



Research Article

Volume 8 Issue 4 - September 2026
DOI: 10.19080/APBJ.2026.08.555742

Anatomy Physiol Biochem Int J

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Gastric Cancer Neurovascular Invasion Syndrome (GC-NVIS): Proposal and Initial Clinicopathological Evidence for a Distinct Neural-Vascular Invasive Phenotype



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Submission: September 24, 2026; **Published:** September 30, 2026

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Abstract

Background: Perineural Invasion (PNI) and Lymphovascular Invasion (LVI) are familiar adverse findings in gastric cancer, but they are generally interpreted as separate routes of spread. Their frequent co-occurrence raises the question of if they sometimes represent a broader invasive state in which tumor cells participate in both the neural and vascular compartments. We propose the use of the term Gastric Cancer Neurovascular Invasion Syndrome (GC-NVIS) for this candidate clinicopathological phenotype and define a staged pathway for its testing.

Methods: We performed a secondary analysis of clinicopathological data from 300 patients in the HMU-GC histopathology cohort. PNI was evaluable in 248 patients and LVI in 250, with 239 having complete data for both. Patients were classified as PNI-/LVI-, PNI+/LVI-, PNI-/LVI+, or PNI+/LVI+. High nodal burden (pathological N2-N3 disease) was selected as the principal external biological validator. Multivariate logistic regression adjusted for age, sex, pathological T category, and tumor infiltration pattern.

Results: PNI and LVI were strongly associated (OR 4.51, 95% CI 2.58-7.88; $P < 0.000001$). The dual-positive phenotype was present in 102/239 evaluable patients (42.7%). N2-N3 disease occurred in 23.0% of PNI-/LVI-, 32.6% of PNI+/LVI-, 26.7% of PNI-/LVI+, and 65.7% of PNI+/LVI+ tumors. After adjustment, the dual-positive phenotype strongly associated with high nodal burden (aOR 4.68, 95% CI 2.17-10.12). A 0-2 invasion-burden measure showed a gradient association with N2-N3 disease. The PNI×LVI interaction was suggestive but not conventionally significant ($P = 0.059$).

Conclusion: Concomitant PNI and LVI identify an unusually aggressive gastric-cancer phenotype and provide a reasonable starting point for syndrome discovery. We propose candidate GC-NVIS and hypothesize that its microscopic substrate is a spatially organized Neurovascular Invasive Niche (NVIN) at the invasive front. However, the current data do not establish a new syndrome and require direct whole-slide spatial validation, evidence of incremental information beyond binary PNI/LVI, and independent replication.

Keywords: Gastric cancer; Perineural invasion; Lymphovascular invasion; Neurovascular invasion; Neurovascular invasive niche; Lymph-node metastasis; Tumor microenvironment; Syndrome discovery

Novelty Statement

Concurrent PNI/LVI and its adverse prognostic significance are neither novel nor are we the first to describe their appearance in resected and preoperatively treated gastric cancer; recent imaging works have even targeted combined LVI/PNI prediction [1,2]. The contribution of the proposed work is the disentanglement of a known Dual-Positive Screen Phenotypic (DCIP) from a spatially defined syndrome. In this framework, GC-NVIS is not diagnosed by PNI+LVI but requires non-random convergence of invaded nerves and lymphovascular channels at a same invasive front, quantified by a locked spatial metric such

as PLSCI and demonstrated to add information beyond the two conventional pathology variables. As far as we know, this spatial requirement has not been operationalized previously as a gastric-cancer syndrome.

Introduction

Gastric cancer is still one of the leading causes of cancer-related deaths in the world, despite a significant number of treatment options, including surgery, systemic therapies, molecular-targeted treatments, and immunotherapy. The most critical factors determining the tumor's stage are the depth

of invasion and the involvement of regional lymph nodes for decision-making; however, a precise anatomical description of individual tumors' biological behavior is not always evident [3,4].

Histopathological features of the invasive tumor front can provide additional information about these mechanisms. The most important of these are Perineural Invasion (PNI) and Lymphovascular Invasion (LVI). PNI is a sign of malignant involvement of neural structures and has been repeatedly associated with deeper tumor invasion, lymphatic and vascular invasion, lymph-node metastasis, advanced stage, and adverse survival in gastric cancer [5].

The biological interpretation of PNI also has changed substantially. Cancer neuroscience increasingly recognizes peripheral nerves as active components of the tumor microenvironment rather than passive anatomical structures. Neural signals have potential to influence cancer-cell proliferation, migration, angiogenesis, immune regulation, stromal remodeling, and therapeutic resistance [6-8]. In gastric cancer, experimental studies implicate cholinergic signaling, nerve-growth-factor pathways, neuronal outgrowth, and reciprocal communication between tumor cells and Schwann cells [9-12].

LVI is a complementary pathway of malignant dissemination. Histologically, LVI is characterized by the presence of tumor cells within lymphatic or vascular channels as well as endothelia and provides direct morphological evidence of cancer cell access to transportation routes, which are relevant on the local or systemic levels. LVI is consistently linked to lymph-node metastasis, deeper invasion, recurrence, and poor prognosis in gastric cancer [13,14]. Lymphangiogenic mechanisms involving VEGF-C, VEGF-D, endothelial remodeling and increased lymphatic-vessel density, provide probable biological mechanisms linking the invasive tumor microenvironment to dissemination [15-17].

Although PNI and LVI are distinct anatomical entities, they often occur together. Previous studies have demonstrated that concurrent PNI and LVI were associated with particularly poor outcomes in gastric cancer [1,2]. However, the majority of studies still treat PNI and LVI as independent adverse pathological variables or mainly combine them for prognosis stratification. Far fewer studies have focused on whether their coexistence constitutes a consistent invasive phenotype with a common tumor-microenvironmental basis.

The invasive front could be the most plausible site for such convergence, since here malignant cells are exposed to the extracellular matrix, fibroblasts, immune cells, peripheral nerves, blood vessels, and lymphatic channels, in close vicinity. Spatially resolved analyses of gastric cancer are increasingly revealing that clinically relevant cellular ecosystems and regions within the tumor microenvironment can be distinguished beyond the conventional histological classification [18,19].

On this basis, we propose the designation of Gastric Cancer Neurovascular Invasion Syndrome (GC-NVIS) for a candidate clinicopathological phenotype consisting of concomitant PNI and LVI. We posit that the histological substrate of the syndrome may represent a spatially organized Neurovascular Invasive Niche (NVIN) comprising malignant cells, peripheral nerves, lymphovascular structures, and associated stromal elements at the invasive tumor front. The intent is not to nominate an already established syndrome but to introduce a falsifiable syndrome-development framework and determine whether available clinicopathological evidence supports progression to direct histological and spatial testing.

Rationale for Proposing GC-NVIS as a New Clinicopathological Syndrome

Proposing a new clinicopathological syndrome demands more than demonstrating that two adverse findings occur within the same tumor. Both PNI and LVI are well-established in gastric-cancer pathology, and the prognostic importance of each is not questioned; rather, the issue is whether their repeated coexistence represents a reproducible biological state that cannot be captured by either alone. In other words, GC-NVIS is only useful to the extent that it can capture organizational information, not mere accumulation.

The HMU-GC data provide a reason to ask the question. Neural and lymphovascular invasion co-occurred strongly, yet each retained an independent association with high nodal burden when taken together. The double-positive group was also distinct from both the single-positive groups: nearly two thirds of PNI+/LVI+ tumors had N2-N3 disease, whilst high nodal burden was much less frequent when only one compartment was involved. The ordinal 0-2 invasion measure added a further clue by highlighting a graded increase in nodal burden as the number of involved compartments increased.

These observations are consistent with a higher-order invasive phenotype, but they are insufficient to prove that such a phenomenon exists. A deeply invasive tumor may simply have more opportunities to encounter nerves and vessels, and a larger specimen may offer more opportunities for a pathologist to detect both PNI and LVI. The syndrome hypothesis must therefore withstand explanations that relate these findings to tumor depth, sampling intensity, tissue area, nerve density, and vessel density. This is why the proposed construct deliberately isolates its diagnostic components from its validating outcomes.

We also avoid equating a syndrome with a statistical interaction. The PNI×LVI interaction estimate found in this cohort was biologically suggestive but did not cross the conventional significance threshold. GC-NVIS is thus not presented as proof that neural invasion causes vascular invasion (or the reverse), but rather as a proposal for a new syndrome that may underlie both. A more plausible early model is that both phenomena may emerge

from a permissive invasive-front microenvironment generated by loss of cohesion, stromal remodeling, neurotrophic signaling, lymphangiogenesis, and local cellular interactions.

The decisive question is anatomical: when PNI and LVI occur together, are they distributed randomly across an aggressive tumor, or do they repeatedly converge within the same microscopic neighborhoods? The Neurovascular Invasive Niche (NVIN) is proposed to test this very idea. If invaded nerves, involved vessels, tumor, and associated stroma show reproducible spatial organization (and if that organization adds information beyond binary PNI and LVI), then the case for GC-NVIS is substantially strengthened. If they do not, the more appropriate interpretation would be a high-risk composite marker than a new syndrome.

Working Definition of GC-NVIS

At this point in time, GC-NVIS is best viewed as a working research construct, and for the clinicopathological phase candidate GC-NVIS (GC-NVIS-v1) is defined by concurrent histologically documented PNI and histologically documented LVI. This deliberately simple definition is consistent with the use of the existing HMU-GC annotations without importing clinical outcome into the syndrome. The definition is intentionally narrower than the biological hypothesis; PNI and LVI are the anchors of the construct while tumor stage, nodal burden, metastasis, recurrence, and survival remain external validators. The exclusion of these outcomes is critical to avoid circular analysis: if one were to import N2-N3 disease into GC-NVIS and then report that the syndrome predicted N2-N3 disease, the analysis would be circular.

The proposed second version (GC-NVIS-v2) requires an additional histological layer, demonstrable NVIN. This would translate to requiring a tumor to have PNI, LVI, and evidence that the neural and lymphovascular events are spatially coupled in the tumor's invasive-front microenvironment. The spatial criteria should be data-derived, not specified in advance; distances, local densities, co-localization, and repeated clustering should be measured continuously, and a parsimonious threshold or score can be chosen and validated in a separate set. Only once reproducibility, incremental value, and external replication have been demonstrated should one consider using the unqualified term GC-NVIS. Until then, the terminology to use in this manuscript (candidate, proposed, or provisional GC-NVIS) should be viewed as qualifying the construct as a hypothesis being tested.

Primary Study Hypothesis

Our central hypothesis is that concurrent PNI and LVI in gastric carcinoma may reflect a coordinated neurovascular invasive state, rather than the coincidence of two adverse histopathological findings. We propose to refer to this state as GC-NVIS for the present, on the provisional basis that the hypothesis has not been contradicted by further evidence. The hypothesis does not assume that one pathway causes the other but proposes that a

subset of tumors can develop an invasive-front environment that will support both neural tropism and lymphovascular invasion.

Our second, more demanding hypothesis is that this state has a measurable spatial substrate. We therefore predict candidate GC-NVIS tumors will be enriched for a Neurovascular Invasive Niche in which invaded nerves, involved lymphovascular channels, tumor cells, and remodeled stroma occur in non-random proximity. If quantitative features of this niche improve biological or prognostic discrimination beyond conventional binary PNI and LVI, then this would be evidence for a progression from a composite phenotype to a distinct clinicopathological syndrome.

Objectives

Primary objective: To determine whether concomitant PNI and LVI define a reproducible clinicopathological and spatially organized invasive phenotype that can serve as the basis for proposing GC-NVIS.

- To characterize the clinicopathological phenotype associated with concomitant PNI and LVI.
- To compare candidate GC-NVIS tumors with PNI-only, LVI-only and neurovascular-negative gastric cancers.
- To determine whether candidate GC-NVIS is independently associated with high regional nodal burden after adjustment for established clinicopathological variables.
- To evaluate whether increasing neurovascular invasion burden demonstrates a biological gradient with tumor aggressiveness.
- To identify a histological Neurovascular Invasive Niche using whole-slide images from the HMU-GC cohort.
- To quantify tumor-nerve, tumor-vessel and nerve-vessel spatial relationships within the invasive tumor front.
- To determine whether the neurovascular spatial organization provides information beyond PNI and LVI considered separately.
- To develop a preliminary quantitative Neurovascular Invasion Burden Score.
- To establish preliminary diagnostic criteria for candidate, spatially supported and validated GC-NVIS.
- To provide a framework for subsequent external validation in independent gastric-cancer cohorts.

Materials and Methods

Study Design

We designed the project as a sequential syndrome-discovery, followed by histopathological-validation study. The first stage takes the clinical portion of HMU-GC to ask whether PNI and

LVI are related but non-redundant dimensions of invasion, and co-exist as a distinct clinicopathological subgroup. The second stage then proceeds from labels to tissues: targeted whole-slide analysis will test whether the double-positive phenotype has a reproductions spatial architecture consistent with the proposed NVIN.

This sequence reflects a deliberate choice. By starting with the clinical spreadsheet, we can identify the most informative patient groups before moving into the laborious process of image review. We also avoid making the image phase an indiscriminate search across thousands of patches: all inferential analyses are grounded at the patient level. Multiple patches or regions from the same tumor may contribute morphometric information, but they will be aggregated within patient so that repeated sampling of one tumor does not masquerade as independent biological replication.

Dataset and Study Population

The HMU-GC-HE-30K dataset includes 300 gastric-cancer whole-slide images associated with the de-identified clinicopathological parameters. The slides were taken from the Harbin Medical University Cancer Hospital's archives and scanned at 20× magnification. The clinical file contained age, sex, pathological T and N categories, M category, overall pathological stage, histological type, Lauren classification, LVI, separate venous-invasion field, PNI, stromal quantity, infiltration pattern, and HER2 score [20].

For the syndrome-discovery analysis, inclusion criteria were the presence of interpretable PNI and LVI fields instead of the disease outcome. Both PNI and LVI were available for 248 and 250 patients, respectively. For 239 patients, the findings were complete. This defines the main four-phenotype population for this study. The variables with incomplete data were kept for secondary analyses that used an open denominator of complete cases. The missing values were neither excluded nor replaced with 'not detected'. The predominance of males among the patients and the single-center origin of the material were considered limitations rather than adjusted for in statistical analyses.

Operational Neurovascular Phenotypes

Patients with complete PNI and LVI information were divided into four mutually exclusive phenotypes. The four-group structure was important because it disentangled the effect of dual invasion from that of either single invasion and compared the dual-positive group with an undifferentiated remainder, rather than an undifferentiated whole.

- a) NV0: PNI-/LVI-.
- b) NV-N: PNI+/LVI-.
- c) NV-V: PNI-/LVI+.
- d) Candidate GC-NVIS-v1: PNI+/LVI+.

The pathological T category, nodal status, M category, overall stage, recurrence, survival, and other outcomes have been excluded from the diagnostic definition. They have been categorized as external validators because they have a potential to test the putative biological relevance of the GC-NVIS to the outcomes but should not be part of the diagnostic definition. The exclusion is particularly relevant in the context of a syndrome-development study where a diagnostic definition that formally includes an outcome variable might generate an apparently strong association through construction.

Neurovascular Invasion Burden

In addition to the four categorical phenotypes, we employ an exploratory ordinal measure that simply counts the number of involved compartments, with 0 indicating neither PNI nor LVI, 1 either PNI or LVI, and 2 both: the purpose of this variable is to test for a biological gradient rather than to construct a clinical score. A monotonic increase in aggressive features from 0 to 1 to 2 involved compartments would be consistent with an increase in invasive capability. However, this measure gives equal weight to PNI and LVI, and contains no information about their extent or spatial relationship. Therefore, it is considered to be a preliminary burden indicator, the later NVIBS being intended to incorporate quantitative neural, vascular, and spatial information.

Outcomes

High regional Nodal burden is the main external biological validator and is defined as pathological N2, N3a, or N3b with N0-N1 as the comparator. The rationale for this choice was the ability to differentiate between the extent of regional spread rather than any positive lymph nodes. This seemed especially pertinent in the initial analysis as the prognostic role of PNI differed between any N+ and N2-N3.

The secondary validators included any nodal metastases, T3-T4, any stage III-IV tumor, M category if adequately populated, infiltration pattern, histology, Lauren classification, and stromal features. Survival, recurrence, and treatment outcomes were unavailable in the current clinical file and thus could not be utilized for the current analysis; these will instead be used in an external longitudinal cohort to validate the proposed PNI-associated syndrome.

Clinicopathological Syndrome Discovery

Stage I comprises four questions. First, is an association between PNI and LVI more frequent than expected by chance? Second, when both are included in the same adjusted model, is each variable informative on the outcome of interest, namely high nodal burden, suggesting that they are not interchangeable? Third, do the four neurovascular categories differ clinically, for example, in the context of double-positive versus single-positive phenotypes? Fourth, is there a gradient in the number of involved compartments? Categorical associations were evaluated by

contingency tables and Pearson chi-square tests, with Fisher exact testing when the expected cell count was less than 5; effect sizes were expressed by odds ratios with 95% confidence intervals instead of P values for dichotomous outcomes. Multivariable logistic regression analysis adjusted for possible confounders such as age, sex, pathological T category, and infiltration pattern. Finally, note that the analysis was not strictly confirmatory but rather aimed to explore whether the observed pattern was sufficiently coherent to warrant a detailed study of the tissue level.

Interaction Analysis

A PNI×LVI product term is included to investigate multiplicative interaction. We pose a tighter statistical question than that defined by the syndrome hypothesis: whether the joint association of PNI and LVI with the outcome is greater than would be predicted by a multiplicative model. We therefore treat interaction as supportive evidence, not a precondition for GC-NVIS. Such a distinction is important because a set of complementary features may constitute a biological syndrome without fulfilling a particular mathematical definition of synergy. By contrast, a significant interaction term would per se not define a new syndrome. The interaction estimate must be evaluated in context of the effect sizes, confidence limits, the four-group pattern, the invasion gradient, and (in a later step) the direct spatial morphology. Larger external cohorts should additionally consider interaction on an additive scale.

Extreme-Phenotype Histological Discovery

The initial slide-based discovery phase is enriched for extremes of biology. For example, approximately 67 patients are PNI+/LVI+ with N2-N3 disease versus approximately 31 being PNI-/LVI- with N0 disease. Comparing these extremes increases the contrast and, thus, makes it more practical to discover candidate microscopical features before reviewing the entire image set.

Nodal status is used for discovery enrichment only and is not part of the GC-NVIS definition; as such, it cannot be carried forward as a diagnostic requirement. Features discovered in the extreme groups (e.g., short nerve vessel distances, clustered PNI/LVI foci, increased density of nerve/vessel, or a particular stromal pattern) must be locked before they are tested in the remaining patients. The validation set should specifically include double-positive cases with low nodal burden and double-negative cases with nodal disease since these discordant tumors provide the most insight on if the spatial phenotype adds any information beyond what was used for the discovery.

Whole-Slide Histological Assessment

The invasive tumor front is the main region of interest because this is where malignant cells meet nerves, lymphatic channels, blood vessels, fibroblasts, immune cells, and remodeled extracellular matrix in the same tissue neighborhood — the tumor center may be sampled as a comparator but cannot substitute if

the relevant interface is absent. Whole-slide or region-of-interest sampling will preserve the connection to the source patient and repeated image patches from the same case will be summarized to patient-level measures to avoid pseudoreplication.

Neural assessment should move beyond a binary PNI label. Candidate variables include the number and density of PNI foci, diameter of involved nerves, percentage of nerve circumference involved by tumor; intra- vs. perineural locations, minimum tumor-nerve distance, local nerve density, and evidence of neural remodeling. Reviewers should distinguish true PNI from simple tumor-nerve apposition and from tangential sections or retraction artifacts, using accepted morphological principles [21-23].

The vascular assessment should similarly quantify LVI focus count and density, tumor-embolus size, involved-channel caliber, tumor-vessel distance, and local vascular density. On routine H&E, lymphatic and venous channels may be hard to separate reliably. The source file further complicates this distinction because its venous-invasion field is perfectly concordant with LVI in jointly evaluable records. For that reason, the present clinical phase uses the broader term LVI. If tissue is available, D2-40/podoplanin, endothelial markers such as CD31/CD34, and elastic stains could later refine the lymphatic and venous components [24-26].

Definition and Quantification of NVIN

NVIN is a provisional definition of a spatial domain within the invasive tumor front in which malignant cells, neural and lymphovascular structures are found in proximity and display coordinated pathological involvement. It's a spatial concept, not molecular, at least for the time being. A niche should be demonstrable on tissue architecture before more speculative signaling pathways are invoked to explain it.

The core morphometric variables would be minimum tumor-nerve distance (D_TN), tumor-vessel distance (D_TV), and nerve-vessel distance (D_NV). These distances need to be supplemented by local nerve and vessel densities, nearest-neighbor relationships, counts of PNI and LVI events within the same region, co-localization statistics, tumor budding, stromal quantity, and a qualitative description of the invasive interface. Repeated occurrence of the same pattern in multiple locations within a tumor would be needed to argue that NVIN represents organization not just chance juxtaposition.

No distance threshold is pre-specified. A rule such as “within 100 micrometers” would be easy to apply, but would be entirely arbitrary before the data are examined. Distances will need to be evaluated continuously first. Candidate cut points might then be identified using distributional analysis, restricted cubic splines, ROC methods, mixture models, or clustering. Any threshold identified during discovery needs to be fixed before internal validation, and its performance should be reported with confidence intervals rather than as a definitive biological boundary.

Proposed Quantitative Neurovascular Invasion Score

If the slide review identified reproducible quantitative features, these could be combined into a Neurovascular Invasion Burden Score (NVIBS). This score could be a compact summary of three domains: neural invasion burden, vascular invasion burden, and spatial coupling. Candidate inputs could include PNI density, LVI density, nerve and vessel density, involvement of larger nerves or vessels, and the degree of neurovascular proximity or co-localization. Weights should be estimated empirically, rather than assigned by intuition, but the resulting model should not be so complex as to be uninterpretable. A highly complex score based on dozens of unstable image features would be extremely challenging to validate and unlikely to translate into pathology practice. Penalized regression or dimensional-reduction methods could be valuable tools during development, followed by simplification and bootstrap assessment of optimism. The goal would be not only discrimination, but a biologically interpretable measure amenable to reproduction by pathologists or computational systems.

Unsupervised Phenotype Discovery

A complementary exploratory analysis might test the hypothesis that a neurovascular phenotype can occur independent of nodal status, stage, or the proposed syndrome label. Hierarchical clustering, latent-class analysis, or mixture modeling could be applied to standardized morphometric variables including PNI density, LVI density, nerve and vessel density, D_NV, tumor budding and stromal features.

This analysis would be productive given the opportunity it provides for the data to contradict the proposed construct. If a cluster independently derived on the basis of these variables proves to be enriched for PNI, LVI, short neurovascular distances and poor outcome, construct validity would be enhanced. On the other hand, failure to find a coherent cluster or evidence that the dominant clusters reflect differences in histological subtypes or depth of tumor invasion rather than neurovascular features would require re-evaluation of the syndrome hypothesis.

Blinded Review and Reproducibility

Histological reviews should be performed blinded to N category, overall pathological stage, and metastatic status. Blindness is critically important since knowing that a case has extensive nodal disease could subconsciously influence interpretation of equivocal PNI, LVI, or spatial proximity. At least two observers should independently assess a representative subset before final criteria are locked.

Agreement for categorical findings should be summarized using kappa statistics, whereas continuous morphometric measurements should be evaluated using intraclass correlation coefficients. Disagreements should be dissected to determine which features are intrinsically ambiguous and which can be clarified through operational definitions or ancillary stains. A

syndrome that cannot be consistently recognized is unlikely to be of value, regardless of its statistical association with outcome.

Statistical Models and Incremental Value

The adjusted main analysis for N2-N3 disease includes GC-NVIS phenotype, pathological T category, infiltration pattern, age, and sex. The extended models explore the prognostic role of Lauren classification, histological type, stromal amount, and HER2, if the number of cases allows a reliable estimation of the treatment effect. The effect estimates are reported as adjusted odds ratios with 95% confidence intervals.

We evaluated the incremental value of the novel prognostic variable using a sequence of nested models instead of calculating the AUC. First, the traditional clinicopathological model was compared with the same model supplemented with the PNI, then with the addition of LVI, then with the addition of both PNI and LVI. We also tested the model with the four-group GC-NVIS phenotype replacing the PNI and LVI, and, if applicable, explored the value of supplementing the clinicopathological model with the four-group GC-NVIS phenotype with additional PNI or LVI. Thus, we formulate the research question: does the new model provide additional prognostic information over and above the information provided by its elements?

The comparison of nested models should be based on likelihood-ratio tests or other model selection criteria such as AIC, BIC, ROC-AUC, calibration intercept and slope, and Brier score. Due to the limited size of the study cohort, bootstrap resampling with optimism correction is recommended for internal validation. Improving the discrimination power of the model (measured by ROC-AUC) is not enough to claim that a syndrome has clinical value. The calibration slope and the shrinkage of coefficients should also be acceptable, and the results of different analyses should agree within reasonable limits.

Sensitivity Analyses and Missing Data

Sensitivity analyses will be undertaken to assess the dependence of the main findings on a particular outcome definition or case mix. This includes an ordinal analysis of N category, exclusion of M1 disease, stratification by Lauren classification and T category, and re-analysis within major pathological stages. Analyses within pathological stage are particularly important as they test the hypothesis that GC-NVIS captures biological heterogeneity that goes beyond mere anatomical extent.

Missingness will be reported for each variable used in modeling. The primary phenotype requires observation of PNI and LVI status, so that complete-case analysis is performed for that definition. For the slide phase, reasons for unevaluable are to be explicitly recorded, including inadequate invasive front, image artifact, absence of assessable neural structures, or inability to classify a vascular space. Unevaluable tissue should never be silently recoded as negative.

Evidence Levels

A four-level evidence framework is used to avoid premature naming: an intent of clarifying the current evidence base while also delineating the evidence needed in support of future statements. Advancement from one level to the next in this hierarchy requires additional evidence beyond more assertive language applied to the same body of evidence.

- Level 1 - Candidate GC-NVIS: PNI+ and LVI+ with a distinct clinicopathological phenotype
- Level 2 - Histologically supported GC-NVIS: PNI+ + LVI+ + NVIN+
- Level 3 - Internally validated GC-NVIS: reproducible and incrementally informative beyond binary PNI/LVI
- Level 4 - Externally validated GC-NVIS: replicated in independent cohorts with outcome validity

This staging allows for a failure of the hypothesis in a

constructive sense: a phenotype that demonstrates prognostic utility but lacks reproducible spatial organization could still be deserving of recognition as a combined high-risk marker but would fall short of the criteria we propose for a neurovascular syndrome.

Ethical Considerations

This work is a secondary analysis of publicly available, de-identified clinical data and histological material. The original HMU-GC study reported approval by the Research Ethics Committee of Harbin Medical University Cancer Hospital (KY2024-16) [20]. The present analysis does not involve any new patient contact, intervention, or collection of identifiable information. Due to the project's intention to propose a new pathological construct, responsible interpretation is also an ethical consideration. GC-NVIS should not be presented as a clinically actionable diagnosis until independent validation demonstrates reproducibility and meaningful incremental value. The terminology used in this manuscript is therefore deliberately provisional (Figure 1).

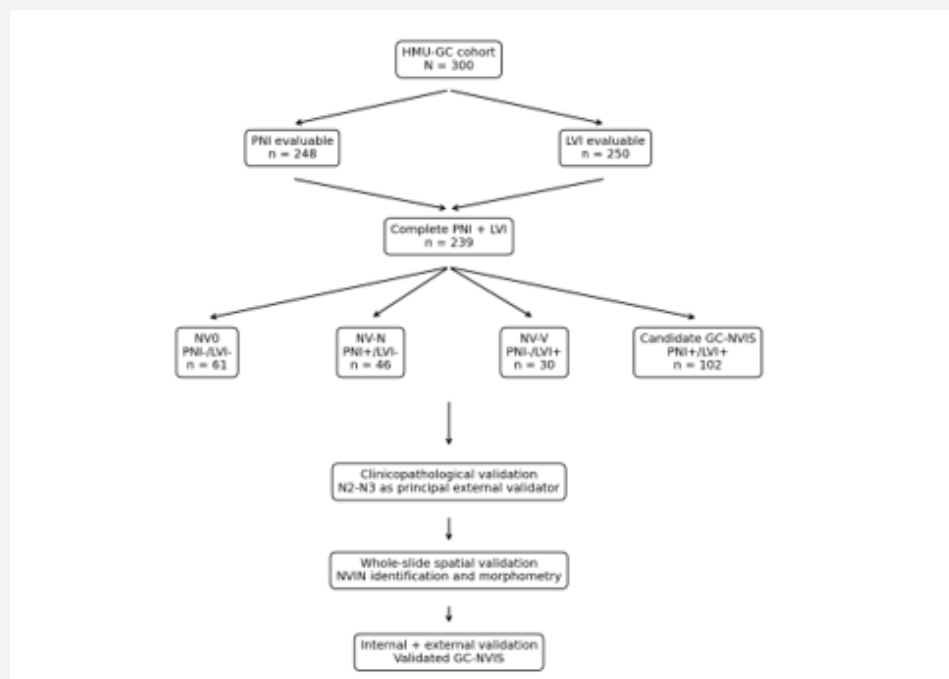


Figure 1: Development pathway for GC-NVIS. The HMU-GC cohort is first classified according to PNI and LVI, followed by clinicopathological validation, whole-slide spatial testing of the proposed NVIN, and subsequent internal and external validation.

Results

Study Population

The number of unique patients in our data set was 300. The mean age of the participants was 60.77 ± 9.09 , with 236 (78.7%) being male. The data set included PNI information on 248 patients, including 157 positive cases (63.3%). Additionally, it

included LVI data from 250 patients, of which 142 were positive (56.8%). In total, 198/300 (66.0%) patients presented with N+ disease, 128/300 (42.7%) presented with N2-N3, 231/300 (77.0%) presented with T3-T4, and 156/300 (52.0%) presented with stage III-IV. Finally, the data set included complete PNI/LVI information for 239 patients (Table 1).

Table 1: Baseline clinicopathological characteristics of the HMU-GC cohort relevant to GC-NVIS development.

Characteristic	Result
Total patients	300
Age, mean $\pm$ SD	60.77 $\pm$ 9.09 years
Male	236 (78.7%)
Female	64 (21.3%)
PNI evaluable	248
PNI positive	157/248 (63.3%)
LVI evaluable	250
LVI positive	142/250 (56.8%)
Complete PNI + LVI	239
Any N+	198/300 (66.0%)
N2-N3	128/300 (42.7%)
T3-T4	231/300 (77.0%)
Stage III-IV	156/300 (52.0%)

Table 2: Relationship of perineural and lymphovascular invasion with aggressive pathological characteristics.

Comparison	N	OR	95% CI	P value
PNI vs LVI	239	4.51	2.58-7.88	<0.000001
PNI vs any N+	248	2.27	1.31-3.94	0.003
PNI vs N2-N3	248	3.7	2.09-6.57	<0.00001
PNI vs T3-T4	248	6.24	3.01-12.97	<0.000001
PNI vs stage III-IV	248	4.02	2.33-6.95	<0.000001
LVI vs any N+	250	3.99	2.26-7.05	<0.000001
LVI vs N2-N3	250	3.95	2.30-6.78	<0.000001

Table 3: Clinicopathological characteristics according to neurovascular invasion phenotype.

Characteristic	NV0 PNI-/LVI- n=61	NV-N PNI+/LVI- n=46	NV-V PNI-/LVI+ n=30	Candidate GC-NVIS PNI+/LVI+ n=102	P value
Age, mean $\pm$ SD	61.2 $\pm$ 9.1	58.9 $\pm$ 10.1	64.4 $\pm$ 8.2	61.8 $\pm$ 8.6	0.072
Male	47 (77.0%)	41 (89.1%)	24 (80.0%)	77 (75.5%)	0.287
T3-T4	37 (60.7%)	40 (87.0%)	23 (76.7%)	96 (94.1%)	<0.00001
Any N+	30 (49.2%)	27 (58.7%)	22 (73.3%)	84 (82.4%)	<0.0001
N2-N3	14 (23.0%)	15 (32.6%)	8 (26.7%)	67 (65.7%)	<0.000001
Stage III-IV	18 (29.5%)	28 (60.9%)	13 (43.3%)	74 (72.5%)	<0.000001
INFc	9 (14.8%)	4 (8.7%)	7 (23.3%)	30 (29.4%)	0.073

Table 4: Multivariable logistic regression for high nodal burden (N2–N3 disease).

Variable	Adjusted OR	95% CI	P value
PNI positive	2.41	1.24-4.68	0.01
LVI positive	2.38	1.29-4.40	0.006
INFb vs INFa	1.96	1.01-3.80	0.047
INFc vs INFa	2.18	1.04-4.58	0.04
T category, per-category increase	1.34	0.88-2.05	0.175
Age, per year	1.02	0.99-1.06	0.18
Male sex	1.15	0.57-2.33	0.702

Table 5: Current evidence supporting GC-NVIS as a candidate clinicopathological syndrome.

Proposed criterion	Evidence from HMU-GC	Status
Strong PNI-LVI co-occurrence	OR 4.51; P<0.000001	Supported
Components not completely redundant	Both independently associated with N2-N3	Supported
Distinct dual-positive phenotype	N2-N3 65.7% vs 23.0-32.6%	Supported
Independent combined association	aOR 4.68 for N2-N3	Supported
Biological invasion gradient	aOR 2.39 per compartment	Supported
Proven PNI×LVI multiplicative synergy	Interaction P=0.059	Not established
Reproducible neurovascular morphology	Whole-slide analysis required	Untested
Neurovascular spatial coupling	Spatial morphometry required	Untested
Incremental value beyond binary PNI/LVI	Spatial model required	Untested
Interobserver reproducibility	Independent review required	Untested
External replication	Independent cohort required	Untested
Survival validation	Unavailable in current CSV	Untested

Table 6: Preliminary diagnostic classification of Gastric Cancer Neurovascular Invasion Syndrome.

Classification	PNI	LVI	NVIN	Interpretation
NV0	-	-	-/NA	Neurovascular-negative
NV-N	+	-	NA	Neural-dominant invasion
NV-V	-	+	NA	Vascular-dominant invasion
GC-NVIS-v1	+	+	Not as- sessed	Candidate syndrome
GC-NVIS-v2	+	+	+	Spatially supported syndrome
Validated GC-NVIS	+	+	+	Externally reproduced syndrome

Note: TNM stage, nodal burden, metastasis, recurrence, and survival are external validators and are not included in the diagnostic definition

Neural-Vascular Co-occurrence

Among the 239 patients with complete PNI and LVI information, the group was distributed as 61 PNI-/LVI-, 46 PNI+/LVI-, 30 PNI-/LVI+, and 102 PNI+/LVI+. Thus, the dual positive group accounted for 42.7% of the evaluable population that fulfilled the clinicopathological criteria for inclusion in the GC-NVIS-v1 candidate repository. The association between the two invasion pathways was strong. PNI-positive tumors had an increased odds ratio for LVI of 4.51 (95% CI 2.58-7.88; P<0.000001). Odds ratio of this magnitude provide a first empirical justification to consider their co-occurrence in a multivariate analysis, albeit without definitive answers to whether they reflect shared biological features, increased tumor depth, or greater sampling opportunities (Table 2).

Four Neurovascular Phenotypes

Among the 239 patients with complete PNI and LVI information, the group was distributed as 61 PNI-/LVI-, 46 PNI+/LVI-, 30 PNI-/LVI+, and 102 PNI+/LVI+. Thus, the dual positive group accounted for 42.7% of the evaluable population that fulfilled the clinicopathological criteria for inclusion in the GC-NVIS-v1 candidate repository. The association between the two invasion

pathways was strong. PNI-positive tumors had an increased odds ratio for LVI of 4.51 (95% CI 2.58-7.88; P<0.000001). Odds ratio of this magnitude provide a first empirical justification to consider their co-occurrence in a multivariate analysis, albeit without definitive answers to whether they reflect shared biological features, increased tumor depth, or greater sampling opportunities (Table 3) (Figure 2).

Magnitude of the Dual-Invasion Phenotype

Using NV0 as the reference, candidate GC-NVIS was associated with notably higher unadjusted odds for several adverse pathological outcomes: any nodal metastasis (OR 4.82, 95% CI 2.36-9.86), N2-N3 disease (OR 6.43, 95% CI 3.12-13.25), T3-T4 disease (OR 10.38, 95% CI 3.93-27.42), and stage III-IV disease (OR 6.31, 95% CI 3.13-12.73). All four associations were highly statistically significant. The particularly strong association with N2-N3 disease is central to the proposed construct. It suggests that the dual phenotype may be more related to the quantity of regional dissemination, rather than a simple binary presence/absence of nodal involvement. Given that these are unadjusted comparisons, however, they have been treated as descriptive evidence and proceeded to multivariable modeling.

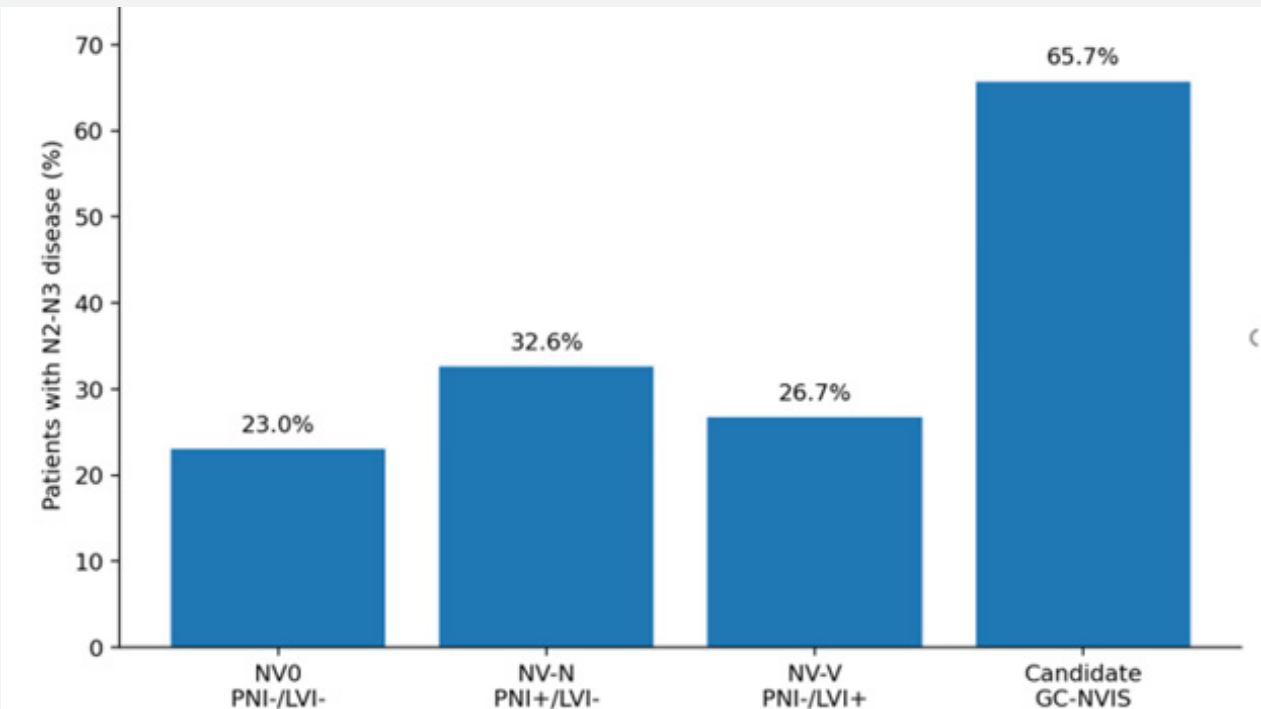


Figure 2: Frequency of high regional nodal burden according to neurovascular phenotype. N2-N3 disease was markedly enriched in candidate GC-NVIS compared with the other groups.

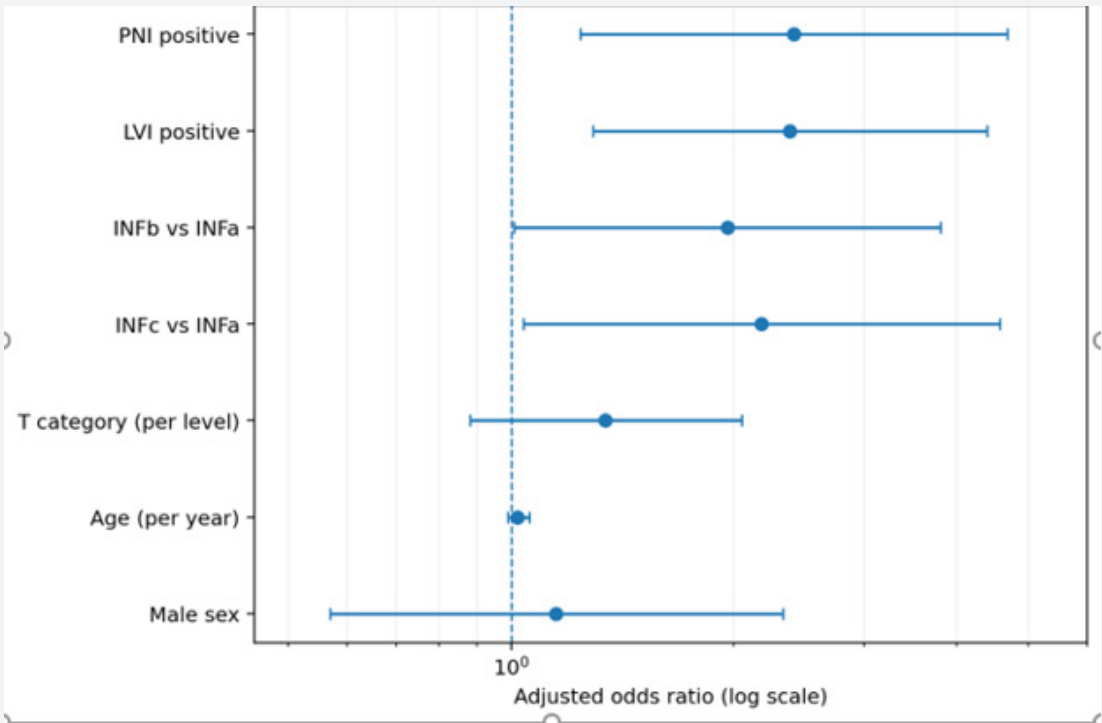


Figure 3: Forest plot of adjusted associations with N2-N3 nodal disease. PNI and LVI retained independent associations after adjustment for age, sex, T category, and infiltration pattern.

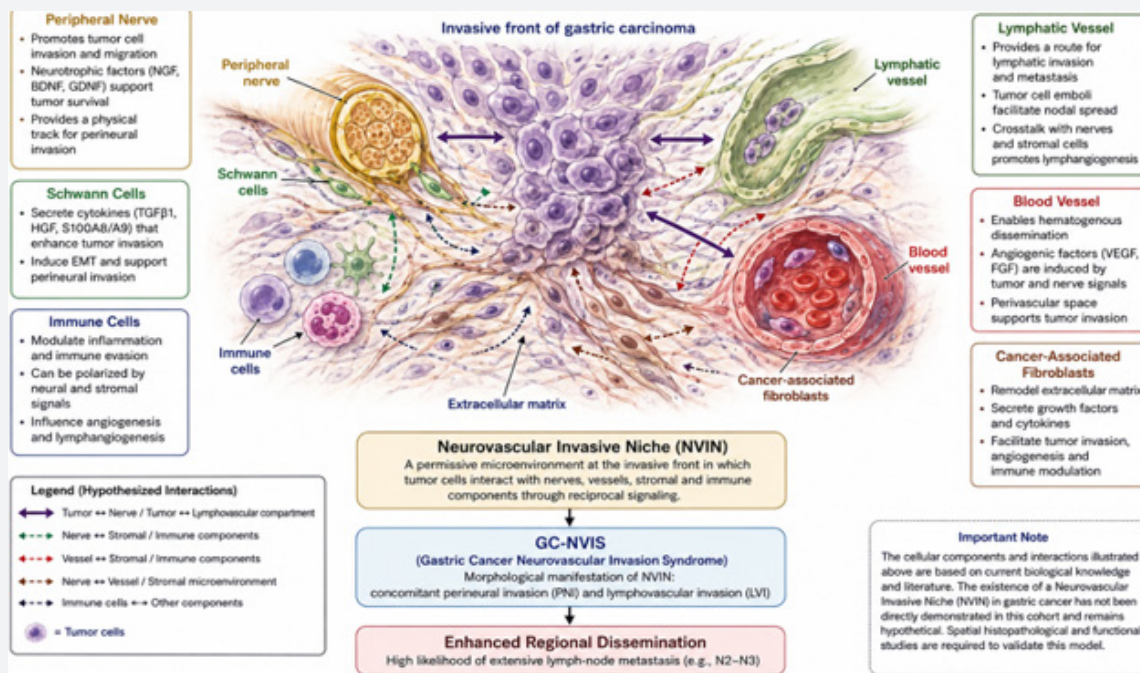


Figure 4: Proposed Neurovascular Invasive Niche underlying GC-NVIS. Conceptual model illustrating a putative invasive-front microenvironment in which gastric carcinoma cells interact with peripheral nerves, Schwann cells, lymphatic and blood vessels, stromal cells, immune cells, and extracellular matrix. The illustrated molecular and directional interactions are hypothetical and require direct spatial and functional validation.

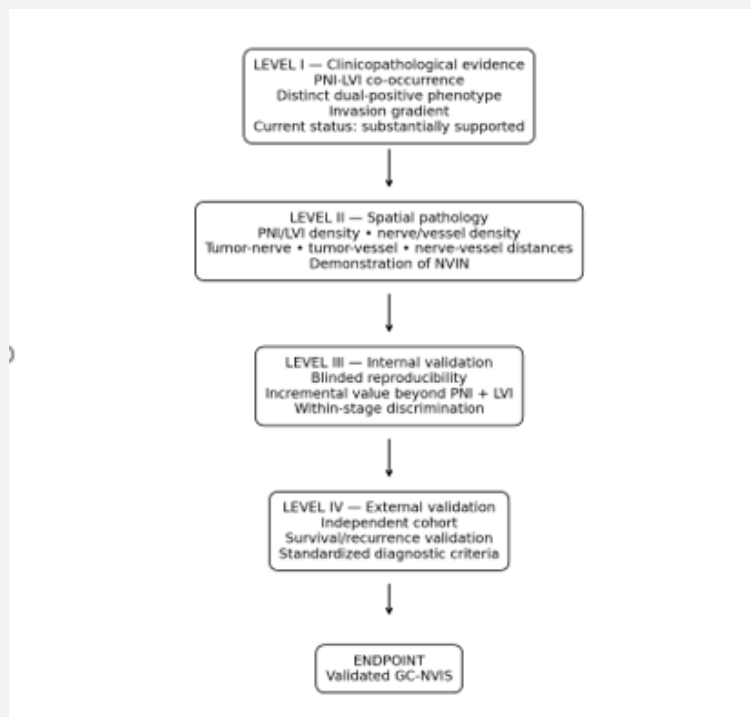


Figure 5: Proposed evidence pathway for validation of GC-NVIS. Current HMU-GC findings provide Level I clinicopathological evidence; spatial pathology, reproducibility, incremental value, and independent cohort validation remain required.

Independent Contributions of PNI and LVI

Using NV0 as the reference, candidate GC-NVIS was associated with notably higher unadjusted odds for several adverse pathological outcomes: any nodal metastasis (OR 4.82, 95% CI 2.36-9.86), N2-N3 disease (OR 6.43, 95% CI 3.12-13.25), T3-T4 disease (OR 10.38, 95% CI 3.93-27.42), and stage III-IV disease (OR 6.31, 95% CI 3.13-12.73). All four associations were highly statistically significant. The particularly strong association with N2-N3 disease is central to the proposed construct. It suggests that the dual phenotype may be more related to the quantity of regional dissemination, rather than a simple binary presence/absence of nodal involvement. Given that these are unadjusted comparisons, however, they have been treated as descriptive evidence and proceeded to multivariable modeling (Table 4) (Figure 3).

Combined Phenotype, Gradient, and Interaction

When the four-category phenotype was used, the candidate GC-NVIS had an adjusted OR of 4.68 and 4.68 for N2-N3 disease versus the NV0 (95%CI: 2.17-10.12; $P < 0.001$). Single positive groups did not show significant differences from the double negative reference after adjustment, which adds weight to the finding as an interesting clinicopathological observation worthy of further consideration.

The ordinal burden analysis yielded an additional insight into the pattern of association. An increase by one category from zero to one to two invasion compartment positives was associated with a 2.39-fold increased adjusted odds of N2-N3 disease (95%CI: 1.63-3.52; $P < 0.00001$). Thus, the data indicate a dose response pattern, rather than simply a presence or absence of any adverse invasion feature.

Finally, the formal multiplicative interaction analysis, while more conservative, did not contradict the findings. The PNI×LVI interaction OR was 3.60 (95%CI: 0.95-13.63; $P = 0.059$). Given the uncertainty around the point estimate, ranging from close to null to very large positive value, we do not interpret the interaction as evidence of synergy, rather strong co-occurrence of two separate effects, with possibly distinct mechanisms.

Any Nodal Metastasis Versus High Nodal Burden

A different pattern was observed for any nodal metastasis compared to N2-N3 disease. LVI remained independently associated with N+ status (aOR 2.61, 95% CI 1.33-5.09; $P = 0.005$), whereas PNI did not (aOR 1.22, 95% CI 0.60-2.46; $P = 0.585$). This finding suggests that the two invasion compartments may provide distinct biologic insight in specific subgroups depending on the extent of regional dissemination. It is plausible that LVI reflects a proximity to lymphatic or vascular channels and therefore has a greater impact on the transition from N0 to any N+ than PNI. In contrast, PNI may indicate a more diffuse invasive process that accumulates risk when nodal disease is extensive. This hypothesis

is consistent with a recent report that nodal burden modulates the prognostic effect of PNI in stage I-III gastric cancer [27].

Evidence Supporting the Proposed GC-NVIS Construct

Taken together, the clinicopathological results satisfy several of the early criteria for syndrome discovery: strong co-occurrence, partial non-redundancy, a distinct dual-positive phenotype and a graded invasion-burden relationship. However, they do not yet satisfy the morphological and validation criteria that would be needed for an established syndrome. Table 5 separates the evidence already present in HMU-GC from the questions that remain open (Table 5).

Preliminary Diagnostic Classification

Taken together, the clinicopathological results satisfy several of the early criteria for syndrome discovery: strong co-occurrence, partial non-redundancy, a distinct dual-positive phenotype and a graded invasion-burden relationship. However, they do not yet satisfy the morphological and validation criteria that would be needed for an established syndrome. Table 6 separates the evidence already present in HMU-GC from the questions that remain open.

Discussion

Principal Findings

The most impressive finding in this study is not PNI/LVI as adverse features, which are already well-known to gastric cancer pathologists. Rather the relationship between the two was intriguing. While they are very closely associated (strong co-occurrence), neither is rendered redundant by the presence of the other in this adjusted analysis. Furthermore, this double-positive category appears to be clearly distinct from the single-positive groups, with almost two thirds showing N2-N3 disease.

This provides a good foundation to consider this syndrome hypothesis further. This entity would need to be recognizable (check), not subsumed into one of its components (check), and represent a meaningful biological gradient (check). The PNI+/LVI+ subgroup met these early requirements as it was strongly associated with high nodal burden after adjustment (the adjusted odds ratio was strong) and the 0-2 invasion count showed an increasingly rising odds of N2-N3 disease (PNI+ had increased odds, PNI+/LVI+ had increased odds beyond that).

At the same time, this analysis raises important limitations for this specific claim. The interaction term was borderline (survival information is not available), and the current variables are binary annotations rather than quantitative histology. Most importantly, there is no proof yet that nerves and vessels are spatially organized within the same invasive microenvironment. For this reason, we regard it as Level I evidence - sufficient for driving targeted histological discovery, but not sufficient to declare a new established syndrome at this point.

Why PNI Plus LVI Alone Is Not Sufficient

PNI and LVI have been subjected to extensive investigations both independently and in connection for many years. Meta-analyses and cohort studies have already established an association of PNI with deeper invasion, lymphatic and vascular invasion, node metastasis and decreased survival, while LVI is similarly associated with nodal spread and adverse outcomes [5,13,14]. In addition, Hwang et al. [1] and Zhang et al. [2] showed that the coincidence of PNI and LVI brings additional adverse prognostic information [1,2]. The fact of the coincidence of these two invasions cannot be presented as a novelty.

The proposed work goes deeper into the analysis of the relationship between PNI and LVI. The question is whether such a coincidence always reflects an organized state with a characteristic spatial structure, consistent with a detectable tumor burden and biological behavior. In this formulation, PNI and LVI act as entry criteria for the search for a new syndrome rather than its definition.

This formulation avoids the pitfall of naming diseases by simply combining the names of two known risk factors without providing evidence of a new entity. If the whole-slide analysis reveals that double-positive tumors do not differ significantly from other malignant tumors in morphology, this is not a reason to abandon the concept of GC-NVIS and consider the combination of PNI and LVI as a single entity. This would be the case if reproducible morphological indicators were found in the double-positive tumors, which would carry additional information over and beyond the initial two invasions.

Why Use the Term Syndrome?

The word 'syndrome' is a hypothesis in this context, and not a conclusion. In the domain of clinical medicine and pathology, syndromes are frequently recognizable patterns of findings that arise before the mechanism has been established fully. Justification for this word is therefore not necessarily a single explanatory causal pathway, but a recurring association of features that define a coherent state and can be recognized reproducibly.

GC-NVIS is proposed in this restricted sense. The core of the observable is neural invasion and lymphovascular invasion; the organizing feature is spatial neurovascular coupling. We are not suggesting that GC-NVIS represents a new histological category, a new TNM category, or a diagnosis that should currently change treatment. The word is valuable because it transforms an observation – PNI and LVI often coincide – into a set of explicit, falsifiable questions about tissue organization and clinical relevance.

The qualifier remains crucial. Until spatial morphology, reproducibility, incremental value and independent replication are demonstrated, candidate or provisional GC-NVIS is more accurate than syndrome without qualification. This wording is not just cautious but defines the scientific status of the construct

by making explicit what evidence would be required to move forward.

Relationship to Existing PNI and LVI Evidence

The present findings closely follow the existing literature, rather than contradict it. PNI in gastric cancer has previously been associated with diffuse or poorly differentiated morphology, lymphatic and vascular invasion, deeper tumor penetration, nodal metastasis, and adverse survival [5]. LVI similarly reflects a biologically important step in dissemination and has been linked to nodal involvement, recurrence, and poor prognosis [13,14]. These prior studies provide the background against which GC-NVIS should be interpreted; they are not evidence that this study discovered the individual associations.

What the present analysis adds is the emphasis on the combined phenotype and on high nodal burden. The observation that PNI and LVI remain independently associated with N2-N3 disease suggests that the two findings are not merely interchangeable labels for advanced stage. The four-group comparison further shows that the most pronounced nodal burden is concentrated in tumors with both forms of invasion.

The vascular component will need refinement in future work. Lymphatic and venous invasion are not biologically identical, and routine H&E can underestimate or misclassify both. D2-40/podoplanin can improve recognition of lymphatic channels, while endothelial and elastic stains can clarify blood-vessel invasion [24-26]. A mature GC-NVIS framework may therefore need to distinguish neural-lymphatic, neural-venous, and combined neural-lymphatic-venous subtypes rather than treating all vascular invasion as one compartment.

The Proposed Neurovascular Invasive Niche

NVIN is the critical element differentiating the syndrome hypothesis from merely a PNI/LVI score. We define it as a spatially organized region at the invasive front, in which malignant cells, peripheral nerves, and lymphovascular structures demonstrate reproducible proximity and coordinated pathological involvement. In this model, NVIN represents the microscopic substrate, while the clinicopathological expression at the level of the patient is represented by GC-NVIS.

The idea has biological plausibility, due to the growing appreciation for the role of nerves in cancer as active microenvironmental participants. Tumor cells can exert effects on axonal outgrowth and altered neural signaling, while the neural/Schwann-cell pathway can modulate migration, survival, stromal effects, immunological responses, and angiogenesis [6,8,27,28]. Vascular and lymphatic structures are also dynamic players, which can respond to growth factors, hypoxia, inflammatory cascades, and matrix modulation. The invasive front can thus represent a location where neural and vascular biology might interact, without an assumed simplistic causal chain linking individual nerves to the vasculature.

A true niche must also be more than simply visual proximity in one field. We would expect to see repeated local organizational motifs, such as involved vessels clustering near PNI foci, invaded nerves occurring in vascular-rich regions, characteristic stromal or budding patterns linking the two, or shorter distances between nerve-vessel pairs compared to what would be expected based on local structural density. Ideally, the pattern should repeat itself in different regions of the same tumor as well as across patients.

The most informative test of this would be to look within the double-positive group itself. If PNI+/LVI+ tumors with a strong NVIN signature showed a higher nodal burden or worse longitudinal outcome than PNI+/LVI+ tumors without spatial coupling, then NVIN would represent an additional biological element that the two binary labels could not capture. There must be that incremental step to demonstrate that the niche is more than an attractive concept.

Gastric-Cancer-Specific Mechanistic Support

Gastric cancer provides some exceptionally compelling biological rationale for considering the neural component. Experimental studies of denervation indicate that neural signaling can affect gastric tumorigenesis, and investigation of nerve growth factor uncovered a self-perpetuating loop linking the tumor and aberrant cholinergic innervation [9,10]. Tumor-neural communication was also reported through the mechanism of VCAM1 and neurotropic invasion, associated with increased metastatic potential [11]. More recent studies highlight Schwann cells as key players in the cross-talk, beyond the supportive role, by identifying prosaposin-GPR37/TGF-beta1 paracrine loop promoting PNI progression in gastric cancer [12].

The vascular component of the model is also supported by the literature since increased lymphatic vessel density and VEGF-C expression are both linked to enhanced lymphatic invasion and nodal metastasis [15-17]. Additionally, tumor budding, infiltrative patterns of growth, extracellular matrix remodeling, and stromal activation are all recognized mechanisms facilitating access to a neural or vascular compartment.

It is important to note that while these findings cannot confirm GC-NVIS causation, they greatly support the credibility of the link. Significantly, the suggested relationship does not necessarily propose a mechanistic connection between LVI and PNI. Rather, it is possible that both are the results of a concerted 'invasive front program' including neurotrophic, lymphangiogenic, stromal, and cellular components.

Shared-Cause Versus Interactive Models

Three broad explanations can account for the observed pattern, and the study should remain open to all of them. The easiest to consider is an independent-invasion model: as tumors become deeper and more aggressive, they encounter more anatomical

structures and therefore accumulate both PNI and LVI. Under this model, the dual-positive phenotype is a marker of advanced disease but not a distinct biological state. A second possibility is a shared-microenvironment model. Here, loss of cohesion, matrix remodeling, fibroblast activation, inflammatory signaling, and local growth factors create a permissive invasive front. Tumor cells do not need nerves and vessels to communicate directly; the same microenvironment simply makes invasion of both structures more likely. The third is the interactive-niche model, in which tumor cells, nerves, Schwann cells, immune cells, fibroblasts, and vessels participate in reciprocal signaling that helps maintain an integrated invasive ecosystem. This is the most biologically ambitious version of NVIN and will ultimately require more than morphology - for example multiplex immunohistochemistry, spatial transcriptomics, or functional experimentation.

The current dataset cannot choose among these models. The value of the GC-NVIS framework is that it turns them into competing explanations that can be tested rather than assuming from the outset that the most complex mechanism is correct.

Why High Nodal Burden Is Informative

Difference between any nodal metastasis and a high nodal burden is one of the more informative findings in the cohort. While LVI remained independently associated with the transition from N0 to any N+ and PNI did not, both factors became independently associated when the endpoint was N2-N3 disease. This suggests that the two features may not contribute identical information across the course of regional spread.

A plausible interpretation is that LVI is closely related to gaining access to conduits that can seed lymph nodes, making it relevant to the presence of nodal disease itself. PNI may instead mark a broader invasive program that becomes particularly visible once dissemination is extensive. The strong enrichment of N2-N3 disease in the double-positive group is consistent with that possibility.

This interpretation should remain cautious as nodal burden is influenced by tumor depth, lymphadenectomy, pathological examination, and tumor subtype. However, recent work showing that nodal burden modifies the prognostic significance of PNI offers external support for considering the extent of nodal disease rather than treating all N+ patients as biologically equivalent [29].

Is There Evidence of Synergy?

Not yet. The multiplicative interaction OR was 3.60, but the 95% confidence interval was wide and included the null, and the P value was 0.059 [30-33]. The point estimate is interesting, particularly in a cohort of this size, but it does not support a claim of statistical synergy.

This is an important place to avoid overinterpretation. The fact that the double-positive group has worse disease than the

double-negative group does not prove that PNI and LVI amplify one another biologically. Similarly, the lack of a conventionally significant multiplicative interaction does not disprove a syndrome. Coherent phenotypes can arise from additive effects, shared upstream drivers, or spatial co-organization without satisfying a particular interaction model.

Larger cohorts should evaluate both multiplicative and additive interaction, paying attention to measures such as relative excess risk due to interaction and attributable proportion where appropriate. Mechanistic synergy, however, will ultimately require tissue-level and experimental evidence rather than a regression coefficient alone [34].

Alternative Explanations

The most obvious alternative explanation is that more advanced tumors invade more things. A T4 tumor has a bigger and deeper invasive front than a small early lesion, and hence is more likely to sample nerves, lymphatics, and veins. Were this an exhaustive explanation of the association, GC-NVIS would be a surrogate marker of advanced invasion, not a separate biological phenomenon.

Sampling opportunity could also generate a similar effect. Larger resections will contain more tumor blocks, more invasive-front length, and more opportunities for a pathologist to spot PNI and LVI. Number of examined sections is rarely recorded in public clinical datasets, but could influence the ability to detect these phenomena. Future slide-based analyses should therefore record the area and invasive-front length of examined tissue, nerve and vessel density, and number of sampled regions, and normalize PNI and LVI burden where feasible rather than interpreting raw counts.

Residual confounding represents another alternative explanation. Tumor size, exact depth of penetration, molecular subtype, treatment exposure, histological heterogeneity, and host stromal response could all influence neurovascular invasion and nodal dissemination. Within-stage analyses, stratification by Lauren type, and external validation can ameliorate but not eliminate these concerns.

These alternative explanations need not be viewed as weaknesses to conceal or explain away, but rather as the controls that the syndrome hypothesis must overcome to be taken seriously. The hypothesis' ability to withstand these challenges are what will give it its eventual credibility. If the proposed spatial pattern holds after adjustment for structure density and tumor extent, then the hypothesis is considerably strengthened. Likewise, if the pattern fails to appear, the hypothesis should be re-evaluated in that light.

Spatial Analysis as the Critical Experiment

The next decisive experiment is spatial rather than statistical. Candidate GC-NVIS tumors should be examined to determine

whether invaded nerves and involved vessels are closer than expected, whether LVI clusters around PNI foci, whether PNI is enriched in vascular rich regions and whether both events repeatedly occur at the same invasive fronts. Observations should be quantified, not selected from visually impressive fields.

Distance alone may not be enough. A nerve and vessel could lie close together simply because the local tissue is densely innervated and vascularized. Spatial analyses should compare observed proximity to the underlying densities of nerves, vessels and tumor. Nearest-neighbor methods, local cross-type clustering, region-based co-occurrence, and permutation approaches may distinguish organization from structural crowding.

The most persuasive comparison is not candidate GC-NVIS versus an obviously favorable control. It is PNI+/LVI+/NVIN+ versus PNI+/LVI+/NVIN-. If those two differ in nodal burden, recurrence or survival after adjustment for stage, the spatial component is doing real work. That result would provide the clearest bridge from an established pair of pathological findings to a new higher order phenotype.

Spatial and Computational Pathology

Recent spatial studies in gastric cancer demonstrate that clinically relevant microenvironmental regions can be identified and associated with their biological state, thus, technically viable to search for NVIN [18,19]. The HMU-GC resource is particularly attractive since the clinical annotations and whole-slide images are related at the patient level.

The computational pathology could help by segmenting the tumor, nerves, vessels, stroma, and invasive front, and then deriving quantitative features, such as PNI density, LVI density, D_TN, D_TV, D_NV, local structure density, and spatial co-occurrence. In addition, automated methods would allow sampling of much larger tissue areas than possible with manual review only.

The role of such an AI should be subordinate to the biological question. A black-box model that correctly predicts N stage would not validate the GC-NVIS if it cannot demonstrate that the neurovascular organization contributes to the prediction. A more useful objective would be explainable morphometry: identify a reproducible architecture, quantify it transparently, and then test whether it adds value beyond conventional pathology.

Potential Clinical Significance

If proven in an external validation, the most immediate clinical relevance for the construct would be its potential to refine the within-stage patient stratification. For instance, there is a known heterogeneity in terms of recurrence risks and invasiveness for patients with identical TNM stages. Thus, the GC-NVIS/NVIN construct might help to identify a sub-population with the most aggressive forms of multidirectional invasion at a given level of anatomical staging.

The other possible clinical utility might be in directing the pathological assessment and research endeavors. For instance, if the neurovascular phenotype is proven to have prognostic significance, the importance of assessing and reporting the full extent of PNI/LVI would be emphasized. Additionally, such a finding could help to prioritize the research efforts by highlighting the cases with the highest neurotrophic infiltration, which would be relevant for studying the molecular pathways of the tumor neuroinvasion, Schwann cell recruitment, and related pro-metastatic effects. As opposed to using PNI or LVI as surrogates for biological aggressiveness, the GC-NVIS construct would allow to define the sample with tumors having the most complex patterns of neurovascular interactions, thus probably possessing the most diverse mechanisms of invasion.

That said, the proposed clinical relevance should not be overestimated as of now. There is no sufficient evidence to adjust the treatment algorithms or surveillance schedules based on the GC-NVIS findings at this stage. The TNM staging and GC-NVIS scoring systems can be used as independent but complementary tools in the clinical oncology practice. The former was developed to assess the tumor extension and plan the treatment, while the latter, if validated, will serve to dissect tumor biology.

Strengths and Limitations

The study has several strengths that contribute to its value for hypothesis generation. The clinical dataset is tethered to whole-slide histology, so that the initial statistical signal can be taken back to the tissue and not merely remain an abstract spreadsheet association. PNI and LVI are independently evaluated, jointly evaluated as four phenotypes, and then analyzed as an ordinal burden metric. The use of N2-N3 as the principal validator offers increased biological resolution than a simple N0/N+ comparator, while the planned extreme-phenotype design affords a tangible starting point for slide review without treating thousands of patches as independent observations.

The limitations are equally informative. The cohort is retrospective, single-institutional, skewed male-dominant, and has missing PNI/LVI in some patients. The source clinical file offers binary information only (not quantitative), lacks survival, recurrence, or treatment data, and cannot directly support a prognostic argument. The venous-invasion field is entirely redundant with LVI in jointly evaluable records, and thus lymphatic and blood-vessel invasion cannot be reliably untangled from the spreadsheet.

The most important limitation is that the tissue has not yet been independently re-reviewed for this specific hypothesis. Binary source annotations cannot tell us how many nerves were involved, or whether invasion affected large or small nerves, or to what extent LVI occurred, or whether these two processes were localized to the same microscopic region. H&E has known

limitations for subtle lymphatic and venous invasion, and ancillary markers are unavailable in the current public image set.

Finally, proposing a new syndrome runs the additional risk of confirmation bias. Once you've proposed a name and neat conceptual figure, it's natural to interpret subsequent findings in that light. The staged evidence framework, blinded slide review, locked validation criteria, and explicit falsifiability conditions are all intended to combat that temptation.

Falsifiability and Recognition Criteria

A useful syndrome hypothesis must be capable of failing. GC-NVIS would be substantially weakened if invaded nerves and vessels showed no meaningful spatial organization, or if apparent clustering disappeared after accounting for local nerve and vessel density, or a spatial score did not improve discrimination beyond ordinary PNI and LVI. Poor interobserver reproducibility would be another concern; a pathological construct that cannot be consistently recognized is unlikely to move beyond one research group.

External replication is important too; the association could be specific to the HMU-GC population, its pathology practices, or its case mix. A second cohort should be able to reproduce the phenotype using locked criteria and preferably provide recurrence or survival data; validation across laboratories would test robustness to differences in sectioning, staining, scanning, and pathological interpretation.

We propose four levels of evidence: clinicopathological association, spatial/morphological validation, internal reproducibility and incremental outcome validation, and independent external replication. The current study reaches level 1. To reach level 2 would require direct demonstration of NVIN. Level 3 would require that this spatial phenotype be reliable and clinically informative; level 4 would require independent external replication.

If these requirements are not met, the appropriate response would not be to say that the study 'failed', but rather that concurrent PNI and LVI are best understood as a marker for high risk rather than a syndrome. The framework is designed to distinguish between these possibilities (Figures 4 & 5).

Conclusion

This analysis finds a distinct double-compartment invasive phenotype of gastric cancer. PNI and LVI were closely linked but not entirely interchangeable, and tumors positive for both represented a group with significantly greater burden of N2-N3 disease than either singly positive or double negative tumors. The progressive increase in high nodal burden across zero, one, and two involved compartments further suggests that neural and lymphovascular invasion may represent increasing dimensions of invasive competence.

These data therefore support a testable proposition, but not a settled conclusion. We propose that the candidate positive for both PNI and LVI be termed Gastric Cancer Neurovascular Invasion Syndrome (GC-NVIS), and hypothesize that the spatial substrate for this pattern of invasion may take the form of a Neurovascular Invasive Niche (NVIN). The distinction is critical, given that the current data support the candidacy of the phenotype, whereas the putative niche must still be demonstrated directly.

The next logical steps in this work should therefore focus on the slides rather than further reanalysis of the same binary variables. If whole slide morphometry demonstrates consistent tumor-nerve-vessel organization, if that arrangement serves to distinguish discordant cases within the same PNI/LVI category, and if these associations can be reproduced externally, then GC-NVIS may represent a previously unrecognized dimension of gastric cancer invasion. Conversely, if these tests come up negative, it would provide a more defensible basis for interpreting this pattern of invasion as a combined high-risk pathological marker. Either outcome would help advance understanding of the biology by which neural and lymphovascular invasion intersect in the context of gastric cancer.

Declaration

Data availability: The clinical and histological data are publicly available as part of the HMU-GC-HE-30K gastric cancer histopathology dataset.

Ethics statement: This study represents secondary analysis of publicly available de-identified data. The original HMU-GC study reported institutional ethical approval (KY2024-16).

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DOI: [10.19080/APBIJ.2025.08.555742](https://doi.org/10.19080/APBIJ.2025.08.555742)

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