

NAO "Karaganda Medical University"

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**

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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. 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. 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 (HMGB1, NSE, UCH-L1) in pediatric patients with drug-resistant epilepsy undergoing VNS therapy in comparison with a conservative medical management cohort.

Research Tasks:

1. To determine baseline (pre-treatment) plasma levels of specific biomarkers of neuroinflammation and neurodegeneration (HMGB1, NSE, UCH-L1) in pediatric patients with DRE in the intervention group (prior to VNS system implantation) and in the comparison group (maintained on optimized standard anti-seizure medication).
2. To investigate the longitudinal dynamics of these biomarker concentrations over a 12-month period following VNS implantation (at predefined intervals: 3, 6, and 12 months) in comparison with the control cohort receiving exclusively conservative pharmacotherapy over identical time frames.
3. To assess the existence, direction, and magnitude of correlations between alterations in biomarker concentrations and the clinical efficacy of VNS therapy (degree of seizure frequency reduction), thereby providing pathogenetic validation for the role of systemic neuroimmunomodulation.
4. To justify VNS therapy as the primary minimally invasive surgical modality in the pediatric cohort at the present stage, based on the evaluation of initial domestic

experience with targeted intracranial interventions (SEEG, DBS ANT) and the delineation of their clinical role within the broader structure of invasive diagnostics and neuromodulation.

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, rigorous correlation analyses demonstrated that the cumulative clinical efficacy of VNS therapy is directly associated with objectively quantifiable systemic immunomodulation, manifested by an early decline in UCH-L1 and a subsequent, delayed suppression of DAMP signaling (attenuation of circulating HMGB1 levels).

Practical Significance of the Results:

Verification of Subclinical Biological Response: The established positive correlation between clinical seizure reduction and declining concentrations of HMGB1 and UCH-L1 supports the application of these proteins as supplementary objective laboratory indicators of VNS efficacy, verifying that seizure control is mediated, in part, via suppression of chronic neuroinflammation and restoration of barrier integrity.

Objectification of Disease-Modifying Effects: Given that VNS currently represents the only feasible surgical neuromodulation option for pediatric patients (<18 years) in the Republic of Kazakhstan, evaluating therapeutic efficacy warrants objective biochemical corroboration. In scenarios featuring equivocal clinical-electrophysiological presentations (unreliable seizure diaries and unstable EEG patterns), this biomarker profile enables quantitative validation of the neuroprotective and anti-inflammatory effects of stimulation. Detecting declines in inflammatory markers equips the clinician with objective evidence that the intervention is exerting a disease-modifying effect.

Framework for Predictive Stratification: The longitudinal profiles of neurospecific proteins established in this study lay the methodological foundation for future translational investigations. This offers clinical practice a pathogenetically validated biomarker panel for developing early patient stratification algorithms (responders vs. non-responders) and personalizing stimulation parameters.

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): strong for UCH-L1 ($r = 0.79$; $p < 0.05$) and moderately strong for the pro-inflammatory alarmin HMGB1 ($r = 0.63$; $p < 0.05$). These observed regularities serve as an objective laboratory indicator of the pathogenetic response to neuromodulation. The persistence of stably high concentrations of these biomarkers 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 is an uninformative marker for monitoring treatment outcomes in the long term.

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 primary provisions and findings of the dissertation research were presented and discussed at international scientific conferences:

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 (INS 2026, Lisbon, Portugal, May 9–14, 2026).

Scope and Structure of the Dissertation.

The dissertation comprises 88 pages of typed manuscript prepared using Microsoft Word. It is organized into an introduction, 3 main chapters, a discussion, a conclusion, and a list of references. The work is illustrated with 8 tables and 8 figures. The bibliography includes 199 sources published in Russian and English.

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 care. During the multi-stage filtration process, strict inclusion and exclusion criteria were applied to the pool of 145 individuals, yielding a homogeneous cohort of 53 candidates. Prior to allocation, the legal representatives of 13 patients declined randomization due to reluctance to expose the child to potential surgical intervention, opting instead to continue pharmacological management. Sample size calculation was performed using G*Power software (version 3.1.9.7) for a repeated-measures design across 4 time points at $\alpha = 0.05$, statistical power $1 - \beta = 0.80$, and a medium effect size $f = 0.28$, incorporating a 5% correction for power loss in the non-parametric Friedman test, which established a minimum threshold of 19 patients. The study cohorts were ultimately formed with 20 children each in the VNS therapy cohort and the comparison group (1:1 ratio, total N = 40).

Using simple computer-generated randomization (MS Excel pseudo-random number generator), patients were allocated in a 1:1 ratio into two comparable groups:

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

Comparison group (n = 20): Continuation of optimized anti-seizure therapy alone (exclusively pharmacological 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 2025 at the Neurosurgical Department of the "Multidisciplinary Regional Children's Hospital" (Karaganda, Kazakhstan). Laboratory analyses were conducted in partnership with the accredited diagnostic facilities of "CDL OLYMP" LLP (Karaganda).

Inclusion Criteria:

- **Age:** 4 to 9 years at initial screening;
- **Epilepsy Classification:** Clinically verified drug-resistant focal epilepsy (according to ILAE 2017 classification);
- **Seizure Semiology:** Documented focal seizures progressing to bilateral tonic-clonic events (focal to bilateral tonic-clonic seizures with rapid propagation, ILAE 2017);
- **Pharmacoresistance:** Confirmed failure of at least two tolerated and appropriately chosen ASM schedules (as monotherapy or combination regimens), fulfilling the consensus definition of drug-resistant epilepsy established by the ILAE;
- **Therapeutic Stability:** Maintained on stable ASM dosing regimens for at least 3 months prior to enrollment;
- **Ineligibility for Resection:** Absence of an identifiable, surgically resectable epileptogenic lesion (e.g., non-lesional MRI), involvement of eloquent cortical structures, or multifocal epileptogenic networks;
- **Informed Consent:** Signed written informed consent obtained from parents or legal guardians.

Exclusion Criteria:

- **Progressive CNS Disorders:** Confirmed neurodegenerative or inborn

- metabolic conditions, or intracranial space-occupying lesions;
- **Psychogenic Events:** Documented history of psychogenic non-epileptic seizures (PNES);
- **Confounding Inflammatory Conditions:** Active systemic infections, autoimmune disorders, or acute exacerbations of chronic inflammatory processes (including neuroinfections and connective tissue diseases) capable of biasing systemic HMGB1, NSE, or UCH-L1 concentrations;
- **Severe Somatic Pathology:** Decompensated cardiovascular disorders (specifically cardiac dysrhythmias or conduction defects), or end-stage hepatic or renal dysfunction;
- **Prior Surgical Interventions:** Previous resective epilepsy surgery or prior implantation of other neuromodulation devices;
- **Anatomical Constraints:** Prior cervical vagotomy or anatomical anomalies precluding safe electrode placement on the left vagus nerve;
- **Compliance Issues:** Anticipated non-compliance of the patient or caregivers with study protocols and follow-up schedules.

The clinical protocol received formal ethical clearance from the Bioethics Committee 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.

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 $1,000 \times g$ 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.

Data analysis was conducted utilizing Statistica 8.0 (StatSoft Inc., USA) and Microsoft Excel.

The normality of quantitative variables was verified using the Shapiro–Wilk test. Given the cohort size ($n=20$ per group) and non-normal distributional characteristics, descriptive statistics are presented as medians and interquartile ranges (Me [Q1; Q3]).

Within-group longitudinal dynamics of biomarker levels across the four linked assessment intervals (baseline, 3, 6, and 12 months) were evaluated using Friedman's non-parametric repeated-measures ANOVA.

Where significant overall differences were identified ($p < 0.05$), Dunn's post-hoc pairwise comparisons with corrections for multiple testing were applied. Intergroup differences were assessed using the Mann–Whitney U test. The association between relative biomarker fluctuations and clinical seizure reduction was examined using Spearman's rank correlation coefficient (r). Two-tailed p -values < 0.05 were defined as statistically significant.

Results and discussion.

This study represents a prospective longitudinal analysis of circulating 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 sustained stimulation. 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, this parameter declined to 9.0 [7.0; 16.0], reaching 7.5 [4.75; 11.0] by month 12, reflecting an overall median reduction of 44.4% relative to baseline. This clinical alleviation was accompanied by progressive reorganization and stabilization of cerebral background activity on EEG. Conversely, the control group maintained on standard ASM therapy exhibited no meaningful longitudinal clinical improvement. This divergence substantiates that the observed clinical benefit represents a specific therapeutic consequence of VNS.

Longitudinal assessment of circulating peripheral biomarkers revealed a

chronologically coordinated and pathogenetically consistent sequence of molecular alterations:

1. **Down-Regulation of Structural Injury Marker (UCH-L1):** A statistically significant decrease in the concentration of the neuronal cytoplasmic deubiquitinating enzyme UCH-L1 was observed by treatment month 6, declining from a baseline median of 5.29 [5.09; 5.72] ng/mL to 3.73 [3.26; 4.91] ng/mL ($p < 0.001$). Because the extravasation of this brain-specific protein into the systemic bloodstream reflects ongoing neuronal stress and blood–brain barrier disruption, its sustained down-regulation signifies marked attenuation of cerebral excitotoxic stress. Within contemporary neurobiological paradigms, this trajectory serves as a systemic laboratory indicator of stabilized neurovascular and barrier integrity.
2. **Kinetics of Sterile Neuroinflammation Driver (HMGB1):** The concentration profile of the ubiquitous DAMP alarmin HMGB1 showed a delayed reduction, becoming prominent by month 12 (declining from 7.65 [6.88; 8.36] ng/mL at baseline to 5.26 [4.99; 5.86] ng/mL, $p \leq 0.001$). This delayed kinetic profile mirrors the clinically recognized cumulative latency of VNS efficacy. In light of established evidence regarding the pivotal role of the HMGB1–TLR4 axis in driving epileptogenesis, the marked depletion of this circulating danger signal corroborates sustained dampening of pro-epileptogenic neuroinflammatory cascades. The demonstrated direct correlations between clinical seizure reduction and molecular declines (UCH-L1: $r = 0.79$, $p < 0.05$; HMGB1: $r = 0.63$, $p < 0.05$) support the utility of this combined panel as an objective translational monitoring tool.

Assessment of the Validity and Reliability of the Results.

The validity and scientific integrity of the experimental conclusions and practical recommendations are ensured by the prospective, randomized, controlled clinical design, inclusion of a parallel control cohort, and a statistically justified sample size ($n=40$) established through multi-tiered screening with rigorous inclusion/exclusion criteria.

Randomization was conducted via pseudo-random number generation in MS Excel in a 1:1 allocation ratio. The trial was executed in full accordance with the principles of the Declaration of Helsinki, received formal approval from the Bioethics Committee of NCJSC "Karaganda Medical University" (Protocol No. 3, February 27, 2024), and was accompanied by written informed consent from all participants' legal guardians.

The analytical reliability of the bioassays was assured through testing within an accredited laboratory setting ("CDL OLYMP" LLP, Karaganda) utilizing commercially validated ELISA kits (Thermo Fisher Scientific, USA). Duplicate determinations, strict compliance with quality control parameters ($CV < 10\%$ and $< 15\%$), and avoidance of repeated freeze-thaw cycles further guaranteed data precision.

Statistical evaluation adhered strictly to robust non-parametric methodologies suited for the sample size and distributional characteristics (Shapiro–Wilk test, Friedman ANOVA, Dunn's post-hoc test, and Spearman 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 inclusion in clinical protocols 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 inadvisable, 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. Baseline (pre-treatment) concentrations of neurospecific biomarkers were determined in the blood plasma of pediatric patients with drug-resistant epilepsy prior to surgical intervention (Point 0). Multiple-fold elevations above

physiological reference values were identified for the evaluated proteins in both cohorts: the median concentration of the neuronal membrane injury marker 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 the control group (2–3 times above normal), while the pro-inflammatory alarmin HMGB1 reached 7.65 [6.88; 8.52] ng/mL and 9.75 [9.27; 10.80] ng/mL, respectively (4–5 times above normative values). This molecular profile objectifies the presence of severe persistent sterile neuroinflammation and blood–brain barrier compromise in intractable epilepsy. The documented baseline values served as an individual reference point for each patient, which enabled subsequent longitudinal monitoring to neutralize the confounding effect of pronounced inter-individual biological variability and rigorously evaluate intra-group therapeutic shifts induced by vagus nerve stimulation.

2. Longitudinal analysis revealed a statistically significant reduction in plasma biomarker concentrations among patients undergoing VNS therapy, in contrast to the cohort receiving exclusively conservative medical therapy. Plasma UCH-L1 levels in the VNS cohort exhibited a progressive decline (Friedman test, $\chi^2 = 41.34$, $p < 0.001$) from a baseline median of 5.29 ng/mL to 3.73 ng/mL by month 6 (adjusted $p = 0.009$) and to 2.66 ng/mL by month 12 (adjusted $p < 0.001$). Following an initial period of stability, the concentration of the alarmin HMGB1 demonstrated a significant reduction by month 12, dropping from 7.65 to 5.26 ng/mL ($\chi^2 = 27.19$, adjusted $p < 0.001$). NSE dynamics did not attain statistical significance ($\chi^2 = 5.34$, $p = 0.149$), retaining marked inter-individual dispersion. In the comparison group maintained on conservative therapy, circulating levels of all biomarkers at the 3-, 6-, and 12-month follow-up points persisted at a plateau without statistically significant changes (UCH-L1: $p = 0.494$; HMGB1: $p = 0.385$; NSE: $p = 0.856$).
3. Correlation analysis demonstrated a statistically significant direct relationship between changes in neuroimmune biomarker levels and the clinical efficacy of VNS therapy. The reduction in seizure frequency (from a baseline median of 13.5 to 7.5 episodes per month, representing a 44.4% reduction) showed a strong direct correlation with the reduction in UCH-L1 levels ($r = 0.79$; $p < 0.05$) and the decrease in HMGB1 concentration ($r = 0.63$; $p < 0.05$). No correlation was observed with NSE levels ($r = -0.04$; $p > 0.05$).
4. Vagus nerve stimulation (VNS) is currently established as the primary and preferred modality for surgical neuromodulation in pediatric drug-resistant epilepsy in the Republic of Kazakhstan. Targeted intracranial interventions (SEEG, DBS ANT), despite their successful translation into domestic clinical practice in adult cohorts, remain restricted in pediatric care due to anatomical, developmental, and institutional factors. This objectively substantiates the necessity of in-depth investigations into the molecular mechanisms and biomarkers governing the efficacy of VNS therapy.

Practical recommendations.

The findings of this dissertation research provide an objective foundation for refining clinical strategies in managing pediatric drug-resistant epilepsy via vagus nerve stimulation. The established longitudinal trajectories of UCH-L1 and HMGB1 support the integration of this biomarker panel as an accessible, minimally invasive laboratory tool for evaluating therapeutic responsiveness. Practically, monitoring these proteins provides clinicians with an objective basis for confirming biological disease modification, guiding stimulation parameter titrations, and developing early patient stratification algorithms (responders vs. non-responders) within clinical protocols.

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