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Antistreptolysin O Titers, Symptom Burden, and Multidimensional Correlates in Children with Suspected Streptococcal-Associated Neuropsychiatric Presentations: A Cross-Sectional Observational Study in a Clinically Referred Cohort

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Abstract

Pediatric acute-onset neuropsychiatric presentations occurring in the context of prior streptococcal exposure remain clinically important but diagnostically inconclusive, particularly at the interface between Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) and Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS). This cross-sectional observational study examined whether serological, psychometric, and electroencephalographic findings converged within a clinically referred pediatric psychiatric cohort at the index assessment. Children and adolescents evaluated for neuropsychiatric symptoms in the context of caregiver- or record-supported history suggestive of prior streptococcal exposure were recruited over a 12-month period through inpatient and outpatient child psychiatric services. Out of 154 screened cases, 96 with analyzable index assessment data were retained and stratified by antistreptolysin O (ASO) status. Symptom burden was quantified using the Pediatric Acute-onset Neuropsychiatric Syndrome 31-Item Symptom Rating Scale (PANS-31) which was treated primarily as a continuous dimensional outcome and examined in relation to ASO titers, time since the last reported streptococcal infection, electroencephalography (EEG) findings, and selected developmental and clinical-history variables. Higher ASO values were associated with greater PANS-31 symptom burden, whereas a shorter interval since the last reported streptococcal infection was associated with both higher ASO titers and higher symptom scores. PANS-31 showed good total scale internal consistency and meaningful domain-level convergence with age-appropriate Child Symptom Inventory-4 (CSI-4) and Adolescent Symptom Inventory-4 (ASI-4) domains. Exploratory within-cohort stratification using an operational PANS-31 threshold and alternative post hoc thresholds suggested that higher symptom burden subgroups were largely confined to the ASO-positive stratum; however, these threshold-based patterns were interpreted descriptively and not as diagnostic performance or predictive classification. These findings do not support a disease-specific biomarker model, but suggest that higher anti-streptococcal serology, more recent

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streptococcal exposure, and greater neuropsychiatric burden may cluster as a clinically interpretable association pattern in a selected real-world psychiatric sample.

Keywords: PANS; PANDAS; antistreptolysin O; pediatric neuropsychiatry; streptococcal exposure; electroencephalography; PANS-31; symptom burden

1. Introduction

Pediatric acute-onset neuropsychiatric presentations occurring in temporal relation to streptococcal exposure continue to challenge clinicians across child psychiatry, neurology, immunology, and infectious diseases. Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS) was originally proposed to describe children with abrupt onset of obsessive–compulsive symptoms and/or tics linked to group A streptococcal infection, whereas the broader Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) framework expanded this clinical space to include additional infectious and non-infectious triggers [1,2]. Despite sustained interest, the field remains marked by uncertain nosological boundaries, heterogeneous case definitions, and ongoing debate regarding pathophysiology, diagnostic reliability, and clinical utility [3,4].

Beyond obsessive–compulsive symptoms, tics, or food restriction, affected children may show anxiety, irritability, emotional lability, behavioral dysregulation, sleep disturbance, cognitive decline, somatic symptoms, or other acute neuropsychiatric changes not fully captured by categorical diagnoses [5]. The shift from PANDAS to PANS acknowledged this broader phenotype while widening the etiological frame [6,7]. Diagnostic reasoning therefore remains clinical and depends on symptom timing, infectious history, abruptness of onset, neurological features, and exclusion of alternative explanations [8].

From a mechanistic perspective, PANDAS has often been discussed in conceptual continuity with Sydenham's chorea, the neurological manifestation of acute rheumatic fever. In both conditions, group A streptococcal exposure is hypothesized to trigger cross-reactive immune responses through molecular mimicry, whereby antibodies directed against streptococcal epitopes may recognize neuronal targets, particularly within basal ganglia-related circuits. Experimental and clinical studies have suggested that antibodies generated after streptococcal infection may interact with neuronal antigens and alter dopaminergic or cortico-striatal signaling, although these mechanisms remain incompletely validated and are not diagnostic at the individual-patient level [9]. This biological plausibility supports careful phenotyping, but does not remove the need for conservative interpretation of serology and exclusion of alternative diagnoses.

A major source of controversy is the absence of a specific and reproducible biomarker for PANS/PANDAS. Antistreptolysin O (ASO) and anti-DNase B antibodies are commonly used to support evidence of prior streptococcal exposure, yet they are not diagnostic on their own and require cautious clinical interpretation. Their kinetics are variable, elevated titers may reflect previous exposure rather than disease-specific neuropsychiatric involvement and isolated serological findings cannot resolve the distinction between association and causation [10]. Other proposed biomarkers and auxiliary tools, including the Cunningham Panel, neuroimaging, cerebrospinal fluid analysis, and broader immunological profiling, have not shown sufficient consistency, specificity, or feasibility for routine clinical use [11,12]. Biological assessment is therefore better understood as supportive rather than decisive for diagnosis.

Electroencephalography (EEG) occupies a similarly limited but potentially useful position in this landscape. It is not a disease-specific tool in PANS or PANDAS, and its

findings should not be overinterpreted. Nevertheless, EEG remains widely available in routine practice and may provide complementary evidence of functional neurophysiological disturbance when considered together with clinical presentation, infectious history, and laboratory context [13]. A similar rationale applies to dimensional symptom assessment. In clinically heterogeneous syndromes where categorical labels may flatten the actual burden of illness, structured symptom scales can offer a more informative representation of symptom severity. The Pediatric Acute-Onset Neuropsychiatric Syndrome 31-Item Symptom Rating Scale (PANS-31) was developed to quantify symptom burden across multiple clinically relevant domains and may therefore be particularly useful in cohorts extending beyond narrow obsessive–compulsive or tic-based presentations [14].

The practical relevance is especially evident in settings where standardized national pathways remain limited. Across Europe, approaches to PANS/PANDAS remain uneven, with some consensus-oriented groups endorsing broader clinical frameworks and others maintaining a more cautious position because of the heterogeneity and quality limitations of the available evidence [15]. In Romania, the evidence base remains scarce, and clinical decisions continue to rely largely on extrapolation from the international literature rather than locally generated pediatric psychiatric data [16,17]. This creates a recurring practice tension: clinicians encounter children with abrupt neuropsychiatric deterioration and a suggestive infectious history, yet must interpret these presentations in the absence of robust local cohorts or protocols, integrating symptom scales, serology, and accessible neurophysiological measures [18].

Within this context, the present study examined the relationship between ASO titers and dimensional neuropsychiatric symptom burden, measured with PANS-31, in a clinically screened pediatric psychiatric cohort with a history suggestive of prior streptococcal exposure. Secondary objectives were to examine the relationships among ASO titers, recency of reported streptococcal infection, EEG abnormalities, selected demographic, developmental, and clinical-history variables, and the domain-level convergence of PANS-31 with the Child Symptom Inventory-4 (CSI-4) and Adolescent Symptom Inventory-4 (ASI-4). Sex, prematurity, and in vitro fertilization (IVF) history were not primary etiological hypotheses; they were retained as exploratory cohort-characterization variables because they may reflect demographic, developmental, perinatal, or referral-related vulnerability within clinically referred pediatric psychiatric populations. These analyses were intended to describe association patterns within the cohort, not to test a causal developmental model. We formulated three hypotheses grounded in the existing literature: that higher ASO titers would be associated with greater PANS-31 symptom burden, that shorter time since the last reported streptococcal infection would show the same directional relationship with symptom severity, and that PANS-31 would demonstrate meaningful domain-level convergence with age-appropriate ASI-4/CSI-4 symptom dimensions.

Accordingly, this cross-sectional observational study used a predominantly univariate analytical approach to examine whether serological, symptomatic, temporal, and neurophysiological findings showed a clinically interpretable association pattern within routine Romanian child psychiatric practice, without attempting to validate a disease-specific biomarker model, establish a causal pathway, define an independent clinical phenotype, or generate confounder-adjusted effect estimates.

2. Materials and Methods

2.1. Study Design

This cross-sectional observational study was conducted in Romanian child and adolescent psychiatric inpatient and outpatient settings. It examined whether children and adolescents evaluated for neuropsychiatric symptoms in the context of a history suggestive

of prior streptococcal exposure showed a recognizable pattern across clinical, serological, psychometric, and neurophysiological assessments available in routine practice. The study was not designed to validate PANDAS as a stand-alone diagnosis, but to examine whether a streptococcal-associated neuropsychiatric presentation could be described in a clinically coherent way within routine assessment.

The primary objective was to examine the association between ASO titers and neuropsychiatric symptom burden measured by PANS-31. Secondary objectives were to examine the relationships among ASO titers, recency of streptococcal exposure, EEG abnormalities, and selected demographic and clinical variables, and to evaluate the psychometric convergence of PANS-31 with CSI-4 and ASI-4 domains.

Because the study protocol followed routine clinical procedures, a broader range of clinical and developmental variables was recorded during cohort construction; however, the present report focuses on the variables most directly related to the predefined study aims.

The present report is based on a single index clinical assessment of the retained cohort and does not include longitudinal follow-up analyses. The manuscript therefore reports cross-sectional association patterns within a clinically assembled cohort, not incidence, temporal evolution, treatment response, or symptom trajectories. The reporting of this cross-sectional observational study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement; the completed STROBE checklist is provided as Supplementary Materials.

2.2. Setting, Recruitment, and Study Duration

Participants were recruited over a 12-month period through both inpatient and outpatient child and adolescent psychiatric care pathways. Inpatient evaluations were conducted at the Institute of Psychiatry "Socola" Iași, whereas outpatient cases were assessed in an ambulatory psychiatric facility operating under contract with the Romanian National Health Insurance House. The routine clinical workflow included psychiatric interview, caregiver-informed history taking, psychometric assessment, blood testing with ASO determination, EEG evaluation and additional investigations when considered necessary and feasible.

The initial screening pool comprised 154 children and adolescents referred for neuropsychiatric assessment in the context of a clinically significant psychiatric or neurobehavioral presentation and caregiver- or record-supported history suggestive of prior Group A streptococcal exposure. This recruitment pathway defined the cohort as clinically referred and psychiatric/neuropsychiatric in nature. The study was not designed to screen all children exposed to streptococcal infection, to estimate the prevalence of neuropsychiatric symptoms after streptococcal exposure, or to determine the absolute risk of later psychiatric or neurological vulnerability in the general pediatric population. To preserve the real-world character of the cohort, no additional research-specific age limits were imposed beyond the usual eligibility for child and adolescent psychiatric services. After applying the eligibility criteria and restriction to cases with analyzable core data, the final analytical cohort comprised 96 participants. Of the 58 cases not retained, 24 had associated neurological, developmental, organic, or other medical conditions considered likely to confound phenotype interpretation, 18 had incomplete core data, and 16 withdrew from the study or did not complete the assessment process. No formal a priori sample-size calculation was performed. The analytical sample size was determined by the number of consecutively screened cases meeting the predefined eligibility criteria during the 12-month recruitment period and by the availability of analyzable core data. For analytical purposes, this cohort was subsequently stratified according to ASO status into an ASO-positive group (n = 50)

and an ASO-negative group (n = 46). The participant selection process and final cohort stratification are summarized in Figure 1.

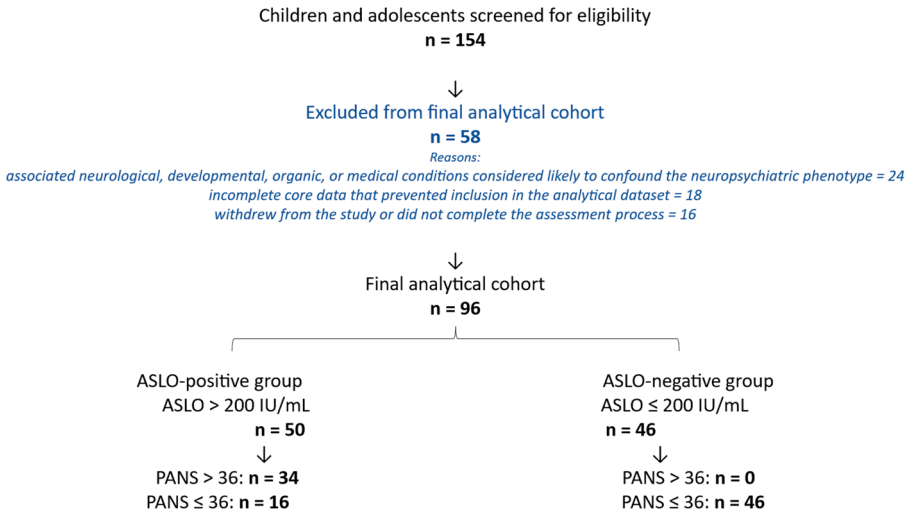


Figure 1. Flow diagram of participant selection and final cohort stratification. Of the 154 screened cases, 96 were retained in the final analytical cohort. The 58 non-retained cases included 24 children with associated neurological, developmental, organic, or other medical conditions considered likely to confound symptom interpretation, 18 cases with incomplete core data, and 16 children who withdrew from the study or did not complete the assessment process.

2.3. Eligibility Criteria

2.3.1. Inclusion Criteria

Patients were eligible if they were evaluated in child and adolescent psychiatric services for neuropsychiatric symptoms and had caregiver- or record-supported history suggestive of prior streptococcal exposure. History suggestive of prior streptococcal exposure was established from caregiver reports and available medical history and included at least one of the following: a previously documented streptococcal infection, recurrent tonsillitis or pharyngitis considered suggestive of streptococcal etiology, or a history of cutaneous, renal, or other clinically compatible infection with possible Group A β-hemolytic streptococcal involvement.

Abrupt onset or abrupt clinical worsening was recorded separately as a clinical-history variable, not as a universal eligibility requirement. It was coded when caregiver report or available documentation supported a sudden or clearly abrupt change in neuropsychiatric symptoms. This allowed the study to examine whether abrupt onset differentiated higher ASO or higher PANS-31 subgroups within the clinically selected cohort.

The inclusion strategy was intended to preserve the typical heterogeneity of routine child and adolescent psychiatric practice. Children were therefore not excluded solely because they had a pre-existing psychiatric diagnosis, provided that the index episode was marked by an abrupt and clinically meaningful change in symptom profile or severity. This allowed inclusion of cases in which a PANS/PANDAS-like presentation was raised during routine assessment despite an already established psychiatric background. A broader set of clinical and developmental variables was recorded during cohort construction, but the present manuscript focuses on those most relevant to the predefined study objectives.

2.3.2. Exclusion Criteria

Children were excluded when major neurological, developmental, or organic conditions were considered likely to confound the neuropsychiatric phenotype or substantially limit diagnostic interpretability within the framework of the study. Exclusion criteria there-

fore included intellectual disability, genetic neurological disorders, neurodevelopmental disorders severe enough to preclude meaningful psychiatric phenotyping, epilepsy, known structural central nervous system pathology, and other major neurological or medical conditions capable of independently accounting for the observed presentation or substantially overlapping with it.

This exclusion strategy was intended to preserve clinical interpretability rather than to create an artificially homogeneous cohort. In selected cases, cranial computed tomography (CT) was considered as an additional structural exclusion tool; however, because compliance was very low, CT could not be incorporated into the core analytical design and was not used as a mandatory inclusion or exclusion procedure. Structural exclusion therefore relied primarily on clinical history, psychiatric and neurological evaluation, and available medical documentation. Also, children were excluded if parents/caregivers refused to provide informed consent and participation, retracted the consent during the study or they did not complete the clinical and paraclinical investigations required. Participants were not followed longitudinally within the framework of the present report. Each child contributed a single index assessment, and no loss-to-follow-up analysis was applicable to the present manuscript.

2.4. Clinical and Anamnestic Assessment

All participants underwent a structured clinical psychiatric interview, caregiver-informed anamnesis, and psychometric assessment. In addition, caregivers completed a study questionnaire designed to capture, in a standardized format, the main clinical and historical variables relevant to the suspected streptococcal-associated neuropsychiatric phenotype. This combined approach allowed the assessment to retain the depth of routine clinical evaluation while improving the consistency of variable recording across cases.

The recorded variables included age, sex, residential environment, family context, care setting, symptom onset, school functioning, infectious history, developmental and perinatal antecedents, somatic complaints, and prior medical or psychiatric diagnoses. The questionnaire and caregiver-informed history documented abrupt clinical change, school decline, attentional difficulties, obsessive symptoms, anxiety, aggression, impulsivity, behavioral dysregulation, mood symptoms, suicidal ideation, tics, psychotic-like symptoms, enuresis, sleep disturbance, changes in eating habits, infectious history, digestive symptoms, allergies, autoimmune conditions, pregnancy-related factors, early postnatal problems, and in vitro fertilization (IVF) history.

Psychiatric diagnoses were coded according to the International Classification of Diseases, 10th Revision (ICD-10), the operational diagnostic framework in the Romanian healthcare system. For presentation in tables and figures, database codes were replaced by diagnostic labels to improve readability. Because the present investigation focused on dimensional symptom burden as measured by PANS-31, the clinical assessment also documented the presenting neuropsychiatric phenotype in detail rather than relying exclusively on categorical diagnoses.

A broader range of clinical and developmental variables was collected during cohort construction than could be examined in equal detail in the present manuscript. These variables will be explored in greater depth in subsequent analyses from the same research project. For the final analyses, emphasis was placed on variables most directly related to the predefined study objectives. The harmonized dataset retained age, sex, residential environment, family setting, outpatient versus inpatient source, abrupt onset, recurrent infections, prematurity, IVF history, functional digestive symptoms, bacterial infections other than streptococcal infection, viral infections, autoimmune pathology, penicillin treatments and history compatible with rheumatic fever, chorea, or endocarditis. Time since

the last reported streptococcal infection was recorded in months whenever it could be reconstructed with sufficient confidence from the available history. For the purposes of the present analyses, ASO value and ASO status were treated as the principal exposure-related variables, total PANS-31 score as the main dimensional clinical outcome, and time since the last reported streptococcal infection, age, sex, care source, recurrent infections, prematurity, IVF history, and functional digestive symptoms as predefined clinical correlates examined in relation to serological and symptomatic burden. These variables were analyzed as candidate correlates and were not treated as formally adjusted confounders in a multivariable model.

2.5. Psychometric Assessment

Neuropsychiatric symptom burden was assessed primarily with the Pediatric Acute-onset Neuropsychiatric Syndrome 31-Item Symptom Rating Scale (PANS-31), which served as the main dimensional outcome measure in the study. The principal analytical endpoint was the total PANS-31 score, retained as a continuous indicator of overall multidomain symptom burden.

For exploratory subgroup analyses, a secondary categorical variable was derived using PANS-31 total score > 36 to identify a subgroup with higher symptom burden within the cohort. This value was selected before formal analysis, within the lower-mild portion of the PANS-31 scale, as an internal operational separator and was not intended to represent a validated diagnostic or severity cutoff. The rationale was methodological rather than diagnostic: in a real-world pediatric psychiatric setting, lower-range PANS-31 scores may overlap substantially with nonspecific psychiatric, behavioral, anxiety-related, somatic, or developmental presentations. Preliminary clinical interviews and pilot scale administrations conducted before the formal recruitment, suggested that a threshold above this lower-overlap range would better identify the more clearly expressed PANS-like symptom burden. The continuous PANS-31 total score therefore remained the primary outcome, while PANS-31 > 36 was used only for exploratory within-cohort stratification.

Age-appropriate external comparator instruments were the Child Symptom Inventory-4 (CSI-4) and the Adolescent Symptom Inventory-4 (ASI-4). These Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)-based scales were used to characterize parallel symptom domains across childhood and adolescence and to examine cross-scale convergence with PANS-31 [19]. Because CSI-4 and ASI-4 are developmentally stratified instruments, they were not treated as a single pooled global measure. Accordingly, convergence analyses were planned at the level of theoretically corresponding symptom domains rather than combined total scores. Within this framework, PANS-31 was treated as the principal syndrome-oriented dimensional measure, whereas CSI-4 and ASI-4 served as external reference instruments and did not replace standard clinical diagnosis. PANS-31 and the age-appropriate ASI-4/CSI-4 measures were completed during the routine clinical assessment process using caregiver-informed reporting, self-reporting in adolescents and with integration of clinician-guided clarification when needed to ensure accurate symptom-domain scoring.

2.6. ASO Assessment

ASO determinations were performed as part of the routine clinical laboratory work-up in the hospital and outpatient laboratory settings, working under National Health Insurance House contracts and using latex-based ASO assay methods. The investigators did not perform, repeat, or reanalyze the laboratory assays. Numerical ASO results, as reported by the respective laboratories in IU/mL, were extracted from the original laboratory records and entered into the study database without recalculation or transformation. Detailed

reagent, lot, and analyzer information was not consistently retained in the source clinical records. For analytical purposes, laboratory-reported ASO values were treated both as a continuous variable and as a binary exposure-related variable, using >200 IU/mL to define the ASO-positive stratum.

ASO determination formed part of the routine clinical work-up together with standard blood testing and inflammatory assessment.

For analytical purposes, ASO was treated both as a continuous laboratory variable and as a binary exposure-related variable. Continuous ASO values were used in correlation and group-comparison analyses, whereas a threshold of >200 IU/mL was used to define the ASO-positive (>200 IU/mL) and ASO-negative ($\leq$200 IU/mL) strata retained in the final cohort. This threshold represents the cutoff value for latex agglutination systems in Romanian laboratories. Therefore, it was used for analytical stratification and should not be interpreted as a stand-alone diagnostic criterion for PANDAS. Within the framework of the present study, ASO was regarded as an accessible marker of prior or recent streptococcal exposure, whose interpretation required integration with symptom timing, psychiatric presentation, and the broader clinical context. At the index psychiatric assessment, none of the enrolled children presented clinical signs suggestive of active streptococcal infection, such as acute pharyngitis, tonsillitis, skin infection, fever, or other acute infectious symptoms. There was also no clinical or documented evidence of systemic post-streptococcal complications, including post-streptococcal glomerulonephritis, carditis, pericarditis, endocarditis, or Sydenham chorea. For this reason, throat swab and streptococcal culture were not systematically collected as part of routine clinical care. ASO values were therefore interpreted as supportive markers of previous streptococcal exposure in a suspected post-infectious neuropsychiatric context, rather than as an indicator of systemic post-streptococcal disease.

2.7. Electroencephalographic Assessment

Electroencephalography (EEG) was included as a complementary neurophysiological assessment within the routine index evaluation of the retained cohort. EEG was not selected after reviewing ASO status or PANS-31 scores and was not used as a disease-specific marker for PANS or PANDAS. Its inclusion reflected the clinical workflow of the participating psychiatric setting, where EEG was available as part of routine neuropsychiatric assessment and could provide functional information in children with complex or substantially changed psychiatric presentations.

Structural and functional neuroimaging were not systematically available within this routine care pathway. Therefore, magnetic resonance imaging, functional magnetic resonance imaging, diffusion imaging, positron emission tomography, and single-photon emission computed tomography were not included as study variables. EEG was not considered a substitute for neuroimaging of basal ganglia or cortico-striatal circuits, but rather an accessible complementary measure to be interpreted together with clinical presentation, infectious history, serology, and dimensional symptom burden.

EEG recordings were performed at the Institute of Psychiatry "Socola" Iași for inpatient and outpatient cases. Recordings were obtained using a Neuron-Spectrum-4/P system (Neurosoft®, Neurosoft LLC, Ivanovo, Russia), with a standard 21-electrode montage based on the international 10–20 system and bilateral auricular references.

For analysis, EEG information was coded from the available clinical reports. In addition to the global distinction between normal and abnormal EEG, the harmonized dataset retained predefined pattern-based variables: low-voltage alpha activity in temporo-parieto-occipital regions, dominant alpha activity in temporo-parieto-occipital regions, polymorphic theta activity, paroxysmal theta activity, spike–wave complexes, and other

nonspecific EEG changes. These variables were used as descriptive neurophysiological correlates rather than diagnostic indicators or elements of a validated EEG severity scale.

2.8. Data Processing and Cohort Harmonization

After completion of clinical recruitment and eligibility screening, the retained cases were entered into a structured analysis spreadsheet and prepared for statistical processing. For analytical organization, the final eligible cohort was arranged into two ASO-defined strata, ASO-positive (n = 50) and ASO-negative (n = 46), which were then harmonized and analyzed together as a combined cohort of 96 participants. This spreadsheet structure reflected post-recruitment analytical stratification, not the initial recruitment process.

Before statistical analysis, column names, coding conventions, and variable labels were standardized across the source sheets to ensure structural consistency of the merged dataset. The resulting analytical file included demographic variables, care setting variables, ICD-10-coded psychiatric diagnoses, symptom-domain variables, clinical-history variables, ASO-related variables, EEG-derived variables, and psychometric measures. Selected categorical variables were additionally recoded into clinically interpretable formats.

Derived variables included binary ASO status and a secondary binary symptom burden variable based on PANS-31 total score. Elevated ASO was defined as >200 IU/mL. For exploratory subgroup analyses, PANS-31 total score > 36 was used to identify a higher symptom burden subgroup within the cohort, as described in Section 2.5. This threshold was used for internal stratification only and should not be interpreted as a validated diagnostic or severity cutoff. For the variable representing time since the last reported streptococcal infection, entries coded as “>12” months were recoded to 12 months in order to preserve an ordinal numerical structure for analysis.

Missing or incomplete values were handled during preprocessing before construction of the analysis-ready dataset. No statistical imputation was performed. When data were incomplete for secondary variables, cases were retained in the cohort and contributed to analyses for which the required variables were available. In the psychometric analysis matrices, no missing values remained at the time of computation.

Local dataset labels used during statistical processing, including “ASLO” and “aslo_value”, were harmonized in the manuscript text as “ASO value.” Where these labels remain visible in generated figure axes, they refer to antistreptolysin O values reported in IU/mL and reflect the Romanian laboratory/statistical dataset terminology.

2.9. Statistical Analysis

Because several study variables showed non-Gaussian distributions, descriptive data are reported primarily as medians and interquartile ranges (IQRs) for continuous variables, and as counts and percentages for categorical variables. The main continuous outcomes were total PANS-31 score and ASO value. Associations between numerical predictors and these outcomes were assessed using Spearman rank correlation coefficients with two-sided p values. Where reported, 95% confidence intervals (CIs) were used to describe the precision of selected estimates. Confidence intervals were interpreted descriptively and were not used to infer diagnostic classification, predictive performance, or causal effects. Continuous outcomes were compared across binary categorical strata using the Mann–Whitney U test. For categorical variables with three ordered levels, exploratory omnibus comparisons were performed using Kruskal–Wallis tests, followed, where appropriate, by pairwise Mann–Whitney U tests with Bonferroni adjustment for multiple comparisons. One-way ANOVA results generated during the initial exploratory analysis were retained only as exploratory parametric consistency checks and were not used as the primary inferential basis.

Binary-derived variables, namely ASO status (>200 IU/mL) and the higher symptom burden subgroup defined by PANS-31 > 36, were used for descriptive stratification and exploratory subgroup analyses. A nominal two-sided significance threshold of $p < 0.05$ was used for the main univariate analyses. Multiple-testing correction was applied only within the psychometric convergence analyses described separately below.

Graphical representations included scatterplots with fitted trend lines and annotated Spearman coefficients and p values for continuous associations, boxplots with overlaid individual observations and annotated p values for group comparisons, a Spearman correlation heatmap integrating ASO, PANS-31, and selected numerical domains in order to visualize the overall association structure among the principal outcome measures and key covariates.

All analyses were interpreted conservatively in light of the observational design, the clinically selected nature of the cohort, and the predominantly univariate statistical approach. The study was intended to identify clinically meaningful association patterns within the cohort rather than to support causal inference or to evaluate ASO as a stand-alone diagnostic biomarker.

Because the study was exploratory and the sample size was modest in relation to the number of candidate correlates, the primary analytical strategy remained predominantly univariate. No multivariable regression models were fitted, no formal confounder-adjusted estimates were generated, and no prespecified interaction terms were tested. Post hoc sensitivity analyses were added using alternative PANS-31 thresholds (>30, >32, >34, >38, >40 and >42), in addition to the predefined >36 operational threshold, to examine whether the ASO/PANS-31 pattern depended on a single cut point.

2.10. Psychometric Evaluation of PANS-31

The psychometric performance of PANS-31 was examined to support its use as the principal dimensional measure of neuropsychiatric symptom burden in the present cohort. These analyses were not intended to validate PANS-31 as a diagnostic instrument or to establish interchangeability with standard psychiatric scales. Rather, they assessed whether PANS-31 showed acceptable internal consistency, coherent dimensional structure, and meaningful convergence with age-appropriate external symptom measures.

Internal consistency was assessed using Cronbach's alpha for the total PANS-31 scale and for clinically defined symptom-domain groupings. Structural coherence was explored using principal component analysis of the 31-item matrix, together with the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy.

Cross-scale convergence was evaluated by calculating Spearman correlations between PANS-31 domain scores and theoretically corresponding domains from the Child Symptom Inventory-4 (CSI-4) or Adolescent Symptom Inventory-4 (ASI-4). Because CSI-4 and ASI-4 are developmentally stratified instruments, they were not combined into a single pooled global score. Interpretation, therefore, focused on domain-level correspondences rather than total score equivalence.

The PANS-31 domains considered in these analyses included obsessive–compulsive symptoms, eating-related symptoms, anxiety, mood/affective symptoms, behavioral dysregulation, cognitive symptoms, motor symptoms, vocal symptoms, somatic symptoms, urinary symptoms, and psychotic-like symptoms. The CSI-4/ASI-4 comparator domains included obsessive–compulsive symptoms, anxiety symptoms, affective symptoms, conduct/behavioral symptoms, eating-related symptoms, motor symptoms, vocal symptoms, psychotic symptoms, enuresis/urinary symptoms, addiction/substance-use symptoms, and the CSI pervasive/developmental domain. The theoretically expected cross-scale pairings were obsessive–compulsive with obsessive–compulsive, anxiety with anxiety,

mood/affective with affective, behavioral dysregulation with conduct/behavioral symptoms, eating-related with eating-related, motor with motor, vocal with vocal, psychotic-like with psychotic, and urinary with enuresis/urinary symptoms. Addiction/substance-use and CSI pervasive/developmental domains were retained as broader external comparator domains rather than direct PANS-31 equivalents.

To account for multiple domain-level correlations, p values from the convergence analyses were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure. Correlations were interpreted according to both statistical significance and effect size, with greater emphasis placed on the strength and direction of theoretically expected associations.

Finally, the pattern of convergent and discriminant relationships was examined using a multitrait–multimethod (MTMM) framework based on Campbell and Fiske's approach. This analysis was used descriptively to determine whether theoretically related domains showed stronger cross-method associations than unrelated domains. All psychometric analyses were conducted in the full analytical cohort of 96 participants and were intended to support dimensional interpretation of PANS-31 within this sample, not to establish general psychometric validity.

2.11. Processing Environment

Statistical analyses and figure generation were performed in Python 3.13.5 (Python Software Foundation, Wilmington, DE, USA), using pandas, matplotlib, and additional Python statistical libraries for multiple-testing correction and psychometric analyses.

3. Results

3.1. Cohort Overview

The final analytical cohort comprised 96 children and adolescents. Of these, 50 participants (52.1%) had ASO values > 200 IU/mL and 46 (47.9%) had ASO values $\leq$ 200 IU/mL. Using the predefined operational threshold, 34 participants (35.4%) had PANS-31 total scores > 36, whereas 62 (64.6%) scored $\leq$ 36. The cross-distribution of these two binary classifications showed that all participants with PANS-31 > 36 belonged to the ASO-positive stratum, while ASO-negative participants were confined to the lower PANS-31 category. This complete separation was interpreted descriptively and was not considered evidence of diagnostic classification, predictive accuracy, or individual-level biomarker performance. Descriptive distributions of the principal numerical and categorical variables according to ASO status are presented in Tables 1 and 2, while the corresponding distributions according to PANS-31 symptom burden subgroup are presented in Tables 3 and 4.

Table 1. Selected numerical features by ASO status (median [IQR]).

Feature	ASO-Negative ($\leq$200 IU/mL; n = 46)	ASO-Positive (>200 IU/mL; n = 50)
Age, years	14 [10–15]	12 [11–13]
Time since last streptococcal infection, months	12 [12–12] *	3 [2–5]
ASO value, IU/mL	116 [74–160]	648 [527–764]
PANS-31 total score	22 [18–25]	40 [34–50]

* Values coded as 12 include intervals of 12 months or longer.

Table 2. Selected categorical features by ASO status.

Variable	Category/Summary	ASO-Negative (≤200 IU/mL; *n* = 46)	ASO-Positive (>200 IU/mL; *n* = 50)
Sex	Female	23 (50.0%)	12 (24.0%)
	Male	23 (50.0%)	38 (76.0%)
Residential environment	Rural	23 (50.0%)	30 (60.0%)
	Urban	23 (50.0%)	20 (40.0%)
Family setting	Family	39 (84.8%)	39 (78.0%)
	Foster care	7 (15.2%)	11 (22.0%)
Care source	Ambulatory	17 (37.0%)	11 (22.0%)
	Inpatient	29 (63.0%)	39 (78.0%)
Recurrent infections	No	39 (84.8%)	35 (70.0%)
	Yes	7 (15.2%)	15 (30.0%)
Sudden onset	No	41 (89.1%)	7 (14.0%)
	Yes	5 (10.9%)	43 (86.0%)
Prematurity	No	44 (95.7%)	31 (62.0%)
	Yes	2 (4.3%)	19 (38.0%)
IVF history	No	43 (93.5%)	41 (82.0%)
	Yes	3 (6.5%)	9 (18.0%)
Functional digestive symptoms	None	40 (87.0%)	13 (26.0%)
	One symptom	5 (10.9%)	27 (54.0%)
	Two or more symptoms	1 (2.2%)	10 (20.0%)
Psychiatric record	None recorded	33 (71.7%)	36 (72.0%)
	Substance-use/addictive disorders	1 (2.2%)	0 (0.0%)
	Autism spectrum/developmental disorders	1 (2.2%)	3 (6.0%)
	Depressive disorder	3 (6.5%)	1 (2.0%)
	Attention-deficit/hyperactivity disorder	4 (8.7%)	9 (18.0%)
	Conduct disorder	4 (8.7%)	1 (2.0%)
Higher symptom burden (PANS-31 > 36)	No	46 (100.0%)	16 (32.0%)
	Yes	0 (0.0%)	34 (68.0%)

Table 3. Selected numerical features according to PANS-31 symptom burden status.

Feature	PANS-31 ≤ 36 (*n* = 62)	PANS-31 > 36 (*n* = 34)
Age, years	14 [10–15]	12 [11–14]
Time since last streptococcal infection, months	12 [6–12]	3 [2–4]
ASO value, IU/mL	150 [90–283]	674 [549–788]
PANS-31 total score	24 [20–30]	45 [40–54]

Values coded as 12 include intervals of 12 months or longer.

Similarly, at the index assessment, no clinical evidence suggested acute post-streptococcal glomerulonephritis, carditis/pericarditis, endocarditis, Sydenham chorea, or another systemic streptococcal/post-streptococcal condition that could independently account for the presentation.

Core variables used in the main analyses were available for the full analytical cohort unless otherwise specified; no statistical imputation was performed.

Table 4. Selected categorical clinical features according to PANS-31 symptom burden status.

Variable	Category	PANS-31 ≤ 36 (*n* = 62)	PANS-31 > 36 (*n* = 34)
Sex	Female	27 (43.5%)	8 (23.5%)
	Male	35 (56.5%)	26 (76.5%)
Residential environment	Rural	35 (56.5%)	18 (52.9%)
	Urban	27 (43.5%)	16 (47.1%)
Family setting	Family	54 (87.1%)	24 (70.6%)
	Foster care	8 (12.9%)	10 (29.4%)
Care source	Ambulatory	21 (33.9%)	7 (20.6%)
	Inpatient	41 (66.1%)	27 (79.4%)
Recurrent infections	No	52 (83.9%)	22 (64.7%)
	Yes	10 (16.1%)	12 (35.3%)
Sudden onset	No	41 (66.1%)	7 (20.6%)
	Yes	21 (33.9%)	27 (79.4%)
Prematurity	No	55 (88.7%)	20 (58.8%)
	Yes	7 (11.3%)	14 (41.2%)
IVF history	No	58 (93.5%)	26 (76.5%)
	Yes	4 (6.5%)	8 (23.5%)
Functional digestive symptoms	None	44 (71.0%)	9 (26.5%)
	One symptom	14 (22.6%)	18 (52.9%)
	Two or more symptoms	4 (6.5%)	7 (20.6%)
Psychiatric record	None recorded	46 (74.2%)	23 (67.6%)
	Substance-use/addictive disorders	1 (1.6%)	0 (0.0%)
	Autism spectrum/developmental disorders	2 (3.2%)	2 (5.9%)
	Depressive disorder	3 (4.8%)	1 (2.9%)
	Attention-deficit/hyperactivity disorder	5 (8.1%)	8 (23.5%)
	Conduct disorder	5 (8.1%)	0 (0.0%)
ASO status	Negative (≤200 IU/mL)	46 (74.2%)	0 (0.0%)
	Positive (>200 IU/mL)	16 (25.8%)	34 (100.0%)

3.2. Association Between ASO Value and PANS-31 Total Score

A strong positive monotonic association was observed between ASO value and total PANS-31 score (Spearman ρ = 0.806, two-sided $p = 3.68 \times 10^{-23}$; $n = 96$; 95% CI: [0.74–0.86]). This association was interpreted dimensionally within the clinically selected cohort and should not be read as evidence of diagnostic classification, causal effect, or individual-level biomarker performance. The individual-level distribution of this association, including visualization on both linear and logarithmic ASO scales, is shown in Figure 2.

Post hoc sensitivity analyses using alternative PANS-31 thresholds showed a directionally stable ASO/PANS-31 pattern. At PANS-31 >30, 45 of 47 participants above the threshold were ASO-positive and 2 were ASO-negative. At PANS-31 >32, 40 of 41 participants above the threshold were ASO-positive and 1 was ASO-negative. From thresholds >34 upward, all participants above the threshold belonged to the ASO-positive stratum: 37/37 at >34, 34/34 at the predefined >36 threshold, 30/30 at >38, 24/24 at >40, and 23/23 at >42. These threshold-based results support the descriptive stability of the ASO/PANS-31

pattern across adjacent cut points. The predefined >36 threshold retained a substantial higher-burden subgroup while avoiding the borderline ASO-negative cases observed at lower thresholds; however, these findings remain exploratory and should not be interpreted as diagnostic accuracy, predictive classification, or individual-level biomarker performance.

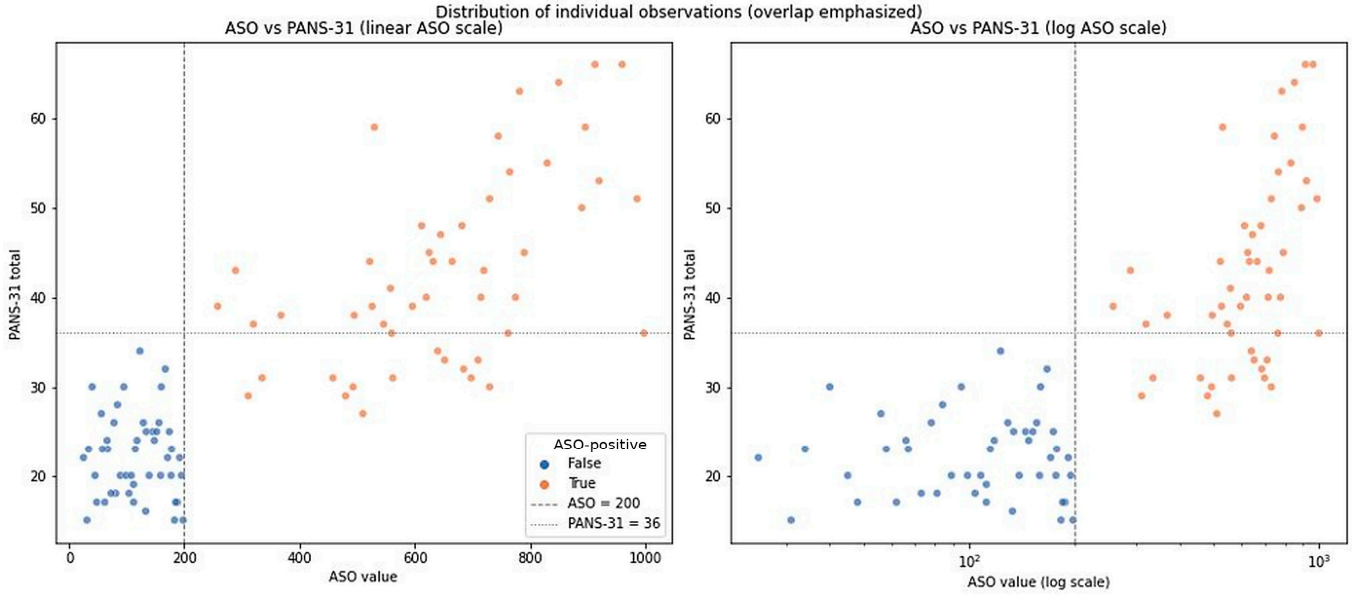


Figure 2. Distribution of individual ASO and PANS-31 observations with overlap emphasized. The left panel shows the ASO–PANS-31 association using a linear ASO scale, and the right panel shows the same association using a logarithmic ASO scale to improve visualization of the ASO distribution. Each point represents one participant and is colored according to ASO status. The vertical reference line marks the ASO threshold of 200 IU/mL, and the horizontal reference line marks the predefined operational PANS-31 threshold of 36. The figure illustrates the positive monotonic association between ASO value and dimensional neuropsychiatric symptom burden, while showing the degree of overlap between ASO strata at lower and intermediate symptom scores. The reference lines are shown for descriptive visualization only and should not be interpreted as diagnostic classification boundaries or evidence of individual-level biomarker performance.

3.3. Recency of Reported Streptococcal Infection

Time since the last reported streptococcal infection, expressed in months, showed strong inverse correlations with both ASO value and total PANS-31 score. A shorter interval since the last reported streptococcal infection was associated with higher ASO titers (Spearman $\rho = -0.787$, $p = 1.89 \times 10^{-21}$; $n = 96$) and with higher PANS-31 total scores (Spearman $\rho = -0.768$, $p = 7.34 \times 10^{-20}$; $n = 96$).

The same pattern was reflected in the subgroup distributions. The ASO-positive group had a median interval of 3 [2–5] months since the last reported streptococcal infection, compared with 12 [12–12] months in the ASO-negative group. The higher PANS-31 symptom burden subgroup had a median interval of 3 [2–4] months, compared with 12 [6–12] months in the lower-burden subgroup. The corresponding mean intervals in the PANS-defined subgroups were 3.3 ± 2.2 months and 9.1 ± 3.9 months, respectively.

Because values above 12 months were recoded to 12 during preprocessing, the upper range of this variable should be read as 12 months or more. The distribution of these associations is shown in Figure 3.

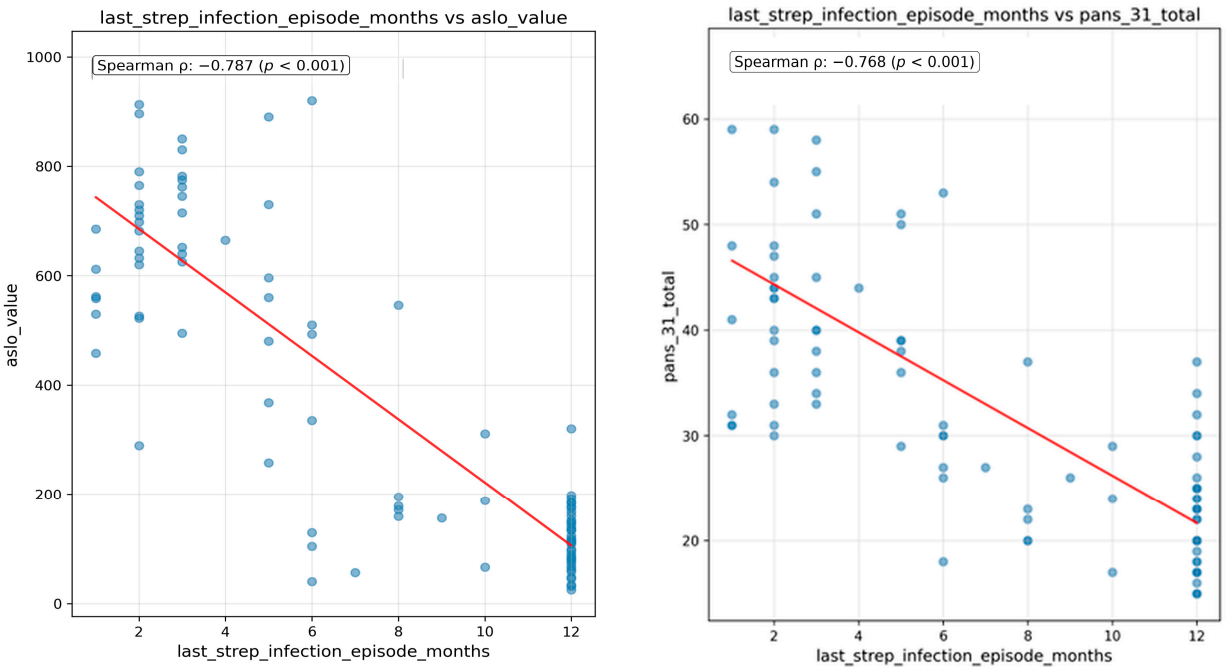


Figure 3. Scatterplot panel showing the relationship between time since the last reported streptococcal infection and the main study endpoints. (**A**) Association between time since the last reported streptococcal infection (months) and ASO value. Note: In the generated figures, "aslo_value" reflects the Romanian laboratory/statistical dataset label for antistreptolysin O and corresponds to ASO value reported in IU/mL. (**B**) Association between time since the last reported streptococcal infection (months) and total PANS-31 score. Blue circles represent individual participant observations; points may overlap when participants have identical or very similar values. The red solid line indicates the fitted linear trend and is included as a visual guide only.

3.4. Age-Related Associations

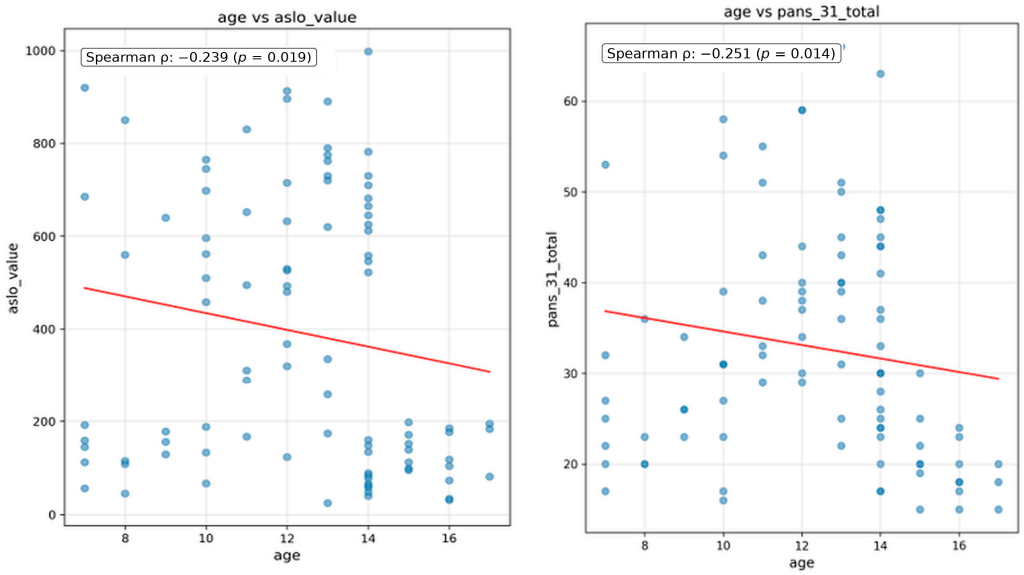
Age showed modest inverse associations with both main study endpoints. Younger participants tended to have higher ASO values (Spearman $\rho = -0.239$, $p = 0.019$) and higher total PANS-31 scores (Spearman $\rho = -0.251$, $p = 0.014$).

This pattern was also reflected in the subgroup distributions. The corresponding mean ages were 11.9 ± 1.9 years in the ASO-positive group and 12.2 ± 1.7 years in the higher PANS-31 subgroup. The distributions of these associations are shown in Figure 4.

3.5. Sex-Related Associations

Male participants showed higher values on both main study endpoints. Median ASO was 526 IU/mL in boys, compared with 174 IU/mL in girls (Mann–Whitney U test, $p = 0.00807$). Median total PANS-31 score was also higher in boys than in girls (33 vs. 23, $p = 0.00177$).

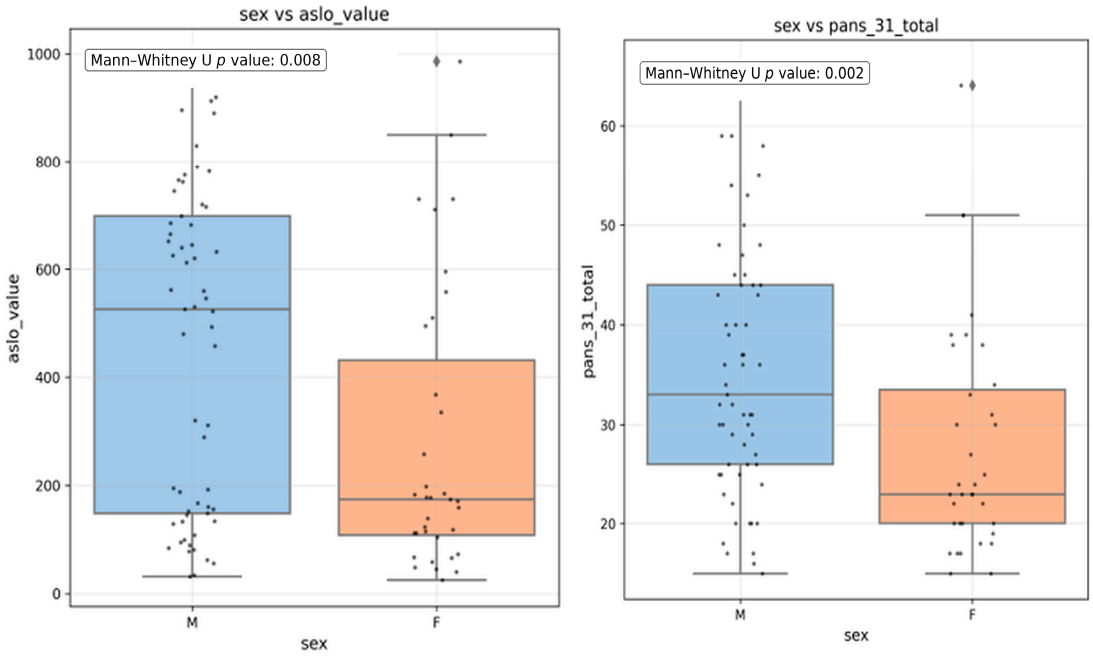
The subgroup distributions were consistent with these findings. In the ASO-stratified cohort, boys accounted for 76.0% of the ASO-positive group and 50.0% of the ASO-negative group. In the PANS-31 subgroup analysis, boys represented 76.5% of the higher symptom burden subgroup and 56.5% of the lower-burden subgroup.



(**A**) (**B**)

Figure 4. (**A**) Scatterplot showing the association between age and ASO value. Each point represents one participant. The fitted trend line illustrates the inverse association between age and ASO titers. (**B**) Scatterplot showing the association between age and total PANS-31 score. Each point represents one participant. The fitted trend line illustrates the inverse association between age and dimensional neuropsychiatric symptom burden.

The distributions of ASO values and total PANS-31 scores according to sex are shown in Figure 5.



(**A**) (**B**)

Figure 5. (**A**) Boxplot of ASO value according to sex. ASO titers are shown for male and female participants. (**B**) Boxplot of total PANS-31 score according to sex. PANS-31 total scores are shown for male and female participants.

3.6. Clinical Correlates of Higher ASO Values

Several categorical clinical variables were associated with higher ASO values in the cohort. The strongest difference was observed for abrupt symptom onset: children with sudden onset had higher ASO titers than those without abrupt onset (median 622.5 vs. 133.5 IU/mL, Mann–Whitney U test, $p = 1.39 \times 10^{-9}$) (Figure 6).

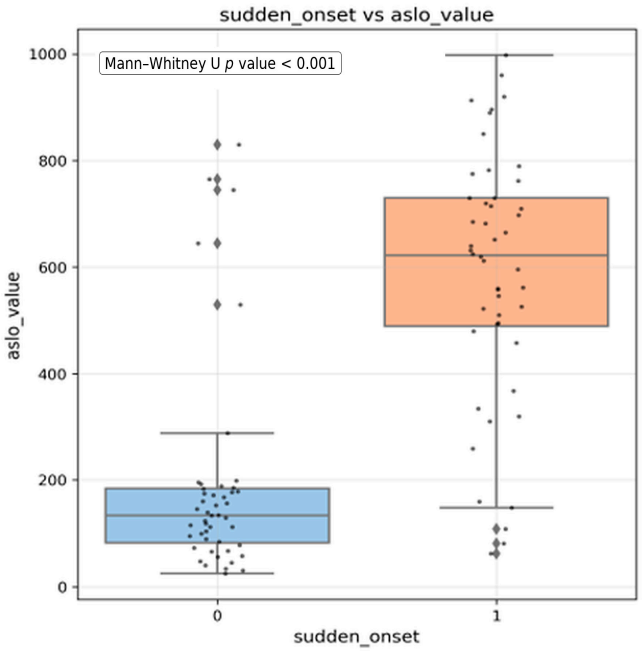
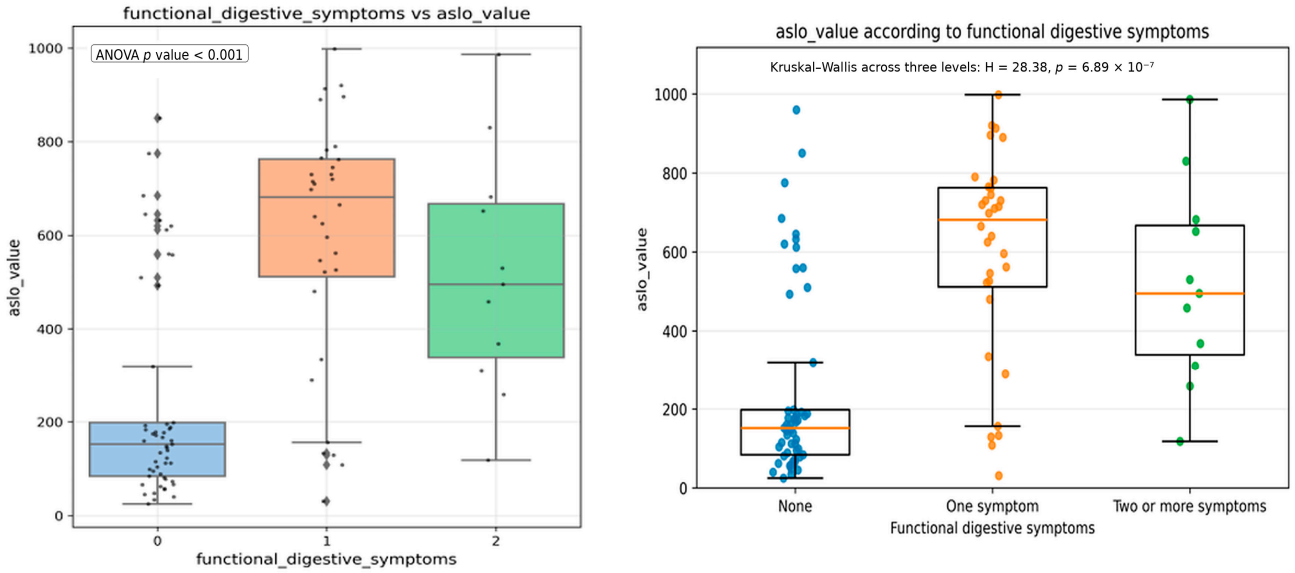


Figure 6. Boxplot of ASO value according to sudden onset status. ASO titers are compared between children with and without abrupt symptom onset.

Functional digestive symptoms were also associated with ASO level. Because this variable had three ordered categories and ASO values were non-Gaussian, the primary omnibus comparison was performed using the Kruskal–Wallis test. ASO values differed significantly across digestive symptom categories (H = 28.38, $p = 6.89 \times 10^{-7}$), as shown in Figure 7B. The originally generated one-way ANOVA result, retained only as an exploratory parametric comparison for visual/statistical continuity, showed the same direction of association ($p = 2.43 \times 10^{-8}$), as shown in Figure 7A. Bonferroni-adjusted pairwise Mann–Whitney U tests showed significant differences between no digestive symptoms and one symptom (p_adj $= 2.97 \times 10^{-6}$), and between no digestive symptoms and two or more symptoms (p_adj = 0.00461), but not between one symptom and two or more symptoms (p_adj = 0.744).

Higher ASO titers were further observed in children with a history of prematurity and in those with recurrent infections. Median ASO was 652 IU/mL in participants with prematurity, compared with 178 IU/mL in those without prematurity ($p = 5.12 \times 10^{-4}$), and 657 IU/mL in participants with recurrent infections, compared with 193.5 IU/mL in those without recurrent infections ($p = 0.00467$). The prematurity comparison is shown in Figure 8.



(**A**) (**B**)

Figure 7. ASO values according to functional digestive symptom burden. (**A**) Exploratory one-way ANOVA visualization across the three digestive symptom categories. This parametric analysis was retained only as a descriptive consistency check. (**B**) Primary nonparametric Kruskal–Wallis analysis, appropriate for the ordinal digestive symptom variable and non-Gaussian ASO distribution, showed significant differences across groups (H = 28.38, $p = 6.89 \times 10^{-7}$).

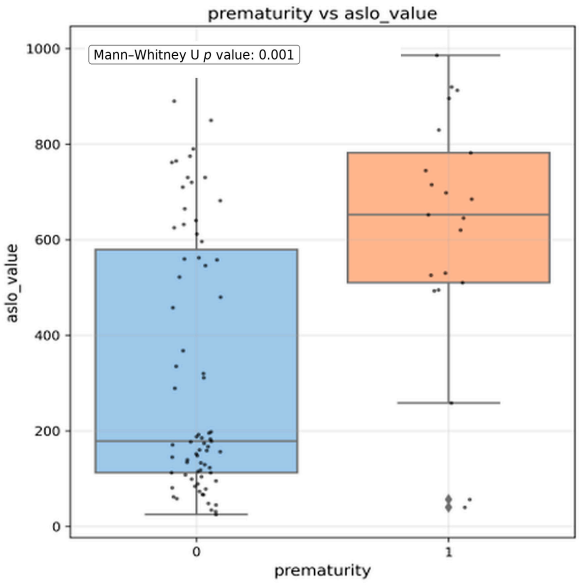


Figure 8. Boxplot of ASO value according to prematurity status. ASO titers are compared between children with and without a history of prematurity. Mann–Whitney U $p = 5.12 \times 10^{-4}$; median ASO 652 IU/mL in children with prematurity versus 178 IU/mL in those without prematurity.

The subgroup distributions were consistent with these findings. The ASO-positive subgroup included higher proportions of children with abrupt onset (86.0% vs. 10.9%), prematurity (38.0% vs. 4.3%), and recurrent infections (30.0% vs. 15.2%). Functional digestive symptoms were also more frequent in the ASO-positive subgroup: 26.0% had no digestive symptoms, compared with 87.0% in the ASO-negative subgroup.

IVF history showed a nonsignificant tendency toward higher ASO values ($p = 0.106$). Other examined variables, including residential environment, foster care background,

bacterial infections other than streptococcal infection, viral infections, and autoimmune pathology, did not show similarly clear associations in the main univariate analyses.

3.7. Clinical Correlates of Higher PANS-31 Symptom Burden

Several categorical clinical variables were associated with higher total PANS-31 scores in the present cohort. The strongest difference was observed for abrupt symptom onset: children with sudden onset had markedly higher PANS-31 total scores than those without abrupt onset (median 38 vs. 23, Mann–Whitney U test, $p = 3.40 \times 10^{-7}$) (Figure 9).

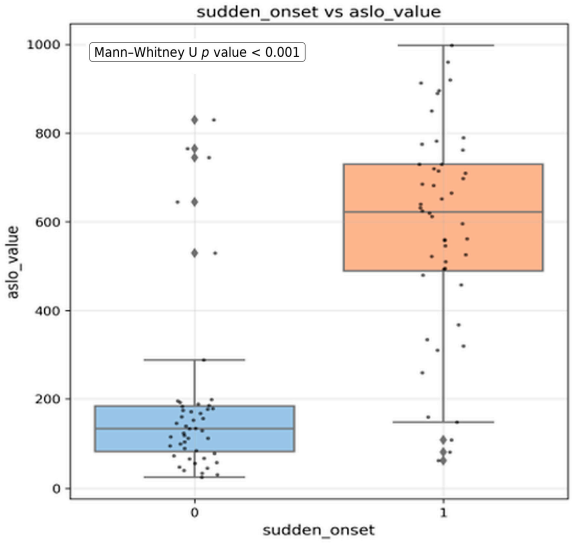
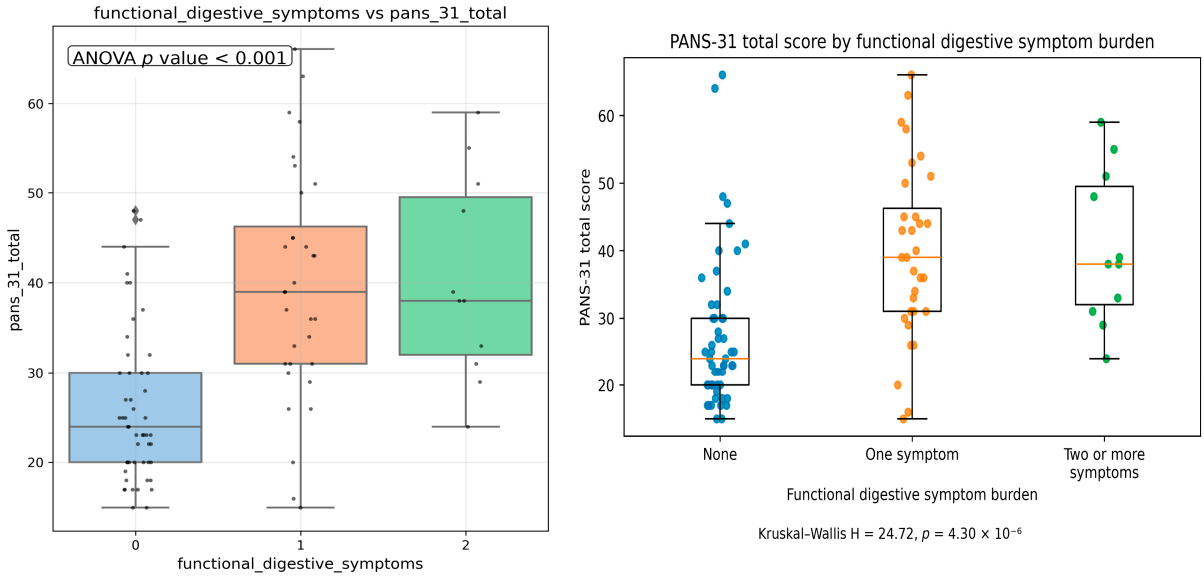


Figure 9. Boxplot of PANS-31 total score according to sudden onset status. Children with abrupt symptom onset showed higher PANS-31 total scores than those without sudden onset. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations. Mann–Whitney U $p = 3.40 \times 10^{-7}$; median PANS-31 total score 38 in children with sudden onset versus 23 in those without sudden onset.

Functional digestive symptoms were also related to dimensional symptom burden. Because this variable had three ordered categories and PANS-31 scores were non-Gaussian, the primary omnibus comparison was performed using the Kruskal–Wallis test. PANS-31 total scores differed significantly across digestive symptom categories ($H = 24.72$, $p = 4.30 \times 10^{-6}$), as shown in Figure 10B. The originally generated one-way ANOVA result, retained only as an exploratory parametric comparison for visual/statistical continuity, showed the same direction of association ($p = 7.56 \times 10^{-6}$) (Figure 10A). Bonferroni-adjusted pairwise Mann–Whitney U tests showed significant differences between no digestive symptoms and one symptom ($p_adj = 3.86 \times 10^{-5}$), and between no digestive symptoms and two or more symptoms ($p_adj = 0.00193$), but not between one symptom and two or more symptoms ($p_adj = 1.000$).

Higher PANS-31 scores were further observed in children with a history of prematurity, in vitro fertilization, and recurrent infections. Median total PANS-31 score was 40 in participants with prematurity compared with 26 in those without prematurity ($p = 3.58 \times 10^{-5}$), 42 in participants with IVF history compared with 29.5 in those without IVF history ($p = 0.00793$), and 40.5 in participants with recurrent infections compared with 29.5 in those without recurrent infections ($p = 0.0267$) (Figures 11 and 12).



(**A**) (**B**)

Figure 10. PANS-31 total score according to functional digestive symptom burden. (**A**) Exploratory one-way ANOVA visualization across the three digestive symptom categories. This parametric analysis was retained only as a descriptive consistency check. (**B**) Primary nonparametric Kruskal–Wallis analysis, appropriate for the ordinal digestive symptom variable and non-Gaussian PANS-31 distribution, showed significant differences across groups (H = 24.72, $p = 4.30 \times 10^{-6}$).

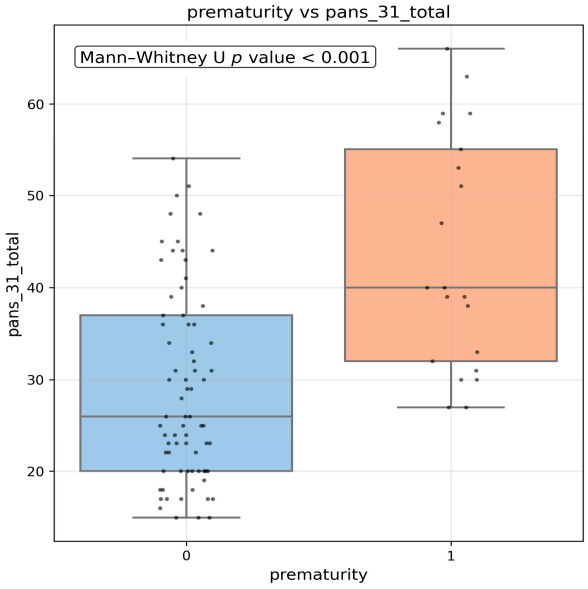


Figure 11. Boxplot of PANS-31 total score according to prematurity status. Children with a history of prematurity showed higher PANS-31 total scores than those without prematurity. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations. Mann–Whitney U $p = 3.58 \times 10^{-5}$; median PANS-31 total score 40 in children with prematurity versus 26 in those without prematurity.

The subgroup distributions were consistent with these findings. The higher PANS-31 subgroup more often included children with abrupt onset (79.4% vs. 33.9%), prematurity (41.2% vs. 11.3%), IVF history (23.5% vs. 6.5%), recurrent infections (35.3% vs. 16.1%), functional digestive symptoms, and male sex (76.5% vs. 56.5%). Functional digestive symptoms were less often absent in the higher-burden subgroup (26.5% vs. 71.0%).

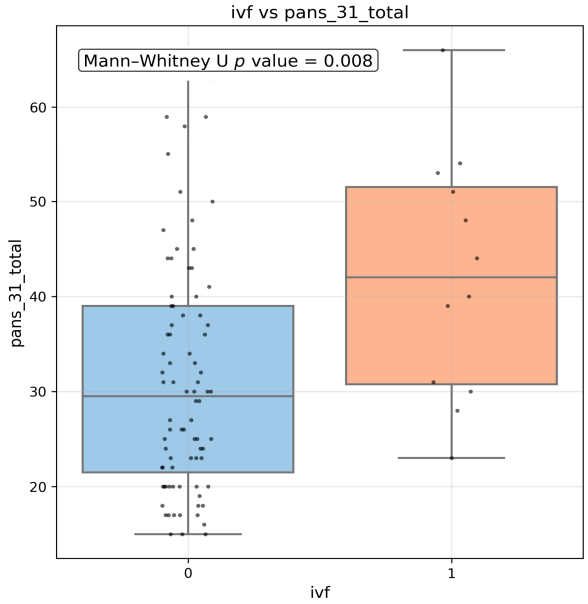


Figure 12. Boxplot of PANS-31 total score according to IVF history. Children with a history of in vitro fertilization (IVF) are compared with those without IVF history in relation to total PANS-31 score. Mann–Whitney U $p = 0.00793$; median PANS-31 total score 42 in children with IVF history versus 29.5 in those without IVF history.

Inpatient referral and foster care background were somewhat more frequent in the higher PANS-31 subgroup, whereas other examined variables, including residential environment, bacterial infections other than streptococcal infection, viral infections, and autoimmune pathology, did not show similarly clear associations with PANS-31 total score in the main univariate analyses.

3.8. EEG Associations with ASO and PANS-31

Selected EEG abnormalities were associated with higher ASO values. The clearest differences were observed for low-voltage alpha activity in temporo-parieto-occipital regions, dominant alpha activity in temporo-parieto-occipital regions, paroxysmal theta activity, and spike–wave complexes, all of which were associated with higher ASO values (all $p < 0.001$). By contrast, polymorphic theta activity was not significantly associated with ASO level ($p = 0.088$), and nonspecific EEG changes showed no significant association with ASO ($p = 0.425$), as seen in Figure 13.

A similar but not identical pattern was observed for dimensional neuropsychiatric symptom burden. Total PANS-31 scores were higher in children with low-voltage alpha temporo-parieto-occipital activity, dominant alpha temporo-parieto-occipital activity, paroxysmal theta activity, and spike–wave complexes (all $p < 0.001$). Polymorphic theta activity ($p = 0.026$) and nonspecific EEG changes ($p = 0.048$) were also associated with higher PANS-31 scores, although these associations were weaker than those observed for the other predefined EEG patterns. These distributions are shown in Figure 14.

Because EEG findings were coded from routine clinical reports as predefined pattern-based variables, the results are presented according to the presence or absence of each EEG pattern rather than as a composite EEG severity score. No validated EEG severity hierarchy was available in the dataset; therefore, participants were not classified into mild, moderate, or severe EEG-abnormality groups. This approach was used to avoid overinterpreting heterogeneous EEG findings while still allowing their descriptive associations with ASO values and PANS-31 symptom burden to be examined.

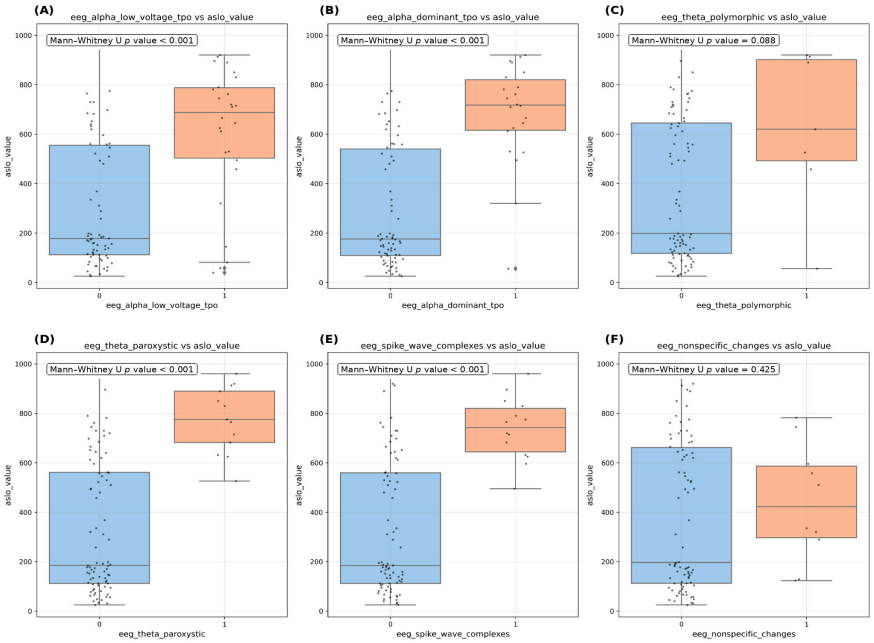


Figure 13. Boxplots showing the distribution of ASO values according to the absence (0) or presence (1) of predefined EEG abnormalities. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations. (**A**) Low-voltage alpha activity in temporo-parieto-occipital regions; (**B**) dominant alpha activity in temporo-parieto-occipital regions; (**C**) polymorphic theta activity; (**D**) paroxysmal theta activity; (**E**) spike–wave complexes; (**F**) nonspecific EEG changes.

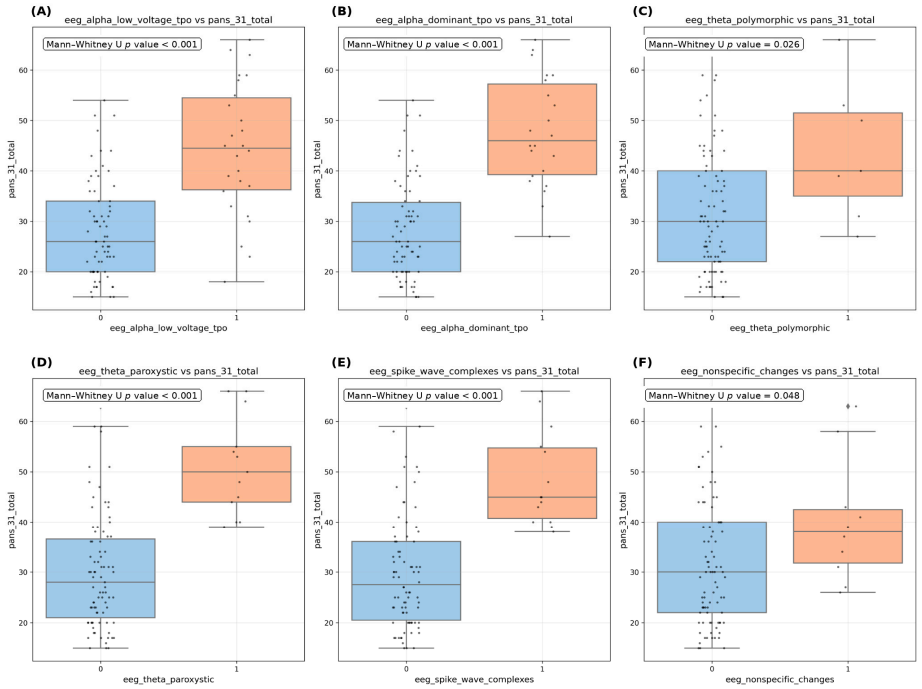


Figure 14. Boxplot panel showing the distribution of PANS-31 total scores according to predefined EEG abnormalities. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations. (**A**) Low-voltage alpha activity in temporo-parieto-occipital regions; (**B**) dominant alpha activity in temporo-parieto-occipital regions; (**C**) polymorphic theta activity; (**D**) paroxysmal theta activity; (**E**) spike–wave complexes; (**F**) nonspecific EEG changes.

3.9. Psychometric Convergence Between PANS-31 and ASI-4/CSI-4 Domains

A Spearman correlation heatmap integrating ASO value, total PANS-31 score, age, time since the last reported streptococcal infection, and selected ASI/CSI domain maxima showed a structured pattern of associations. The strongest positive correlation in the matrix was observed between ASO value and total PANS-31 score ($\rho = 0.81$). The strongest inverse correlations involved time since the last reported streptococcal infection, both with ASO value ($\rho = -0.79$) and with total PANS-31 score ($\rho = -0.77$). Among the external symptom domains, total PANS-31 score showed its highest positive correlations with ASI/CSI anxiety ($\rho = 0.56$) and enuresis ($\rho = 0.52$), whereas the CSI pervasive domain showed a moderate negative correlation with total PANS-31 score ($\rho = -0.44$). The full correlation structure is shown in Figure 15.

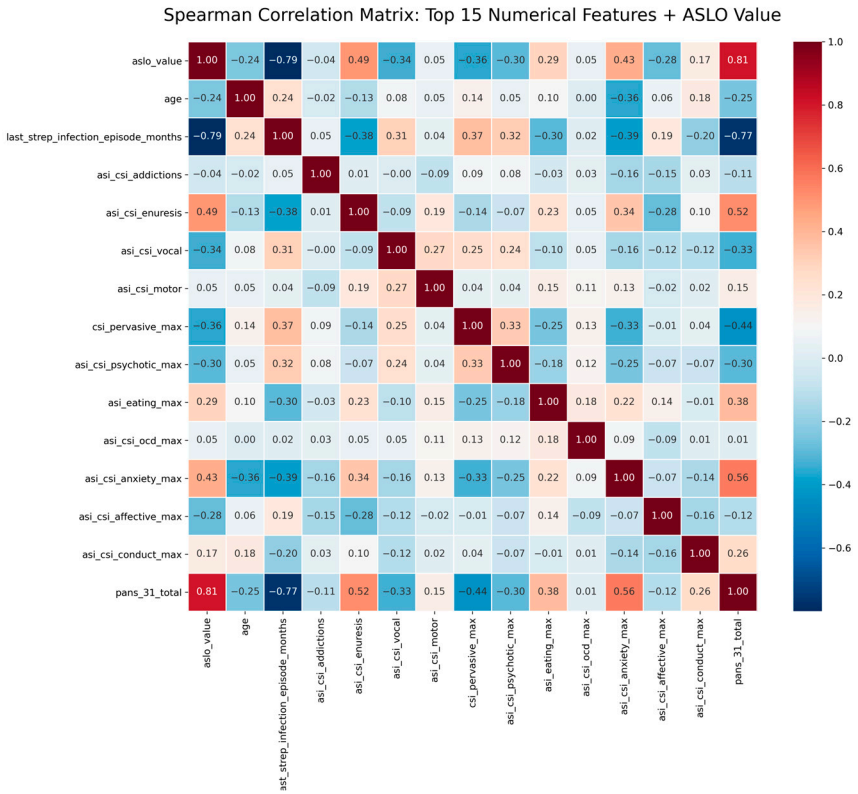


Figure 15. Spearman correlation heatmap including ASO value, total PANS-31 score, time since the last reported streptococcal infection, age, and selected ASI/CSI domain maxima.

3.9.1. Psychometric Validation Results: PANS-31 vs. ASI/CSI

Reliability and Internal Consistency

The PANS-31 total scale demonstrated good internal consistency (Cronbach's $\alpha = 0.835$), meeting clinical research standards ($\alpha > 0.70$) and supporting the composite use of all 31 items as a unified severity measure. Domain-level analyses were used descriptively to explore symptom organization and cross-scale correspondence, rather than to treat each PANS-31 domain as an independently validated subscale. The relevant findings are summarized in Appendix A, Table A1.

Construct Validity: Factor Structure

Principal component analysis supported a structured dimensional organization of PANS-31. At the domain level, four components accounted for 72.7% of cumulative variance, with a Kaiser–Meyer–Olkin coefficient of 0.766 (Figure 16). At the item level,

five components accounted for 72.5% of cumulative variance, and the first component explained 38.0% of total variance.

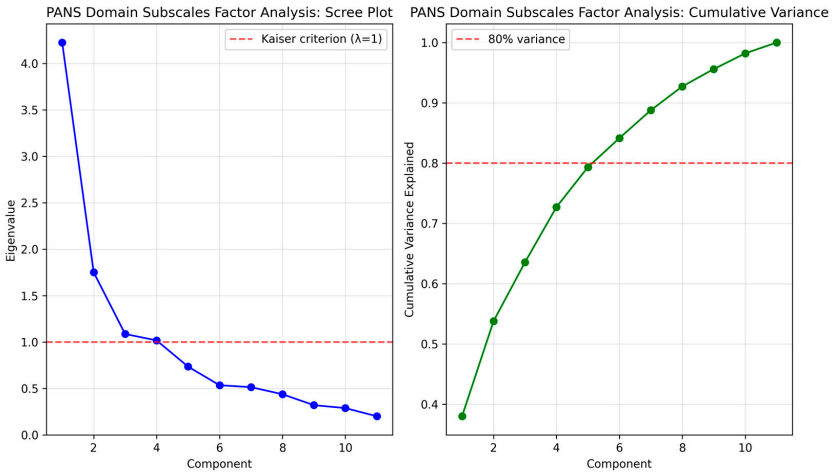


Figure 16. Scree plot showing eigenvalue distribution and cumulative variance explained across principal components; bar chart with KMO adequacy coefficient.

Domain-Level Convergence: Cross-Scale Convergence with ASI/CSI

Cross-scale associations were stronger at the domain level than at the total score level. When theoretically corresponding domains were paired directly across instruments, the highest inter-scale correlations were observed for anxiety (r = 0.899), eating symptoms (r = 0.801), behavioral/conduct symptoms (r = 0.765), and obsessive–compulsive symptoms (r = 0.719). Additional positive correlations were observed for motor symptoms (r = 0.693), enuresis/urinary symptoms (r = 0.684), psychotic symptoms (r = 0.626), vocal symptoms (r = 0.550), and affective/mood symptoms (r = 0.464). These findings were interpreted as supportive domain-level convergence within the present cohort, not as full criterion validation, diagnostic equivalence, or interchangeability between instruments.

Correlations Between ASI/CSI Domains and Total PANS-31 Score

Correlations between individual ASI/CSI domains and total PANS-31 score showed a non-uniform pattern. The strongest positive associations were observed for enuresis (r = 0.581, $p < 0.001$) and anxiety (r = 0.566, $p < 0.001$), followed by eating-related symptoms (r = 0.379, $p = 0.001$), motor symptoms (r = 0.328, $p = 0.003$), and conduct symptoms (r = 0.287, $p = 0.008$). The CSI pervasive domain showed a moderate inverse correlation with total PANS-31 score (r = −0.310, $p = 0.005$). Associations with addictions, vocal symptoms, psychotic symptoms, and affective symptoms were not significant, while the OCD domain showed a borderline association (r = 0.215, $p = 0.055$). The full results are presented in Table 5.

Table 5. Spearman correlations between PANS-31 total score and corresponding ASI/CSI domains with significance levels. (*, **, *** for $p < 0.05$, 0.01, 0.001).

ASI/CSI Domain	Spearman r	*p* Value
ASI/CSI addictions *	−0.057	0.583
ASI/CSI enuresis ***	0.581	<0.001
ASI/CSI vocal *	−0.127	0.241
ASI/CSI motor **	0.328	0.003
CSI pervasive max **	−0.310	0.005
ASI/CSI psychotic max *	−0.188	0.083

Table 5. *Cont.*

ASI/CSI Domain	Spearman r	*p* Value
ASI eating max ***	0.379	0.001
ASI/CSI OCD max */**	0.215	0.055
ASI/CSI anxiety max ***	0.566	<0.001
ASI/CSI affective max *	−0.187	0.083
ASI/CSI conduct max **	0.287	0.008

Domain Correspondence Analysis

In 8 of 11 ASI/CSI domains, the empirically strongest correlation matched the theoretically expected PANS domain. The highest inter-scale correlations were observed for anxiety (|r| = 0.899), eating-related symptoms (|r| = 0.801), behavioral/conduct symptoms (|r| = 0.765), and obsessive–compulsive symptoms (|r| = 0.719). Additional matched correlations were observed for psychotic symptoms (r = 0.626) and affective/mood symptoms (r = 0.464). The full PANS × ASI/CSI domain correlation matrix is shown in Figure 17.

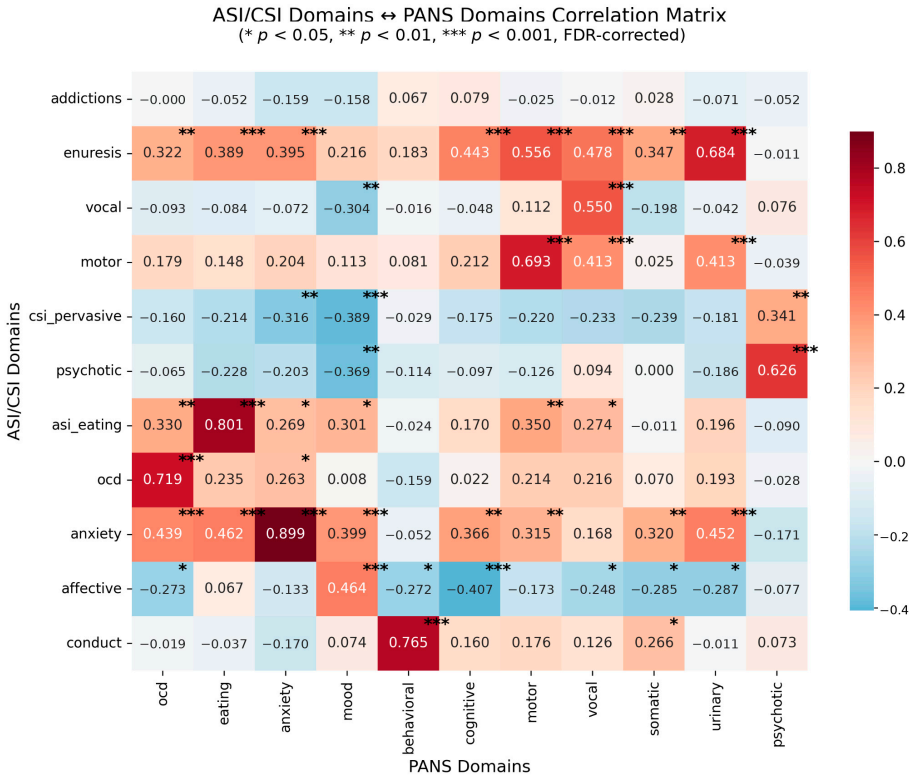


Figure 17. Heatmap showing PANS × ASI/CSI domain correlation matrix with significance markers (*, **, *** for $p < 0.05$, 0.01, 0.001).

Differential validity analysis showed that each PANS domain correlated at |r| > 0.3 with at least one ASI/CSI domain, supporting meaningful alignment with established child and adolescent psychiatric constructs.

Multitrait–Multimethod (MTMM) Validation

Campbell and Fiske criteria were applied to validate the relationship between PANS and ASI/CSI as separate but related measurement methods:

- Convergent validity exceeded discriminant validity: Mean convergent correlation (|r| = 0.689) significantly exceeded mean discriminant validity coefficients within

methods (ASI/CSI within-scale |r| = 0.126; PANS within-scale |r| = 0.301), with convergent–discriminant differences.
- Convergent correlations stronger than heterotrait–heteromethod: Mean |r| = 0.689 for convergent validity vs. mean |r| = 0.186 for uncorrelated constructs across methods.
- Pattern consistency: The ordering and relative magnitudes of correlations remained consistent across the multimethod matrix, supporting a single underlying construct structure.

The observed MTMM pattern was broadly consistent with the Campbell and Fiske framework and supports meaningful convergence between PANS-31 and age-appropriate ASI/CSI domains in this clinically enriched sample. However, these findings should be interpreted cautiously and replicated in larger, more diverse cohorts before stronger claims are made about cross-method equivalence or general psychometric generalizability.

Clinical Classification Agreement

Binary classification using median-split cutoffs on PANS and ASI/CSI total scores yielded 53.1% overall accuracy (Cohen's $\kappa = 0.062$, 95% CI: [−0.20 to 0.32]). The modest kappa value indicated only slight agreement, suggesting that PANS-31 and pooled ASI/CSI totals do not classify participants in equivalent ways when reduced to binary groupings. This divergence is expected given that the instruments measure overlapping but distinct symptom domains and that different clinical thresholds may apply.

4. Discussion

4.1. Principal Findings

The present study examined the relationship between antistreptolysin O titers and PANS-31 symptom burden with a secondary focus on selected clinical-history variables, EEG findings, and dimensional psychiatric symptom domains in a realistic, clinically selected pediatric psychiatric cohort. The main finding was a strong association between higher ASO values and higher PANS-31 total scores. Importantly, this association did not occur in isolation. Higher ASO values clustered with a shorter reported interval since the last streptococcal infection, more frequent abrupt onset, recurrent infections, functional digestive symptoms, male predominance, selected developmental history variables such as prematurity and IVF history, and selected EEG abnormalities. Taken together, these findings suggest that, in this real-world psychiatric cohort, higher anti-streptococcal serology and greater multidomain neuropsychiatric burden formed a clinically interpretable association pattern rather than a purely serological finding.

This association pattern should therefore be understood as multidimensional and cohort-specific. The clinically informative signal appeared to emerge from the convergence of exposure history, symptom timing, dimensional symptom burden, somatic vulnerability, developmental background, and neurophysiological context. However, this interpretation should remain cautious. These findings remain exploratory: ASO cannot be treated as a disease-specific biomarker, and the observed convergence does not establish either a distinct diagnostic entity or a causal link between streptococcal exposure and neuropsychiatric symptoms. Current reviews and the consensus-oriented literature emphasize that PANS and PANDAS remain clinically heterogeneous with debated conditions in which biological markers may support, but cannot replace clinical reasoning [20–23]. The present results are therefore best understood as evidence of clinical convergence: in this cohort, serological burden, reported infection timing, abrupt symptom change, somatic and developmental vulnerability markers, EEG findings, and dimensional psychiatric severity tended to align.

4.2. ASO, Infection Timing, Abrupt Onset, Age, and Sex

The ASO–PANS-31 association is clinically meaningful because it was supported by the temporal structure of the data. The inverse association between ASO value and time since the last reported streptococcal infection is not unexpected; ASO and anti-DNase B are dynamic markers of previous group A streptococcal exposure, and single absolute titers may remain elevated for months or be misleading without paired measurements. Therefore, the relevant observation in the present cohort is not simply that ASO values were higher closer to the reported infection, which is biologically plausible, but this expected serological gradient aligned with higher PANS-31 burden and more frequent abrupt onset. This pattern strengthens the plausibility of a post-infectious or immune-associated clinical presentation, while still falling short of causal inference. As such, ASO should remain interpreted as a marker of prior or recent streptococcal exposure.

This distinction is important in light of the existing literature. Diagnostic and conceptual reviews consistently caution that ASO and anti-DNase B are supportive markers and cannot diagnose PANS/PANDAS by themselves [24]. At the same time, serological signals may be more informative in narrower streptococcal-associated phenotypes than in heterogeneous acute-onset neuropsychiatric presentations [25]. Conversely, previous clinical and review data indicate that ASO titers alone have limited discriminatory value outside a carefully defined clinical and temporal context [26,27]. The present study fits between these positions: ASO was not treated as diagnostic, but its strong association with symptom burden, recency of reported infection, and abrupt onset suggests that it may help identify a clinically enriched subgroup when interpreted dimensionally rather than categorically.

A small but clinically important interpretive issue concerns children with elevated ASO despite a remote reported streptococcal episode, including intervals approaching or exceeding 12 months. Such findings should not be interpreted automatically as evidence of persistent active infection. They may reflect repeated unrecognized exposures, asymptomatic carriage, prolonged antibody persistence, incomplete reconstruction of infection timing, or individual differences in immune kinetics [28,29]. In future cohorts, these children should be analyzed separately, ideally through serial ASO/anti-DNase B measurements, contemporaneous throat or site-specific cultures when symptomatic, and explicit assessment of carrier status.

Age and sex showed a weaker but directionally consistent pattern. Younger age and male sex were more frequent among participants with higher ASO values and higher PANS-31 scores. This is compatible with classical PANDAS descriptions and retrospective clinical cohorts in which onset commonly occurs in school-age children and boys are overrepresented [30,31]. However, these demographic features should not be overinterpreted. The cohort was recruited through child psychiatric services and does not represent the general pediatric population. Age and sex, therefore, help characterize the referral-shaped cohort observed here, but they do not independently explain the ASO–PANS relationship.

4.3. Clinical Correlates: Recurrent Infections, Functional Digestive Symptoms, Prematurity, and IVF History

Recurrent infections and functional digestive symptoms add an important nonserological layer to the observed association pattern. Recurrent infections may reflect repeated infectious exposure or repeated immune challenge, although the present design cannot directly test this mechanism. Functional digestive symptoms were exploratory but clinically relevant because they were associated with both ASO values and PANS-31 scores in the nonparametric and parametric analyses. These findings suggest that, in some children, the more clearly expressed clinical presentation extended beyond narrowly psy-

chiatric symptoms and included a broader somatic context. Because the digestive symptom variable had three ordered categories, these associations should be interpreted as analytical group differences.

Current microbiome-oriented studies and reviews describe altered gut microbiota composition, mucosal immune activation, intestinal permeability, and gut–brain signaling as plausible contributors to PANS/PANDAS heterogeneity [32–34]. However, the literature remains preliminary. The present findings should, therefore, not be interpreted as evidence of a gastrointestinal cause, but rather as compatible with a gut–immune–brain vulnerability context in which infectious exposure, immune reactivity, somatic symptoms, and psychiatric burden may become clinically intertwined.

Prematurity and IVF history should be interpreted cautiously. Neither variable is syndrome-specific, and the present study cannot support a causal relationship between early developmental factors and PANS-like symptom burden. Their inclusion was exploratory and intended to characterize whether developmental or perinatal-history variables clustered with higher symptom burden within this clinically referred cohort. Prematurity was associated with both higher ASO values and higher PANS-31 scores, whereas IVF history was more clearly related to PANS-31 burden than to ASO values. A more defensible interpretation is that IVF history may identify a subgroup with broader developmental, perinatal, familial, or referral-related vulnerability in whom abrupt neuropsychiatric decompensation becomes more visible or clinically significant. This interpretation is consistent with broader developmental and immune-programming models, but direct evidence linking IVF to PANS/PANDAS is limited. These findings should therefore remain hypothesis-generating rather than causal or syndrome-specific.

Equally important are the variables that did not clearly separate the groups. Residential environment, bacterial infections other than streptococcal infection, viral infections, and autoimmune pathology did not show gradients comparable to those observed for abrupt onset, recurrent infections, functional digestive symptoms, prematurity, or ASO-related burden. This argues against a nonspecific accumulation of medical complexity explanation. The higher-burden subgroup was not defined by indiscriminate comorbidity, but by a more selective clustering of features: elevated ASO, shorter infection interval, abrupt onset, recurrent infectious history, functional digestive symptoms, and selected developmental vulnerability markers.

4.4. EEG Findings

The EEG findings should be interpreted as complementary neurophysiological correlates and not as disease-specific markers. In the present cohort, several abnormalities—particularly low-voltage alpha activity, dominant alpha activity in temporo-parieto-occipital regions, paroxysmal theta activity, and spike–wave complexes—were associated with both higher ASO values and higher PANS-31 scores. Other findings, including polymorphic theta activity and nonspecific EEG abnormalities, appeared to track symptom burden more consistently than serological status. This pattern argues against a single electrophysiological signature of PANS/PANDAS, but also against a completely random distribution of EEG changes across the cohort.

The clinical relevance of these findings lies less in diagnostic specificity than in their contribution to broader clinical and neurophysiological characterization. EEG may help identify children whose neuropsychiatric presentation is accompanied by measurable functional neurophysiological disturbance, but such findings do not diagnose a streptococcal-triggered syndrome and should not be interpreted as equivalent to neuroimaging evidence. The absence of systematic MRI-based neuroimaging is therefore an important limitation

of the present study, but it reflects the real-world clinical pathway from which the cohort was assembled.

MRI-based approaches could provide complementary information on basal ganglia morphology, cortico-striatal circuitry, white-matter connectivity, structural abnormalities, and functional network organization. EEG, by contrast, provides a more accessible functional measure of cortical activity. These methods answer different questions and should be viewed as complementary.

This interpretation is consistent with the diagnostic and neuropsychiatric literature emphasizing that EEG may be useful in selected evaluations but remains nonspecific and context-dependent [35]. Broader neurobiological models of PANS/PANDAS have implicated immune-mediated mechanisms, antineuronal antibodies, basal ganglia-related pathways, sleep disruption, and network-level dysfunction rather than a single lesion-specific mechanism [36,37]. Therefore, the EEG findings in the present cohort should be viewed as clinically convergent with broader neurobiological models of network-level involvement, but not as a diagnostic electrophysiological marker.

Based on the present findings, EEG should not be recommended solely on the basis of elevated ASO titers. A more appropriate interpretation is that EEG may be considered in clinically selected children with abrupt or substantially changed neuropsychiatric presentations, especially when symptoms are severe, atypical, fluctuating, associated with cognitive or sleep disturbance, or accompanied by neurological features such as paroxysmal events, altered awareness, motor abnormalities, or concern for seizure activity. In such cases, EEG may help refine the neuropsychiatric assessment, but its findings must remain anchored in the full clinical, developmental, and psychiatric context.

4.5. PANS-31, ASI/CSI Domains, and Operational Thresholding

The psychometric findings support the use of PANS-31 as a dimensional measure of syndrome-oriented symptom burden in this cohort. PANS-31 showed meaningful convergence with age-appropriate ASI/CSI domains, including anxiety, obsessive–compulsive symptoms, eating-related symptoms, conduct/behavioral dysregulation, motor symptoms, urinary/enuresis symptoms, and psychotic-like symptoms. This is clinically important because PANS-31 did not behave as an isolated or arbitrary scale; rather, it overlapped with established psychiatric symptom domains while retaining a broader multidimensional structure.

This pattern should be interpreted as dimensional convergence, not diagnostic equivalence. ASI and CSI are DSM-oriented symptom inventories designed to capture broad psychiatric domains, whereas PANS-31 was developed to quantify the multidomain symptom burden typical of acute-onset neuropsychiatric presentations [38,39]. The present results suggest that PANS-31 may be useful in routine psychiatric settings where categorical diagnoses alone may flatten clinically meaningful symptoms. The scale appears to capture a syndrome-oriented burden that intersects with, but is not reducible to, conventional anxiety, obsessive–compulsive, behavioral, somatic, urinary, or motor symptom domains.

The use of PANS-31 > 36 should be interpreted as an internal operational approach for exploratory subgrouping, not as a diagnostic boundary or externally validated severity cutoff. The threshold-sensitivity analysis supported the operational defensibility of this predefined cut point: lower exploratory thresholds still included a small number of ASO-negative borderline cases, whereas thresholds from the mid-30s upward showed a stable ASO-positive pattern. Because the present cohort did not include a broad distribution of very high PANS-31 scores, more restrictive thresholds would have reduced the analyzable higher-burden subgroup without materially changing the observed ASO/PANS-31 pattern. Thus, PANS-31 > 36 was retained as a conservative but not overly restrictive cohort-

specific separator. More broadly, the issue is not where clinically relevant symptoms begin, but where syndrome-oriented discrimination becomes more informative in routine psychiatric practice. This interpretation is consistent with the psychometric principle that clinically useful measures should show convergence with related domains while retaining discriminant value from nonspecific psychopathology [40,41].

4.6. Romanian Clinical Context, Prior Antibiotic Exposure, and Antibiotic Stewardship

The Romanian clinical context is relevant to the interpretation of the findings. Child and adolescent psychiatric care, pediatric services, primary care, and specialized neurological or immunological assessment are not always integrated through standardized national pathways. More broadly, Romanian healthcare has been described as facing persistent challenges related to access, coordination, resource distribution, and patient navigation [42,43]. In such a setting, children with abrupt neuropsychiatric deterioration and a suggestive infectious history may reach psychiatric services after heterogeneous medical itineraries, prior laboratory testing, and various family-led attempts to identify an explanatory medical framework.

In the present cohort, referral narratives frequently suggested that families had already explored multiple clinical evaluations before psychiatric assessment, including infectious, immune-mediated, developmental, psychosocial, and non-medical interpretations of the child's symptoms. Although these pathways were not systematically quantified, they are clinically relevant because online communities and parent-to-parent information networks may shape expectations regarding PANS/PANDAS as an identifiable organic condition, sometimes encouraging prolonged and costly diagnostic investigations before integrated psychiatric evaluation. This context does not invalidate the observed ASO–PANS-31 association, but it underscores the need to interpret the findings within a real-world care environment characterized by fragmented pathways, high parental uncertainty, and variable access to coordinated multidisciplinary assessment. Local pediatric experience with immune-mediated and rheumatological presentations, as well as emerging Romanian clinical reporting on PANS-like presentations, further supports the relevance of interpreting the cohort within its national care context [44,45].

This context may also explain why some parents reported previous antibiotic exposure, including penicillin or macrolides, either after a clinically suspected streptococcal infection or later in response to elevated ASO titers. In some cases, families reported repeated benzathine benzylpenicillin injections administered as prophylaxis in the context of persistently elevated ASO values. Such practices appear to reflect real-world care pathways, informal medical traditions, and parental attempts to obtain an explanatory and treatable organic diagnosis, rather than evidence supporting a specific therapeutic approach. These observations should therefore be interpreted through an antibiotic stewardship framework. Current infectious diseases and PANS/PANDAS management recommendations support antibiotic treatment when group A streptococcal infection is documented or clinically suspected, whereas isolated ASO elevation should not be considered proof of active infection or a stand-alone indication for antimicrobial therapy [6,24,46].

Prior antibiotic exposure also represents an important interpretive limitation. Antibiotics administered before microbiological assessment may reduce subsequent culture yield, shorten antigenic exposure, and potentially attenuate or reshape the ASO/anti-DNase B trajectory, thereby limiting the temporal interpretability of a single ASO value [28,29]. In the present cohort, however, previous penicillin exposure did not appear to organize the main statistical pattern and was not a dominant explanatory factor for ASO level, PANS-31 burden, ASO-stratified group membership, or functional digestive symptoms. This argues against the interpretation that the observed characteristics merely reflect previous empirical

antibiotic use. Rather, ASO should be viewed as one component of a broader exposure-linked cluster: it marked prior anti-streptococcal immune response and gained clinical relevance only when interpreted together with infection timing, abrupt onset, symptom burden, EEG findings, and selected vulnerability markers.

4.7. Clinical Positioning of the Cohort

The present cohort appears to occupy an intermediate position between highly selective classical descriptions of PANDAS and broader, more heterogeneous PANS-like pediatric acute neuropsychiatric presentations. Abrupt onset remained central, serology and timing were not randomly distributed, the higher-burden subgroup showed a more coherent developmental and medical context, and selected EEG abnormalities added a functional layer. At the same time, the absence of very severe PANS-31 scores, the lack of preschool-aged children, and the psychiatric recruitment pathway argue against treating this sample as a prototype of the most severe or biologically "pure" form of the syndrome.

This intermediate position may be one of the study's strengths. Much of the uncertainty in this field arises not only from rare and dramatic cases, but also from intermediate presentations that do not fit neatly into either a conventional primary psychiatric model or a sharply defined neuroimmune category. The present cohort likely belongs to that middle territory, where dimensional assessment, cautious biological contextualization, and complementary EEG information may be most useful. Also, in this context, there is another important question for future studies: whether similar multidimensional clustering would emerge if the infectious axis were defined not by anti-streptococcal serology, but by other infectious or post-infectious markers implicated in broader PANS-like presentations. The PANS framework allows for potential non-streptococcal triggers, including *Mycoplasma pneumoniae*, *Borrelia burgdorferi*, and selected viral or neurotropic infections, although the strength and specificity of evidence might differ across pathogens.

4.8. Strengths, Limitations, and Future Directions

A major strength of this study is its integrative real-world design. In a field in which isolated biomarkers have repeatedly shown limited diagnostic value, the present analysis did not examine ASO, symptom burden, EEG findings and developmental or clinical-history variables as disconnected observations. Instead, it examined whether these elements showed a clinically interpretable association pattern within routine child psychiatric practice. This approach reflects the way complex acute-onset neuropsychiatric presentations are actually evaluated: through the accumulation of convergent clinical, temporal, biological, psychometric, and neurophysiological evidence rather than through a single decisive marker.

In children meeting the abrupt onset criterion, clinical assessment was usually obtained within approximately one week from the sudden neuropsychiatric change, reflecting the acute referral pattern of the participating services. This allowed the study to examine whether abrupt onset differentiated higher ASO or higher PANS-31 subgroups within the clinically selected cohort. The study also benefits from the use of structured dimensional assessment through PANS-31, age-appropriate ASI/CSI comparator domains, and routinely available EEG data. This allowed the cohort to be characterized beyond categorical diagnosis alone and provided a more granular description of symptom burden, functional neurophysiological correlates, and clinical-history patterns.

Another important strength is the selective structure of the findings. The higher-burden subgroup was not defined by a nonspecific accumulation of medical complexity. Rather, abrupt onset, shorter time since reported streptococcal infection, ASO elevation, recurrent infections, functional digestive symptoms, prematurity, greater PANS-31 burden,

and selected EEG abnormalities showed more coherent gradients than residential environment, non-streptococcal bacterial infections, viral history, or autoimmune pathology. This selective clustering supports the clinical interpretability of the observed signal, but it should be understood as a cohort-specific association pattern rather than as evidence of a validated vulnerability profile, independently defined phenotype, or distinct diagnostic subgroup.

Several limitations must also be acknowledged. First, the cohort was clinically selected and recruited from child psychiatric services, and the analytical sample was derived from predefined ASO strata. This recruitment pathway limits epidemiological interpretation. Because participants had already come to psychiatric or neuropsychiatric attention, the study cannot estimate the prevalence of neuropsychiatric symptoms among all children exposed to streptococcal infection, nor the later psychiatric or neurological risk associated with such exposure. Because the analyses were predominantly univariate, no control group was included, and ASO was measured at a single index assessment, the findings cannot support causal, diagnostic, or predictive interpretation. The strong ASO–PANS-31 association and the complete separation of higher PANS-31 cases into the ASO-positive group should therefore be interpreted as descriptive patterns within a clinically selected cohort, not as evidence of an independently validated clinical phenotype or disease-specific biomarker.

Children with mild, transient, delayed, subclinical, or unrecognized symptoms may not have been captured due to low addressability, while children with more severe acute presentations may have entered pediatric, neurological, infectious diseases, or immunological pathways instead. The results are, therefore, most informative for clinically enriched psychiatric settings, rather than for unselected community, primary care, or post-infectious pediatric populations.

Second, the study was cross-sectional, index assessment-based, and predominantly univariate. Although post hoc sensitivity analyses using alternative PANS-31 thresholds were added, these analyses do not replace comprehensive multivariable modeling, prospective validation, or external replication. The observed associations cannot establish causality, temporal direction beyond reconstructed clinical history, or independent effects after adjustment for potential confounders such as age, sex, care setting, baseline psychiatric vulnerability, developmental history, previous treatment exposure, referral pathway, or caregiver recall. The complete separation of higher PANS-31 cases into the ASO-positive stratum may partly reflect the clinically enriched recruitment pathway, referral context, caregiver- or record-supported suspicion of streptococcal exposure, and circular clinical ascertainment. Recent imbalance-aware and demography-aware biomedical modeling work further highlights that signals observed in selected clinical datasets may be affected by class imbalance, demographic imbalance, and subgroup-specific instability. Methodological approaches such as GAN-based imbalance correction and demography-aware federated learning illustrate the importance of validating findings from selected clinical samples in larger, prospectively recruited, demographically balanced, and externally validated cohorts [47,48]. The findings of the present study should therefore be interpreted as hypothesis-generating association patterns within a selected clinical cohort, not as causal, diagnostic, predictive, or individual-level classification evidence.

Third, exposure characterization was limited. Time since the last reported streptococcal infection was reconstructed from caregiver-informed history and available medical documentation, which may be affected by recall bias, incomplete records, or differential care-seeking. In addition, no participant presented with clinical signs of active pharyngitis, tonsillitis, skin infection, fever, or another acute infectious condition requiring throat swab or culture at index assessment. Therefore, ASO was interpreted as a marker of prior or recent streptococcal exposure, not as evidence of active infection or proof of a neuropsychiatric trigger. The absence of systematic throat swab/culture and the lack of parallel

anti-DNase B testing further limited the precision of streptococcal characterization. Prior antibiotic exposure represents an additional limitation. Because antibiotic timing was not uniformly documented relative to infection onset, ASO measurement, and psychiatric worsening, the study could not determine whether treatment occurred early enough to modify throat-culture positivity or the magnitude and timing of the anti-streptococcal antibody response. This limits the interpretation of both ASO-negative and moderately ASO-positive cases.

Fourth, the psychometric and neurophysiological findings should be interpreted within their methodological boundaries. PANS-31 was used as a dimensional measure of symptom burden and showed meaningful convergence with ASI/CSI domains, but this does not establish diagnostic equivalence between instruments or validate PANS-31 as a stand-alone diagnostic tool in this setting. Similarly, EEG abnormalities were clinically informative as complementary neurophysiological correlates, but they are nonspecific and context-dependent, and should not be interpreted as electrophysiological biomarkers of PANS/PANDAS.

Fifth, several clinical-history variables, including prematurity, IVF history, recurrent infections, and functional digestive symptoms, were exploratory correlates. Although they helped characterize the higher-burden subgroup, the study was not designed to determine whether these variables represent causal risk factors, developmental vulnerability markers, referral-related features, or consequences of broader medical complexity. Their interpretation should therefore remain hypothesis-generating.

Finally, the study has contextual limitations relevant to external validity. Because the sample was assembled within Romanian child psychiatric services, referral pathways, access to pediatric neurology, infectious diseases, or immunological assessment, prior laboratory testing, prior antibiotic exposure, parental explanatory models, and local healthcare-navigation patterns may have shaped which children entered the cohort. In a debated area such as PANS/PANDAS, some families may reach specialist care after previous medical consultations and exposure to explanatory frameworks linking infection, immunity, and abrupt psychiatric deterioration. These features increase the ecological relevance of the study for routine Romanian specialist practice, but indicate that the sample should be interpreted as naturalistic and referral-shaped rather than population-based. Accordingly, the findings are locally informative and hypothesis-generating, but their generalizability to unselected pediatric populations or to healthcare systems with different referral structures remains limited.

Future studies should move beyond larger samples alone and use prospective, multidisciplinary, multimodal, and staged assessment protocols. Such protocols should be developed and implemented collaboratively by child psychiatrists, pediatric neurologists, pediatricians, infectious diseases specialists, immunologists or rheumatologists, microbiologists, neuroradiologists, psychologists, and biostatisticians, so that clinical, infectious, immunological, neurophysiological, imaging, and psychometric data are collected in a standardized and temporally meaningful manner. Prospective studies should also follow children with microbiologically documented streptococcal infection, including those not referred to psychiatric services, using repeated behavioral, neuropsychiatric, serological, and developmental assessments over time. Ideally, children with abrupt onset presentations should be assessed as early as possible after symptom emergence, with acute-phase documentation obtained within the first days after onset whenever feasible, and followed longitudinally through worsening, partial remission, and recovery or recurrence phases.

Beyond conventional epidemiological validation, future larger-scale studies may also require dedicated modeling-oriented designs if the aim shifts from descriptive association analysis to prediction, risk stratification, or individualized clinical decision support.

In such settings, recent methodological work from other biomedical fields may be informative. Zhao et al. [47] proposed DiGAN, a generative adversarial network-based imbalance correction framework for diabetic data analysis that combines synthetic data generation, Laplacian Score feature selection, and downstream classification to address severe class imbalance. In a separate clinical risk-prediction context, Zhao et al. [48] proposed a demography-aware personalized federated learning framework designed to balance predictive accuracy, demographic fairness, privacy preservation, and communication efficiency across distributed healthcare datasets. These approaches are not directly applicable to the present descriptive cross-sectional analysis, but they illustrate the type of methodological infrastructure that may be needed in future multi-center PANS/PANDAS or post-infectious neuropsychiatric studies involving larger datasets, class imbalance, demographic heterogeneity, privacy constraints, subgroup fairness assessment, and external validation.

A feasible core multidisciplinary panel would include structured psychiatric and neurological examination, standardized documentation of abrupt onset or abrupt worsening, infectious history, throat swab or culture when acute infection is clinically suspected, ASO and anti-DNase B titers with repeated measurements, inflammatory markers, clinically indicated pathogen-specific testing, and dimensional symptom assessment using PANS-31 together with age-appropriate obsessive–compulsive, tic, anxiety, affective, behavioral, sleep, urinary, and eating-related measures. Pathogen-specific testing could be guided by pediatric and infectious diseases evaluation and, in selected cases, include non-streptococcal infectious or post-infectious markers such as *Mycoplasma pneumoniae*, *Borrelia burgdorferi*, Epstein–Barr virus, cytomegalovirus, or other neurotropic agents, depending on clinical context and local epidemiology. This would allow future studies to test whether the multidimensional clustering observed in streptococcal-enriched presentations also appears in non-streptococcal PANS-like cohorts, or whether different infectious agents generate partially distinct clinical–neurophysiological profiles.

EEG could be included as a functional neurophysiological measure, particularly in children with atypical episodes, altered awareness, regression, paroxysmal symptoms, marked fluctuation, or concern for seizure activity. Repeated EEG assessment across phases of worsening and partial remission could help clarify whether observed abnormalities behave more like state markers of symptomatic load or stable features of a vulnerable subgroup [49]. Future work should also evaluate EEG severity using predefined and reproducible scoring procedures rather than relying only on the presence or absence of heterogeneous EEG patterns. An expanded research panel would add structural MRI, diffusion imaging, functional MRI, and, in selected cases, positron emission tomography or single-photon emission computed tomography to examine basal ganglia, cortico-striatal, white-matter, and network-level abnormalities. Immunological profiling, cerebrospinal fluid studies, and genetic or microbiome assessment may also be considered in specialized research settings, but these should be interpreted as exploratory rather than diagnostic until validated in larger prospective cohorts.

5. Conclusions

In this clinically selected pediatric psychiatric cohort, ASO elevation did not function as an isolated diagnostic signal. Its relevance emerged when it converged with shorter time since reported streptococcal infection, greater PANS-31 symptom burden, abrupt onset, recurrent infections, selected developmental correlates, functional digestive symptoms, and EEG abnormalities. This pattern suggests that serology should not be interpreted in isolation; rather, the clinically informative signal in this cohort was the convergence of serological, temporal, symptomatic, developmental, somatic, and neurophysiological findings.

The study does not establish ASO as a disease-specific biomarker and does not prove causality. However, it shows that, in routine Romanian child psychiatric practice, suspected streptococcal-associated PANS-like presentations may be described with greater coherence when serological, temporal, clinical, psychometric, and neurophysiological data are interpreted together. This integrative approach may be particularly valuable for the intermediate cases that are often most difficult to classify: presentations that are too abrupt, complex, and biologically suggestive to be reduced to conventional psychiatric categories, yet insufficiently specific to support a narrow biomarker-based diagnosis.

Within the limits of an observational and predominantly univariate design, PANS-31 appeared useful as a dimensional measure of neuropsychiatric burden and as a practical framework for structuring assessment. Future prospective studies should determine whether the pattern of convergence identified here represents a transient state-related pattern or a reproducible subgroup of children with post-infectious neuropsychiatric vulnerability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/psychiatryint7040155/s1, Completed STROBE checklist.

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Institutional Review Board Statement: 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 (parents, tutors and children, as stated by the Romanian law).

Data Availability Statement: The study protocol and analytical framework were registered on the Open Science Framework (OSF) on 13 April 2026. The registration is currently under embargo until 30 September 2026. A view-only link can be provided for peer review: https://osf.io/vdezu/overview?view_only=6b2267a51fd7466abae3e58f80148ce8, accessed on 26 April 2026. The data presented in this study are available on reasonable request from the corresponding author, subject to institutional and ethical approval where applicable. The data are not publicly available due to privacy and ethical restrictions, as the dataset contains sensitive clinical information from minors.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of Variance
ASI-4	Adolescent Symptom Inventory-4

ASO	Antistreptolysin O
ASLO	Equivalent to ASO (Romanian term used for statistical dataset)
CSI-4	Child Symptom Inventory-4
CT	Computed Tomography
CI	Confidence Interval
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
EEG	Electroencephalography
FDR	False Discovery Rate
ICD-10	International Classification of Diseases, Tenth Revision
IQR	Interquartile Range
IVF	In Vitro Fertilization
KMO	Kaiser–Meyer–Olkin
MTMM	Multitrait–Multimethod
OCD	Obsessive–Compulsive Disorder
PANDAS	Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections
PANS	Pediatric Acute-onset Neuropsychiatric Syndrome
PANS-31	Pediatric Acute-onset Neuropsychiatric Syndrome 31-Item Symptom Rating Scale
SD	Standard Deviation
TPO	Temporo-Parieto-Occipital

Appendix A

Table A1. Psychometric performance of PANS-31 and convergence with ASI-4/CSI-4 domains.

Section	Measure/Domain	Comparator/Mapping	Result	Interpretation
A. Reliability and construct validity	PANS-31 total scale	Cronbach's α	0.835	Good internal consistency
	Combined ASI-4/CSI-4 domain set	Cronbach's α	0.221	Poor total scale consistency
	ASI-4/CSI-4 domains	Factors retained	5	65.7% cumulative variance explained
	ASI-4/CSI-4 domains	KMO	0.586	Modest sampling adequacy
	PANS domain subscales	Factors retained	4	72.7% cumulative variance explained
	PANS domain subscales	KMO	0.766	Good sampling adequacy
	PANS-31 items	Factors retained	5	72.5% cumulative variance explained
	PANS-31 items	KMO	0.662	Acceptable sampling adequacy
	PANS-31 items	First factor variance	38.0%	Strong general factor
B. ASI-4/CSI-4 domains vs. total PANS-31 score	Enuresis	PANS-31 total	0.581	Good
	Anxiety	PANS-31 total	0.566	Good
	Eating	PANS-31 total	0.379	Moderate
	Motor	PANS-31 total	0.328	Moderate
	Conduct	PANS-31 total	0.287	Weak
	CSI pervasive	PANS-31 total	−0.310	Inverse, moderate
C. Theoretical domain-level convergence	Anxiety	Anxiety	0.899	Excellent
	Eating	Eating	0.801	Excellent
	Conduct	Behavioral	0.765	Excellent
	OCD	OCD	0.719	Excellent
	Motor	Motor	0.693	Good
	Enuresis	Urinary	0.684	Good
	Psychotic	Psychotic	0.626	Good
	Vocal	Vocal	0.550	Good
	Affective	Mood	0.464	Moderate

Note: Domain-level convergence was classified as excellent for $|r| \geq 0.70$, good for 0.50–0.69, moderate for 0.30–0.49, and weak for <0.30. Addictions and pervasive developmental symptoms were not included in the primary theoretical mapping because they had no direct PANS equivalents. The qualitative labels used for domain-level convergence refer to effect-size categories of the observed correlations and should not be interpreted as formal diagnostic-validity grades.

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