Karaganda Medical University named after Petr Pospelov

ABSTRACT

of the dissertation work for the degree of Doctor
of Philosophy (PhD) on the topic:

The Effect of Controlled Neuromodulation on Biomarker Levels in Patients with Pharmacoresistant Epilepsy

Specialty: 8D10100 "Medicine"

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Introduction.

Relevance of the Problem. Epilepsy represents one of the most common, heterogeneous, and socially disabling chronic diseases of the central nervous system, affecting, according to WHO estimates, approximately 50 million people worldwide [1]. The condition is associated with a substantial economic burden, high comorbidity, and an increased risk of premature mortality. Despite the continuous advancement of pharmacotherapy, approximately 30% of patients constitute a cohort with drug-resistant epilepsy (DRE). It is precisely this category of patients, experiencing intractable severe seizures and the persistent threat of sudden unexpected death in epilepsy (SUDEP), that poses the most challenging clinical problem [2, 3]. The situation is further compounded by the fact that pediatric patients represent the most vulnerable demographic with DRE. Chronic paroxysmal activity exerts a devastating impact on the developing brain, disrupting neuro-ontogenetic processes, which consistently leads to psychomotor developmental delay, persistent cognitive deficits, and profound social maladaptation for both the child and their family [4].

Limitations of Surgery and the Priority of VNS in Children. Historically, resective surgery has served as the primary radical method for treating DRE; however, its application is constrained by the localization of epileptogenic foci within eloquent brain regions, multifocality of lesions, and the intrinsic complexity of the epileptogenic network [5, 6]. Controlled neuromodulation modalities provide an effective alternative. It is crucial to emphasize that although advanced invasive techniques (such as deep brain stimulation — DBS, and stereoelectroencephalography — SEEG) are currently being successfully integrated into domestic clinical practice (including at our center) for adult patients, their translation to pediatric cohorts remains hindered by significant anatomical, age-related, and methodological barriers. Consequently, vagus nerve stimulation (VNS) remains the fundamental, and frequently the only available, surgical option for this vulnerable pediatric cohort at present [7, 8].

Pathophysiology of the Delayed Effect of VNS and Neuroinflammation. Shifting the clinical focus toward VNS therapy introduces a fundamental challenge: its therapeutic response exhibits considerable variability and possesses an inherently delayed (cumulative) character. Elucidating the mechanisms underlying this gradual accumulation of efficacy necessitates a shift in the pathophysiological paradigm. Contemporary research demonstrates that the pathogenesis of pharmacoresistance is underpinned by chronic sterile neuroinflammation and breakdown of the blood–brain barrier (BBB). Within this framework, the delayed clinical response to VNS is substantiated by the premise that stimulation functions not merely as an electrophysiological desynchronizer, but also as a systemic immunomodulatory mechanism operating via activation of the cholinergic anti-inflammatory pathway [9–14].

Statement of the Scientific Problem. In the context of this delayed therapeutic onset, traditional evaluative criteria encounter critical objective limitations. Clinical monitoring reliant on parent-reported seizure diaries is inevitably susceptible to subjectivity, incomplete documentation, and the omission of atypical or

subtle seizures. Instrumental diagnostic modalities similarly present inherent constraints. Structural neuroimaging (MRI) lacks informational value for tracking the dynamic response to neuromodulation, as VNS does not produce macroscopic morphological alterations in brain tissue. Meanwhile, routine scalp EEG, although remaining a standard modality, does not consistently correlate with the true neurobiological state of the patient. Clinical practice frequently reveals marked dissociations: significant clinical seizure reduction may not be accompanied by immediate resolution of epileptiform activity on EEG, and vice versa. Thus, established clinical tools cannot objectively capture the dynamics of neuroinflammation and the stabilization of cellular integrity that constitute the underlying biological tissue response to therapy.

Justification of the Proposed Solution. Consequently, there is an urgent demand for the integration of molecular biomarkers that can serve as minimally invasive, rapid, reliable, and accessible instruments for objective monitoring. Longitudinal assessment of neurospecific markers of cellular injury (UCH-L1, NSE) and the principal pro-inflammatory alarmin driving neuroinflammation (HMGB1)—whose elevation and extravasation into the systemic circulation directly reflect compromised functional integrity of the blood–brain barrier—offers an innovative translational solution [15–25]. Profiling these markers will not only advance fundamental insight into the anti-inflammatory properties of VNS, but will also establish an objective metric for evaluating cumulative biological responses. This establishes the foundation for detecting therapeutic responsiveness prior to the manifestation of definitive EEG improvements, thereby facilitating the individualized optimization of stimulation parameters for each patient.

Objective of the Study:

To evaluate the pathogenetic significance and longitudinal dynamics of peripheral biomarkers of neuroinflammation and neurodegeneration (NSE, UCH-L1, HMGB1) in pediatric patients with drug-resistant epilepsy undergoing VNS therapy in comparison with a conservative medical management cohort.

Research Tasks:

1. Based on the analysis of structural brain alterations (according to MRI data) and bioelectrical activity disturbances (according to EEG data), to determine clinical criteria for the inadvisability of radical resective surgery in pediatric patients of the study cohort and, taking into account the limitations of intracranial interventions (SEEG, ANT-DBS) in domestic pediatric practice, to evaluate the clinical and electrophysiological efficacy of VNS therapy.

2. To determine baseline (pre-treatment — at the time of study enrollment) plasma levels of specific biomarkers of neuroinflammation and neurodegeneration (NSE, UCH-L1, HMGB1) in pediatric patients with DRE in the intervention group (prior to VNS system implantation) and in the comparison group (maintained on baseline anti-seizure pharmacotherapy).

3. To investigate the longitudinal dynamics of these biomarker concentrations over a 12-month period following VNS system implantation (at predefined follow-up intervals: 3, 6, and 12 months) in comparison with the cohort receiving exclusively

conservative medical therapy over identical time frames.

4. To evaluate the presence, direction, and strength of the correlation between alterations in biomarker levels and the clinical efficacy of VNS therapy (degree of seizure frequency reduction), thereby providing pathogenetic validation for the role of systemic neuroimmunomodulation.

Scientific Novelty:

For the first time within a prospective controlled clinical investigation, a comprehensive 12-month longitudinal profile of a specific biomarker panel reflecting neuroinflammation and neurodegeneration was evaluated in pediatric patients with drug-resistant epilepsy undergoing vagus nerve stimulation (VNS).

For the first time, a comprehensive correlation analysis was performed demonstrating that the cumulative clinical efficacy of VNS therapy is directly associated with objectively quantifiable systemic immunomodulation: an early decline in UCH-L1 and a subsequent, delayed suppression of DAMP signaling (attenuation of circulating HMGB1 levels).

A method for assessing biological response and laboratory monitoring of the efficacy of vagus nerve stimulation (VNS) in children with drug-resistant epilepsy was developed and introduced into clinical practice (Certificate of Entry into the State Register of Rights to Objects Protected by Copyright of the Republic of Kazakhstan No. 78900), enabling objective verification of therapeutic response at the molecular level (Appendix P).

Practical Significance of the Results:

Verification of subclinical biological response to therapy. The established direct correlation between clinical improvement and declining concentrations of HMGB1 and UCH-L1 supports the application of these proteins as supplementary objective laboratory indicators of VNS efficacy, verifying that the anticonvulsant effect of VNS is mediated, in part, via suppression of chronic neuroinflammation and restoration of blood–brain barrier integrity.

Objectification of disease-modifying effect. Given that VNS currently represents the primary available surgical neuromodulation modality for pediatric patients (<18 years) in the Republic of Kazakhstan, expecting therapeutic clinical efficacy requires objective substantiation. In scenarios featuring equivocal clinical-electrophysiological presentations (unreliable seizure diaries and unstable EEG patterns), the developed biomarker profile enables quantitative validation of the neuroprotective and anti-inflammatory effects of stimulation. Detecting declines in inflammatory markers provides the clinician with evidence that the intervention is exerting its disease-modifying effect.

Framework for predictive analytics. Based on the longitudinal dynamic data of neurodegenerative and neuroinflammatory proteins obtained for the first time, a methodological foundation has been laid for further investigations. This equips clinical practice with a pathogenetically substantiated panel for the future development of algorithms for early patient stratification (responders vs. non-

responders) and personalization of neurostimulation parameters.

Developed scientific and practical provisions, implementations in practical healthcare and educational activities (Appendix P)**:**

— Implementation Act into clinical practice of Regional Multidisciplinary Children's Hospital, Karaganda;

— Implementation Act into clinical practice of Multidisciplinary Hospital named after Prof. Kh.Zh. Makazhanov, Karaganda — ANT-DBS method;

— Implementation Act into clinical practice of Multidisciplinary Hospital named after Prof. Kh.Zh. Makazhanov, Karaganda — SEEG method;

— Implementation Act into educational process of NCJSC «Karaganda Medical University».

Key provisions submitted for defense:

1. Long-term vagus nerve stimulation (VNS) in children with drug-resistant epilepsy exerts a neuroimmunomodulatory effect, manifested by a statistically significant reduction in systemic circulating levels of the biomarker of neurodegeneration and BBB permeability (UCH-L1) by 29.5% by month 6, with a two-fold reduction by month 12 of therapy (by 49.7%; $p < 0.001$), as well as a delayed reduction of the key inducer of sterile neuroinflammation (alarmin HMGB1) by 31.2% ($p < 0.001$) by the end of the follow-up year. The absence of comparable dynamics in the conservative therapy group confirms the specific disease-modifying influence of VNS on restoring the functional integrity of the blood–brain barrier and suppressing the neuroinflammatory cascade.

2. Longitudinal reductions in the concentrations of the molecular biomarkers HMGB1 and UCH-L1 demonstrated a statistically significant direct correlation with the clinical efficacy of VNS therapy (degree of seizure frequency reduction), displaying a strong direct association for UCH-L1 ($r = 0.79$; $p < 0.05$) and a moderate association for the pro-inflammatory alarmin HMGB1 ($r = 0.63$; $p < 0.05$). These established regularities serve as an objective laboratory indicator of the pathogenetic response to administered neuromodulation. The persistence of biomarker concentrations without statistically significant downward dynamics in the conservative therapy group ($p > 0.05$) confirms the pathogenetic specificity of VNS action.

3. The systemic marker of acute injury (neuron-specific enolase, NSE) is characterized by high transient dispersion and does not reflect the cumulative disease-modifying effect of VNS therapy. The absence of a statistically significant decline in marker levels over 12 months of observation (Friedman test, $\chi^2 = 5.34$; $p = 0.149$) and the lack of correlation with seizure frequency reduction ($r = -0.04$; $p > 0.05$) prove that NSE in the long term is an uninformative marker for monitoring treatment outcomes.

Approbation and publications.

A total of 3 scientific articles have been published on the topic of the dissertation in the international peer-reviewed journal Frontiers in Human Neuroscience, indexed in the first quartile (Q1) of Scopus and Web of Science

databases.

Approbation of the work.

The materials and findings of the dissertation research were presented and discussed at international scientific forums:

— The 4th Interim Meeting of the International Neuromodulation Society (INS 4th Interim Meeting, Almaty, Kazakhstan, October 2–4, 2025);

— The 17th World Congress of the International Neuromodulation Society (17th World Congress of the International Neuromodulation Society — INS 2026, Lisbon, Portugal, May 9–14, 2026).

The work was presented at an extended meeting of the Department of Surgical Diseases on May 26, 2026.

Scope and structure of the dissertation:

The dissertation comprises 98 pages of the main text prepared using Microsoft Word. It is organized into an introduction, 3 main chapters, a conclusion, and a list of references. The work is illustrated with 13 tables and 8 figures. The bibliography includes 178 sources published in Russian and English. The dissertation is supplemented with 13 appendices.

Materials and methods.

Study Design:

This investigation was structured as a prospective, randomized, controlled clinical study.

Object of the Clinical Study.

Due to the absence of a specialized personalized national registry for pediatric patients with drug-resistant epilepsy (DRE) in the Republic of Kazakhstan, the target population size was determined using epidemiological extrapolation. The primary clinical screening pool over the study period comprised 145 pediatric patients presenting for specialized medical care. During the multi-stage filtration process, strict inclusion and exclusion criteria were applied to the pool of 145 individuals, yielding a strictly verified cohort of 53 candidates.

Prior to allocation, the legal representatives of 13 of the 53 selected patients refrained from participating

in the randomization protocol, opting instead to continue current conservative medical treatment and declining the potential for surgical intervention. The remaining 40 patients were allocated in a 1:1 ratio using simple computer-generated randomization (MS Excel pseudo-random number generator) into two comparable groups: the VNS therapy cohort (n = 20) and the comparison group (n = 20).

To confirm that the actually formed sample size (N = 40) possessed sufficient statistical power to achieve reliable results, an a priori mathematical modeling was performed using G*Power software (version 3.1.9.7). The methodological foundation was based on the prospective longitudinal study design aimed at evaluating the within-group dynamics of key clinical and laboratory parameters (seizure frequency and concentrations of specific proteins UCH-L1, HMGB1, and NSE) across four linked assessment time points: baseline, 3, 6, and 12 months (number of measurement

points k = 4, degrees of freedom for repeated comparisons df = 3). A one-way repeated-measures analysis of variance model (ANOVA: Repeated measures, within factors) was selected as the calculation base, serving as the direct parametric analog of the non-parametric Friedman rank test.

The calculation was based on conservative requirements of evidence-based biostatistics: establishing a standard two-tailed significance level ($\alpha = 0.05$) and a target statistical power of not less than 80% ($1 - \beta = 0.80$). A moderate within-subject correlation among repeated observations ($\rho = 0.5$) was assumed with a sphericity coefficient $\varepsilon = 1.0$. Based on clinical premises, a moderate (medium) effect size according to J. Cohen's classification ($f = 0.30$) was incorporated into the detection model.

As a result of the modeling, the minimum required calculated sample size was 17 patients per group. However, relying on the methodological principle of E. Lehmann — E. Pitman regarding the asymptotic relative efficiency of rank tests, an adjustment for potential power loss (approximately 15%) when transitioning from parametric analysis to the non-parametric Friedman test was incorporated. Accounting for this correction, the required sample size was 20 patients in each group (with non-centrality parameter $\lambda = 12.24$; critical F-value = 2.80; error degrees of freedom df = 48; and final calculated power $1 - \beta = 81.5\%$), which completely corresponds to the size of the recruited clinical cohort.

Intervention group (n = 20): Implantation of a vagus nerve stimulation (VNS) system combined with optimized anti-seizure pharmacotherapy;

Comparison group (n = 20): Continuation of optimized anti-seizure pharmacotherapy alone (exclusively medical treatment).

The compared groups were initially homogeneous regarding sex, age, and baseline epileptic seizure frequency.

Study sites and timeline. The study was carried out between 2023 and 2026 at the Neurosurgical Department of the Regional Multidisciplinary Children's Hospital of Karaganda. The laboratory phase of the study was conducted in partnership with the laboratory of «CDL OLYMP» LLP (Karaganda).

Inclusion criteria:

Age: 4 to 9 years at initial screening;
Epilepsy type: Confirmed diagnosis of drug-resistant focal epilepsy (according to the ILAE 2017 classification); documented failure of two or more appropriately chosen and administered anti-seizure medication regimens (as monotherapy or in combination), meeting the ILAE consensus definition of drug-resistant epilepsy;
Seizure semiology: Documented focal seizures progressing to bilateral tonic-clonic seizures (with rapid bilateralization, ILAE 2017);
Stable therapeutic regimen: Maintenance on stable doses of anti-seizure medications for at least 3 months prior to study enrollment;
Ineligibility for resective surgery: Absence of a clearly delineated epileptogenic focus amenable to surgical resection, presence of foci within functionally eloquent brain structures, or presence of multifocal lesions;
Informed consent: Signed written informed consent obtained from the legal

representatives (guardians) of the patient.

Exclusion criteria:

Progressive CNS disorders: Diagnosed neurodegenerative or inborn metabolic disorders, as well as intracranial mass lesions;
Psychogenic seizures: Documented history of psychogenic non-epileptic seizures (PNES);
Factors biasing biomarker levels: Presence of acute infectious pathology, autoimmune disorders, or chronic inflammatory processes in acute exacerbation (including neuroinfections and systemic connective tissue diseases) capable of distorting systemic levels of HMGB1, NSE, or UCH-L1;
Severe somatic pathology: Decompensated cardiovascular disorders (in particular, cardiac rhythm or conduction abnormalities), as well as severe hepatic or renal impairment;
Surgical history: Prior resective surgery for epilepsy or prior implantation of other neurostimulation systems;
Anatomical contraindications: Prior cervical vagotomy or anatomical anomalies precluding safe electrode placement on the left vagus nerve;
Compliance: Anticipated inability of the patient or their legal representatives to adhere to the study protocol and follow-up visit schedule.

The study protocol received formal approval from the Bioethics Commission of NCJSC «Karaganda Medical University» (Protocol No. 3, dated February 27, 2024).

Data Collection and Case Report Forms (CRF).

For each enrolled subject, a standardized Case Report Form (CRF) was maintained, incorporating:

- Demographic and Anthropometric Parameters: Patient identification code (ID), sex, age, body weight;
- Clinical History and Neurological Status: Age at epilepsy onset, seizure semiology and baseline frequency, suspected etiology, comprehensive neurological assessment, and documentation of comorbidities;
- Neuroimaging Findings: Preoperative brain MRI data;
- Electrophysiological Data: Serial video-EEG monitoring records;
- Treatment Protocols: Operative notes on VNS implantation and programmatic stimulation parameters (intervention group); detailed ASM regimens and dosage adjustments (both groups);
- Laboratory Profiles: Serial concentrations of HMGB1, NSE, and UCH-L1 measured in plasma at baseline and at 3, 6, and 12 months; standard complete blood counts and biochemical panels;
- Clinical Outcomes: Frequency, severity, and duration of seizures recorded during protocol-defined follow-up visits.
- Conclusions of the joint examination by a neurosurgeon, neurologist, and pediatrician at follow-up visits, including the assessment of general condition, objective status, and neurological status.

Laboratory Analysis Methods.

Quantitative measurements of targeted neurospecific biomarkers in blood plasma were performed using solid-phase enzyme-linked immunosorbent assays (ELISA). The biomarker panel comprised:

Ubiquitin C-terminal hydrolase L1 (UCH-L1);

High-mobility group box 1 protein (HMGB1);

Neuron-specific enolase (NSE).

All laboratory assays were performed in strict adherence to manufacturers' protocols (Thermo Fisher Scientific, USA).

Sample Preparation and Quality Assurance: Peripheral venous blood was drawn into EDTA-coated tubes and centrifuged at 1,000 × g for 15 minutes at 2–8 °C within 30 minutes of venipuncture. Separated plasma was divided into single-use aliquots and stored at –80 °C until batch testing, preventing freeze-thaw degradation. All specimens were evaluated in duplicates. The intra-assay and inter-assay coefficients of variation (CV) were verified at <10% and <15%, respectively.

Methods of Statistical Analysis.

Statistical data analysis was conducted using the Statistica 8.0 software package (StatSoft Inc., USA), G*Power (version 3.1.9.7), and MS Excel.

Normality of quantitative variable distribution was tested using the Shapiro–Wilk test. Given the sample size (n = 20) in the study groups and non-normal data distribution, descriptive statistics are presented as medians and interquartile ranges (Me [Q1; Q3]).

To analyze the longitudinal dynamics of neurospecific biomarker concentrations (UCH-L1, HMGB1, NSE) across four linked time points (baseline, 3, 6, and 12 months) in each group, the non-parametric Friedman test was employed.

When statistically significant differences were detected ($p < 0.05$), post-hoc pairwise comparisons were conducted using Dunn's test with correction for multiple comparisons. To evaluate the direction and strength of the relationship between alterations in biomarker levels and the degree of seizure reduction, Spearman's rank correlation coefficient was calculated. Differences were considered statistically significant at $p < 0.05$.

Results and discussion.

This study represents a prospective longitudinal analysis of systemic biomarkers aimed at deciphering the pathogenetic mechanisms underlying the clinical efficacy of vagus nerve stimulation (VNS) in children with drug-resistant epilepsy. The findings validate the concept that therapeutic neuromodulation extends beyond mere acute electrophysiological desynchronization. Clinical seizure reduction significantly correlates with the emergence of a cumulative neuroimmunomodulatory effect, objectively validated via laboratory monitoring.

Summary and Evaluation of Research Results.

Primary clinical responsiveness was tracked through longitudinal monitoring of

monthly seizure frequency, alongside qualitative evaluation of serial EEG recordings at baseline and after 12 months of continuous therapy. Patients receiving active VNS therapy exhibited a statistically significant, progressive decrease in seizure frequency ($\chi^2 = 42.85$, $p < 0.001$). The baseline median seizure count was 13.5 [10.75; 16.25] episodes per month. By month 6 of therapy, the median declined to 9.0 [7.0; 16.0], and by month 12 it reached 7.5 [4.75; 11.0], representing an overall reduction of 44.4% relative to baseline. This clinical alleviation was accompanied by positive reorganization of cerebral bioelectrical activity according to EEG data. In the comparison group maintained on exclusively conservative medical therapy, no longitudinal clinical dynamics were observed. This contrast convincingly substantiates that the observed outcome represents a specific consequence of neuromodulation.

Profiling of peripheral biomarkers revealed a consistent and pathogenetically substantiated chronology of their alterations in blood plasma:

1. Reduction of structural injury marker (UCH-L1): A statistically significant decrease in the concentration of the intracellular neuronal enzyme UCH-L1 was documented (declining from a baseline median of 5.29 [5.09; 5.72] to 3.73 [3.26; 4.91] ng/mL) by treatment month 6 ($p = 0.009$). Because the extravasation of this brain-specific protein into the systemic bloodstream reflects cellular distress and compromised blood–brain barrier integrity, its reliable reduction signifies marked attenuation of cerebral excitotoxic stress. Within contemporary neurobiological paradigms, such dynamics are regarded as a systemic laboratory hallmark of blood–brain barrier stabilization.

2. Dynamics of sterile neuroinflammation marker (HMGB1): The concentration profile of the pro-inflammatory DAMP protein HMGB1 was characterized by a delayed reduction by month 12 (from 7.65 [6.88; 8.36] to 5.26 [4.99; 5.86] ng/mL, $p \leq 0.001$). This delayed decline is consistent with the well-recognized clinical cumulative latency of VNS. Grounded in literature data regarding the pivotal role of the HMGB1–TLR4 axis in driving epileptogenesis, the reduction of this circulating alarmin reflects systemic suppression of pro-epileptogenic neuroinflammatory cascades. The demonstrated statistically significant direct correlation (for UCH-L1: $r = 0.79$, $p < 0.05$; for HMGB1: $r = 0.63$, $p < 0.05$) between the reduction of these markers and the percentage of seizure reduction supports the utility of the UCH-L1 and HMGB1 panel as a promising translational monitoring tool.

Assessment of the Validity and Reliability of the Results.

The validity and scientific integrity of the experimental conclusions, findings, and practical recommendations are ensured by the prospective, randomized, controlled clinical study design incorporating a parallel comparison cohort, alongside a scientifically justified sample size ($n = 40$) formed through multi-stage screening with rigorous inclusion and exclusion criteria.

The randomization procedure was executed via computer-generated pseudo-random numbers (in MS Excel) in a 1:1 ratio. The study was conducted in full compliance with the principles of the Declaration of Helsinki, approved by the

Bioethics Commission of NCJSC «Karaganda Medical University» (Protocol No. 3, dated February 27, 2024), and accompanied by mandatory written informed consent from participants' legal representatives.

The analytical reliability of the laboratory phase is guaranteed by performing investigations at the accredited diagnostic facilities of «CDL OLYMP» LLP (Karaganda) using commercially validated test systems (Thermo Fisher Scientific, USA), performing analyses in duplicates with adherence to standard coefficients of variation (CV <10% and <15%), and strictly avoiding repeated freeze-thaw cycles of biospecimens.

Statistical processing was carried out using appropriate non-parametric methods strictly matching the group sizes and data distribution characteristics (Shapiro–Wilk test, Friedman ANOVA, post-hoc Dunn's test, Spearman rank correlation), with key clinical and biological shifts reaching high levels of statistical confidence ($p < 0.001$).

Comparison with Similar Results of Domestic and Foreign Studies.

The cumulative clinical response to VNS documented herein aligns closely with large-scale international registry data (CORE-VNS) and published meta-analyses. The initial domestic implementation of DBS ANT in Kazakhstan reported by our group demonstrates comparable therapeutic outcomes to landmark clinical trials (SANTE, MORE), substantiating the utility of deep thalamic stimulation for multifocal epilepsy phenotypes.

Nevertheless, longitudinal profiling of this dual biomarker panel (UCH-L1 and HMGB1) in a pediatric clinical cohort undergoing VNS is described here for the first time. Our clinical findings validate translational hypotheses previously derived from preclinical experimental models, providing direct clinical evidence for the engagement of the cholinergic anti-inflammatory pathway in attenuating persistent neuroinflammation and secondary cellular degradation.

Justification of the Need for Additional Studies and Proposed Future Directions:

1. Multi-Center Prospective Trials with Expanded Cohorts: Expanding sample sizes across multiple clinical sites is warranted to definitively validate this dual biomarker panel (HMGB1, UCH-L1), paving the way for its integration as an objective tool for pathogenetic monitoring and patient stratification (responders vs. non-responders).
2. Assessment of Long-Term Neuroimmunomodulatory Durability: Given that this study tracked patients over a 12-month interval, extended longitudinal monitoring spanning 3–5 years of continuous therapy will be valuable to establish whether the disease-modifying benefits persist without therapeutic habituation or tachyphylaxis.

Negative Results Leading to the Discontinuation of Certain Directions.

During longitudinal follow-up, an explicit negative finding was obtained concerning systemic neuron-specific enolase (NSE) levels. Unlike UCH-L1 and HMGB1, NSE failed to demonstrate statistically significant longitudinal trends in

either cohort following VNS initiation. Measured values displayed marked inter-individual variance and sporadic outliers (reaching 25.24 ng/mL), reflecting the pronounced sensitivity of this enzyme to transient confounding events (such as subclinical paroxysms or unrecorded seizures occurring shortly prior to sampling).

This negative outcome indicates that NSE is characterized by high physiological volatility and lacks adequate predictive stability, rendering it uninformative for tracking cumulative neuroprotection and disease modification during VNS therapy. Consequently, routine reliance on NSE as an isolated biomarker of long-term therapeutic response in pediatric DRE is unadvisable, and diagnostic focus should center upon the targeted UCH-L1 and HMGB1 panel.

Conclusion.

Summary Conclusions Based on the Results of the Dissertation Research:

1. According to neuroimaging and electrophysiological monitoring data, it was established that 100% of the evaluated children (n = 40) were not candidates for open resective surgery due to perinatal CNS lesions (40%, n = 16), cortical malformations (30%, n = 12), bilateral hippocampal sclerosis and combined defects (30%, n = 12), as well as the complexity of the epileptogenic network — presenting as secondary bilateralization in 85% (n = 34) and independent asynchronous foci in 15% (n = 6). The initial domestic experience with intracranial interventions (SEEG, ANT-DBS) confirmed their clinical feasibility in adult patients; however, it revealed anatomical and institutional barriers to their translation into pediatric practice. This determined VNS therapy as the method of choice in pediatric patients, achieving a 44.4% seizure reduction.

2. Baseline biomarker levels in the studied cohort objectify persistent neuroinflammation and blood–brain barrier compromise: the median UCH-L1 was 5.29 [5.09; 5.72] ng/mL in the intervention group and 7.79 [6.94; 8.61] ng/mL in controls, while HMGB1 was 7.65 [6.88; 8.36] ng/mL and 9.75 [9.27; 10.80] ng/mL, respectively, exceeding normal threshold values for a healthy population according to the literature. Baseline values of neuron-specific enolase (NSE) were characterized by pronounced inter-individual variability, with a median of 1.34 [0.88; 5.20] ng/mL in the intervention group and 2.41 [1.88; 2.67] ng/mL in the comparison group.

3. During VNS therapy, a progressive decline in UCH-L1 was identified from 5.29 to 3.73 ng/mL by month 6 ($p = 0.009$) and to 2.66 ng/mL by month 12 ($p < 0.001$; Friedman test, $\chi^2 = 41.34$), along with a delayed reduction in HMGB1 by month 12 from 7.65 to 5.26 ng/mL ($\chi^2 = 27.19$; $p < 0.001$). NSE dynamics were statistically non-significant ($\chi^2 = 5.34$; $p = 0.149$). In the control group, biomarker levels remained without statistically significant dynamics ($p > 0.05$).

4. Seizure frequency reduction during VNS (by 44.4%) exhibits a direct correlation with the reduction in UCH-L1 ($r = 0.79$; $p < 0.05$) and HMGB1 ($r = 0.63$; $p < 0.05$), demonstrating that the anticonvulsant effect is realized via systemic neuroimmunomodulation. Concurrently, no correlation was found with NSE levels ($r = -0.04$; $p > 0.05$).

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