Article

Not peer-reviewed version

Antistreptolysin O Titers, Symptom Burden, and Multidimesional Correlates in Children with Suspected Streptococcal-Associated Neuropsychiatric Presentations: An Observational Cohort Study

Cristina-Gabriela Schiopu , Cristina Elena Dobre , Ovidiu Alexinschi , Dan-Catalin Oprea , Alexandra Bolos * , Oriana-Maria Onicescu , Carmen Gabriela Lupusoru , Adriana Gurbet , Marcel-Alexandru Gaina , Cristinel Stefanescu

Posted Date: 6 May 2026

doi: 10.20944/preprints202605.0375.v1

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

 doi:10.20944/preprints202605.0375.v1

Article

Antistreptolysin O Titers, Symptom Burden, and Multidimesional Correlates in Children with Suspected Streptococcal-Associated Neuropsychiatric Presentations: An Observational Cohort Study

Cristina-Gabriela Schiopu [1,2], Cristina Elena Dobre [2], Ovidiu Alexinschi [2], Dan-Catalin Oprea [1,2], Alexandra Boloș [1,2,*], Oriana-Maria Onicescu [3], Carmen Gabriela Lupusoru [1,2], Adriana Gurbet [2], Marcel-Alexandru Gaina [1] and Cristinel Stefanescu [1,2]

[1] Grigore T. Popa University of Medicine and Pharmacy Iasi, 16 Universității Str., 700115 Iași, Romania

[2] "Socola" Institute of Psychiatry, 36 Bucium St., 700282 Iași, Romania

[3] Faculty of Computer Science, Alexandru Ioan Cuza University of Iași, 16 General Berthelot St., 700483 Iași, Romania

* Correspondence: alexandra.bolos@umfiasi.ro; Tel.: +40-744-479-885

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 PANS and PANDAS. This observational cohort study examined whether serological, psychometric, and electroencephalographic findings converged within a clinically selected pediatric psychiatric sample. Children and adolescents presenting with acute-onset or abruptly worsened neuropsychiatric symptoms and a history suggestive of prior streptococcal exposure were recruited over a 12-month period through inpatient and outpatient child psychiatric services. Of 154 screened cases, 96 with analyzable baseline data were retained and stratified by ASO status. Symptom burden was quantified using the Pediatric Acute-onset Neuropsychiatric Syndrome 31-Item Symptom Rating Scale (PANS-31) and examined in relation to ASO titers, time since the last reported streptococcal infection, EEG findings, and selected developmental and clinical-history variables. Higher ASO values were strongly 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 CSI-4 and ASI-4 domains. These findings do not support a disease-specific biomarker model, but suggest that higher antistreptococcal serology, more recent streptococcal exposure, and greater neuropsychiatric burden may cluster within a more clearly expressed clinical phenotype in a real-world psychiatric environment.

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 disease. 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 PANS framework expanded this clinical space to include additional infectious and non-infectious triggers [1,2]. Despite sustained interest, the field remains marked by

 doi:10.20944/preprints202605.0375.v1

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, urinary 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 phenotype 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 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].

 doi:10.20944/preprints202605.0375.v1

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 acute-onset or abruptly worsened neuropsychiatric presentations and 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 and clinical-history variables; also, this study examines the domain-level convergence of PANS-31 with the Child Symptom Inventory-4 (CSI-4) and Adolescent Symptom Inventory-4 (ASI-4). We prespecified our hypotheses, related to actual literature and research: 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. Rather than attempting to validate a disease-specific biomarker model, the study aimed to determine whether, within routine Romanian child psychiatric practice, serological, symptomatic, and neurophysiological findings tend to cluster into a more clearly expressed clinical pattern.

The study was observational and predominantly univariate by design; it was intended to characterize association patterns within a clinically assembled cohort not to support causal inference or confounder-adjusted effect estimation.

2. Materials and Methods

2.1. Study Design

This observational cohort study was conducted in Romanian child and adolescent psychiatric inpatient and outpatient settings. It examined whether children presenting with acute-onset or abruptly worsened neuropsychiatric symptoms, together with 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.

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

The present report is based on the index clinical assessment of the retained cohort and does not include longitudinal follow-up analyses. In this sense, the manuscript reports association patterns within a clinically assembled cohort at the time of baseline psychiatric evaluation.

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.

 doi:10.20944/preprints202605.0375.v1

The initial screening pool comprised 154 children and adolescents referred for neuropsychiatric assessment because of acute symptom onset, abrupt symptom worsening, or a clinically significant change in psychiatric presentation, together with a history suggestive of prior Group A streptococcal exposure. 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 application of 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 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.

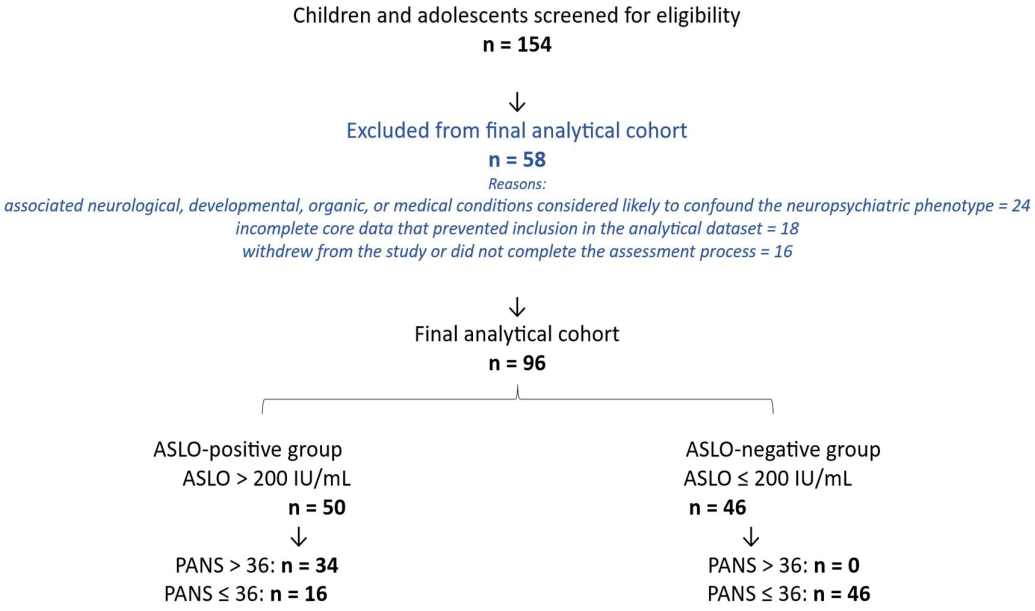


Figure 1. Flow diagram of participant selection and final cohort stratification. Of the 154 screened cases, 96 were retained in the final analytical cohort. The 58 non-retained cases included 24 children with associated neurological, developmental, organic, or medical conditions considered likely to confound phenotype 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, presented with acute-onset or abruptly worsened neuropsychiatric symptoms, with the index change occurring within 7 days and had a history suggestive of prior streptococcal exposure. Acute presentation was defined as either new-onset psychiatric or neurobehavioral symptoms or a rapid and clinically meaningful worsening of a pre-existing psychiatric condition, clearly exceeding the patient's previous level of functioning. History suggestive of prior streptococcal exposure was established from caregiver report and available medical history and included at least one of the

 doi:10.20944/preprints202605.0375.v1

following: a previously documented streptococcal infection, recurrent tonsillitis or pharyngitis considered suggestive of streptococcal etiology, or a history of cutaneous, renal infection or other compatible entities with Group A β-hemolytic streptococcal involvement.

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 therefore 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 was not 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 sign the informed consent and participation or 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, eating changes, infectious history, digestive symptoms, allergies, autoimmune conditions, pregnancy-related factors, early postnatal problems, and IVF history.

Psychiatric diagnoses were coded according to ICD-10 (the operational framework in Romania Health care system) using three-character disease codes and were retained primarily for standardized cohort characterization rather than as the main analytical framework of the study. Because the present investigation focused on dimensional symptom burden as measured by PANS-31, the clinical

 doi:10.20944/preprints202605.0375.v1

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 and they will be debated further along the research project. For the final analyses, emphasis was placed on variables most directly related to the predefined study objectives. The harmonized dataset therefore 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 not as formally adjusted confounders within 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 symptom burden across the multidomain phenotype under investigation. For exploratory subgroup analyses, a secondary categorical variable was derived using a threshold of PANS-31 > 36 in order to identify a subgroup with more clearly expressed symptom burden within the cohort. The rationale was methodological rather than diagnostic. Because the cohort was recruited from a real-world pediatric psychiatric setting, scores near the lower mild range may still reflect symptom constellations with substantial overlap with nonspecific psychiatric presentations. Before the formal start of the study, we conducted preliminary feasibility observations that concluded these observations. As such, a threshold of 36 was therefore retained as a more conservative within-cohort separator, intended to identify the more clearly expressed portion of the PANS-like phenotype rather than to define an externally validated severity category.

Age-appropriate external comparator instruments were the Child Symptom Inventory-4 (CSI-4) and the Adolescent Symptom Inventory-4 (ASI-4). These DSM-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

Antistreptolysin O (ASO) titers were used as the principal serological marker of prior streptococcal exposure in the present study. In inpatient cases, ASO testing was performed in the hospital laboratory, whereas in outpatient cases it was performed in a private laboratory using the

 doi:10.20944/preprints202605.0375.v1

same semi-quantitative latex agglutination method, as the most laboratories in contract with National Health Insurance, use this same system. 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 (≤200 IU/mL) strata retained in the final cohort. This threshold represents the significance border for latex-aglutination 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 baseline, 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 diagnostic work-up. It was not used as a disease-specific marker for PANS or PANDAS, but as an accessible clinical measure that could be interpreted alongside symptom burden, infectious history, and serological findings.

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

For analysis, EEG data were coded from the available clinical reports. In addition to the global distinction between normal and abnormal EEG, the harmonized dataset retained several 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 complementary descriptors of neurophysiological context rather than as markers of diagnostic specificity.

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 rather than 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.

 doi:10.20944/preprints202605.0375.v1

Derived variables included binary ASO status and a secondary binary symptom-burden variable based on the PANS-31 total score. Elevated ASO was defined as >200 IU/mL. For exploratory subgroup analyses, a PANS-31 total score > 36 was used to identify a higher symptom-burden subgroup within the cohort. This threshold was used pragmatically for descriptive stratification and should not be interpreted as a validated diagnostic 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.

2.9. Statistical Analysis

Because several study variables showed non-Gaussian distributions, descriptive data are reported primarily as medians and interquartile ranges 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. Continuous outcomes were compared across binary categorical strata using the Mann–Whitney U test. For categorical variables with three ordered levels, omnibus group comparisons were treated as exploratory and interpreted cautiously in light of sample size, ordinal structure, and distributional heterogeneity.

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, and 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 relative to the number of candidate correlates, the main 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. Sensitivity analyses were not performed. The reported estimates should therefore be interpreted as unadjusted association measures within a clinically selected cohort.

2.10. Psychometric Evaluation of PANS-31

Before being retained as the principal dimensional outcome measure in the present cohort, PANS-31 was examined through a multicomponent psychometric framework addressing internal consistency, structural coherence, and cross-scale convergence with age-appropriate psychiatric symptom measures. The aim of these analyses was not to position PANS-31 as a replacement for standard clinical diagnosis, but to determine whether it functioned as a sufficiently coherent dimensional measure of neuropsychiatric symptom burden in this clinically selected pediatric sample.

 doi:10.20944/preprints202605.0375.v1

Internal consistency was assessed using Cronbach's alpha for the total PANS-31 scale and for theory-informed domain groupings derived from the item structure. Structural coherence was explored by principal component analysis of the full 31-item matrix together with the Kaiser–Meyer–Olkin coefficient, in order to examine dimensionality and the extent to which the item set supported a common symptom-burden dimension.

Criterion-related convergence was evaluated by calculating Spearman correlations between PANS-31 domain scores and theoretically corresponding CSI-4 or ASI-4 domains. Multiple testing correction was applied using the Benjamini–Hochberg false discovery rate procedure, and convergence was interpreted primarily according to effect size rather than dichotomized significance alone. Because CSI-4 and ASI-4 are developmentally stratified instruments and did not behave as a single pooled total measure in the present dataset, cross-scale interpretation was conducted mainly at the level of corresponding symptom domains rather than combined total scores.

To further examine cross-scale structure, differential-validity analysis was used to test whether the strongest empirical correspondences matched the theoretically expected domain pairings. In addition, a multitrait–multimethod approach based on the Campbell and Fiske framework was applied to compare convergent and discriminant validity patterns within and across methods. These analyses were conducted in the full sample (N = 96) and were intended to support the use of PANS-31 as the main dimensional measure in the subsequent association analyses, rather than to imply full interchangeability with ASI/CSI-based assessment approaches.

2.11. Processing Environment

Statistical analyses and figure generation were performed in Python 3.13.5, using the pandas and matplotlib packages.

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 ≤200 IU/mL. Using the predefined operational threshold, 34 participants (35.4%) had PANS-31 total scores > 36, whereas 62 (64.6%) scored ≤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. Descriptive distributions of the principal numerical and categorical variables according to ASO status are presented in Tables 1A and 1B, while the corresponding distributions according to PANS-31 symptom-burden subgroup are presented in Tables 2A and 2B.

Similarly, no baseline 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.

 doi:10.20944/preprints202605.0375.v1

Table 1. Selected numerical features by ASO status (Median [IQR]). **B.** Selected categorical features by ASO status.

(A)		
Feature	**ASO-Negative (≤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]

(B)			
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 (database code)	None recorded	33 (71.7%)	36 (72.0%)
	114	1 (2.2%)	0 (0.0%)
	119	1 (2.2%)	3 (6.0%)
	321	3 (6.5%)	1 (2.0%)
	350	4 (8.7%)	9 (18.0%)
	351	4 (8.7%)	1 (2.0%)
Clinically significant symptom burden (PANS-31 > 36)	No	46 (100.0%)	16 (32.0%)
	Yes	0 (0.0%)	34 (68.0%)

* ICD-10 three-character codes: 114, addictions; 119, spectrum disorders; 321, depressive disorder; 350, AD/HD; 351, conduct disorder. *Values coded as 12 include intervals of 12 months or longer.

 doi:10.20944/preprints202605.0375.v1

Table 2. Selected numerical features according to PANS-31 symptom-burden status. **B**. Selected categorical clinical features according to PANS-31 symptom-burden status.

(A)		
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]

(B)			
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 (database code)	None recorded	46 (74.2%)	23 (67.6%)
	114	1 (1.6%)	0 (0.0%)
	119	2 (3.2%)	2 (5.9%)
	321	3 (4.8%)	1 (2.9%)
	350	5 (8.1%)	8 (23.5%)
	351	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%)

*Values coded as 12 include intervals of 12 months or longer. * ICD-10 three-character codes: 114, addictions; 119, spectrum disorders; 321, depressive disorder; 350, AD/HD; 351, conduct disorder.

 doi:10.20944/preprints202605.0375.v1

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

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 $\rho = 0.806$, $p = 3.68 \times 10^{-23}$; n=96; 95% CI: [0.74–0.86]). The effect size substantially exceeds typical thresholds for "strong" correlation ($\rho > 0.70$), explaining ~65% of shared variance (ρ^2). The distribution of this association is shown in Figure 2.

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).

Figure 2. Scatterplot showing the association between ASO value and total PANS-31 score. Each point represents one participant; the fitted line illustrates the positive monotonic relationship between ASO value and dimensional neuropsychiatric symptom burden.

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.

 doi:10.20944/preprints202605.0375.v1

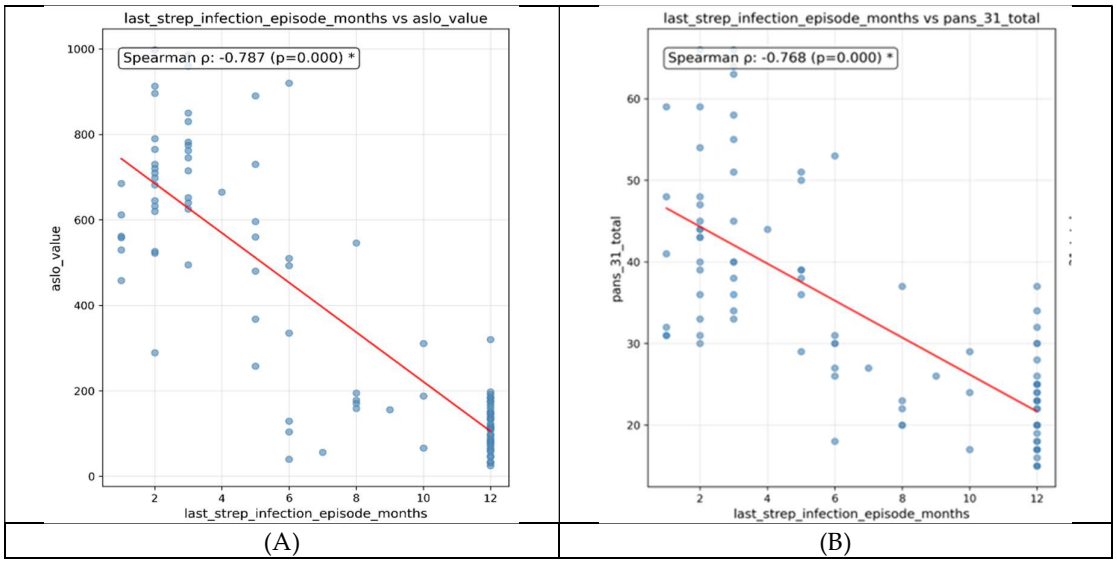


Figure 3. Scatterplot panel showing the relationship between time since the last reported streptococcal infection and the main study endpoints. (A) Association between time since the last reported streptococcal infection (months) and ASO value. (B) Association between time since the last reported streptococcal infection (months) and total PANS-31 score.

3.4. Age-Related Associations

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

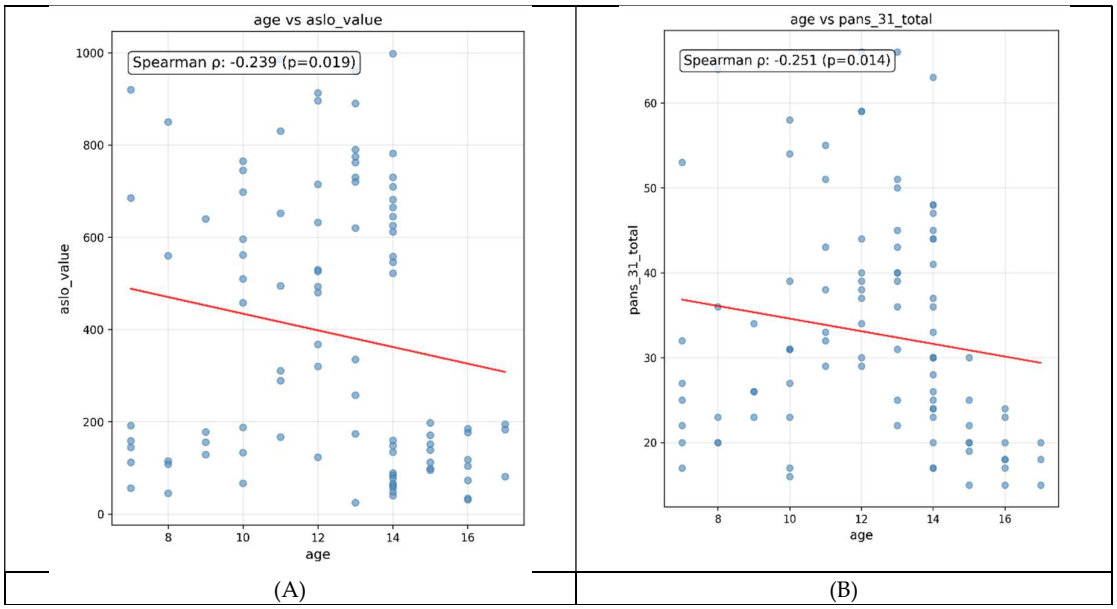


Figure 4. (A) Scatterplot showing the association between age and ASO value. Each point represents one participant. The fitted trend line illustrates the inverse association between age and ASO titers. **(B) Scatterplot showing the association between age and total PANS-31 score.** Each point represents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

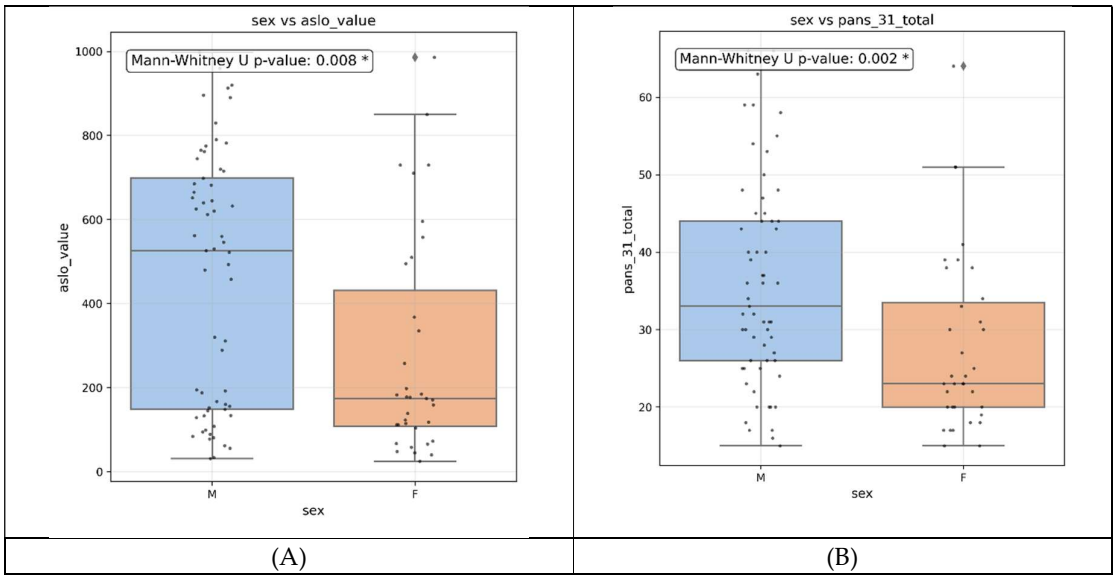
 doi:10.20944/preprints202605.0375.v1

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.

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 = 1.39×10^{-9}) (Figure 6).

 doi:10.20944/preprints202605.0375.v1

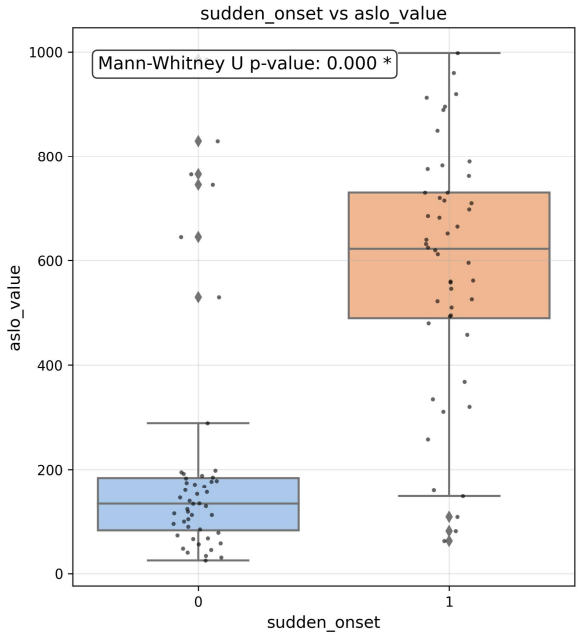


Figure 6. Boxplot of ASO value according to sudden onset status. ASO titers are compared between children with and without abrupt symptom onset.

Functional digestive symptoms were also associated with ASO level. In an exploratory omnibus comparison across the three predefined categories of digestive symptom burden, ASO values differed significantly (one-way ANOVA, $p = 2.43 \times 10^{-8}$) (Figure 7).

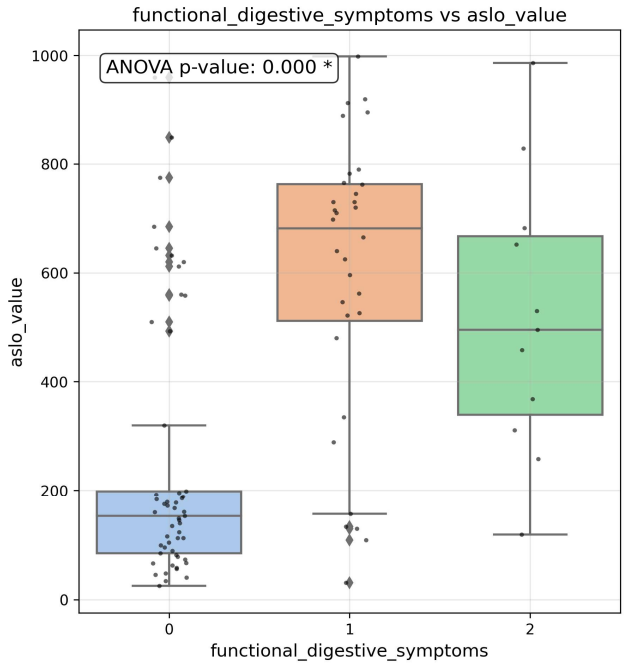


Figure 7. Boxplot of ASO value according to functional digestive symptom burden. ASO values are shown across the three predefined categories of functional digestive symptoms: none, one symptom, and two or more symptoms. Functional digestive symptoms: One-way ANOVA across three levels, $p = 2.43 \times 10^{-8}$.

 doi:10.20944/preprints202605.0375.v1

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.

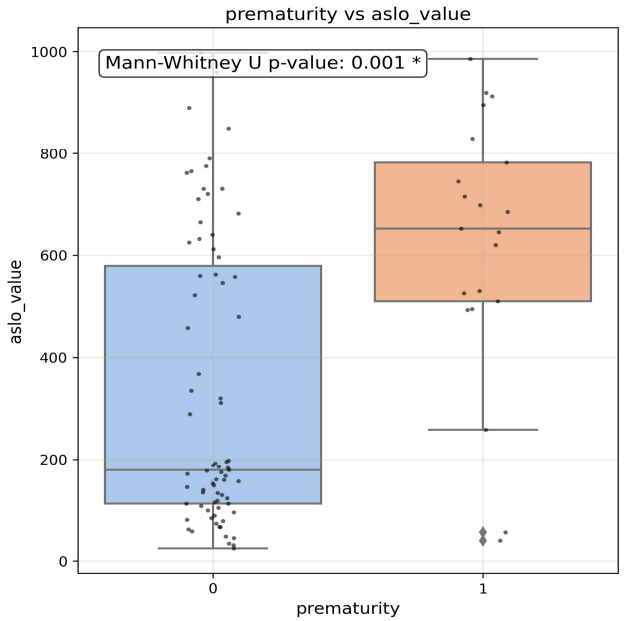


Figure 8. Boxplot of ASO value according to prematurity status. ASO titers are compared between children with and without a history of prematurity**.** Mann–Whitney U p = 5.12×10^-4; median ASO 652 IU/mL in children with prematurity versus 178 IU/mL in those without prematurity.

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).

 doi:10.20944/preprints202605.0375.v1

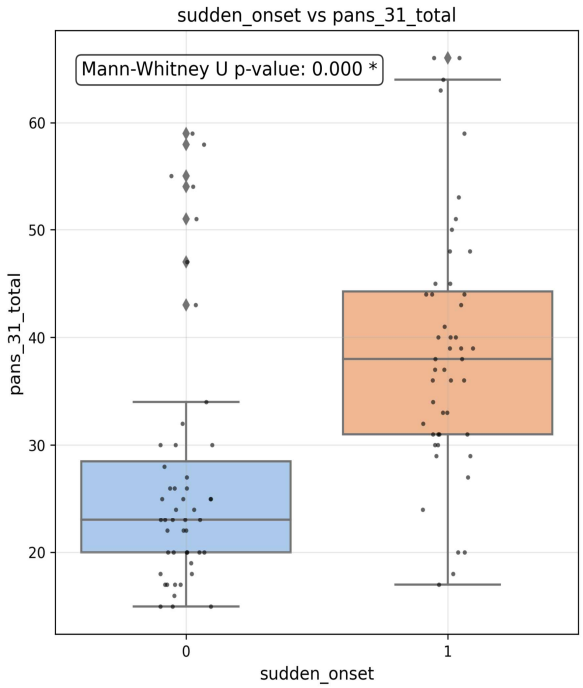


Figure 9. Boxplot of PANS-31 total score according to sudden onset status. Children with abrupt symptom onset showed higher PANS-31 total scores than those without sudden onset. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations.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

Functional digestive symptoms were also strongly related to dimensional symptom burden. Across the three predefined categories of gastrointestinal symptom burden, PANS-31 total scores differed significantly in an exploratory omnibus comparison (one-way ANOVA, $p = 7.56 \times 10^{-6}$), indicating a graded increase in symptom burden across higher digestive-symptom categories (Figure 10).

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 ($p = 0.0267$) (Figure 11 and 12).

 doi:10.20944/preprints202605.0375.v1

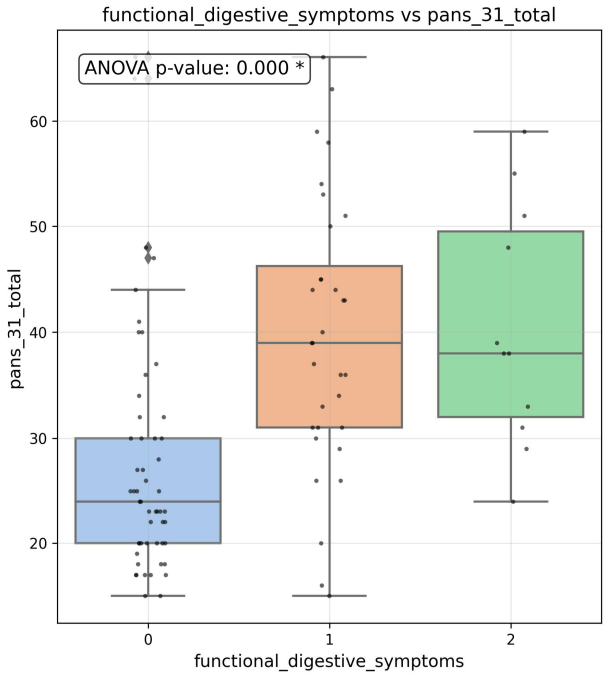


Figure 10. Boxplot of PANS-31 total score according to functional digestive symptoms. PANS-31 total score is shown across the three predefined categories of functional digestive symptoms: none, one symptom, and two or more symptoms. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations. One-way ANOVA across three levels, $p = 7.56 \times 10^{-6}$, indicating graded differences in symptom burden according to gastrointestinal symptom burden.

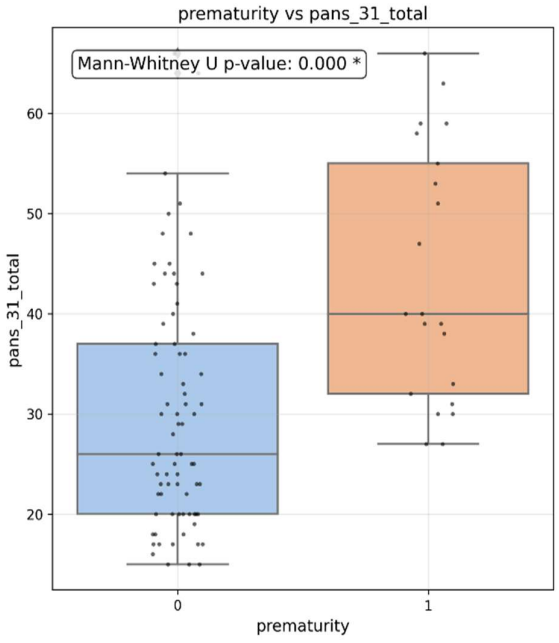


Figure 11. Boxplot of PANS-31 total score according to prematurity status. Children with a history of prematurity showed higher PANS-31 total scores than those without prematurity. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual 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.

 doi:10.20944/preprints202605.0375.v1

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%).

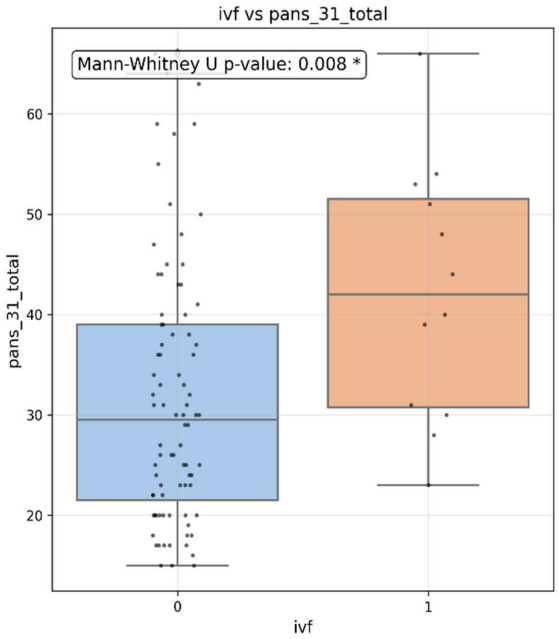


Figure 12. Boxplot of PANS-31 total score according to IVF history. Children with a history of in vitro fertilization (IVF) are compared with those without IVF history in relation to total PANS-31 score**.** Mann–Whitney U p = 0.00793; median PANS-31 total score 42 in children with IVF history versus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

The pattern was similar for 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 less pronounced as observed in Figure 14.

 doi:10.20944/preprints202605.0375.v1

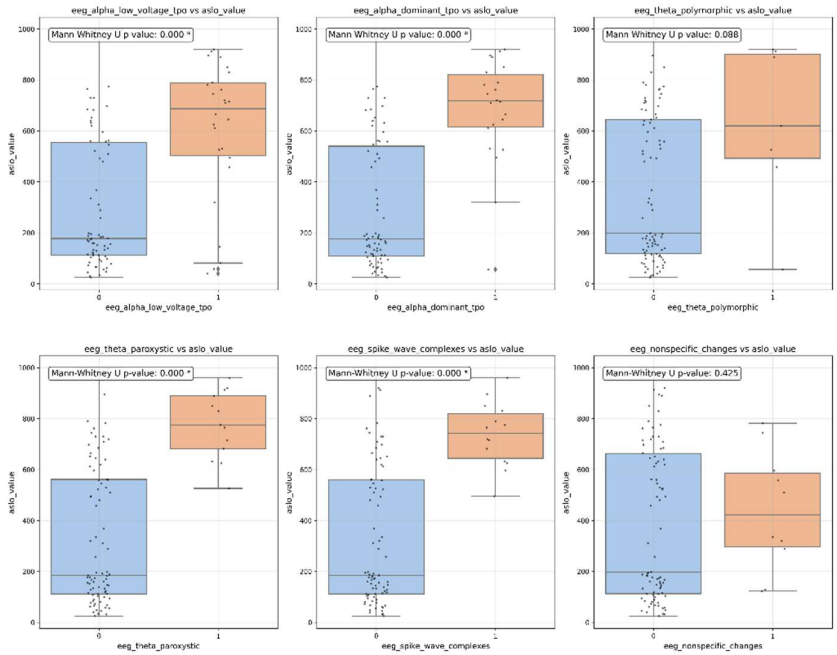


Figure 13. Boxplots showing the distribution of ASO values according to the absence (0) or presence (1) of predefined EEG abnormalities. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations. (A) low-voltage alpha activity 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.

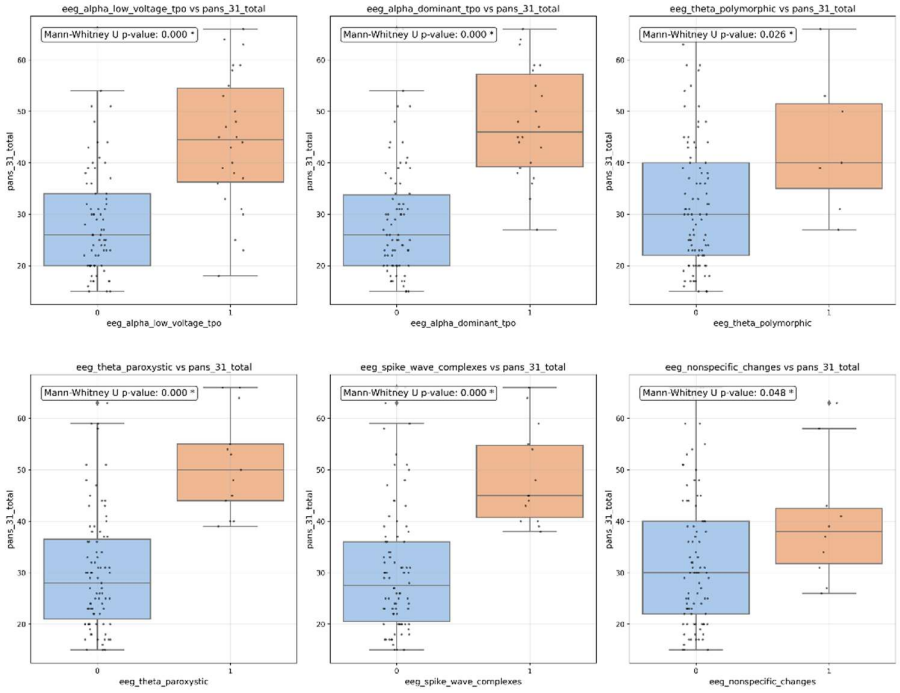


Figure 14. Boxplot panel showing the distribution of PANS-31 total scores according to predefined EEG abnormalities. Boxes represent the interquartile range, center lines the median, whiskers the distribution range, and points individual observations. (A) low-voltage alpha activity in temporo-parieto-occipital regions; (B)

 doi:10.20944/preprints202605.0375.v1

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 ($\varrho = 0.81$). The strongest inverse correlations involved time since the last reported streptococcal infection, both with ASO value ($\varrho = -0.79$) and with total PANS-31 score ($\varrho = -0.77$). Among the external symptom domains, total PANS-31 score showed its highest positive correlations with ASI/CSI anxiety ($\varrho = 0.56$) and enuresis ($\varrho = 0.52$), whereas the CSI pervasive domain showed a moderate negative correlation with total PANS-31 score ($\varrho = -0.44$). The full correlation structure is shown in Figure 15.

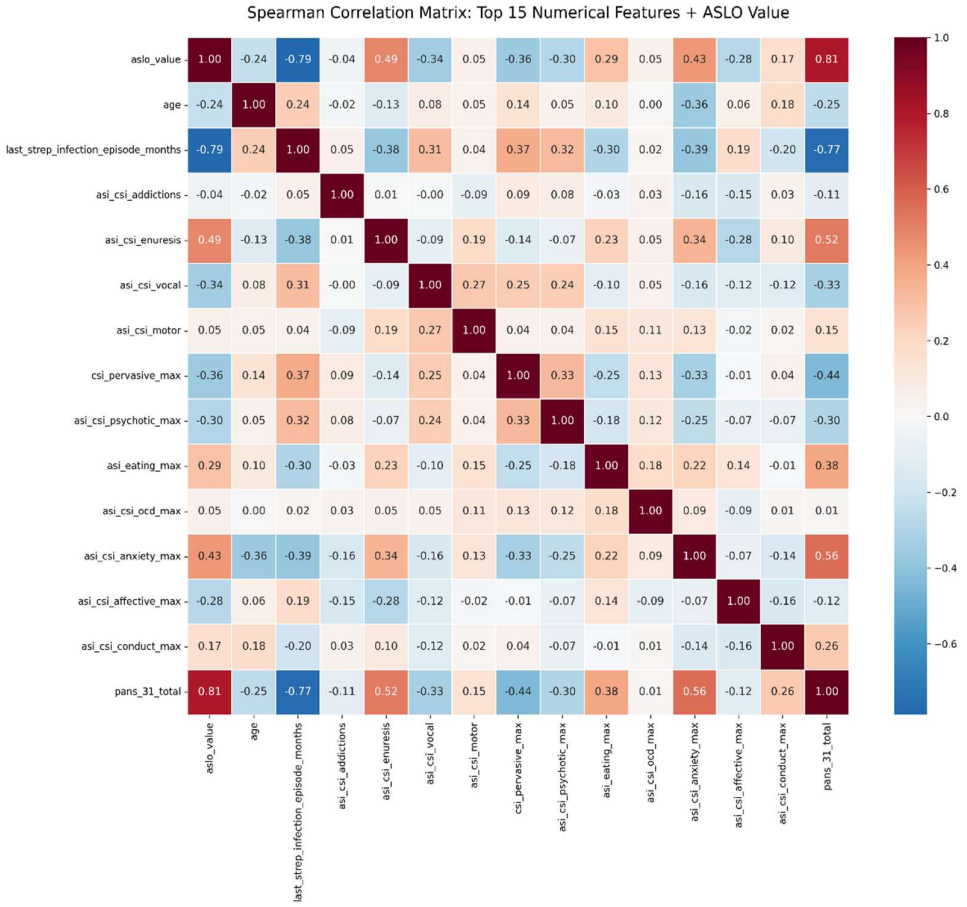


Figure 15. Spearman correlation heatmap including ASO value, total PANS-31 score, time since the last reported streptococcal infection, age, and selected ASI/CSI domain maxima.

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. Individual domain subscales (OCD, eating, anxiety, mood, behavioral, cognitive, motor, vocal, somatic, urinary, psychotic) showed acceptable to good reliability with a median $\alpha = 0.783$). The relevant findings can be observed in Appendix A, Table A1.

 doi:10.20944/preprints202605.0375.v1

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. At the item level, five components accounted for 72.5% of cumulative variance, and the first factor explained 38.0% of total variance.

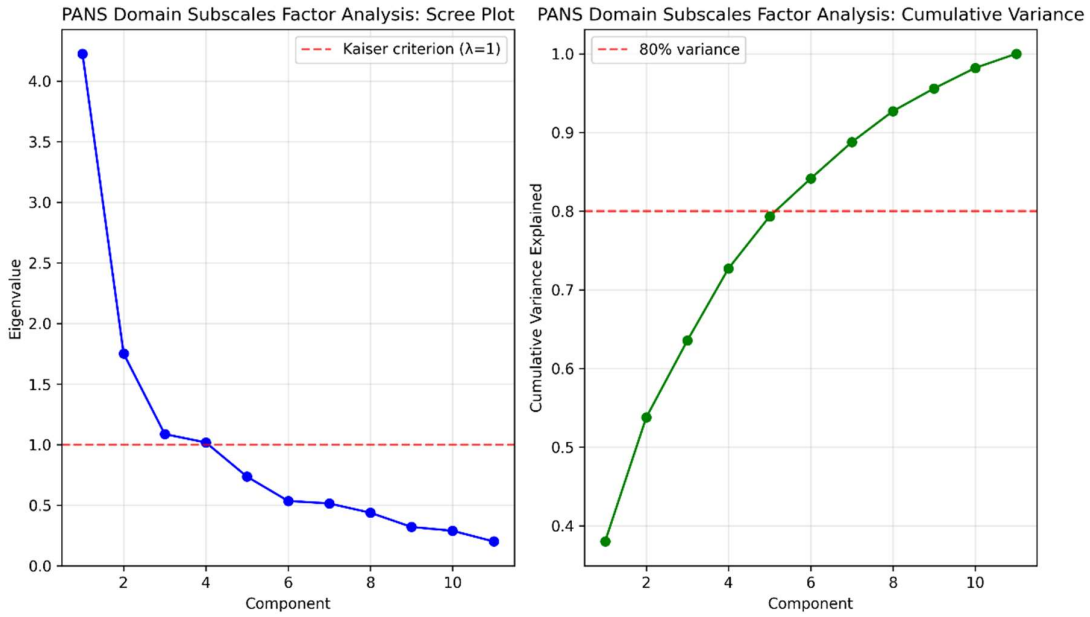


Figure 16. Screen plot showing eigenvalue distribution and cumulative variance explained across principal components; bar chart with KMO adequacy coefficient.

Criterion Validity: 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).

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 3.

 doi:10.20944/preprints202605.0375.v1

Table 3. 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
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.

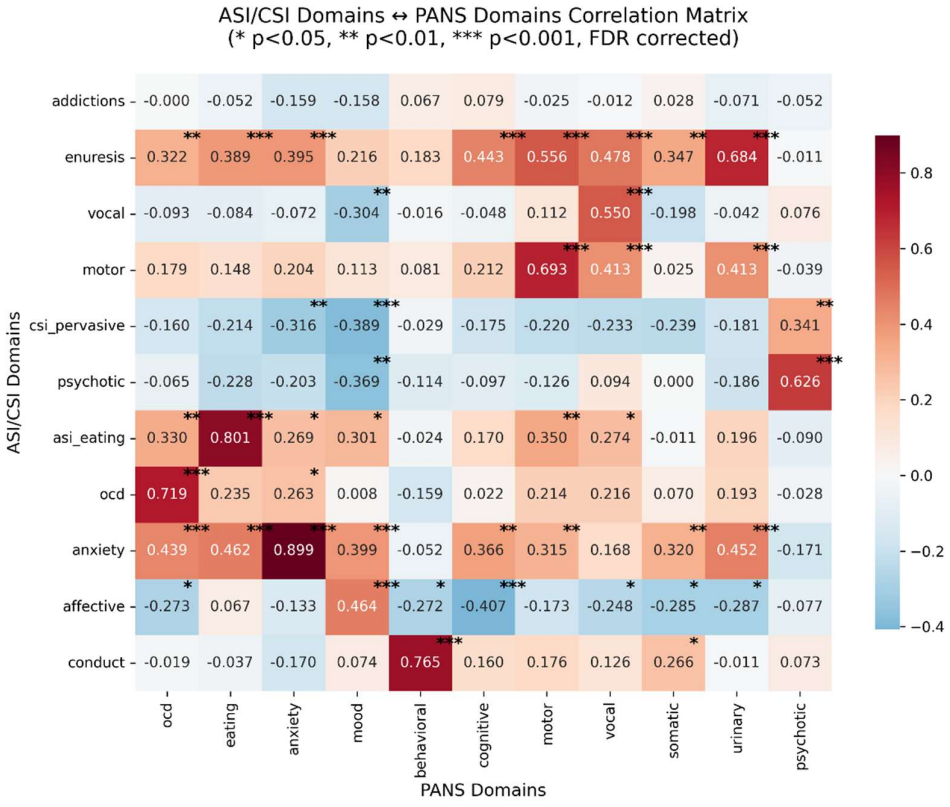


Figure 17. Heatmap showing PANS × ASI/CSI domain correlation matrix with significance markers (*, **, *** for $p < 0.05, 0.01, 0.001$).

 doi:10.20944/preprints202605.0375.v1

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, PANS-31 symptom burden, 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 antistreptococcal serology and greater multidomain neuropsychiatric burden were part of a broader vulnerability profile rather than a purely serological pattern.

This vulnerability profile should therefore be understood as multidimensional. 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. The findings do not establish ASO as a disease-specific biomarker for PANS/PANDAS, do not define a separate diagnostic entity, and do not prove a causal link between streptococcal exposure and neuropsychiatric symptoms. Current reviews and consensus-oriented literature emphasize that PANS and PANDAS remain clinically heterogeneous with debated conditions in which biological

 doi:10.20944/preprints202605.0375.v1

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 that 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 be 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 in the higher ASO/higher PANS-31 profile. 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 phenotype observed here, but they do not independently explain the ASO–PANS relationship.

4.3. Phenotypic Profile: Recurrent Infections, Functional Digestive Symptoms, Prematurity, and IVF History

Recurrent infections and functional digestive symptoms add an important non-serological layer to the observed phenotype. Recurrent infections may reflect repeated infectious exposure or repeated immune challenge, although the present observational 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. This suggests that, in some children, the more clearly expressed phenotype, extended beyond a narrowly psychiatric presentation and included a broader somatic context.

 doi:10.20944/preprints202605.0375.v1

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, this 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 even more cautiously. Neither variable is syndrome-specific, and the present study cannot support a causal relationship between early developmental factors and PANS-like symptom burden. Nevertheless, their pattern is informative. 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, selected developmental vulnerability markers.

4.4. EEG Findings: Clinical Meaning Without Electrophysiological Overstatement

The EEG findings should be interpreted as complementary neurophysiological correlates rather than 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 phenotypic characterization. Alpha-pattern abnormalities may reflect altered background cortical organization, arousal regulation, or network stability rather than a lesion-specific process. Paroxysmal theta activity and spike–wave complexes may indicate intermittent cortical instability or increased excitability. In children presenting with abrupt or abruptly worsened neuropsychiatric symptoms, such findings do not diagnose a streptococcal-triggered syndrome; however, they suggest that the clinical picture is not fully captured by behavioral description alone.

This interpretation is consistent with 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 equivalent to neuroimaging evidence or as a diagnostic electrophysiological marker.

 doi:10.20944/preprints202605.0375.v1

The practical value of EEG in this setting is one of refinement rather than labeling. In routine child psychiatry, EEG abnormalities may help identify children whose abrupt neuropsychiatric deterioration is accompanied by measurable functional neurophysiological disturbance, supporting closer neurological observation, more careful longitudinal follow-up, and caution before reducing the presentation to a conventional primary psychiatric disorder. At the same time, none of the EEG patterns observed here are specific to PANS or PANDAS, and their interpretation 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 it suggests that PANS-31 did not behave as an isolated or arbitrary scale; instead, it overlapped with established psychiatric symptom domains while retaining a broader multidimensional structure.

This finding 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 therefore suggest that PANS-31 may be particularly useful in routine psychiatric settings where categorical diagnoses alone may flatten clinically meaningful symptom constellations. The scale appears to capture a syndrome-oriented burden that intersects with, but is not reducible to, conventional anxiety, obsessive-compulsive, behavioral, somatic, or motor symptom domains.

The use of PANS-31 > 36 also raises a broader methodological point. In highly selected PANS research samples, the lower boundary of mild symptomatology may be sufficient for identifying clinically relevant cases. In a real-world pediatric psychiatric cohort, however, lower scores may overlap substantially with nonspecific psychopathology, including anxiety, behavioral dysregulation, affective symptoms, somatic complaints, and other clinically important but non-specific constellations. Before the formal start of the study, preliminary feasibility observations in our clinical setting suggested that this overlap was particularly relevant at lower PANS-31 scores. Accordingly, the >36 threshold was selected a priori for the formal analysis to improve clinical specificity for the post-infectious phenotype of interest. In this context, the present findings suggest that a slightly more conservative operational threshold may improve phenotypic discrimination for subgrouping purposes. This observation should be read as cohort-based and exploratory rather than as a proposal to revise the original scale cutoff. The issue may be less about where clinically relevant symptoms begin and more about where syndrome-oriented specificity becomes more convincing in routine psychiatric practice. This interpretation is consistent with the broader psychometric principle that clinically useful measures should show convergent validity 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 variable family-led attempts to identify an explanatory medical framework.

 doi:10.20944/preprints202605.0375.v1

In the present cohort, referral narratives frequently suggested that families had already explored multiple explanatory models 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-disease 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 phenotype merely reflects previous empiric antibiotic use. Rather, ASO should be viewed as one component of a broader exposure-linked phenotype: it marked prior antistreptococcal 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.

 doi:10.20944/preprints202605.0375.v1

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 tested whether these elements converged into a clinically meaningful profile 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.

A second strength is the early capture of the index psychiatric presentation. Children were evaluated within 7 days of symptom onset or abrupt worsening, which improves the temporal precision of the psychiatric assessment and reduces recall distortion regarding the acute neuropsychiatric episode. 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 strengthens the clinical credibility of the observed signal and supports the interpretation that the cohort captured a distinguishable vulnerability profile rather than a diffuse pattern of unrelated comorbidity.

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. Therefore, the findings cannot be used to estimate the prevalence of elevated ASO titers, PANS, PANDAS, or streptococcal-associated neuropsychiatric presentations in the general pediatric population. The results are most informative for clinically enriched psychiatric settings, rather than for unselected community or primary-care populations.

Second, the study was observational, baseline-based, and predominantly univariate. No multivariable models were fitted, no formal confounder-adjusted estimates were generated, and no interaction or sensitivity analyses were performed. Consequently, the observed associations cannot establish causality, temporal direction beyond the reconstructed clinical history, or independent effects after adjustment for potential confounders such as age, sex, care setting, baseline psychiatric vulnerability, developmental history, or previous treatment exposure. The findings should therefore be interpreted as association patterns within a selected clinical cohort, not as causal 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 baseline. ASO was therefore 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 antistreptococcal antibody response. This limits the interpretation of both ASO-negative and moderately ASO-positive cases.

 doi:10.20944/preprints202605.0375.v1

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, 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 disease, 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 focus on stronger clinical design. Prospective, multicenter cohorts recruiting children close to symptom onset would allow more precise documentation of infectious events, better longitudinal characterization of symptom trajectories, and clearer separation between acute fluctuation and more stable neuropsychiatric burden. The biological framework should also be strengthened through repeated ASO assessment, parallel anti-DNase B testing, and, where feasible, broader immune markers. Future protocols should also record antibiotic timing in relation to infection onset, symptom onset, and blood sampling, and should include a predefined approach for children with persistently elevated ASO or suspected chronic carrier status. Repeated EEG assessment across phases of worsening and partial remission could help clarify whether the observed abnormalities behave more like state markers of symptomatic load or stable features of a vulnerable subgroup [47]. Finally, the dimensional use of PANS-31 deserves external validation in broader and developmentally diverse pediatric samples, including children recruited through pediatrics, neurology, infectious disease, and community mental health settings rather than psychiatry alone.

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 the clinically meaningful unit of interpretation is not serology alone, but a multidimensional post-infectious vulnerability profile.

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, a streptococcal-associated PANS-like phenotype can be delineated with greater coherence when serological, temporal, clinical, psychometric, and neurophysiological data are interpreted together. This integrative approach may

 doi:10.20944/preprints202605.0375.v1

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 clustered phenotype 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 the website of this paper posted on Preprints.org, Completed STROBE cohort checklist.

Author Contributions: Conceptualization, CGS and CS; methodology, CGS, AB, OO, CGL and CS; software, OO; validation, CGS, AB, OO, MAG and CS; formal analysis, OO and CGS; investigation, CGS, CED, OA, DCO, CGL and AG; resources, CGS, CGL, AG, AB and CS; data curation, CGS, CED, OA, DCO and OO; writing—original draft preparation, CGS; writing—review and editing, CGS, AB, MAG, CGL, AG, OO and CS; visualization, CGS and OO; supervision, AB and CS; project administration, CGS; funding acquisition - not applicable. All authors contributed equally, have read and agreed to the published version of the manuscript**.**

Funding: This research received no external funding.

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 (14090/17.05.2023) and University of Medicine and Pharmacy ”Grigore T. Popa” Iasi, Romania (5036/22.02.2024). 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 13.10.2024; 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 13.04.2026. The registration is currently under embargo until 30.09.2026. A view-only link can be provided for peer review: https://osf.io/vdezu/overview?view_only=6b2267a51fd7466abae3e58f80148ce8. 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.

Acknowledgments: During the preparation of this manuscript, the authors used OpenAI’s custom GPT version “GPT-5.4-Grammar Checker”, for language editing, text organization, and stylistic refinement. Partial figure generation were performed in Python 3.13.5 alongside statistical data annalysis. The authors have reviewed and edited the output and take full responsibility for the content of this publication.”.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of Variance
ASI-4	Adolescent Symptom Inventory-4
ASO	Antistreptolysin O
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

 doi:10.20944/preprints202605.0375.v1

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

Appendix A.1

Table 1. Psychometric performance of PANS-31 and convergence with ASI-4/CSI- 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.

References

1. Macerollo, A.; Martino, D. Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS): An Evolving Concept. Tremor Other Hyperkinet. Mov. 2013, 3, tre-03-167-4158-1. https://doi.org/10.7916/D8ZC81M1

 doi:10.20944/preprints202605.0375.v1

2. Swedo, S.E.; Leckman, J.F.; Rose, N.R. From Research Subgroup to Clinical Syndrome: Modifying the PANDAS Criteria to Describe PANS (Pediatric Acute-Onset Neuropsychiatric Syndrome). Pediatr. Therapeut. 2012, 2, 113. https://doi.org/10.4172/2161-0665.1000113
3. Wilbur, C.; Bitnun, A.; Kronenberg, S.; Laxer, R.M.; Levy, D.M.; Logan, W.J.; Shouldice, M.; Yeh, E.A. PANDAS/PANS in Childhood: Controversies and Evidence. Paediatr. Child Health 2019, 24, 85–91. https://doi.org/10.1093/pch/pxy145
4. Vreeland, A.; Thienemann, M.; Frankovich, J. A Narrative Review: Is Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) a Syndrome? Dev. Neurosci. 2023, 45, 263–278. https://doi.org/10.1159/000534261
5. Swedo, S.E.; Frankovich, J.; Murphy, T.K. Overview of Treatment of Pediatric Acute-Onset Neuropsychiatric Syndrome. J. Child Adolesc. Psychopharmacol. 2015, 25, 372–374. https://doi.org/10.1089/cap.2014.0073
6. Gilbert, D.L.; Mink, J.W.; Singer, H.S. A Pediatric Neurology Perspective on Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infection and Pediatric Acute-Onset Neuropsychiatric Syndrome. J. Pediatr. 2018, 199, 243–251. https://doi.org/10.1016/j.jpeds.2018.04.035
7. de Oliveira, S.K.F.; Pelajo, C.F. Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infection (PANDAS): A Controversial Diagnosis. Curr. Infect. Dis. Rep. 2010, 12, 103–109. https://doi.org/10.1007/s11908-010-0082-7
8. Prato, A.; Gulisano, M.; Scerbo, M.; Barone, R.; Vicario, C.M.; Rizzo, R. Diagnostic Approach to Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS): A Narrative Review of Literature Data. Front. Pediatr. 2021, 9, 746639. https://doi.org/10.3389/fped.2021.746639
9. Kirvan, C.A.; Swedo, S.E.; Kurahara, D.; Cunningham, M.W. Streptococcal mimicry and antibody-mediated cell signaling in the pathogenesis of Sydenham's chorea. Autoimmunity 2006, 39, 21–29. https://doi.org/10.1080/08916930500484757
10. La Bella, S.; Rigante, D.; Mancini, G.; Ferrara, G.; Basilicata, R.; Breda, L. Pediatric Acute-Onset Neuropsychiatric Syndrome: An Updated Literature Review. Microorganisms 2023, 11, 2549. https://doi.org/10.3390/microorganisms11102549
11. Dop, D.; Niculescu, C.E.; Padureanu, V.; Dumitrescu, F.; Padureanu, R.; Niculescu, E.C. PANDAS Syndrome—An Evolving Concept. Exp. Ther. Med. 2021, 21, 94. https://doi.org/10.3892/etm.2020.9526
12. Hesselmark, E.; Bejerot, S. Biomarkers for Diagnosis of Pediatric Acute Neuropsychiatric Syndrome (PANS)—Sensitivity and Specificity of the Cunningham Panel. J. Neuroimmunol. 2017, 312, 31–37. https://doi.org/10.1016/j.jneuroim.2017.09.002
13. Leonardi, L.; Lorenzetti, G.; Carsetti, R.; Cozzolino, D.; Di Stasio, M.; Massaro, G.; et al. Immunological Characterization of an Italian PANDAS Cohort. *Front. Pediatr.* 2024, *11*, 1216282. https://doi.org/10.3389/fped.2023.1216282.
14. De Visscher, C.; Hesselmark, E.; Rautio, D.; Djupedal, I.G.; Monsees, J.; Ramklint, M.; Mataix-Cols, D.; et al. Measuring Clinical Outcomes in Children with Pediatric Acute-Onset Neuropsychiatric Syndrome: Data from a 2–5 Year Follow-Up Study. *BMC Psychiatry* 2021, *21*, 484. https://doi.org/10.1186/s12888-021-03450-5
15. Chang, K.; Frankovich, J.; Cooperstock, M.; Cunningham, M.W.; Latimer, M.E.; Murphy, T.K.; et al. Clinical Evaluation of Youth with Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS): Recommendations from the 2013 PANS Consensus Conference. J. Child Adolesc. Psychopharmacol. 2015, 25, 3–13. https://doi.org/10.1089/cap.2014.0084
16. Pfeiffer, H.C.V.; Wickenhauser, M.; Woodhall, M.; Waters, P.; Dollner, R.; Günther, C.C.; Pinto, F.M.; Staff, A.C.; Vedeler, C.A.; Nossum, R.; et al. Pediatric Acute-Onset Neuropsychiatric Syndrome—PANS: Identification, Clinical Features and Autoantibody Findings. Acta Paediatr. 2021, 110, 3153–3160. https://doi.org/10.1111/apa.15875
17. Petre, I.; Barna, F.; Gurgus, D.; Tomescu, L.C.; Apostol, A.; Purcarea, V.L. Analysis of the Healthcare System in Romania: A Brief Review. *Healthcare* 2023, *11*, 2069. https://doi.org/10.3390/healthcare11142069.

 doi:10.20944/preprints202605.0375.v1

18. Predescu, E.; Calugar, I.; Bibu-Monus, C.; Sipos, R. Trends and Prevalence of Hospital Admissions Related to Deliberate Self-Poisoning and Used Substances in Romanian Adolescents between 2016 and 2022. Children 2023, 10, 790. https://doi.org/10.3390/children10050790American Academy of Pediatrics Board of Directors. Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS): Clinical Report. Pediatrics 2025, 155, e2024070334. https://doi.org/10.1542/peds.2024-070334
19. Tsai AP, Youngstrom EA, Gadow KD, Horwitz SM, Fristad MA, Daughters SB, Young AS, Arnold LE, Birmaher B, Salcedo S, Findling RL. Diagnostic accuracy of the Child and Adolescent Symptom Inventory (CASI-4R) substance use subscale in detecting substance use disorders in youth. Psychological Assessment. 2023 Feb;35(2):178.
20. Nolan, E.E.; Gadow, K.D.; Sprafkin, J. Teacher Reports of DSM-IV ADHD, ODD, and CD Symptoms in Schoolchildren. *J. Am. Acad. Child Adolesc. Psychiatry* 2001, *40*, 241–249. https://doi.org/10.1097/00004583-200102000-00020https://doi.org/10.2147/NDT.S362202.
21. Wald, E.R.; Schmitt, B.D.; Barson, W.J. Evaluation and Management of PANS. *Front. Pediatr.* 2023, *11*, 1170379. https://doi.org/10.3389/fped.2023.1170379.
22. Goren, N.; Benseler, S.; Swedo, S.E.; Gorman, D.; Crncec, R.; Kapur, S. A Pragmatic Approach to PANS: A Narrative Review and Canadian Practice Recommendations. *Paediatr. Child Health* 2025, *30*, 157–163. https://doi.org/10.1093/pch/pxae092.
23. Frankovich, J.; Swedo, S.; Murphy, T.; Dale, R.C.; Agalliu, D.; Williams, K.; Daines, M.; Hornig, M.; Chugani, H.; Sanger, T.; et al. Clinical Management of Pediatric Acute-Onset Neuropsychiatric Syndrome: Part II—Use of Immunomodulatory Therapies. *J. Child Adolesc. Psychopharmacol.* 2017, *27*, 574–593. https://doi.org/10.1089/cap.2016.0148
24. Cooperstock, M.S.; Swedo, S.E.; Pasternack, M.S.; Murphy, T.K. Clinical Management of Pediatric Acute-Onset Neuropsychiatric Syndrome: Part III—Treatment and Prevention of Infections. *J. Child Adolesc. Psychopharmacol.* 2017, *27*, 594–606. https://doi.org/10.1089/cap.2016.0151.
25. Ebrahimi Taj, F.; Seddigh, A.; Moharreri, F.; Talaei, A.; Salehi, M. OCD and Streptococcal Infection: A Systematic Review. *Basic Clin. Neurosci.* 2015, *6*, 38–43.
26. Morris-Berry, C.M.; Pollard, M.; Gao, S.; Thompson, C.; Singer, H.S.; Tourette Syndrome Study Group. Anti-Streptococcal, Tubulin, and Dopamine Receptor 2 Antibodies in Children with PANDAS and Tourette Syndrome: Single-Point and Longitudinal Assessments. *J. Neuroimmunol.* 2013, *264*, 106–113. https://doi.org/10.1016/j.jneuroim.2013.09.010.
27. Murphy, T.K.; Storch, E.A.; Lewin, A.B.; Edge, P.J.; Goodman, W.K. Clinical Factors Associated with Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal

Infections. *J. Pediatr.* 2012, *160*, 314–319. https://doi.org/10.1016/j.jpeds.2011.07.012.

28. Shet, A.; Kaplan, E.L. Clinical use and interpretation of group A streptococcal antibody tests: A practical approach for the pediatrician or primary care physician. Pediatr. Infect. Dis. J. 2002, 21, 420–426.
29. Johnson, D.R.; Kurlan, R.; Leckman, J.; Kaplan, E.L. The human immune response to streptococcal extracellular antigens: Clinical, diagnostic, and potential pathogenetic implications. Clin. Infect. Dis. 2010, 50, 481–490.
30. Hutanu, A.; Iancu, M.; Dredeț, C.; Tripon, R.; Constantin, T.; Bâlici, S.; Bâlici, M.; Maier, S.; Hutanu, A.; Andercou, O. Streptococcal Infection and PANDAS. *Cureus* 2022, *14*, e27729. https://doi.org/10.7759/cureus.27729.
31. Bottoms, K.B.; Rice, D.N.; Veerapandiyan, A. Cognitive, Behavioral, and Psychiatric Presentations of Pediatric Acute-Onset Neuropsychiatric Syndrome. *Front. Behav. Neurosci.* 2024, *18*, 1467469. https://doi.org/10.3389/fnbeh.2024.1467469.
32. Grandinetti, R.; Mussi, N.; Pilloni, S.; Ramundo, G.; Miniaci, A.; Turco, E.; Piccolo, B.; et al. Pediatric Acute-Onset Neuropsychiatric Syndrome and Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infections: A Delphi Study and Consensus Document about Definition, Diagnostic Criteria, Treatment and Follow-Up. Front. Immunol. 2024, 15, 1420663. https://doi.org/10.3389/fimmu.2024.1420663
33. Tagi, V.M.; Quarta, A.; Bosetti, A.; Biffi, M.; Gialuz, J.; Chiorri, C.; Roggero, P.; Vizzari, G.; Mosca, F.; Zuccotti, G.; et al. Nutritional Assessment and Dietary Intervention in Children with Pediatric Acute-Onset

 doi:10.20944/preprints202605.0375.v1

Neuropsychiatric Syndrome (PANS). *Front. Nutr.* 2025, *11*, 1477893. https://doi.org/10.3389/fnut.2024.1477893.

34. Gagliano, A.; Maniaci, M.; Maugeri, M.; Ferrante, M.; Giofrè, C.; Tanca, M.G.; Sotgiu, S.; Carta, A. PANS and Gut Microbiota: A Narrative Review. *Int. J. Mol. Sci.* 2025, *26*, 7767. https://doi.org/10.3390/ijms26167767.
35. Gamucci, A.; Uccella, S.; Sciarretta, L.; D'Apruzzo, M.; Calevo, M.G.; Mancardi, M.M.; Veneselli, E.; De Grandis, E. PANDAS and PANS: Clinical, Neuropsychological, and Biological Characterization of a Monocentric Series of Patients and Proposal for a Diagnostic Protocol. *J. Child Adolesc. Psychopharmacol.* 2019, *29*, 305–312. https://doi.org/10.1089/cap.2018.0087.
36. Gagliano, A.; Puligheddu, M.; Ronzano, N.; Congiu, P.; Tanca, M.G.; Cursio, I.; Carucci, S.; Sotgiu, S.; Grossi, E.; Zuddas, A. Artificial Neural Networks Analysis of Polysomnographic and Clinical Features in Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS): From Sleep Alteration to "Brain Fog". *Nat. Sci. Sleep* 2021, *13*, 1209–1224. https://doi.org/10.2147/NSS.S300818.
37. Leonardi, L.; Criniti, S.; Guerricchio, L.; Pignataro, M.G.; Bernardi, S.; Liotti, L.; Bernabei, S.; Messia, V.; Catania, S.; Pisani, F.; et al. Neurological and Neuropsychological Features of PANDAS: A Systematic Review. *Children* 2024, *11*, 1043. https://doi.org/10.3390/children11091043.
38. Gadow, K.D.; Sprafkin, J. *Adolescent Symptom Inventory-4 Screening Manual*; Checkmate Plus: Stony Brook, NY, USA, 1997.
39. Balaj, A.; Miclea, M.; Albu, M. The Adaptation Of The Child Symptom Inventory-4 (Csi-4) On Romanian Population. *Cogn. Brain Behav.* 2014, *18*, 105–121.
40. Campbell, D.T.; Fiske, D.W. Convergent and Discriminant Validation by the Multitrait-Multimethod Matrix. *Psychol. Bull.* 1959, *56*, 81–105. https://doi.org/10.1037/h0046016.
41. Sprafkin, J.; Gadow, K.D.; Salisbury, H.; Schneider, J.; Loney, J. Further Evidence of Reliability and Validity of the Child Symptom Inventory-4: Parent Checklist in Clinically Referred Boys. *J. Clin. Child Adolesc. Psychol.* 2002, *31*, 513–524. https://doi.org/10.1207/S15374424JCCP3104_10.
42. Vlădescu, C.; Scîntee, S.G.; Olsavszky, V.; Hernández-Quevedo, C.; Sagan, A. Romania: Health System Review. *Health Syst. Transit.* 2016, *18*, 1–170.
43. Brînduşe, L.A.; Chiriac, N.D.; Astărăstoae, V.; Dobrescu, A.I.; Daina, C.M.; Boeriu, E.; Mihuțiu, L.L.; Feier, H. Romanian Patients' Experiences of the Medical System and Suggestions for Improvement. *BMC Health Serv. Res.* 2024, *24*, 1186. https://doi.org/10.1186/s12913-024-11861-9.
44. Pop, T.L.; Pop, C.; Baican, A.; Oros, M.; Gocan, H.; Grama, A. Epidemiology and Characteristics of Rheumatic Diseases in Children from Romania: Experience of a Tertiary Pediatric Rheumatology Centre (2006–2019). *Turk. Pediatri Ars.* 2020, *55* (Suppl. 1), 69–84. https://doi.org/10.14744/TurkPediatriArs.2020.77775.
45. Angheluș, R.; Pop, C.; Miu, N.; Pop, T.L. Pediatric Acute-Onset Neuropsychiatric Syndrome—Clinical Experience in a Tertiary Center from Romania. *Rom. J. Fam. Med.* 2025, *8*, 22.
46. Shulman, S.T.; Bisno, A.L.; Clegg, H.W.; Gerber, M.A.; Kaplan, E.L.; Lee, G.; Martin, J.M.; Van Beneden, C. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin. Infect. Dis. 2012, 55, e86–e102. https://doi.org/10.1093/cid/cis629
47. Pooni, R.; Vreeland, A.; Thienemann, M.; Frankovich, J. Characterizing PANS Exacerbations in Prospective Longitudinal Data. *Front. Behav. Neurosci.* 2024, *18*, 1342486. https://doi.org/10.3389/fnbeh.2024.1342486