sided. Within each prespecified BH-FDR family, $q < 0.05$ was considered statistically significant. Multiplicity correction was applied within conceptually distinct analytical families rather than across every analysis in the manuscript; exploratory findings were therefore interpreted as hypothesis-generating.

Statistical analyses were conducted in CPython 3.12.13 using NumPy 2.3.5, pandas 2.2.3, and SciPy 1.17.0 on Linux x86_64. Fixed random seeds were used for reproducibility. HC3 covariance estimation was implemented explicitly in the reproducible analysis script. Figures were generated in Python from the locked participant-level dataset and corresponding statistical analyses using Matplotlib 3.10.8 and seaborn 0.13.2. Statistical reporting followed STROBE principles for observational studies (von Elm et al., 2007).

3.7. Ethics

All participants provided written informed consent before research procedures. The study received research-ethics approval and the required institutional authorisations before recruitment. Identifying committee, institution, approval-number and approval-date details are supplied separately to the editor to preserve double-blind peer review. Only de-identified analytical identifiers were used in the reproducible dataset.

4. Results

4.1. Data Integrity and Sample Characteristics

The locked analytical cohort included 50 participants and 31 complete symptom items per participant. There were no missing analytical values, item scores outside 0–4, duplicated participant identifiers or mismatches between recalculated item totals, recorded totals and domain sums. Twenty-six participants (52%) had $\text{ASO} \geq 200$ IU/mL, 21 (42%) had a paediatric psychiatric history and 11 (22%) had at least one recorded routine EEG modification. Of those 11, seven had low-voltage alpha activity and four had polymorphic theta activity.

Mean age was 25.56 years (SD 5.32; range 18–34), 30 participants (60%) were male, 23 (46%) lived in rural settings and 34 (68%) were recruited from hospital-based care. Mean ASO was

176.26 IU/mL (SD 63.12), with median 200.50 IU/mL (IQR 122.50–228.25) and range 75–301. The current diagnostic distribution was heterogeneous: emotionally unstable personality disorder, n=14; substance-use/dependence disorder, n=8; schizophrenia, n=8; depressive disorder, n=8; ADHD, n=5; OCD, n=4; and one participant each with acute psychotic disorder, bipolar disorder, and sleep disorder. Table 2 summarises the cohort.

Table 2. Clinical characteristics of the analytical cohort (N=50)

Characteristic	Value
Age, years	25.6 (5.3); median 26.0 [IQR 21.0–30.0]; range 18.0–34.0
Female sex	20 (40%)
Rural residence	23 (46%)
Hospital source	34 (68%)
ASO, IU/mL	176.3 (63.1); median 200.5 [IQR 122.5–228.2]; range 75.0–301.0
ASO $\geq$ 200 IU/mL	26 (52%)
Previous benzathine benzylpenicillin	14 (28%)
Paediatric psychiatric history	21 (42%)
Any routine EEG modification	11 (22%)
Atopic background	9 (18%)
Recorded autoimmune condition	6 (12%)
Functional gastrointestinal condition	21 (42%)
Current psychotropic treatment	50 (100%)
PANS-31 total score	52.5 (7.1); median 53.0 [IQR 46.2–58.8]; range 40.0–65.0

Note. Values are n (%) unless a continuous summary is shown. ASO=antistreptolysin O; EEG=electroencephalography; IQR=interquartile range. Recorded autoimmune conditions were heterogeneous; functional gastrointestinal pathology consisted of irritable bowel syndrome in this dataset.

4.2. Symptom Profile and Measurement Reliability

The mean PANS-31 total score was 52.48 (SD 7.08), with median 53, IQR 46.25–58.75, and a range of 40–65. Because the cohort was selected from psychiatric settings using a pragmatic streptococcal-history criterion, these values are descriptive of this sample and are not population norms or diagnostic cut-offs.

Internal consistency of the 31-item total was low (Cronbach's α =0.535; 95% bootstrap CI 0.356–0.646). Domain alphas ranged from 0.010 for functional regression to 0.578 for affective symptoms; estimates were imprecise, and several confidence intervals included zero.

This pattern supports treating the inventory as a multidomain symptom profile rather than a psychometrically homogeneous adult scale. Domain score distributions and reliability estimates are reported in Table 3.

Table 3. PANS-31-derived score distributions and internal consistency

Scale/domain	Items	Mean (SD)	Median [IQR]	Cronbach's alpha (95% CI)
PANS-31 total	31	52.48 (7.08)	53.0 [46.2–58.8]	0.535 (0.356 to 0.646)
OCD/anxiety	5	9.18 (2.52)	9.0 [7.2–11.0]	0.395 (0.084 to 0.580)
Affective	5	12.34 (2.83)	13.0 [10.2–15.0]	0.578 (0.374 to 0.711)
Behavioural	3	6.78 (1.79)	7.0 [6.0–8.0]	0.272 (-0.182 to 0.523)
Cognitive/motor	4	8.66 (2.26)	9.0 [7.0–10.0]	0.378 (-0.047 to 0.611)
Functional regression	2	4.06 (1.15)	4.0 [3.0–5.0]	0.010 (-0.639 to 0.381)
Somatic/autonomic	4	4.88 (2.00)	5.0 [4.0–6.0]	0.366 (-0.029 to 0.570)
Sensory hypersensitivity	1	1.86 (1.03)	2.0 [1.0–2.0]	Not applicable (single item)
Psychotic	2	0.88 (1.15)	0.0 [0.0–1.0]	0.515 (0.052 to 0.768)
Tics	2	0.48 (0.84)	0.0 [0.0–1.0]	0.283 (-0.146 to 0.661)
Eating	3	3.36 (1.76)	3.0 [2.0–4.0]	0.334 (-0.132 to 0.607)

Note. PANS-31 scores were produced from a Romanian study adaptation completed by adult participants. Alpha is not applicable to the single-item sensory-hypersensitivity domain. Confidence intervals used 5,000 bootstrap replications.

4.3. Primary Analysis: ASO and the PANS-31-Derived Total Score

Continuous ASO showed a modest positive monotonic association with the PANS-31 total score (Spearman $\rho=0.305$; 95% bootstrap CI 0.014–0.568; $p=0.031$). The parametric companion estimate was weaker and did not cross the conventional significance threshold (Pearson $r=0.269$; $p=0.059$), while the confidence interval around the Spearman estimate remained broad.

The locked threshold comparison was not statistically significant. Participants with ASO ≥ 200 IU/mL had a mean PANS-31 total of 53.85 (SD 6.84), compared with 51.00 (SD 7.19) among those below 200 IU/mL. The mean difference was 2.85 points (95% bootstrap CI -0.99 to 6.59), Mann–Whitney $p=0.139$, rank-biserial correlation=0.245, Welch $p=0.159$ and Hedges' $g=0.400$. The continuous and threshold results are not contradictory: categorisation discards information and may reduce power, while the single cut point is not a validated adult neuropsychiatric boundary.

4.4. Secondary Domain-Specific Associations

Across the ten operational domains, the two largest associations that remained below the BH-FDR threshold were tics ($\rho=0.496$; 95% bootstrap CI 0.228–0.696; raw $p=0.00025$; $q=0.0025$) and cognitive/motor symptoms ($\rho=0.421$; 95% CI 0.162–0.640; $p=0.0023$; $q=0.0117$) (Table 4; Figures 1 and 2). Sensory hypersensitivity ($\rho=0.316$; $q=0.0696$) and somatic/autonomic symptoms ($\rho=0.311$; $q=0.0696$) were nominally associated but did not survive correction. Because formal statistical contrasts between domain-specific correlation coefficients were not performed, these results identify the most multiplicity-robust observed associations rather than proving that the underlying effects differ across domains.

Eating, functional regression, psychotic, affective, OCD/anxiety and behavioural domains were not statistically significant after multiplicity correction. The behavioural point estimate was negative ($\rho=-0.190$), further indicating that the total-score association was not accompanied by

uniformly positive domain estimates. Within this dataset, the tic and cognitive/motor domains therefore provided the clearest multiplicity-controlled locations of the observed ASO association.

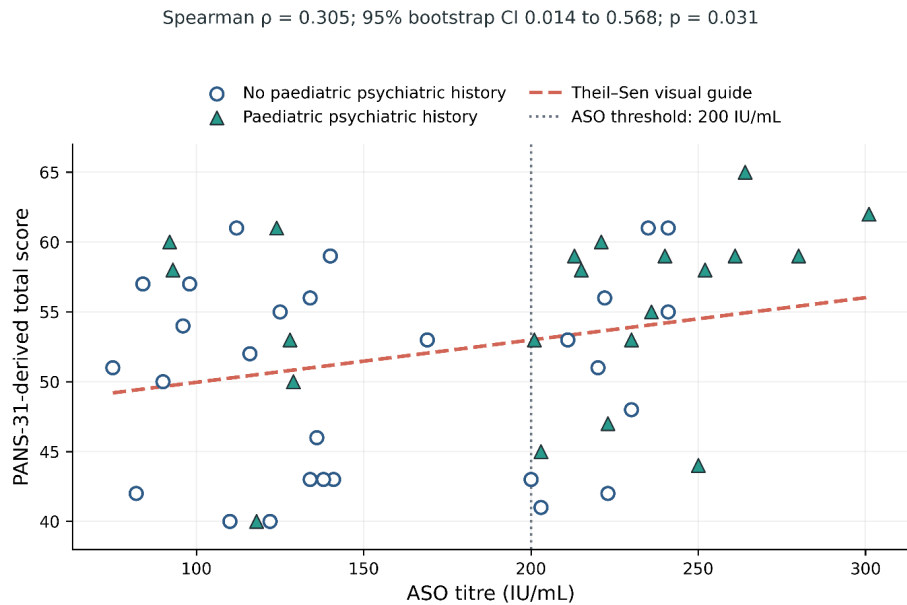


Figure 1. Relationship between continuous ASO and the PANS-31-derived total score. Symbols distinguish participants by documented paediatric psychiatric history; the dashed line is a robust visual guide, and the dotted vertical line marks the locked 200-IU/mL operational threshold. Statistical inference is Spearman-based.

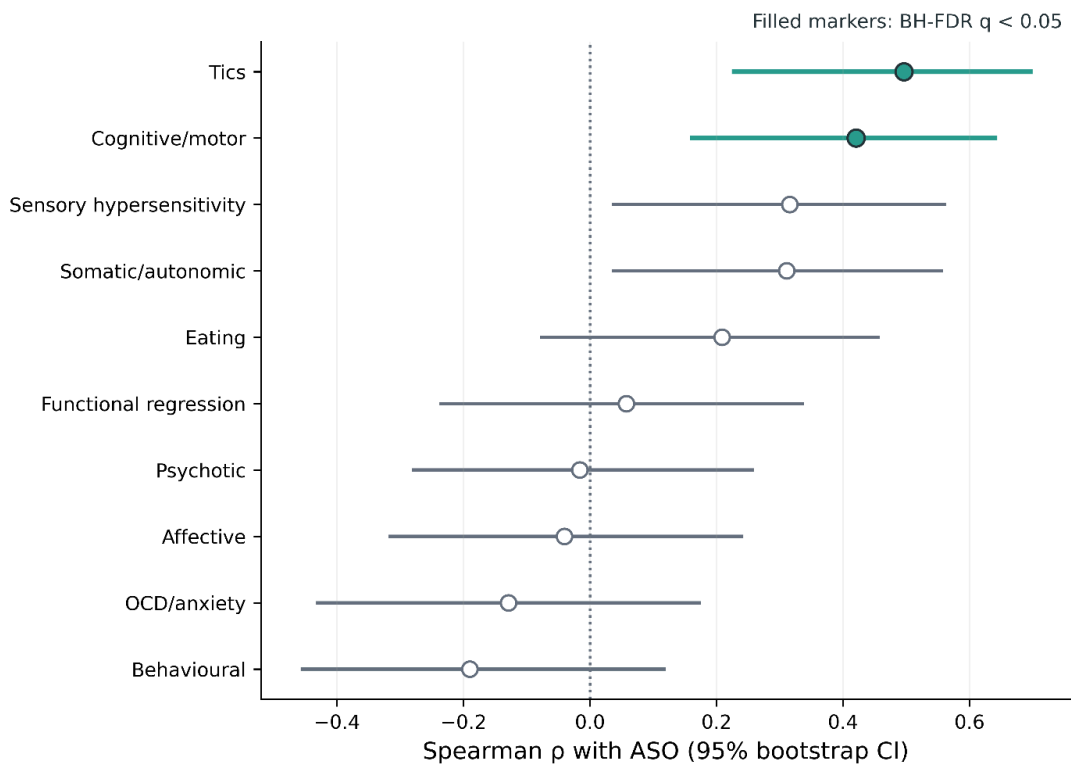


Figure 2. Associations between continuous ASO and ten study-defined operational PANS-31 symptom domains. Points show Spearman rho estimates and horizontal lines show 95% bootstrap confidence intervals. Filled markers identify domains remaining significant after BH-FDR correction across the ten-domain family.

Table 4. Associations between continuous ASO and study-defined operational symptom domains

Domain	Spearman rho (95% bootstrap CI)	Raw p	BH-FDR q
Tics	0.496 (0.228 to 0.696)	0.0002	0.0025
Cognitive/motor	0.421 (0.162 to 0.640)	0.0023	0.0117
Sensory hypersensitivity	0.316 (0.038 to 0.560)	0.0254	0.0696
Somatic/autonomic	0.311 (0.037 to 0.556)	0.0278	0.0696
Eating	0.209 (-0.076 to 0.456)	0.1454	0.2907
Functional regression	0.058 (-0.235 to 0.336)	0.6913	0.8641
Psychotic	-0.016 (-0.279 to 0.256)	0.9116	0.9116
Affective	-0.040 (-0.316 to 0.240)	0.7805	0.8672
OCD/anxiety	-0.129 (-0.430 to 0.173)	0.3729	0.5327
Behavioural	-0.190 (-0.455 to 0.117)	0.1865	0.3108

Note. Confidence intervals are nonparametric percentile bootstrap intervals from 5,000 replications. BH-FDR=Benjamini–Hochberg false-discovery-rate correction across the ten-domain family. The domains are study-defined analytical groupings rather than validated adult PANS subscales.

4.5. Exploratory Item-Level Localisation

Only one of the 31 item-level ASO correlations survived BH-FDR correction: vocal tics (I31; rho=0.484; 95% bootstrap CI 0.270–0.640; p=0.00037; q=0.0115). Urinary incontinence/enuresis (I25; rho=0.407; q=0.0523) and cognitive symptoms/“brain fog” (I22; rho=0.371; q=0.0823) were the next-largest positive results but remained exploratory. Deterioration in graphic activities, motor tics, sensory hypersensitivity, and food refusal/avoidance had nominal p<0.05 but q>0.05. Agitation/hyperactivity/impulsivity had a nominal negative association (rho=-0.306; p=0.031; q=0.119). Because item responses were self-reported, ordinal and tested in a small sample, even the vocal-tic finding requires independent replication.

4.6. Exploratory Clinical Context: Paediatric History, EEG and Other Variables

The four developmental strata showed an ordered descriptive pattern. Mean PANS-31 totals were 50.11 for no paediatric psychiatric history/ASO <200 (n=18), 51.27 for no history/ASO ≥200 (n=11), 53.67 for paediatric history/ASO <200 (n=6), and 55.73 for paediatric history/ASO ≥200 (n=15). However, the adjusted ASO-category × paediatric-history interaction was null (p=0.949). Figure 3 should therefore be read as a descriptive stratification, not as evidence that paediatric history modifies the ASO association or that the apparent ordering represents a biological gradient.

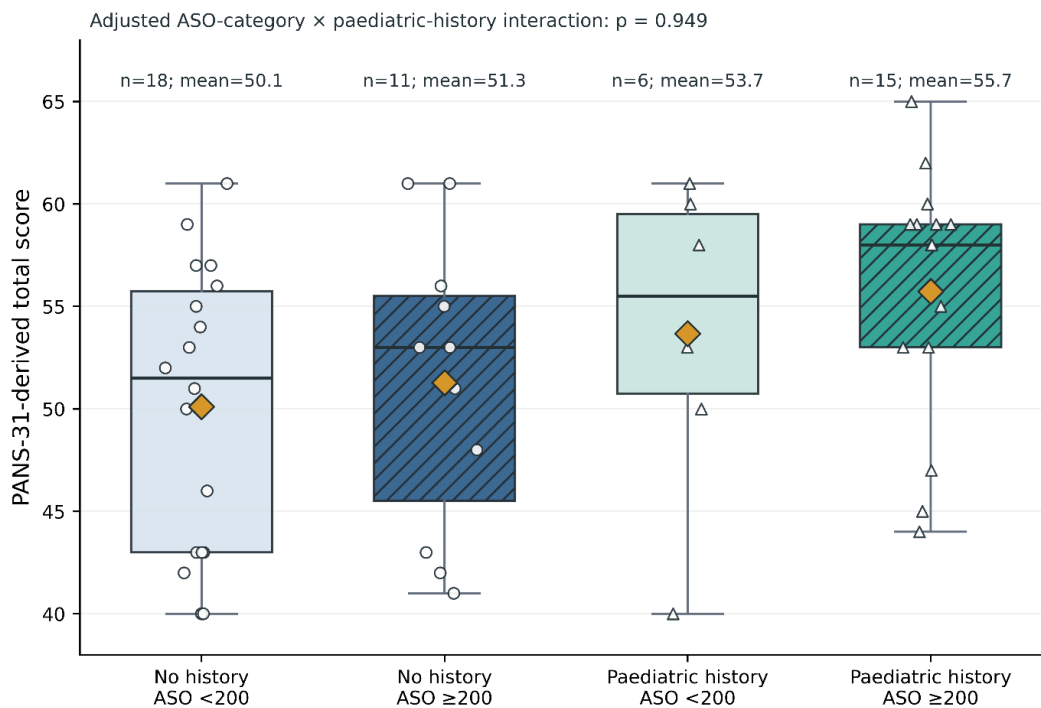


Figure 3. PANS-31-derived total-score distributions across paediatric psychiatric history and ASO strata. Boxes show interquartile ranges and medians, individual points show participants, and diamonds show means. The display is descriptive; the adjusted ASO-category × paediatric-history interaction was not supported ($p=0.949$).

In the nine-variable exploratory family, recorded autoimmune pathology ($n=6$ versus $n=44$), outpatient recruitment source ($n=16$ versus $n=34$) and paediatric psychiatric history ($n=21$ versus $n=29$) each showed nominally higher PANS-31 total scores (Mann–Whitney $p=0.0096$, 0.0131 and 0.0186 , respectively). None met $q<0.05$ after BH-FDR correction; all three had $q=0.0557$. The autoimmune subgroup was particularly small and diagnostically heterogeneous, so its large unadjusted mean difference cannot be interpreted as an autoimmune effect.

Functional gastrointestinal pathology was recorded in 21 participants (42%), but PANS-31-derived total scores were similar in participants with versus without this history (mean 52.29 vs 52.62 ; Mann–Whitney $p=0.8361$; BH-FDR $q=0.8361$).

Any EEG modification was not associated with PANS-31 total score (mean difference 1.13 points; Mann–Whitney $p=0.606$; BH-FDR $q=0.779$) and no EEG-domain comparison survived correction across ten domains. Likewise, no paediatric-history domain comparison survived its ten-test correction; the tic-domain difference was nominal ($p=0.0074$) but $q=0.0737$. These results do not establish absence of neurophysiological or developmental effects; they show that routine nonspecific EEG categories and retrospective paediatric history did not yield multiplicity-robust domain associations in this sample.

4.7. Exploratory ASO Associations Across Developmental, Medical and Diagnostic Context

The exploratory clinical-context analyses addressed a different question from the primary ASO–symptom analysis: whether seroreactivity itself was associated with developmental or medical characteristics. Participants with a documented paediatric psychiatric history had higher ASO levels than those without such history (median 221 versus 138 IU/mL; rank-biserial $r=0.420$; $p=0.012$; $q=0.020$). Higher ASO was also observed among participants with recorded autoimmune pathology (238 versus 140.5 IU/mL; $r=0.682$; $p=0.0075$; $q=0.0188$) and among those with any routine EEG modification (230 versus 138 IU/mL; $r=0.562$; $p=0.0049$; $q=0.0188$). Functional gastrointestinal pathology was not associated with higher ASO; titres were descriptively lower

among participants with this history (median 169 vs 211 IU/mL; rank-biserial $r=-0.202$; $p=0.2305$; $q=0.2305$). Atopic background was likewise not supported after correction. These results describe the distribution of ASO within this selected cohort; they do not establish that the associated clinical features share one autoimmune mechanism (Table 5; Figure 4).

Table 5. Exploratory clinical-context associations with continuous ASO

Clinical feature	n present/absent	ASO present, median [IQR]	ASO absent, median [IQR]	Rank-biserial r (95% CI)	p / q
Paediatric psychiatric history	21/29	221 [129–250]	138 [112–211]	0.420 (0.112 to 0.701)	0.0122 / 0.0203
Autoimmune pathology	6/44	238 [224.5–256]	140.5 [117.5–222.2]	0.682 (0.402 to 0.902)	0.0075 / 0.0188
Atopic background	9/41	220 [200–240]	141 [116–223]	0.339 (–0.019 to 0.669)	0.1174 / 0.1467
Functional GI pathology	21/29	169 [118–213]	211 [124–240]	–0.202 (–0.496 to 0.126)	0.2305 / 0.2305
Any EEG modification	11/39	230 [220.5–244]	138 [114–214]	0.562 (0.259 to 0.828)	0.0049 / 0.0188
Previous BPG treatment*	14/36	126 [101–139.5]	212 [134–237]	–0.538 (–0.778 to –0.254)	0.0035 / —*

Note. Positive rank-biserial values indicate higher ASO in the group with the feature. BH-FDR correction was applied to the five clinical indicators from paediatric psychiatric history through EEG. *BPG=benzathine benzylpenicillin. Previous BPG exposure was analysed separately because it is a therapeutic exposure and potential determinant of ASO; its inverse association is not evidence of a treatment effect. CI=confidence interval; EEG=electroencephalography; IQR=interquartile range.

ASO also differed globally across the nine current psychiatric diagnostic categories (Kruskal–Wallis $H=18.59$; $p=0.0172$; permutation $p=0.0045$). The corresponding association between diagnosis and the binary ASO ≥ 200 -IU/mL threshold did not cross the conventional 0.05 level (exact Fisher–Freeman–Halton $p=0.0630$). No post-hoc diagnostic comparisons were undertaken because three categories contained one participant and several others remained small. The global finding therefore indicates heterogeneity in the serological distribution across the clinical sample, not enrichment of ASO within any specific adult psychiatric diagnosis.

Previous benzathine benzylpenicillin treatment was associated with lower ASO at the bivariate level (median 126 versus 212 IU/mL; rank-biserial $r=-0.538$; $p=0.0035$) but was analysed outside the five-variable clinical-context FDR family. In the exploratory HC3 model with ASO as the dependent variable (adjusted $R^2=0.343$), paediatric psychiatric history ($B=+37.4$ IU/mL; $p=0.023$) and recorded autoimmune pathology ($B=+49.3$ IU/mL; $p=0.018$) retained nominal associations; the EEG coefficient attenuated ($B=+23.7$ IU/mL; $p=0.338$), while previous benzathine benzylpenicillin remained inversely associated ($B=-44.1$ IU/mL; $p=0.023$). Because treatment indication, timing, and interval from infection were not controlled, and because a single ASO titre is not a disease-activity measure, the penicillin result cannot be interpreted as antibiotic efficacy or as a longitudinal serological response to treatment.

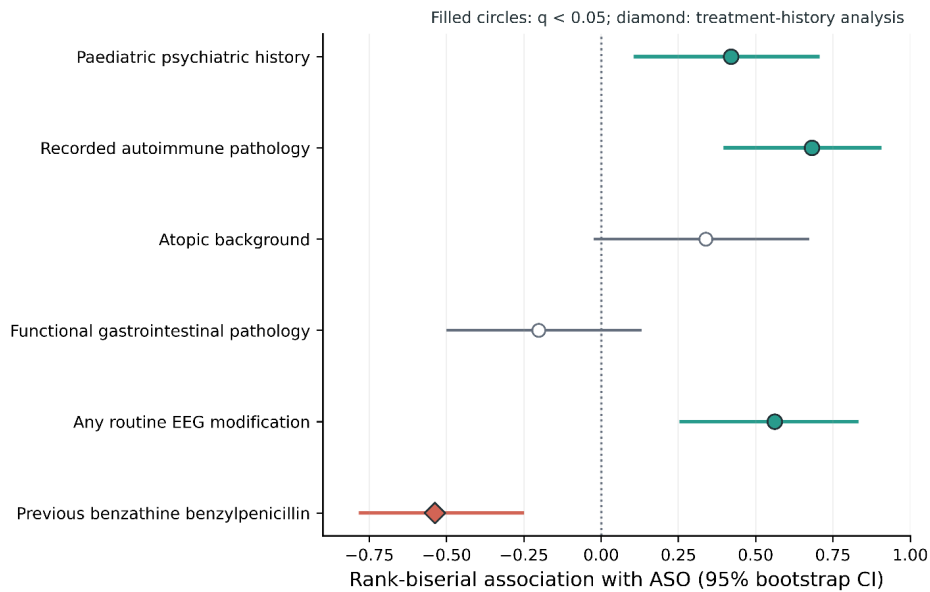


Figure 4. Exploratory associations between continuous ASO and selected clinical-context variables. Points show rank-biserial effect estimates and horizontal lines show bootstrap 95% confidence intervals. Filled circles identify $q < 0.05$ within the five-variable clinical-context FDR family. Previous benzathine benzylpenicillin is shown separately with a diamond because treatment exposure was not included in that FDR family. Effect direction is associational and does not imply causality.

4.8. Adjusted Sensitivity Analyses

Table 6 and Figure 5 show the model sequence. In unadjusted OLS with HC3 standard errors, each 50-IU/mL increase in ASO corresponded to 1.51 additional PANS-31 total points (95% CI -0.08 to 3.10 ; $p=0.0615$). After adjustment for age and sex, the coefficient was 1.42 (95% CI -0.20 to 3.04 ; $p=0.0845$). After paediatric psychiatric history was added, it decreased to 0.83 (95% CI -1.08 to 2.74 ; $p=0.3846$); the paediatric-history coefficient was 3.81 points ($p=0.1196$). After addition of any EEG modification, the ASO coefficient remained nonsignificant ($B=1.00$; $p=0.3627$).

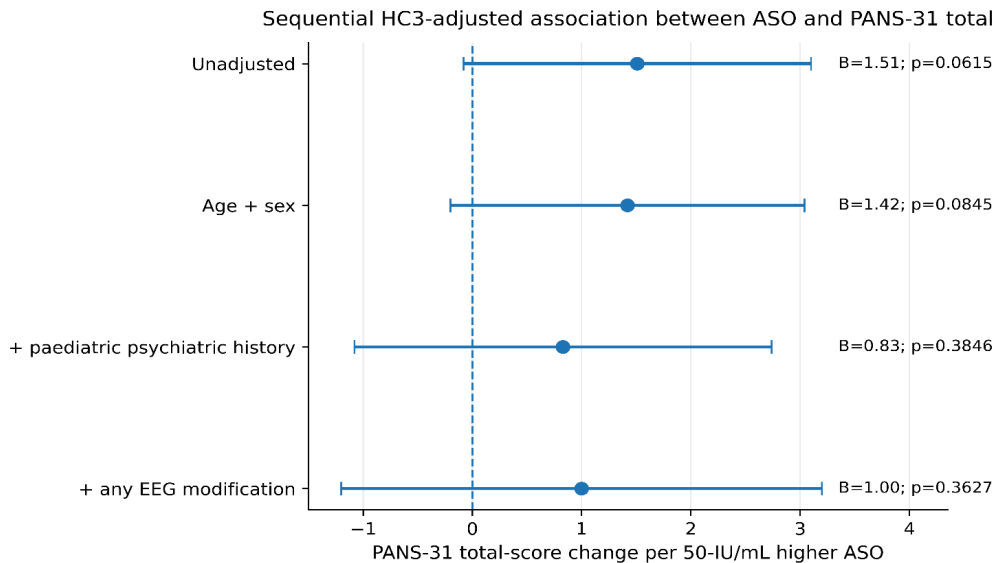


Figure 5. Sequential HC3-adjusted associations between continuous ASO and PANS-31-derived total score. Points represent regression coefficients for the change in PANS-31 total score per 50-IU/mL higher ASO, and horizontal lines represent 95% confidence intervals. Models were sequentially unadjusted, adjusted for age and sex, additionally adjusted for paediatric psychiatric history, and additionally adjusted for any routine

EEG modification. The attenuation after inclusion of paediatric psychiatric history illustrates the sensitivity of the crude association to measured developmental context.

Rank-based sensitivity models showed the same attenuation: the ranked ASO coefficient had $p=0.0535$ after adjustment for age and sex and $p=0.2786$ after paediatric psychiatric history was added. In this sample, the reduction in the ASO coefficient after developmental history was introduced shows that the crude ASO–total association was sensitive to measured developmental context. It does not establish mediation or confounding, and the adjusted confidence intervals remain too wide to define either the presence or absence of a clinically meaningful independent effect.

Table 6. Global ASO associations and adjusted sensitivity models for PANS-31 total score

Analysis	Estimate	95% CI	p
Continuous ASO vs PANS-31 total	Spearman rho 0.305	0.014 to 0.568	0.0312
ASO ≥ 200 vs < 200 IU/mL	Mean difference 2.85; Hedges g 0.40	-0.99 to 6.59	0.1391 (Mann–Whitney)
OLS HC3, unadjusted (per 50 IU/mL)	B 1.51 (HC3 SE 0.79)	-0.08 to 3.10	0.0615
OLS HC3, adjusted for age and sex	B 1.42 (HC3 SE 0.81)	-0.20 to 3.04	0.0845
OLS HC3, plus paediatric psychiatric history	B 0.83 (HC3 SE 0.95)	-1.08 to 2.74	0.3846
OLS HC3, plus any EEG modification	B 1.00 (HC3 SE 1.09)	-1.20 to 3.20	0.3627
ASO-category $\times$ paediatric-history interaction	B -0.33 (HC3 SE 5.17)	-10.76 to 10.09	0.9492

Note. OLS coefficients represent PANS-31 points per 50-IU/mL higher ASO. HC3 denotes heteroskedasticity-consistent standard errors. The categorical interaction used ASO ≥ 200 versus < 200 IU/mL and any paediatric psychiatric history. Analyses are associational.

5. Limits and Discussions

5.1. Principal Findings

Against a literature dominated by paediatric cohorts and isolated adult reports, the present study provides a structured dimensional examination of PANS/PANDAS-relevant symptom patterns in young adult psychiatric patients with a history consistent with previous streptococcal exposure. Continuous ASO showed a modest association with the PANS-31-derived total score, while the strongest multiplicity-robust associations were observed for tic and cognitive/motor symptoms. At item level, vocal tics were the only finding that remained significant after correction across all 31 items.

The binary ≥ 200 -IU/mL contrast did not reproduce the continuous ASO–total association, and the ASO coefficient attenuated after adjustment for paediatric psychiatric history. Exploratory clinical-context analyses also showed higher ASO among participants with paediatric psychiatric history and recorded autoimmune pathology, whereas routine EEG did not independently converge with symptom findings. Taken together, the results identify a selective dimensional pattern within this cohort rather than a uniform serology–symptom–EEG profile.

5.2. A Neuroimmune Interpretation Without Diagnostic Overreach

The selective tic and cognitive/motor pattern is biologically plausible because cortico-striatal and related basal-ganglia circuits are implicated in tic expression and motor regulation, while frontostriatal networks also contribute to executive and attentional processes represented within the cognitive/motor domain. Molecular mimicry, cross-reactive antineuronal antibodies, altered dopamine-receptor signalling, blood–brain barrier disruption, and immune-cell trafficking provide plausible mechanisms through which streptococcal immune responses could influence these circuits (Cunningham, 2019; Cunningham & Cox, 2016; Dileepan et al., 2016; Menendez et al., 2024; Xu et al., 2021, 2024). The present findings are therefore compatible with current mechanistic models, while remaining insufficient to demonstrate any of these mechanisms directly.

This distinction is particularly important for ASO, which reflects an immune response to streptolysin O but cannot identify a pathogenic neuronal antibody, precisely date the relevant infection, or demonstrate central nervous system inflammation (Sen & Ramanan, 2014; Wilbur et al., 2019). The observed findings should therefore be interpreted as serology–symptom covariance rather than evidence of active neuroinflammation. Nevertheless, the concentration of the association in tic and cognitive/motor domains rather than across all symptom dimensions provides a focused target for replication.

The autoimmune-context finding adds a further exploratory layer. Participants with recorded autoimmune pathology had higher ASO, and autoimmune pathology retained a nominal association in the adjusted ASO model. Because this subgroup was small and clinically heterogeneous, the finding cannot support a general autoimmune interpretation; paediatric evidence likewise cautions against assuming a uniform autoimmune diathesis in PANDAS (Stagi et al., 2014). It does, however, support prospective investigation of biological heterogeneity in larger cohorts.

5.3. Persistence, Attenuation or Reorganisation Across Development

If a PANS/PANDAS-relevant developmental trace persists into adulthood in some individuals, it need not reproduce the paediatric phenotype item for item. Neural maturation, improved executive control, tic suppression, changing social demands, and accumulated treatment may alter the expression of earlier vulnerability. Longitudinal studies in Tourette syndrome and paediatric-onset obsessive-compulsive disorder indicate that overt symptoms may diminish while motor, cognitive, or circuit-related vulnerabilities remain clinically relevant, and executive functions continue to mature into early adulthood (Bloch et al., 2005, 2011; Groth et al., 2017; Tervo-Clemmens et al., 2023).

The present domain-specific findings are compatible with this developmental perspective. The strongest ASO associations were concentrated in tic and cognitive/motor features rather than distributed uniformly across the PANS-derived symptom spectrum. Such selectivity may be consistent with attenuation or phenotypic reorganisation rather than simple persistence of a childhood syndrome. Recent longitudinal PANS research has likewise described heterogeneous disease-state and clinical-course trajectories rather than a single uniform progression (Masterson et al., 2025), while isolated adult observations suggest that selected clinical or neurophysiological features may remain detectable beyond adolescence (Mulas et al., 2025).

The cross-sectional design cannot distinguish persistence from recurrence, shared vulnerability, treatment effects, or unrelated adult psychopathology. However, the association between paediatric psychiatric history and higher ASO, together with attenuation of the ASO–total coefficient after adjustment for that history, indicates that developmental context materially influences interpretation and warrants prospective investigation.

5.4. Psychometric Meaning of a Low-Reliability Total

The PANS-31-derived total showed relatively low internal consistency in the present adult cohort (Cronbach's $\alpha=0.535$), compared with the substantially higher value reported for the original caregiver-rated paediatric instrument ($\alpha=0.89$) in children with PANS (Bernstein et al., 2024). This difference should be interpreted in the context of substantial changes in age group,

informant, language, clinical composition, and assessment setting. In the present study, the instrument was used as an investigator-adapted adult self-report measure in a diagnostically heterogeneous psychiatric sample rather than in a clinically defined paediatric PANS cohort.

The lower alpha indicates limited internal consistency of the 31-item total score in this adult cohort. Given that the instrument spans symptom dimensions with potentially different developmental trajectories and clinical relevance, limited internal homogeneity of the total score is not unexpected in a diagnostically heterogeneous adult psychiatric sample.

This finding also supports the decision to examine study-defined symptom domains alongside the global score. The multiplicity-robust associations observed for tic and cognitive/motor symptoms, contrasted with nonsignificant estimates in several other domains, illustrate that the total score may conceal domain-specific heterogeneity. At the same time, these operational domains are not validated adult subscales, several contain few items, and some also showed low internal consistency; they should therefore be interpreted as exploratory analytical groupings rather than established psychometric dimensions.

Because no independent adult validation measure was administered in the present study, the current findings concern internal consistency only and should not be interpreted as establishing or refuting the convergent, construct, or criterion validity of the adult working adaptation. Future studies should formally evaluate the psychometric properties of an adult version using independent validated measures and larger, clinically characterised samples.

5.5. Clinical Context, Diagnostic Heterogeneity, EEG and Treatment

The clinical-context analyses do not support a diagnosis-specific interpretation. ASO varied globally across the nine current psychiatric diagnostic categories, but several groups were small and three contained a single participant. The binary diagnosis-by-ASO analysis did not cross the conventional significance threshold, and diagnosis-specific post-hoc comparisons were not undertaken because they would have been unstable. The observed heterogeneity therefore should not be interpreted as evidence of streptococcal specificity for any individual adult psychiatric diagnosis.

Routine EEG provided only partial convergence with the serological findings. Participants with a recorded EEG modification had higher ASO at the bivariate level, but EEG was not associated with PANS-31-derived total or domain scores after correction, and its coefficient attenuated in the adjusted ASO-context model. Because low-voltage alpha and polymorphic theta are nonspecific routine descriptors, these findings neither confirm nor exclude subtler neurophysiological involvement; they indicate that routine EEG did not independently reproduce the serology–symptom pattern.

Functional gastrointestinal pathology was common in the cohort (42%) but was associated with neither greater PANS-31-derived symptom burden nor higher ASO. This finding is relevant to emerging gut–brain hypotheses in PANS/PANDAS, although paediatric microbiome studies have examined microbial composition and exploratory microbiota–ASO relationships rather than clinical gastrointestinal history (Quagliarello et al., 2018). The present result therefore neither supports nor refutes a microbiome-mediated mechanism.

Previous benzathine benzylpenicillin exposure was associated with lower ASO but not with lower PANS-31-derived symptom burden. Because treatment was not randomised and indication, dose, adherence, timing, reinfection history, and interval to ASO measurement were unavailable, this association cannot be interpreted as evidence of therapeutic efficacy. Instead, it suggests that antimicrobial history should be considered when interpreting later serological findings.

5.6. Strengths and Interpretive Value

Several design and analytical features strengthen the interpretability of this exploratory study. Participants were recruited prospectively across two routine psychiatric-care settings, and the final analytical dataset was locked and cross-checked against participant-level symptom data and

source clinical variables, with complete item-level observations and no score-reconstruction discrepancies.

Continuous ASO was retained as the primary exposure rather than reduced to a single threshold, effect estimates were accompanied by confidence intervals, and multiplicity was controlled within prespecified analytical families. Sparse diagnostic contingency data were handled using an exact test, while null and discordant findings were retained rather than selectively emphasising statistically significant results. The analysis also deliberately separated streptococcal seroreactivity, current symptom dimensions, and formal psychiatric diagnosis, avoiding their conflation.

Finally, modelling was intentionally parsimonious for a sample of 50 participants. Sensitivity analyses were used to examine robustness and the influence of measured developmental context rather than to generate high-dimensional predictive claims. Together, these choices provide a transparent and reproducible framework for identifying focused candidate associations that can be tested in larger longitudinal and multimodal cohorts.

5.7. Limitations and Bias Map

Several limitations constrain interpretation and generalisability. First, the cohort was small and diagnostically heterogeneous. The 50 participants were psychiatric patients selected using a pragmatic history consistent with previous streptococcal exposure and therefore are not representative of the general adult population, all psychiatric patients, or adults with independently verified paediatric PANS/PANDAS. No psychiatric comparator without such history or community control group was available. In addition, nine of the 63 evaluated adults did not complete the ASO/EEG pathway or return for completion, introducing potential attrition bias.

Exposure classification was also heterogeneous. Previous streptococcal infection was not uniformly confirmed by contemporaneous microbiological testing, and the timing of infection relative to symptom onset was not reconstructed. A single ASO determination cannot reliably distinguish remote from more recent immune exposure. Hospital testing used latex agglutination, whereas outpatient determinations used immunoturbidimetric methods, and participant-level assay information was unavailable. Serial ASO measurements, anti-DNase B, and contemporaneous throat culture or PCR were not available.

Outcome measurement requires similar caution. The Romanian PANS-31 was an investigator-prepared adult working adaptation without formal adult or transcultural validation. The original instrument was developed as a caregiver-rated paediatric measure, and its psychometric properties cannot be assumed to transfer directly to adult self-report. The relatively low internal consistency observed in this cohort further indicates heterogeneity of the total score. Moreover, abrupt childhood onset and temporal coupling between infection and symptom exacerbation were not established; consequently, the present study cannot test formal PANDAS criteria or define an adult PANS syndrome.

Residual confounding also remains possible. All participants were receiving psychotropic treatment, but medication class, dose, combinations, duration, and recent changes were not systematically encoded. Psychiatric history, current diagnosis, substance use, recurrent infections, antimicrobial exposure, comorbid disease, and other unmeasured factors may influence ASO or symptom reporting. Several exploratory subgroups were small, including the autoimmune subgroup, and multivariable models remain vulnerable to instability despite parsimonious specification and sensitivity analyses.

Finally, biological specificity was limited by the available clinical data. Routine EEG consisted of qualitative descriptors rather than quantitative electrophysiological biomarkers, and no cytokines, cerebrospinal-fluid inflammatory markers, neuronal autoantibodies, immune-function assays, microbiome profiling, or structural or functional neuroimaging were available. Without longitudinal within-person sampling and appropriate comparator groups, the observed associations cannot establish a temporal or causal pathway linking streptococcal exposure, immune response, neural change, and neuropsychiatric symptoms.

5.8. Future Research: Longitudinal Multimodal Phenotyping and Explainable Modelling

The present findings provide a focused basis for prospective investigation. Future multicentre cohorts should distinguish adults with documented paediatric PANS/PANDAS, psychiatric controls without such history, individuals with recurrent documented streptococcal infection without acute-onset neuropsychiatric syndromes, and community controls. Longitudinal assessment should integrate infection timing, serial ASO and anti-DNase B measurements, inflammatory and neuronal-antibody markers, antimicrobial exposure, validated adult measures of tics, obsessive-compulsive symptoms and executive function, quantitative EEG, and, where feasible, microbiome and neuroimaging measures.

Larger longitudinal datasets could also provide an appropriate foundation for explainable predictive modelling. Rather than attempting to classify an unvalidated adult PANDAS diagnosis, such models could examine trajectories of persistence, remission, or phenotypic reorganisation and determine whether temporally characterised immune and developmental variables add information beyond conventional psychiatric assessment. External validation, calibration, uncertainty, and subgroup performance should be evaluated explicitly, and model explanations should be tested for stability rather than interpreted as mechanisms (Poldrack et al., 2020; Varoquaux, 2018).

The present study helps narrow this future feature space by identifying tic and cognitive/motor dimensions as the strongest multiplicity-robust symptom signals, while also showing that routine EEG did not provide independent convergence and that the continuous ASO association was not reproduced by the single binary threshold.

6. Conclusions

Among 50 young adult psychiatric patients selected using a pragmatic history consistent with previous streptococcal exposure, continuous ASO showed a modest association with PANS-31-derived symptom burden. The strongest multiplicity-robust associations involved tic and cognitive/motor dimensions, and vocal tics were the only individual item surviving correction across all 31 items. In contrast, the binary ≥ 200 -IU/mL comparison was nonsignificant, and the ASO–total association attenuated after adjustment for paediatric psychiatric history.

Exploratory analyses further showed higher ASO among participants with paediatric psychiatric history and recorded autoimmune pathology, but routine EEG did not independently converge with the symptom findings. Overall, these cross-sectional results support a selective, hypothesis-generating association between streptococcal seroreactivity and particular neuropsychiatric symptom dimensions rather than evidence of a uniform adult PANS/PANDAS phenotype, active neuroinflammation, or a diagnostic role for ASO.

Replication in larger longitudinal studies should incorporate comparator groups, documented infection timing, validated adult symptom measures, and multimodal immune and neurophysiological assessment.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of Institute of Psychiatry “Socola” Iași, Romania (14090/17 May 2023) and University of Medicine and Pharmacy “Grigore T. Popa” Iasi, Romania (5036/22 February 2024). Written site permission for participant recruitment and data collection in the private outpatient psychiatric practice was obtained from the legal representative of the practice on 13 October 2024; no separate registration number was assigned.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Consent for publication

The manuscript reports aggregate, de-identified results and contains no identifiable individual participant material.

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Competing interests

The authors declare no conflicts of interest.

Author contributions

Conceptualization, C.-G.S. and C.S.; methodology, C.-G.S., A.B., O.-M.O. and C.S.; software, O.-M.O.; validation, C.-G.S., A.B., O.-M.O. and C.S.; formal analysis, C.-G.S. and O.-M.O.; investigation, C.-G.S.; resources, C.-G.S., A.B. and C.S.; data curation, C.-G.S., D.-C.O. and O.-M.O.; writing—original draft preparation, C.-G.S.; writing—review and editing, C.-G.S., A.B., D.-C.O. and C.S.; visualization, C.-G.S., D.-C.O. and O.-M.O.; supervision, A.B. and C.S.; project administration, C.-G.S. All authors have read and agreed to the final version of the manuscript.

Data and code availability

Participant-level clinical data are not publicly deposited because they contain sensitive health information. De-identified data may be made available by the corresponding author on reasonable request, subject to institutional and ethical requirements. The complete analysis code, aggregate statistical tables, software environment information and publication figures can be provided for editorial or peer-review purposes.

Use of generative artificial intelligence

OpenAI GPT Sol 5.6 Writing Assistant was used under direct author supervision for language editing and document formatting. Statistical analyses and publication figures were generated in Python from the analytical dataset. The authors verified the analytical outputs, references, interpretations and final text and retain full responsibility for the manuscript.

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