Rectal Tumor Height and Neoadjuvant Therapy Inform Interpretation of the Conventional Twelve-Node Benchmark: A Single-Center Colorectal Surgery Cohort

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AIM: The twelve-node benchmark is widely used as a minimum reference for staging adequacy and quality assessment after colorectal cancer resection. This study evaluated whether rectal tumor height and neoadjuvant therapy (NAT) status should be considered when interpreting this benchmark in rectal cancer.

METHODS: This single-center retrospective cohort study included patients with non-metastatic colorectal adenocarcinoma who underwent minimally invasive radical resection with evaluable lymph node counts between 1 June 2019 and 30 June 2024. Rectal cancer constituted the primary analytic cohort; a contemporaneous colon cancer cohort was included as an institutional reference. The primary outcome was low lymph node yield (LNY), defined as fewer than twelve retrieved lymph nodes; lymph node yield (LNY) as a count outcome was analyzed as a complementary outcome. We first performed descriptive comparisons across tumor-height and NAT strata. We then fitted multivariable-adjusted negative binomial and logistic regression models in the rectal cancer cohort, including tumor height, NAT status, and their interaction to estimate adjusted height- and NAT-associated contrasts and test interaction.

RESULTS: The cohort included 4282 patients: 2317 with colon cancer and 1965 with rectal cancer, including 1024 without NAT and 941 with NAT. The colon cancer cohort had a median LNY of 19; the interquartile range (IQR) was 14–26, and the low-yield rate was 8.9%. Rectal cancer without and with NAT each had a median LNY of 15.0 (IQR, 12.0–19.0) and low-yield rates of 17.4% and 21.6%, respectively. Within rectal cancer, low-yield classification increased from high to mid to low rectal tumors (13.5%, 20.4%, and 26.8%). In adjusted rectal interaction models, comparisons with high rectal tumors were estimated at the no-NAT reference level. Low rectal tumors were associated with lower LNY (incidence rate ratio (IRR), 0.83; 95% CI, 0.77–0.89; $p < 0.001$). They also had higher odds of low LNY (odds ratio (OR), 2.20; 95% CI, 1.44–3.35; $p < 0.001$). In the mid-rectal stratum, NAT was associated with higher odds of low LNY (OR, 1.82; 95% CI, 1.24–2.66; $p = 0.002$); however, the overall interaction for low LNY was not statistically significant (p for interaction = 0.331).

CONCLUSIONS: The twelve-node benchmark remains a useful reference for pathological staging and quality review. In the studied minimally invasive, sphincter-preserving rectal cancer cohort, a lymph node count below twelve should be interpreted together with tumor height, NAT exposure, and treatment composition rather than being used as an isolated indicator of inadequate surgical or pathological quality.

Keywords: rectal neoplasms; lymph node yield; twelve-node benchmark; neoadjuvant therapy; tumor location; minimally invasive surgery

Introduction

Adequate lymph node evaluation is fundamental to pathological staging after colorectal cancer resection, because nodal status determines tumor-node-metastasis (TNM) stage, informs adjuvant treatment decisions, and shapes prognostic counseling [1]. The benchmark of at least twelve examined lymph nodes has become a widely used minimum reference for staging adequacy and quality assessment in colorectal and rectal cancer specimens [1,2,3]. However, the twelve-node standard is best understood as a pragmatic minimum reference rather than a biological cut-point at which oncological adequacy changes abruptly [4].

The clinical meaning of lymph node yield (LNY) depends on the context in which it is measured. In colon cancer, contemporary population-based data have linked LNY with nodal positivity and survival, while also suggesting that LNY may reflect tumor biology and institutional context rather than surgical quality alone [5]. Additional population-based data indicate that lymph node evaluation in routine practice varies across institutions and clinical contexts [6]. Evidence derived largely from colon cancer should therefore not be transferred mechanically to rectal cancer, which differs in pelvic anatomy, mesorectal specimen configuration, treatment sequencing, and patterns of nodal regression after preoperative therapy [2,7].

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Neoadjuvant therapy (NAT) is one major reason why rectal cancer requires contextual interpretation. Preoperative chemoradiotherapy can reduce nodal retrieval through treatment-related lymphoid depletion, fibrosis, and nodal atrophy [8,9]. At the same time, reduced LNY after NAT has not consistently been associated with inferior oncological outcomes in rectal cancer cohorts [10,11]. Multicenter data in node-negative rectal cancer after NAT have similarly questioned whether lower node counts should be interpreted as prognostically adverse in all patients [12].

Anatomical heterogeneity within rectal cancer may be equally important. Rectal cancer surgical guidance distinguishes upper rectal tumors from middle and lower rectal tumors in relation to mesorectal excision strategy and treatment planning [2,13]. This distinction reflects differences in pelvic anatomy, distal mesorectal requirements, and technical constraints that may influence specimen characteristics and nodal retrieval expectations [13,14]. Previous rectal cancer series have also linked lymph node yield to tumor location, specimen-related factors, and neoadjuvant treatment exposure [15,16].

This study was designed around a rectal cancer treatment-anatomy framework. Rectal cancer patients formed the primary analytic cohort and were evaluated according to tumor height and NAT status. A contemporaneous colon cancer cohort provided institutional context for nodal retrieval under the same surgical-pathological workflow. We evaluated whether low LNY in rectal cancer should be interpreted in relation to tumor height and treatment context rather than mechanically classified as a surgical or pathological quality deficit.

Methods

Study Design and Setting

This single-center retrospective cohort study analyzed consecutive patients who underwent colorectal cancer surgery at Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, between 1 June 2019 and 30 June 2024. The study protocol was approved by the Ethics Committee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine (Approval No. 2026-2395-01) and was conducted in accordance with the Declaration of Helsinki; informed consent was waived owing to the retrospective design and use of de-identified data. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline (**Supplementary Material**) [17].

Study Population and Cohort Construction

The target population comprised patients with non-metastatic colorectal adenocarcinoma who underwent minimally invasive radical resection, either laparoscopic or robotic, with evaluable lymph node counts. The cohort was restricted to this operative context to reduce heterogeneity in surgical approach and resection pattern. All consecutive patients meeting eligibility criteria during the study period were included; no a priori sample size calculation was performed because all eligible cases during the study period were analyzed.

Patients were excluded for non-target pathology or tumor presentation, preoperative distant metastasis, non-target operative settings, concurrent major non-colorectal organ resection, unevaluable lymph node counts, or incomplete core analytic data. Detailed exclusion categories and counts are shown in Fig. 1. Open and non-restorative procedures were excluded to reduce heterogeneity in operative approach and resection pattern. As a result, the rectal cancer analytic cohort represented a minimally invasive, sphincter-preserving surgical context. No age-based exclusion criterion was applied; all patients included in the final cohort were adults.

Analytic Cohorts and Analysis Hierarchy

The analysis was organized hierarchically. First, a contemporaneous colon cancer cohort was retained as an internal institutional reference under the same surgical-pathological workflow. This reference cohort was not treated as exchangeable with rectal cancer or as a co-equal primary comparison group. Second, rectal cancer patients constituted the primary analytic cohort and were evaluated according to tumor height and NAT status. Third, six rectal treatment-anatomy scenarios were derived by crossing rectal height (high, mid, or low) with NAT status (no NAT or NAT). Because these cohorts represented clinically defined contexts rather than exchangeable groups, baseline characteristics were summarized descriptively without a single overall hypothesis test. The primary adjusted models were restricted to rectal cancer and estimated rectal-height contrasts and NAT-associated contrasts within rectal-height strata.

Rectal Tumor Height and NAT Definitions

Rectal tumor height was categorized as high (>10 cm from the anal verge), mid (5–10 cm), or low (<5 cm). Height classification was based on review of the original clinical records, including pelvic magnetic resonance imaging (MRI), colonoscopy, contrast-enhanced abdominal computed tomography (CT), and pathological or operative notes. For patients receiving NAT, pretreatment MRI or colonoscopy was preferentially reviewed when available to minimize post-treatment reclassification; when pretreatment documentation was incomplete, the last available preoperative assessment before surgery was cross-checked against other available records. Two investigators independently performed height classification, with MRI-based distance prioritized when available. All rectal cancer cases in the final analytic cohort were classifiable as high, mid, or low rectum.

NAT was defined as any preoperative oncological treatment delivered before rectal cancer surgery, including radiotherapy, chemotherapy, immunotherapy, or combinations of these modalities. NAT status was therefore interpreted as a heterogeneous treatment-context variable rather than a single standardized regimen. Consequently, binary NAT sta-

tus was not interpreted as the isolated effect of traditional chemoradiotherapy. Detailed rectal treatment distributions are provided in **Supplementary Table 1**.

Outcomes

The primary outcome was low LNY according to the conventional twelve-node benchmark, defined as fewer than twelve retrieved lymph nodes and analyzed as a binary variable. LNY, defined as the total number of lymph nodes retrieved on postoperative pathology, was analyzed as a complementary count outcome to characterize the underlying distribution of nodal yield. Node-positive detection, defined as the presence of more than zero positive lymph nodes, was summarized descriptively to contextualize clinical scenarios.

Covariates

Adjustment covariates were specified a priori as a fixed set based on clinical relevance and availability before or at the index operation; they were not selected by univariable significance testing or automated stepwise procedures. The set comprised demographic factors (age, sex, body mass index, and American Society of Anesthesiologists grade), preoperative tumor characteristics (carcinoembryonic antigen level, clinical T category, and clinical N category), temporal practice (surgery year), and operative-context variables (procedure type and surgical approach). Demographic and preoperative tumor factors were included to account for patient and disease severity, surgery year to account for secular changes in surgical-pathological practice, and procedure type and surgical approach to account for operative-context heterogeneity in nodal retrieval. Because operative-context variables may partly reflect anatomy- and treatment-related selection, the adjusted estimates were interpreted as conditional observational associations rather than total or causal effects of NAT.

Statistical Analysis

The distribution of continuous variables was assessed using Kolmogorov–Smirnov test with Lilliefors correction. Normally distributed variables were presented as mean ± standard deviation (SD), whereas non-normally distributed variables were presented as median and interquartile range (IQR). Categorical variables were presented as frequencies and percentages. For descriptive group comparisons, LNY distributions were compared using Wilcoxon rank-sum tests for two-group rectal NAT status comparisons and Kruskal-Wallis tests for rectal-height comparisons within NAT strata. Categorical outcomes were compared using Pearson chi-square tests. Pairwise within-height comparisons of low LNY between NAT and no NAT used Fisher's exact tests with Bonferroni correction across the three rectal-height strata. These univariable tests were descriptive and were not treated as the primary inferential analysis.

The primary multivariable analyses were restricted to the rectal cancer cohort. Negative binomial regression was used to model LNY as a count outcome while accommodating overdispersion, with effect estimates reported as incidence rate ratio (IRR) values and 95% CI values. Logistic regression was used to model low LNY, with effect estimates reported as odds ratio (OR) values and 95% CI values. Both models included the full fixed adjustment set described above. During cohort construction, cases with unrecoverable missingness in core analytic variables were excluded. In the final analytic cohort, missingness was less than 2% for each modeled covariate. Before model fitting, continuous covariates were imputed to the median and categorical covariates to the mode. Rectal tumor height was complete among rectal cancer patients and was not applicable to the colon cancer cohort.

To evaluate whether NAT-associated differences in LNY varied by rectal tumor height, tumor height, NAT status, and their interaction were retained in both primary outcome models. Clinically interpretable derived contrasts were reported, including mid versus high rectum, low versus high rectum, low versus mid rectum, and NAT versus no NAT within each height stratum. The overall tumor-height-by-NAT interaction was evaluated separately for each outcome using likelihood-ratio tests comparing the full interaction model with the corresponding main-effects model.

For sensitivity analyses, the same interaction models were repeated after excluding patients who received immunotherapy-containing regimens. Within the NAT cohort, exploratory models compared immunotherapy-containing with non-immunotherapy NAT, adjusting for rectal height and the same covariates; results are reported in **Supplementary Table 2**. All analyses were performed using R software (version 4.5.3, R Foundation for Statistical Computing, Vienna, Austria). Two-sided p values < 0.05 were considered statistically significant.

Results

Study Population

From 5992 candidate surgical records, five-stage exclusion yielded a final analytic cohort of 4282 patients: 2317 with colon cancer, 1024 with rectal cancer without NAT, and 941 with rectal cancer with NAT (Fig. 1). Baseline characteristics are presented descriptively in Table 1.

Descriptive Lymph Node Yield Across Cohorts and Rectal Contexts

The colon cancer cohort had a median LNY of 19 (IQR, 14–26), with 8.9% of cases below the twelve-node benchmark and a node-positive rate of 32.9%. Within rectal cancer, the median LNY was 15.0 (IQR, 12.0–19.0) both without and with NAT, whereas classification as low LNY was more frequent in the NAT group (17.4% versus 21.6%; $p = 0.019$) (Table 2).

Fig. 2 shows patient-level LNY distributions for the colon cancer institutional reference and the six rectal treatment-

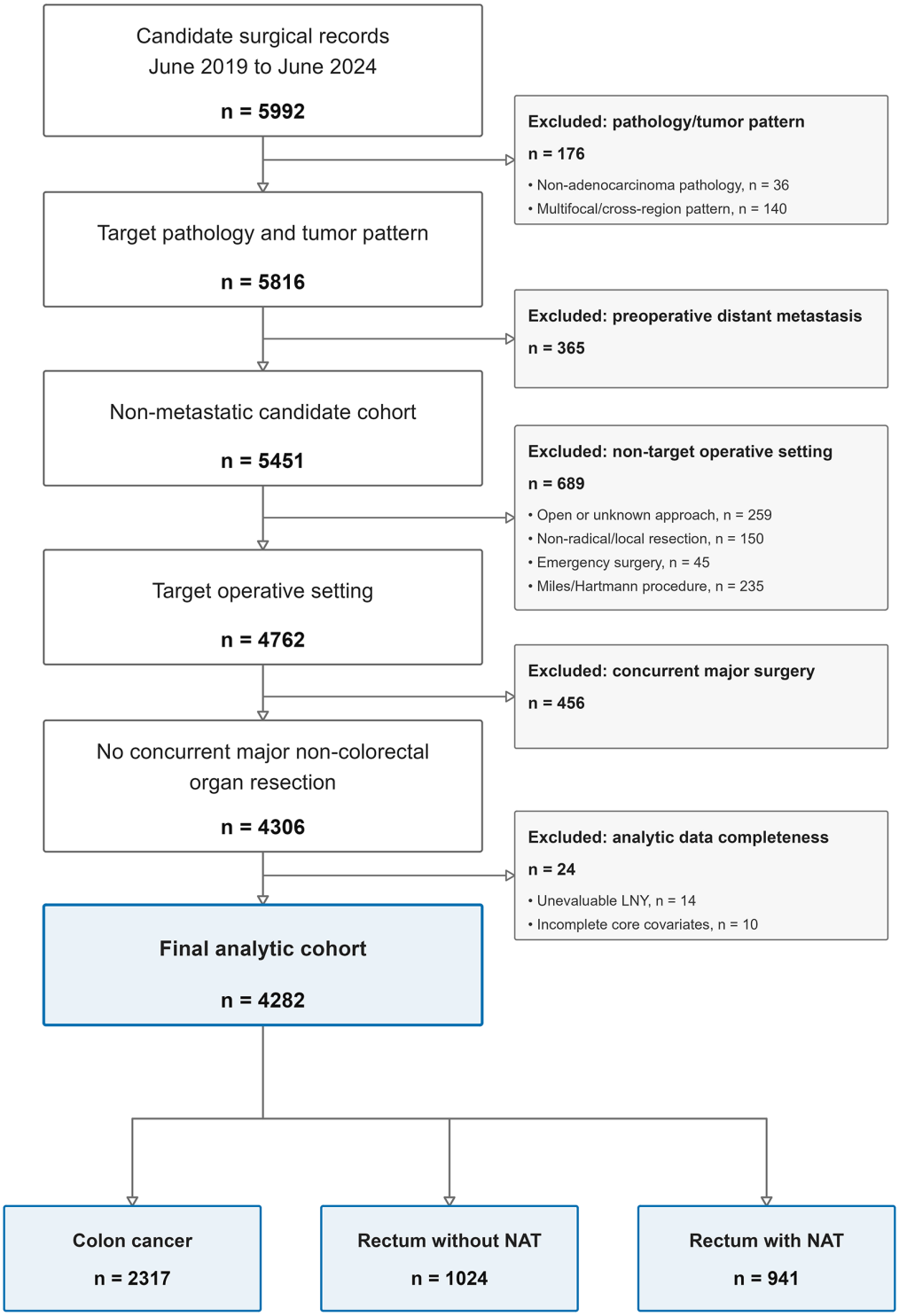

Fig. 1. Study flow diagram. The cohort screening flow shows exclusion counts at each step and the final classification into colon cancer and rectal cancer cohorts. NAT, neoadjuvant therapy; LNY, lymph node yield.

anatomy groups defined by tumor height and NAT status. Among patients without NAT, median LNY was 15.0 (IQR, 13.0–20.0), 15.0 (IQR, 13.0–19.0), and 14.0 (IQR, 11.5–17.0) in high-, mid-, and low-rectal tumors, respectively. Among patients with NAT, the corresponding values were 17.0 (IQR, 13.0–22.0), 15.0 (IQR, 12.0–19.0), and 13.0 (IQR, 11.0–17.0). Within patients without NAT, low-yield classification increased from high to mid to low rectal tumors (13.6%, 17.2%, and 25.1%; $p = 0.001$). Among patients with NAT, the corresponding proportions were 13.3%, 23.8%, and 28.3% ($p < 0.001$) (Table 2).

Within-height NAT versus no-NAT comparisons for low-yield classification showed no significant difference after Bonferroni correction in high rectal cancer (13.3% vs

Table 1. Baseline characteristics of the colon cancer and rectal cancer cohorts.

Characteristic	Colon (n = 2317)	Rectum without NAT (n = 1024)	Rectum with NAT (n = 941)
Demographics			
Age, years, mean (SD)	65.2 (12.1)	66.9 (11.3)	61.8 (10.6)
Sex, n (%)			
Female	988 (42.6%)	397 (38.8%)	306 (32.5%)
Male	1329 (57.4%)	627 (61.2%)	635 (67.5%)
BMI, kg/m^2, mean (SD)	23.0 (3.3)	22.9 (3.2)	22.8 (3.0)
CEA, ng/mL, median (IQR)	3.11 (1.89–6.18)	2.82 (1.83–4.73)	3.16 (2.12–5.26)
ASA grade, n (%)			
I	4 (0.2%)	2 (0.2%)	5 (0.5%)
II	2009 (86.7%)	876 (85.5%)	859 (91.3%)
III	302 (13.0%)	145 (14.2%)	77 (8.2%)
IV	2 (0.1%)	1 (0.1%)	0 (0.0%)
Tumor characteristics			
Clinical T stage, n (%)			
T1	392 (16.9%)	204 (19.9%)	48 (5.1%)
T2	315 (13.6%)	267 (26.1%)	118 (12.5%)
T3	1137 (49.1%)	492 (48.0%)	684 (72.7%)
T4	473 (20.4%)	61 (6.0%)	91 (9.7%)
Clinical N stage, n (%)			
N0	1632 (70.4%)	748 (73.0%)	488 (51.9%)
N1	631 (27.2%)	209 (20.4%)	349 (37.1%)
N2	54 (2.3%)	67 (6.5%)	104 (11.1%)
Rectal tumor height			
Rectal height, n (%)			
High	Not applicable	427 (41.7%)	315 (33.5%)
Mid	Not applicable	378 (36.9%)	357 (37.9%)
Low	Not applicable	219 (21.4%)	269 (28.6%)
Surgical characteristics			
Surgical approach, n (%)			
Robotic	567 (24.5%)	446 (43.6%)	413 (43.9%)
Laparoscopic	1750 (75.5%)	578 (56.4%)	528 (56.1%)

Note: Values are presented as mean (SD), median (IQR), or n (%), as appropriate. Percentages were calculated within each column. Abbreviations: ASA, American Society of Anesthesiologists; BMI, body mass index; CEA, carcinoembryonic antigen; IQR, interquartile range; NAT, neoadjuvant therapy.

13.6%; Bonferroni-adjusted $p = 1.000$), mid rectal cancer (23.8% vs 17.2%; Bonferroni-adjusted $p = 0.084$), or low rectal cancer (28.3% vs 25.1%; Bonferroni-adjusted $p = 1.000$). Thus, after correction for the three comparisons, NAT was not significantly associated with low-yield classification within any rectal-height stratum in these unadjusted analyses. These non-significant comparisons did not establish equivalence and were interpreted separately from the adjusted model-derived contrasts.

Primary Adjusted Rectal Interaction Models

The primary inferential analysis used rectal-only adjusted interaction models including tumor height, NAT status, and their interaction; the resulting clinically interpretable contrasts are summarized in Fig. 3. Rectal-height contrasts were estimated at the no-NAT reference level. Compared with high rectal cancer without NAT, the adjusted IRR for LNY was 0.94 (95% CI, 0.89–1.00; $p = 0.036$) for mid rectal cancer and 0.83 (95% CI, 0.77–0.89; $p < 0.001$) for low rectal cancer. The adjusted OR for low LNY was 2.20 (95% CI, 1.44–3.35; $p < 0.001$) for low versus high rectum. Low versus mid rectum was also associated with higher odds of low LNY (OR, 1.56; 95% CI, 1.03–2.37; $p = 0.035$). In contrast, mid versus high rectum showed a directionally higher but non-significant OR of 1.41 (95% CI, 0.95–2.09; $p = 0.087$).

The overall tumor-height-by-NAT interaction was statistically significant for LNY (p for interaction = 0.044) but not for low LNY classification (p for interaction = 0.331). Within-stratum NAT estimates were as follows. In high rectal cancer, NAT was not significantly associated with LNY (IRR, 1.013; 95% CI, 0.952–1.078; $p = 0.691$) or low LNY classification (OR, 1.197; 95% CI, 0.769–1.864; $p = 0.427$). In mid rectal cancer, NAT was associated with lower LNY (IRR, 0.910; 95% CI, 0.854–0.969; $p = 0.003$) and higher odds of low LNY classification (OR, 1.820; 95% CI, 1.243–

Table 2. Lymph node yield and node-positive detection across rectal cancer contexts.

Panel A. Rectal cancer by NAT status					
Outcome	Rectum without NAT (n = 1024)	Rectum with NAT (n = 941)	*p* value		
LNY, median (IQR)	15.0 (12.0–19.0)	15.0 (12.0–19.0)	0.600		
Low LNY <12, n (%)	178 (17.4%)	203 (21.6%)	0.019		
Node-positive detection, n (%)	158 (15.4%)	395 (42.0%)	<0.001		
Panel B. Height within NAT strata					
NAT status	Outcome	High rectum	Mid rectum	Low rectum	*p* value
No NAT	N	427	378	219	
No NAT	LNY, median (IQR)	15.0 (13.0–20.0)	15.0 (13.0–19.0)	14.0 (11.5–17.0)	<0.001
No NAT	Low LNY <12, n (%)	58 (13.6%)	65 (17.2%)	55 (25.1%)	0.001
No NAT	Node-positive detection, n (%)	85 (19.9%)	50 (13.2%)	23 (10.5%)	0.002
NAT	N	315	357	269	
NAT	LNY, median (IQR)	17.0 (13.0–22.0)	15.0 (12.0–19.0)	13.0 (11.0–17.0)	<0.001
NAT	Low LNY <12, n (%)	42 (13.3%)	85 (23.8%)	76 (28.3%)	<0.001
NAT	Node-positive detection, n (%)	163 (51.7%)	150 (42.0%)	82 (30.5%)	<0.001

Note: Values are median (IQR) or n (%). Percentages were calculated within each subgroup. Low lymph node yield (LNY) was defined as fewer than 12 retrieved lymph nodes. Node-positive detection was defined as at least one positive lymph node on postoperative pathology; after NAT, it represents residual pathological nodal positivity rather than baseline nodal disease. The table is restricted to rectal cancer. Panel A compares NAT status groups, and Panel B compares rectal-height groups within each NAT stratum. *p* values were calculated using Wilcoxon rank-sum tests for two-group LNY comparisons, Kruskal-Wallis tests for rectal-height LNY comparisons, and Pearson chi-square tests for categorical outcomes. For low LNY, Bonferroni-adjusted Fisher's exact *p* values for NAT versus no NAT within high-, mid-, and low-rectal strata were 1.000, 0.084, and 1.000, respectively.

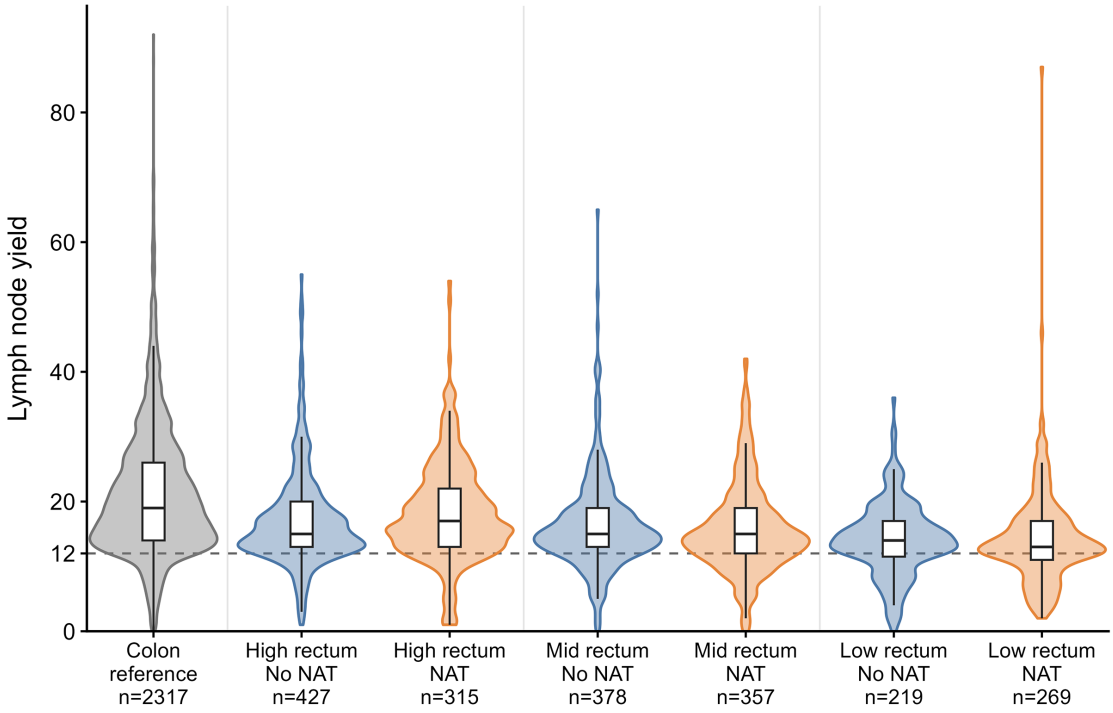

Fig. 2. Lymph node yield across the colon reference and rectal treatment-anatomy groups. Note: Patient-level LNY distributions in the colon cancer institutional reference and six rectal groups formed by crossing tumor height (high, mid, or low) with NAT status (no NAT or NAT). Violin widths show kernel-density estimates using a bandwidth of one node; embedded box plots show the median, interquartile range (IQR), and whiskers extending to the most extreme values within 1.5 times the IQR. Group sample sizes are shown below the x-axis. The dashed line marks the conventional twelve-node benchmark. Values are descriptive; exact numerical summaries and subgroup comparisons are reported in Table 2.

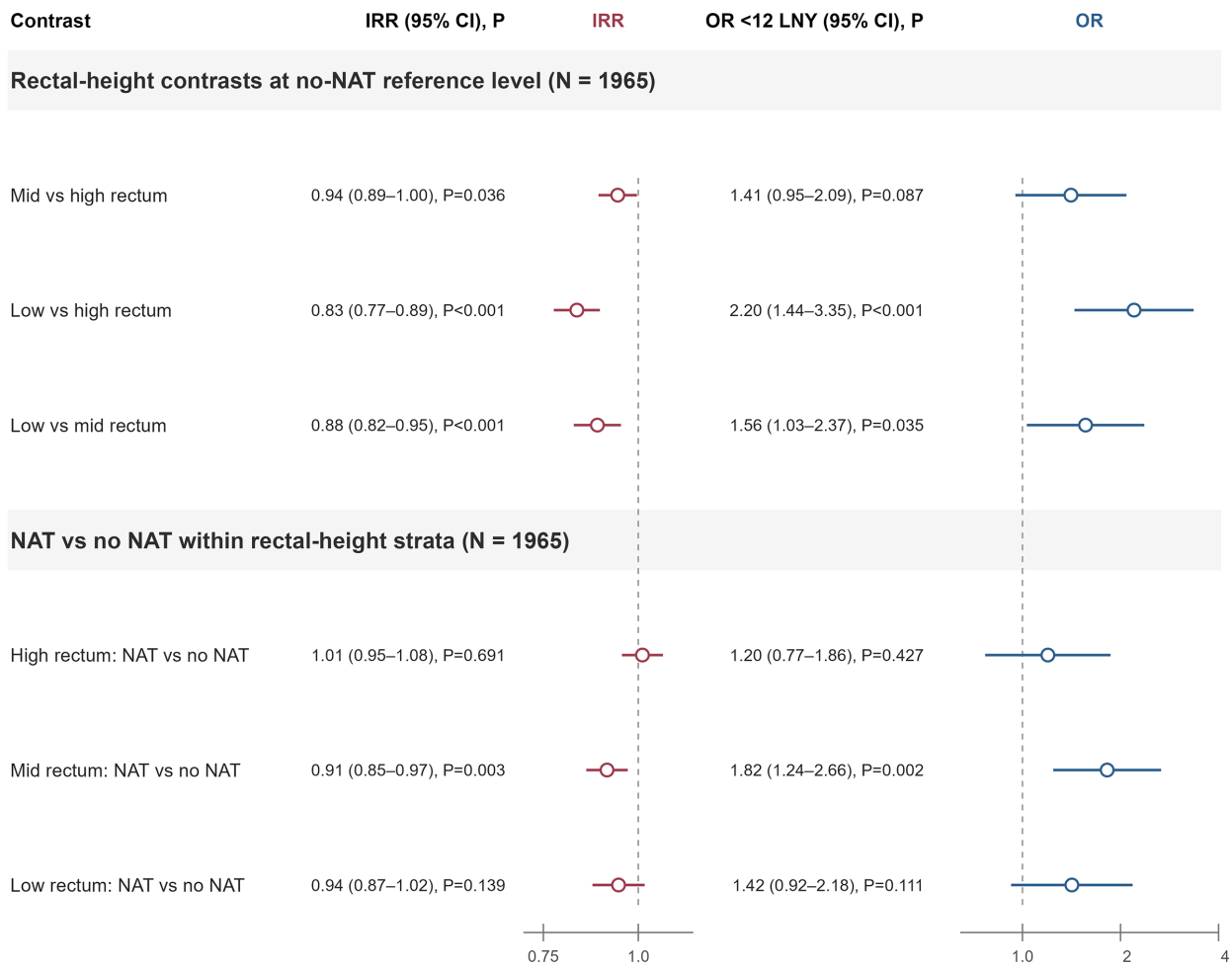

Fig. 3. Primary adjusted rectal contrast estimates for lymph node yield and low-yield classification. Note: The dashed vertical lines mark the null value of 1.0 for IRR and OR estimates. The forest plot separates rectal-height contrasts estimated at the no-NAT reference level from derived NAT-versus-no-NAT contrasts within each rectal-height stratum from the interaction model. NAT-versus-no-NAT rows should not be interpreted as comparisons with high rectum without NAT. Abbreviations: IRR, incidence rate ratio; OR, odds ratio.

2.664; $p = 0.002$). In low rectal cancer, the NAT-associated contrast did not reach statistical significance for LNY (IRR, 0.942; 95% CI, 0.870–1.020; $p = 0.139$) or low LNY classification (OR, 1.418; 95% CI, 0.923–2.179; $p = 0.111$). These derived contrasts are summarized in Fig. 3.

After excluding 110 patients with immunotherapy-containing NAT, the adjusted analysis of 1855 patients yielded similar anatomical and mid-rectal NAT contrasts, whereas the low-rectal NAT contrasts strengthened (LNY: IRR, 0.913; 95% CI, 0.842–0.990; low LNY: OR, 1.631; 95% CI, 1.049–2.536) (**Supplementary Table 2**). Within the NAT cohort, immunotherapy-containing versus non-immunotherapy NAT was not clearly associated with LNY (IRR, 1.034; 95% CI, 0.946–1.129) or low LNY (OR, 0.841; 95% CI, 0.505–1.403).

Discussion

In this single-center cohort, low LNY was more frequent in rectal cancer than in the contemporaneous colon institutional reference and increased from high to low rectal locations. The colon cohort was used only to contextualize nodal retrieval under the same surgical-pathological workflow; primary inference came from rectal-only analyses. These descriptive findings support a context-aware interpretation of the twelve-node benchmark.

The anatomical gradient is consistent with the known location dependence of nodal assessment. Population-based and provider-level studies have shown that LNY varies with tumor location, clinicopathological features, pathology practice, and institutional factors [18,19,20,21]. Low rectal tumors are also managed within a narrower pelvic field and under different distal mesorectal requirements and operative strategies; current guidance distinguishes upper from middle and lower rectal tumors in relation to mesorectal excision [2,13]. Consequently, the same lymph node count may carry different quality-audit implications across rectal locations.

In the stratified descriptive analyses, none of the within-height NAT versus no-NAT differences in low-LNY prevalence remained statistically significant after Bonferroni cor-

rection. The mid-rectal contrast was numerically largest (23.8% vs 17.2%; adjusted $p = 0.084$) but did not meet the prespecified significance threshold. These findings do not support a uniform unadjusted association between NAT and low LNY across height strata; conversely, non-significance should not be interpreted as proof of equivalence.

The adjusted models refined this pattern. At the no-NAT reference level, mid and low rectal tumors had lower LNY than high rectal tumors; low versus high and low versus mid rectal tumors also had higher adjusted odds of low LNY, whereas the mid-versus-high OR was not significant. NAT was associated with lower LNY and higher odds of low LNY in the mid-rectal stratum, but neither outcome reached significance in the high- or low-rectal strata. Importantly, the overall tumor-height-by-NAT interaction was significant for LNY analyzed as a count outcome ($p = 0.044$) but not for binary low LNY ($p = 0.331$). The significant mid-rectal conditional contrast should therefore not be interpreted as conclusive evidence that the NAT association with binary low LNY differs across tumor heights.

After excluding the 110 patients who received immunotherapy-containing NAT, the anatomical and mid-rectal contrasts were similar, while the low-rectal NAT contrasts strengthened. Within the NAT cohort, immunotherapy-containing regimens were not clearly associated with either LNY outcome. These sensitivity analyses suggest that the main pattern was not driven solely by the immunotherapy subgroup, although heterogeneous NAT regimens preclude attribution to a specific treatment component.

These findings have practical implications for multidisciplinary review and surgical quality audit. The twelve-node benchmark remains useful as a simple staging and quality reference, but a count below twelve in rectal cancer should prompt contextual review rather than automatic attribution to inadequate surgery or pathology. Relevant context includes tumor height, NAT exposure and composition, specimen handling, pathological retrieval, and institutional workflow.

Several limitations should be acknowledged. This single-center retrospective study may reflect institutional treatment selection, surgical technique, and pathology processing. Detailed measures of pathological retrieval intensity, specimen length, mesorectal completeness, pathological response, and changes in specimen-processing practice were unavailable, and survival outcomes were not assessed. The cohort was limited to minimally invasive, sphincter-preserving surgery; therefore, the low-rectal subgroup may not represent all low rectal cancers. NAT regimens were heterogeneous, and residual confounding and confounding by indication remain possible. Because the models also conditioned on operative-context variables, the estimates are conditional observational associations rather than total or causal treatment effects. This study addresses contextual interpretation of the twelve-node benchmark rather than a survival-optimal threshold.

Conclusions

The twelve-node benchmark remains useful for pathological staging and surgical quality review, but it should not be applied mechanically in rectal cancer. Within the primary rectal cancer cohort, low LNY was more common in mid and low rectal tumors and varied according to NAT context. These findings indicate that lymph node counts below twelve in rectal cancer should be interpreted in relation to tumor height, treatment exposure, and institutional workflow rather than being equated directly with inadequate surgical or pathological quality. The study does not establish a new threshold or a survival-based measure of oncological adequacy.

Availability of Data and Materials

The data supporting the findings of this study are not publicly available because of institutional data governance and patient privacy restrictions. De-identified analytical data may be made available from the corresponding author upon reasonable request and with appropriate institutional approval.

Author Contributions

FW: conceptualization, methodology, formal analysis, writing – review & editing. SY: data curation. YL: methodology, formal analysis, writing – original draft. SD: conceptualization, resources, supervision. All authors have been involved in revising the manuscript critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine (Approval No. 2026-2395-01) and was conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived owing to the retrospective design and use of de-identified data.

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Conflict of Interest

Sheng Dai was a member of the Editorial Board of this journal at the time of acceptance. Sheng Dai had no involvement in the peer review of this article and has no access to

information regarding its peer review. Other authors declare that they have no conflict of interest.

Declaration of Generative AI and AI-Assisted Technologies in Manuscript Preparation

During the preparation of this manuscript, the authors used AI-assisted tools (OpenAI GPT-5.5) for language editing, formatting assistance, and consistency checking. The authors reviewed and edited all AI-assisted output and take full responsibility for the content of the manuscript.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.62713/aic.4838.

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