Research Square

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Fluorescent lymphography and lymph node retrieval and perioperative outcomes in rectal cancer surgery: a prospective non-randomized cohort study stratified by neoadjuvant chemoradiotherapy

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Abstract

Background

Total mesorectal excision (TME) is critical for mid-to-low rectal cancer. This study evaluated the association of fluorescence lymphangiography (FL) withlymph node (LN) retrieval and perioperative outcomes, as well as its diagnostic performance for detecting metastatic LNs.

Methods

This prospective cohort study included 461 patients undergoing TME between 2019 and 2023. Patients received FL-guided TME (n = 111) or conventional lymphadenectomy (n = 350). After 1:2 propensity score matching, 111 FL and 222 non-FL patients were analyzed. Subgroup analyses were performed according to neoadjuvant chemoradiotherapy (nCRT).

Results

FL was associated with greater LN retrieval (29.3 vs. 26.7, P = 0.049) and a higher proportion of patients with ≥ 25 LNs retrieved (66.7% vs. 51.4%, P = 0.007). Among patients without nCRT, FL was associated with shorter operative time (236.9 vs. 278.8 min, P = 0.001), lower blood loss (42.3 vs. 118.8 mL, P < 0.001), and shorter hospital stay (6.4 vs. 14.6 days, P < 0.001). In nCRT patients, FL was not associated with greater LN retrieval (P = 0.213) but was associated with shorter hospitalization (6.3 vs. 10.4 days, P < 0.001). Sensitivity for metastatic LNs was 37.4% in non-nCRT and 27.8% in nCRT patients, with a negative predictive value of 89.7–91.6%. Survival outcomes were comparable (P ≥ 0.183).

Conclusions

FL was associated with greater LN retrieval and favorable perioperative outcomes, particularly among patients without nCRT. However, its ability to detect metastatic LNs was limited, especially after nCRT. FL may serve as an adjunct for lymphatic mapping and surgical guidance.

Introduction

Total mesorectal excision (TME) remains the gold standard in the surgical treatment of mid-to-low rectal cancer, providing curative intent by ensuring complete tumor resection and minimizing the risk of local recurrence [1, 2]. Central to this approach is lymph node (LN) dissection, as the number and pathological status of retrieved LNs serve as vital indicators for both staging and prognosis [3, 4]. A comprehensive and thorough lymphadenectomy is essential for accurate staging, given that the presence of metastatic LNs is one of the most important prognostic factors in rectal cancer [5]. However, the adequacy of LN retrieval can be influenced by a variety of factors, including tumor size and location, neoadjuvant chemoradiotherapy (nCRT), patient body mass index (BMI), the surgical approach, and the quality of pathological examination.

Fluorescent lymphography (FL), utilizing indocyanine green (ICG) dye, has gained significant attention in recent years as an advanced technique for intraoperative lymphatic mapping [6, 7]. This method allows for the real-time visualization of lymphatic drainage and enables the identification of sentinel LNs that may not be easily detected through conventional methods [8]. The integration of FL into colorectal cancer surgeries, particularly for rectal cancer, has shown promising results by improving the completeness of lymphadenectomy and aiding in the detection of otherwise elusive metastatic nodes [9, 10]. Previous studies have demonstrated that FL-guided lymphadenectomy increases the total number of retrieved LNs, potentially leading to more accurate staging and improved patient outcomes [11]. This technique has shown particular promise in minimally invasive surgery, such as laparoscopic or robotic TME, offering the potential for enhanced surgical precision and reduced patient morbidity.

Despite the increasing use of FL in colorectal cancer surgery, its impact in the context of nCRT remains less well understood. The nCRT is a common preoperative treatment for locally advanced rectal cancer, and its effects on tumor response, lymph node

involvement, and surgical outcomes have been extensively studied [12, 13]. However, the role of FL in improving LN retrieval and its diagnostic performance in both nCRT and non-nCRT populations has not been thoroughly investigated. While some studies suggest that nCRT may alter lymphatic drainage patterns and affect the detection of metastatic nodes [14], the use of FL may still offer an advantage in visualizing and dissecting lymph nodes that might otherwise be overlooked.

This study aims to evaluate the clinical value of FL in rectal cancer surgery by comparing perioperative and oncological outcomes between patients undergoing FL-guided TME and those who received standard lymphadenectomy. Specifically, the study focuses on the impact of FL on LN retrieval, operative efficiency, and staging accuracy in both nCRT and non-nCRT patient populations. By assessing these outcomes, we seek to determine whether FL improves lymph node dissection and enhances surgical results in mid-to-low rectal cancer, with implications for both short-term recovery and long-term oncological prognosis.

Methods

Patients

A prospectively maintained database of patients diagnosed with mid-to-low rectal cancer was analyzed to identify those who underwent curative-intent total TME between January 2019 and December 2023 from three centers. Patients were excluded if they met any of the following criteria: age below 18 or above 80 years, non-adenocarcinoma histology, presence of distant metastasis at initial diagnosis, documented allergy to ICG, or previous colorectal surgery. Based on the utilization of FL during surgery, patients were divided into two groups: the FL group, in which ICG-guided lymphatic mapping was performed, and the non-FL group, in which standard lymphadenectomy was conducted without fluorescence guidance.

Neoadjuvant chemoradiotherapy was administered based on standard institutional protocols, with indications primarily determined by tumor stage and anatomical location. To ensure comparability between groups, propensity score matching (PSM) was employed, adjusting for age, sex, BMI, tumor size, tumor stage, nodal stage, and receipt of nCRT.

Surgical Procedure

All patients underwent minimally invasive TME, either laparoscopic or robotic, based on surgeon preference and patient selection. The institutional protocol for ICG-based fluorescent lymphography has been previously described [15]. Briefly, ICG (2.5 mg/mL) was injected into the submucosal layer at four quadrants surrounding the tumor 24 hours before surgery by an experienced endoscopist. The total dose of ICG was adjusted according to evolving fluorescence imaging technologies: initially, 3 mg ICG was injected, but this was reduced to 1.5 mg as detection sensitivity improved.

During surgery, the presence of fluorescent lymph nodes was evaluated using near-infrared fluorescence (NIRF) imaging. If fluorescent LNs were observed beyond the standard lymphadenectomy extent, additional dissection was performed. After specimen retrieval, fluorescence status was reassessed at the back table, with LNs classified as fluorescent or non-fluorescent. Injection failure—defined as excessive ICG spillage into the abdominal cavity or diffusely stained tissues preventing clear LN identification—resulted in classification within the non-FL group.

Lymph Node Retrieval and Pathological Analysis

All resected specimens were processed by experienced colorectal surgeons and pathologists. Lymph nodes were dissected from the mesorectal tissue and categorized according to the Japanese Classification of Colorectal Cancer. In the FL group, fluorescence detection was performed for each LN station. Stations containing one or more fluorescent LNs were categorized as fluorescent LN stations; otherwise, they were classified as non-fluorescent LN stations. To assess the correlation between fluorescence and nodal metastasis, fluorescence positivity was compared with histopathological results.

Statistical Analysis

To reduce confounding factors, propensity score matching (PSM) was applied to balance baseline characteristics between the FL and non-FL groups. The propensity score was calculated using logistic regression, with variables including age, sex.BM, tumor size, nCRT status, tumor and nodal stages. Matched pairs were selected based on the closest propensity scores between the two groups. Balance between the groups was assessed using standardized mean differences (SMD), with an SMD of less than 0.1 indicating adequate matching.

Continuous variables were compared using Student's *t*-test and are reported as mean ± standard deviation (SD). Categorical variables were analyzed using the chi-square test or Fisher's exact test, with results presented as frequencies and percentages. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of fluorescence for metastatic LN detection were calculated. Sensitivity was defined as TP/ (TP + FN), specificity as TN/ (TN + FP), PPV as TP/ (TP + FP), and NPV as TN/ (TN + FN). Kaplan–Meier survival analysis with log-rank tests was used to compare overall survival (OS) and relapse-free survival (RFS) between FL and non-FL groups, further stratified by nCRT status. A *P* value < 0.05 was considered statistically significant. Statistical analyses were performed using R software (version 4.3.3) SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

Results

Baseline Characteristics Before and After PSM

A total of 501 patients diagnosed with mid-to-low rectal cancer were initially screened for inclusion. After applying exclusion criteria, 40 patients were excluded due to age below 18 or above 80 years (n = 9), a pathological diagnosis other than adenocarcinoma (n = 9), distant metastasis at initial diagnosis (n = 20), and documented allergy to ICG (n = 2). Ultimately, 461 patients were eligible for analysis, comprising 111 patients in the FL group and 350 in the non-FL group (Fig. 1).

Before PSM, significant baseline differences were observed between the FL and non-FL groups. The mean age was 56.3 versus 58.5 years (P = 0.074; SMD = 0.193), and the proportion of patients aged < 60 years was higher in the FL group (57.4% vs. 50.5%, P = 0.238; SMD = 0.140). Tumor staging also differed, with a greater proportion of cT1–2 tumors in the FL group (34.2% vs. 22.9%, P = 0.023; SMD = 0.254) and a higher percentage of pT1–2 tumors (48.6% vs. 33.7%, P = 0.006; SMD = 0.307). Additionally, imbalances were noted in cN and pN status, preoperative carcinoembryonic antigen (CEA) levels, tumor size, differentiation, and invasion markers.

To mitigate selection bias and enhance comparability, PSM was performed in a 1:2 ratio, adjusting for age, sex, BMI, tumor size, nCRT, tumor stage, and nodal stage. Following PSM, 111 patients remained in the FL group, while 222 were included in the non-FL group. The matching process effectively minimized baseline differences, with SMD reduced to < 0.1 for all variables. Post-matching, the mean age was 57.6 versus 58.5 years (P = 0.515; SMD = 0.075), and the proportion of patients aged < 60 years was 52.7% versus 50.5% (P = 0.786; SMD = 0.045). Similarly, cT (28.4% vs. 34.2%, P = 0.332; SMD = 0.092) and pT status (45.5% vs. 48.6%, P = 0.669; SMD = 0.063) were well balanced. Other clinicopathological characteristics, including cN and pN status, preoperative CEA levels, tumor size, differentiation, and nCRT use, were also comparable between the groups after matching (Table 1).

Table 1
Patient demographic characteristics and surgical procedures before and after the PSM

Clinical variables	Before PSM				After PSM			
	Non-FL group (n = 350), %	FL group (n = 111), %	P	SMD	Non-FL group (n = 222), %	FL group (n = 111), %	P	SMD
Age, yr, mean (SD) *	56.27 (11.20)	58.48 (11.62)	0.074	0.193	57.63 (11.05)	58.48 (11.62)	0.515	0.075
< 60	201 (57.4)	56 (50.5)	0.238	0.140	117 (52.7)	56 (50.5)	0.786	0.045
≥ 60	149 (42.6)	55 (49.5)			105 (47.3)	55 (49.5)		
Sex			0.744	0.048			0.783	0.045
Male	210 (60.0)	64 (57.7)			133 (59.9)	64 (57.7)		
Female	140 (40.0)	47 (42.3)			133 (59.9)	64 (57.7)		
BMI, kg/m2, mean (SD) *	24.06 (3.37)	24.06 (3.62)	0.996	< 0.001	24.04 (3.40)	24.06 (3.62)	0.955	0.001
< 24	172 (49.1)	51 (45.9)	0.632	0.064	108 (48.6)	51 (45.9)	0.727	0.054
≥ 24	178 (50.9)	60 (54.1)			114 (51.4)	60 (54.1)		
cT status			0.023	0.254			0.332	0.092
cT 1–2	80 (22.9)	38 (34.2)			63 (28.4)	38 (34.2)		
cT 3–4	270 (77.1)	73 (65.8)			159 (71.6)	73 (65.8)		
cN status			0.683	0.057			0.456	0.086
cN0	126 (36.0)	43 (38.7)			97 (43.7)	43 (38.7)		
cN+	224 (64.0)	68 (61.3)			125 (56.3)	68 (61.3)		
pT status			0.006	0.307			0.669	0.063
pT 1–2	118 (33.7)	54 (48.6)			101 (45.5)	54 (48.6)		
pT 3–4	232 (66.3)	57 (51.4)			121 (54.5)	57 (51.4)		
pN status			0.246	0.138			0.907	0.027
pN0	137 (39.1)	51 (45.9)			105 (47.3)	51 (45.9)		
pN+	213 (60.9)	60 (54.1)			117 (52.7)	60 (54.1)		
Preoperative CEA			0.555	0.076			0.606	0.074
Normal	218 (62.3)	65 (58.6)			138 (62.2)	65 (58.6)		
High	132 (37.7)	46 (41.4)			84 (37.8)	46 (41.4)		
Tumor size, cm, mean (SD) *	4.19 (1.81)	4.03 (1.70)	0.419	0.089	4.25 (1.96)	4.03 (1.70)	0.320	0.119
≤ 4.0	201 (57.4)	62 (55.9)	0.856	0.032	119 (53.6)	62 (55.9)	0.785	0.045
> 4.0	149 (42.6)	49 (44.1)			103 (46.4)	49 (44.1)		
Differentiation			0.319	0.120			0.907	0.027
Well/moderate	210 (60.0)	60 (54.1)			123 (55.4)	60 (54.1)		
Poorly	140 (40.0)	51 (45.9)			99 (44.6)	51 (45.9)		
Lymphatic invasion			0.440	0.967			0.716	0.056

Clinical variables	Before PSM				After PSM			
	Non-FL group (n = 350), %	FL group (n = 111), %	P	SMD	Non-FL group (n = 222), %	FL group (n = 111), %	P	SMD
Negative	201 (57.4)	69 (62.2)			144 (64.9)	69 (62.2)		
Positive	149 (42.6)	42 (37.8)			78 (35.1)	42 (37.8)		
Vascular invasion			0.225	0.146			0.967	0.019
Negative	212 (60.6)	75 (67.6)			152 (68.5)	75 (67.6)		
Positive	138 (39.4)	36 (32.4)			70 (31.5)	36 (32.4)		
Perineural invasion			0.989	0.013			0.365	0.119
Negative	190 (54.3)	61 (55.0)			135 (60.8)	61 (55.0)		
Positive	160 (45.7)	50 (45.0)			87 (39.2)	50 (45.0)		
nCRT			0.012	0.283			0.612	0.072
No	234 (66.9)	59 (53.2)			126 (56.8)	59 (53.2)		
Yes	116 (33.1)	52 (46.8)			96 (43.2)	52 (46.8)		

Values in parentheses are percentages unless indicated otherwise; value is *mean (standard deviation)

SMD standardized mean differences, FL fuorescent lymphography, BMI body mass index, nCRT neoadjuvant chemoradiotherapy.

Operative Findings and Short-Term Outcomes

After PSM, among all patients, the FL group had a shorter mean operative time (255.9 vs. 282.8 minutes, P = 0.007) and lower estimated blood loss (58.9 vs. 105.1 mL, P < 0.001) than the non-FL group (Table 2). The FL group also had a lower incidence of anastomotic leakage (10.0% vs. 13.5%, P = 0.046), shorter time to first flatus (2.1 vs. 2.2 days, P = 0.010), earlier transition to a soft diet (3.3 vs. 3.5 days, P = 0.020), and shorter postoperative hospital stay (6.4 vs. 12.7 days, P < 0.001).

Table 2
Operative fndings and short-term outcomes after PSM

	All patients (n = 333)			Non-nCRT (n = 185)			nCRT (n = 148)		
	Non-FL group (n = 222), %	FL group (n = 111), %	P	Non-FL group (n = 126), %	FL group (n = 59), %	P	Non-FL group (n = 96), %	FL group (n = 52), %	P
Operation time*	282.8 (101.8)	255.9 (74.9)	0.007	278.8(103.8)	236.92(59.9)	0.001	287.9(99.5)	277.5(84.4)	0.521
Blood loss*	105.1 (101.2)	58.9 (81.8)	< 0.001	118.8(87.6)	42.3(39.0)	< 0.001	114.8(87.0)	109.6(77.7)	0.632
Intraoperative bleeding*	12 (5.4%)	5 (4.5%)	0.420	3 (3.0%)	1 (2.0%)	0.700	9 (7.8%)	4 (6.8%)	0.750
Anastomotic leakage	30 (13.5%)	11 (10.0%)	0.046	9 (8.9%)	2 (4.1%)	0.035	21 (18.0%)	9 (15.3%)	0.7620
Gas passing*	2.2 (0.6)	2.1 (0.5)	0.010	1.8 (0.4)	1.7 (0.3)	0.005	2.7 (0.5)	2.6 (0.4)	0.300
Soft diet*	3.5 (0.8)	3.3 (0.7)	0.020	3.1 (0.6)	2.8 (0.5)	0.010	3.9 (0.7)	3.8 (0.6)	0.350
Hospital stays*	12.7 (9.7)	6.4 (2.2)	< 0.001	14.6(9.5)	6.4(2.0)	< 0.001	10.4(9.5)	6.3(2.5)	< 0.001

Values in parentheses are percentages unless indicated otherwise; value is *mean (standard deviation)

FL fuorescent lymphography, nCRT neoadjuvant chemoradiotherapy.

In the non-nCRT subgroup, FL use was associated with shorter operative time (236.9 vs. 278.8 minutes, P = 0.001) and lower estimated blood loss (42.3 vs. 118.8 mL, P < 0.001). The incidence of anastomotic leakage was also lower in the FL group (4.1% vs. 8.9%, P = 0.035), with shorter time to first flatus (1.7 vs. 1.8 days, P = 0.005), earlier transition to a soft diet (2.8 vs. 3.1 days, P = 0.010), and shorter postoperative hospital stay (6.4 vs. 14.6 days, P < 0.001).

In contrast, among patients who received nCRT, no significant differences were observed between the FL and non-FL groups in operative time (277.5 vs. 287.9 minutes, P = 0.521), estimated blood loss (109.6 vs. 114.8 mL, P = 0.632), anastomotic leakage (15.3% vs. 18.0%, P = 0.762), time to first flatus (2.6 vs. 2.7 days, P = 0.300), or transition to a soft diet (3.8 vs. 3.9 days, P = 0.350). However, postoperative hospital stay remained shorter in the FL group (6.3 vs. 10.4 days, P < 0.001)..

Lymph Node Retrieval

The FL group had a greater overall lymph node (LN) retrieval than the non-FL group (Table 3). Among all patients, the FL group had a higher mean total number of retrieved LNs (29.3 ± 12.5 vs. 26.7 ± 12.8, P = 0.049) and a higher proportion of patients with ≥ 25 LNs retrieved (66.7% vs. 51.4%, P = 0.007). However, no significant differences were observed in the numbers of mesenteric lymph nodes (MLNs) or lateral lymph nodes (LLNs) between the two groups.

Table 3
Comparison of retrieved lymph nodes after the PSM

All Patients	Non-FL group	FL group	P value
Patients number	222	111	
Total LN*	26.7 (12.8)	29.3 (12.5)	0.049
MLN*	17.4 (10.2)	19.1 (11.5)	0.168
LLN*	9.3 (6.8)	10.2 (6.2)	0.246
Retrieved LN ≥ 12	206 (92.8)	107 (96.4)	0.192
Retrieved LN ≥ 25	114 (51.4)	74 (66.7)	0.007
Non-nCRT patients			
Patients number	126	59	
Total LN*	26.4 (12.5)	29.4 (13.2)	0.035
MLN*	18.1 (10.6)	19.2 (11.6)	0.527
LLN*	8.3 (5.8)	10.2 (6.5)	0.045
Retrieved LN ≥ 12	120 (95.2)	58 (98.3)	0.308
Retrieved LN ≥ 25	60 (47.6)	40 (67.8)	0.010
nCRT patients			
Patients number	96	52	
Total LN*	27.1 (13.3)	29.2 (11.8)	0.344
MLN*	16.5 (9.6)	19.0 (11.5)	0.155
LLN*	10.7 (7.7)	10.2 (5.8)	0.711
Retrieved LN ≥ 12	86 (89.6)	49 (94.2)	0.340
Retrieved LN ≥ 25	51 (53.1)	34 (65.4)	0.150

Values in parentheses are percentages unless indicated otherwise;
value is *mean (standard deviation)
FL fuorescent lymphography, LN lymph node, nCRT neoadjuvant chemoradiotherapy, LLN Lateral lymph node, MLN mesenteric lymph node

In the non-nCRT subgroup, the FL group had a higher mean total LN count (29.4 ± 13.2 vs. 26.4 ± 12.5, P = 0.035) and a higher proportion of patients with ≥ 25 LNs retrieved (67.8% vs. 47.6%, P = 0.010). The number of retrieved LLNs was also higher in the FL group (10.2 ± 6.5 vs. 8.3 ± 5.8, P = 0.045), whereas no significant difference was observed in MLN retrieval.

In contrast, among patients who received nCRT, no significant differences were observed between the FL and non-FL groups in total LN retrieval, MLN or LLN counts, or the proportions of patients with ≥ 12 or ≥ 25 LNs retrieved.

Overall, these findings suggest that the association between FL and greater LN retrieval was more evident among patients who did not receive nCRT, whereas a similar association was not observed in patients who received nCRT.

Diagnostic Performance of FL for Metastatic LN Detection

Among all patients, FL demonstrated a sensitivity of 34.0% (95% CI, 30.9–37.3) and a specificity of 64.4% (95% CI, 63.4–65.4) for detecting metastatic involvement of total LN stations (Table 4). Sensitivity was 37.4% in the non-nCRT subgroup (P = 0.023) and 27.8% in the nCRT subgroup (P = 0.082). The corresponding NPV ranged from 89.7% to 91.6%. Overall, the NPV was 90.6% (95% CI, 89.8–91.3), whereas the PPV was 8.9% (95% CI, 7.9–9.9).

Table 4
Diagnostic value of fuorescent lymphography for detection of metastatic stations and metastatic lymph nodes

	Total	No. of metastasis	No. of non-metastasis	P	Sensitivity[a] (CI)	Specifcity[b] (CI)	NPV[c] (CI)	PPV[d] (CI)
Diagnostic value of fuorescent lymphography for Total LNs								
All patients				0.362	34.0(30.9–37.3)	64.4 (63.4–65.4)	90.6 (89.8–91.3)	8.9 (7.9–9.9)
Fluorescent	3252	288 (TP)	2964 (FP)					
Non-fuorescent	5927	559 (FN)	5368 (TN)					
Non-nCRT				0.100	37.4 (33.5–41.6)	66.1 (64.7–67.5)	89.7 (88.7–90.7)	11.8 (10.3–13.4)
Fluorescent	1734	204 (TP)	1530 (FP)					
Non-fuorescent	3325	341 (FN)	2984 (TN)					
NCRT				< 0.001	27.8 (23.1–33.1)	62.4 (60.9–64.0)	91.6 (90.5–92.6)	5.5 (4.5–6.8)
Fluorescent	1518	84 (TP)	1434 (FP)					
Non-fuorescent	2602	218 (FN)	2384 (TN)					
Diagnostic value of fuorescent lymphography for Total MLNs								
All patients				0.681	34.8 (31.4–38.3)	64.5 (63.2–65.7)	87.8 (86.7–88.8)	11.8 (10.5–13.3)
Fluorescent	2121	251 (TP)	1870 (FP)					
Non-fuorescent	3862	471 (FN)	3391 (TN)					
Non-nCRT				0.019	37.8 (33.6–42.3)	67.6 (65.9–69.3)	86.9 (85.5–88.2)	16.1 (14.0-18.3)
Fluorescent	1133	182 (TP)	951 (FP)					
Non-fuorescent	2282	299 (FN)	1983 (TN)					
NCRT				< 0.001	28.6 (23.3–34.6)	60.5 (58.5–62.5)	89.1 (87.5–90.6)	7.0 (5.6–8.7)
Fluorescent	988	69 (TP)	919 (FP)					
Non-fuorescent	1580	172 (FN)	1408 (TN)					
Diagnostic value of fuorescent lymphography for Total LLNs								
All patients				0.167	29.6 (22.3–38.1)	64.4 (62.7–66.1)	95.7 (94.8–96.5)	3.3 (2.4–4.5)
Fluorescent	1131	37 (TP)	1094 (FP)					

	Total	No. of metastasis	No. of non-metastasis	P	Sensitivity[a] (CI)	Specifcity[b] (CI)	NPV[c] (CI)	PPV[d] (CI)
Diagnostic value of fuorescent lymphography for Total LNs								
Non-fuorescent	2065	88 (FN)	1977 (TN)					
Non-nCRT				0.712	34.4 (23.9–46.6)	63.4 (60.9–65.7)	96.0 (94.6–97.0)	3.7 (2.4–5.5)
Fluorescent	601	22 (TP)	579 (FP)					
Non-fuorescent	1043	42 (FN)	1001 (TN)					
NCRT				0.108	24.6 (15.5–36.7)	65.5 (63.0-67.8)	95.5 (94.0-96.6)	2.8 (1.7–4.6)
Fluorescent	530	15 (TP)	515 (FP)					
Non-fuorescent	1022	46 (FN)	976 (TN)					

CI confdence interval 95, *TP* true positive, *FP* false positive, *FN* false negative, *TN* true negative

a Sensitivity was calculated by [TP/TP + FN]

b Specifcity was calculated by [TN/TN + FP]

c NPV, negative predictive value was calculated by [TN/TN + FN]

d PPV, positive predictive value was calculated by [TP/TP + FP]

LLN Lateral lymph node, MLN mesenteric lymph node

nCRT, neoadjuvant chemoradiotherapy

In the non-nCRT subgroup, sensitivity was 37.4% (95% CI, 33.5–41.6), with a specificity of 66.1% (95% CI, 64.7–67.5), an NPV of 89.7% (95% CI, 88.7–90.7), and a PPV of 11.8% (95% CI, 10.3–13.4). Among patients who received nCRT, sensitivity was 27.8% (95% CI, 23.1–33.1), specificity was 62.4% (95% CI, 60.9–64.0), NPV was 91.6% (95% CI, 90.5–92.6), and PPV was 5.5% (95% CI, 4.5–6.8).

For metastatic mesenteric lymph nodes (MLNs), the overall sensitivity was 34.8% (95% CI, 31.4–38.3) and PPV was 11.8% (95% CI, 10.5–13.3). In the non-nCRT subgroup, sensitivity was 37.8% (95% CI, 33.6–42.3) and PPV was 16.1% (95% CI, 14.0–18.3), whereas in the nCRT subgroup, sensitivity was 28.6% (95% CI, 23.3–34.6) and PPV was 7.0% (95% CI, 5.6–8.7).

For lateral lymph nodes (LLNs), the overall sensitivity was 29.6% (95% CI, 22.3–38.1) and PPV was 3.3% (95% CI, 2.4–4.5). Sensitivity was 34.4% (95% CI, 23.9–46.6) and PPV was 3.7% (95% CI, 2.4–5.5) in the non-nCRT subgroup, compared with 24.6% (95% CI, 15.5–36.7) and 2.8% (95% CI, 1.7–4.6), respectively, in the nCRT subgroup.

Among fluorescent LNs detected beyond the planned extent of lymph node dissection, the PPV for metastatic involvement was 2.3% (39/1685). Overall, FL demonstrated relatively high NPV but limited sensitivity and low PPV for detecting metastatic LNs, with diagnostic performance generally less favorable among patients who received nCRT.

Survival Outcomes Stratified by Neoadjuvant Chemoradiotherapy Status

With a median follow-up of 32 months, no statistically significant differences were observed in overall survival (OS) or relapse-free survival (RFS) between the FL and non-FL groups (P = 0.189 and P = 0.477, respectively; Fig. 2).

To further assess whether these associations differed according to neoadjuvant treatment status, patients were stratified by receipt of nCRT. In the non-nCRT subgroup, OS and RFS were comparable between the FL and non-FL groups (P = 0.532 and P = 0.374, respectively). Similarly, in the nCRT subgroup, no statistically significant differences were observed in OS (P = 0.346) or RFS (P = 0.183). Overall, no statistically significant differences in OS or RFS were observed between the FL and non-FL groups, either overall or within the nCRT-stratified subgroups.

Discussion

Our study found that FL use was associated with greater LN retrieval and favorable perioperative outcomes in patients with mid-to-low rectal cancer, particularly among those who did not receive nCRT. ICG fluorescence provides real-time visualization of lymphatic channels and may facilitate the identification and dissection of lymphatic structures and LN-bearing tissue, including areas where adipose tissue or altered anatomical planes may make conventional dissection more challenging. However, given the observational nature of this study, these findings should be interpreted as associations rather than evidence of a direct causal effect of FL.

The differences associated with FL were most evident in the non-nCRT subgroup. In this population, FL use was associated with shorter operative time, lower blood loss, and greater retrieval of mesorectal and lateral LNs. These findings suggest that FL may facilitate lymphatic mapping and LN retrieval during rectal cancer surgery, although further prospective studies are warranted to determine whether these associations reflect a direct benefit of fluorescence guidance. This improved efficiency may be attributable to real-time intraoperative mapping of lymphatic structures, which may facilitate the identification of key nodal basins and support more comprehensive lymphatic dissection. In this context, our findings are in agreement with previous reports demonstrating that FL increases the total LN count, which is vital for accurate staging and prognostication [16, 17]. Moreover, achieving the recommended threshold of 12 or more LNs ensures that the pathological evaluation is robust, potentially leading to better-informed treatment decisions [18, 19].

Conversely, the potential benefits of FL were less evident in the nCRT subgroup. Neoadjuvant chemoradiotherapy induces histopathological changes, including fibrosis, lymphocyte depletion, and alterations in vascular and lymphatic structures [20–22]. These treatment-related changes may affect ICG uptake, lymphatic transport, and the resulting fluorescent signal, potentially contributing to the lower sensitivity and PPV observed among patients who received nCRT. Nevertheless, FL demonstrated a relatively high negative predictive value in this subgroup, suggesting that the absence of fluorescence was frequently associated with the absence of nodal metastasis, although this finding should be interpreted in the context of the limited sensitivity and PPV of fluorescence alone. These observations suggest that nCRT-induced tissue alterations may influence the performance of intraoperative fluorescence imaging and highlight the need for further studies to optimize the timing, dose, and administration strategy of ICG in patients undergoing nCRT.

Notably, our study incorporated a stratified analysis comparing the associations of FL with surgical outcomes in patients receiving nCRT and those not receiving nCRT. This approach provides further insight into whether the clinical utility of fluorescence-guided techniques may differ according to preoperative treatment status. In addition, propensity score matching was used to improve baseline comparability between the FL and non-FL groups and to reduce the potential influence of measured confounding factors. Nevertheless, residual confounding cannot be excluded given the non-randomized study design.

However, several limitations should be acknowledged. First, the non-randomized design of this study introduces the possibility of selection bias and residual confounding, which cannot be completely eliminated by propensity score matching despite adjustment for measured baseline characteristics. Second, the combined use of intraoperative and back-table FL precluded assessment of the individual contribution of each approach to lymph node retrieval. Third, the single-center setting and relatively limited sample size may restrict the generalizability of our findings, particularly to patients with different patterns of neoadjuvant treatment or more advanced disease. Finally, the follow-up duration may be insufficient to fully assess the long-term oncologic implications of FL-guided lymphadenectomy. Future prospective, preferably multicenter, studies with larger sample sizes and longer follow-up are warranted to validate these findings and to clarify the clinical and oncologic significance of FL. Further research is also needed to optimize the timing, dose, and administration strategy of ICG in patients receiving nCRT and to determine whether such optimization can improve the diagnostic performance of FL.

In summary, FL was associated with greater LN retrieval and favorable perioperative outcomes during TME for rectal cancer, with these associations being more evident among patients who did not receive nCRT. The association with greater LN retrieval was less evident after nCRT, and the diagnostic performance of FL for identifying metastatic LNs remained limited, despite a relatively high negative predictive value. These findings support the potential role of FL as an adjunctive tool for lymphatic mapping and LN dissection rather than as a standalone method for nodal assessment or staging. Further prospective studies are warranted to optimize FL protocols and clarify its clinical utility in patients receiving nCRT.

Abbreviations

FL
Fluorescence Lymphangiography
ICG
Indocyanine Green
LN
Lymph Node
BMI
Body Mass Index
PSM
Propensity Score Matching
NPV
Negative Predictive Value
PPV
Positive Predictive Value
MLN
Mesenteric Lymph Node
LLN
Lateral Lymph Node
NIR
Near-Infrared
NCRT
Neoadjuvant Chemoradiotherapy

Declarations

Ethics approval and consent to participate

Ethics reference number is NCC4123, which is approved by Ethics Committee of the Clinical Trials Center of National Cancer Center (Phone + 0086 010 87787112). The hyperlink to registration: http://cicams.ctims.com.cn/actmsf/index.action.

Consent for publication

The authors have obtained consent from all participants for the publication of any data contained within the manuscript.

Disclosures

Wenlong Qiu, Cheng Zhou, Wenhao Zhang, Qian Liu have no conflicts of interest or financial ties to disclose.

Competing Interests

The authors declare no competing interests.

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Author Contribution

Wenlong Qiu: Conceptualization, Methodology, Formal analysis, investigation, Writing - original draft. Cheng Zhou: Methodology, Formal analysis, investigation, Writing - review & editing. Wenhao Zhang: Resources, Data curation, Writing - review & editing. Qian Liu: Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration.# Wenlong Qiu, Cheng Zhou and Wenhao Zhang contributed equally to this work. All authors have approved the final version of the article.

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Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to confidentiality agreements with participants, but are available from the corresponding author on reasonable request.

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Figures

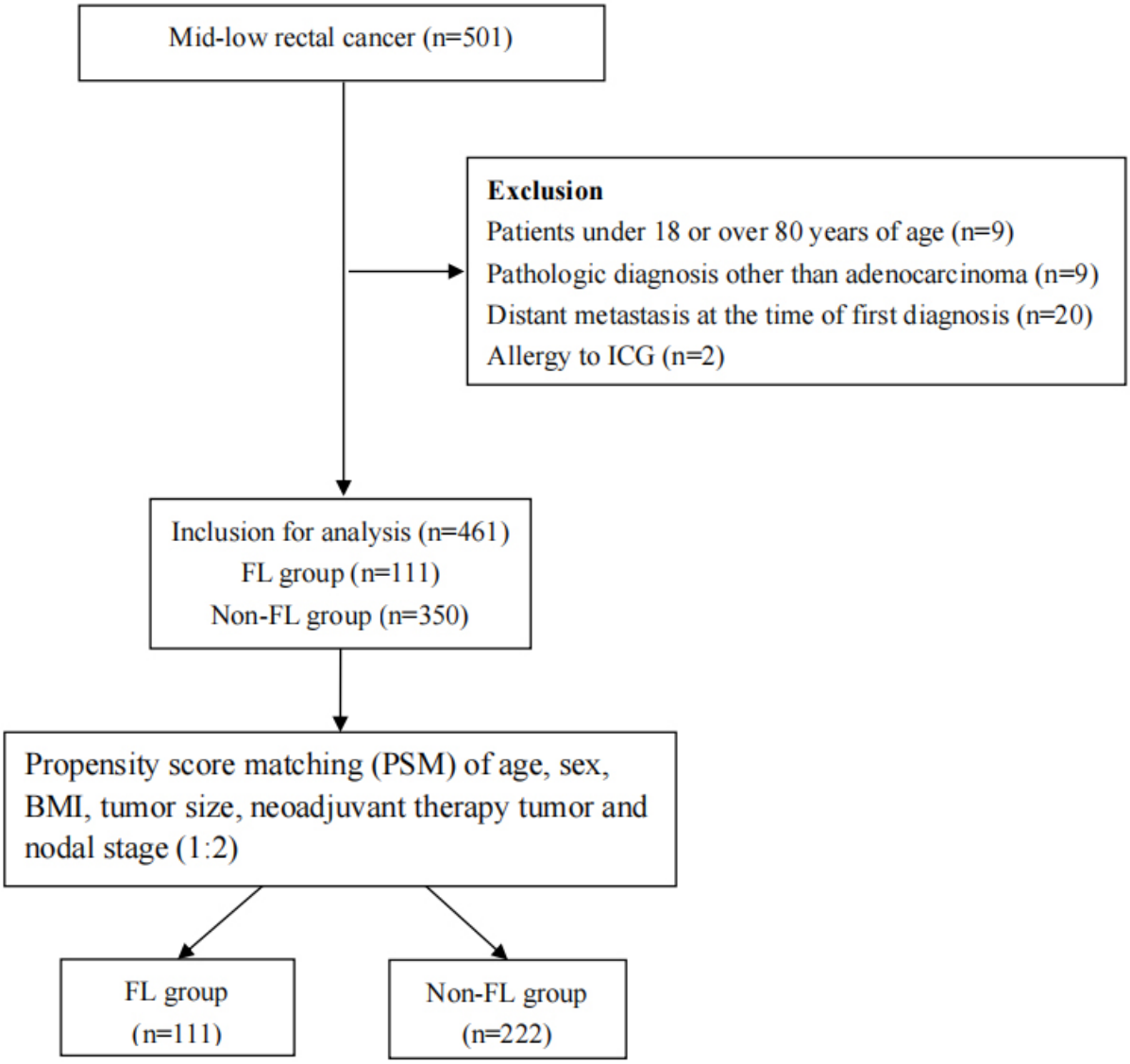

Figure 1

Patient selection flow for the study.

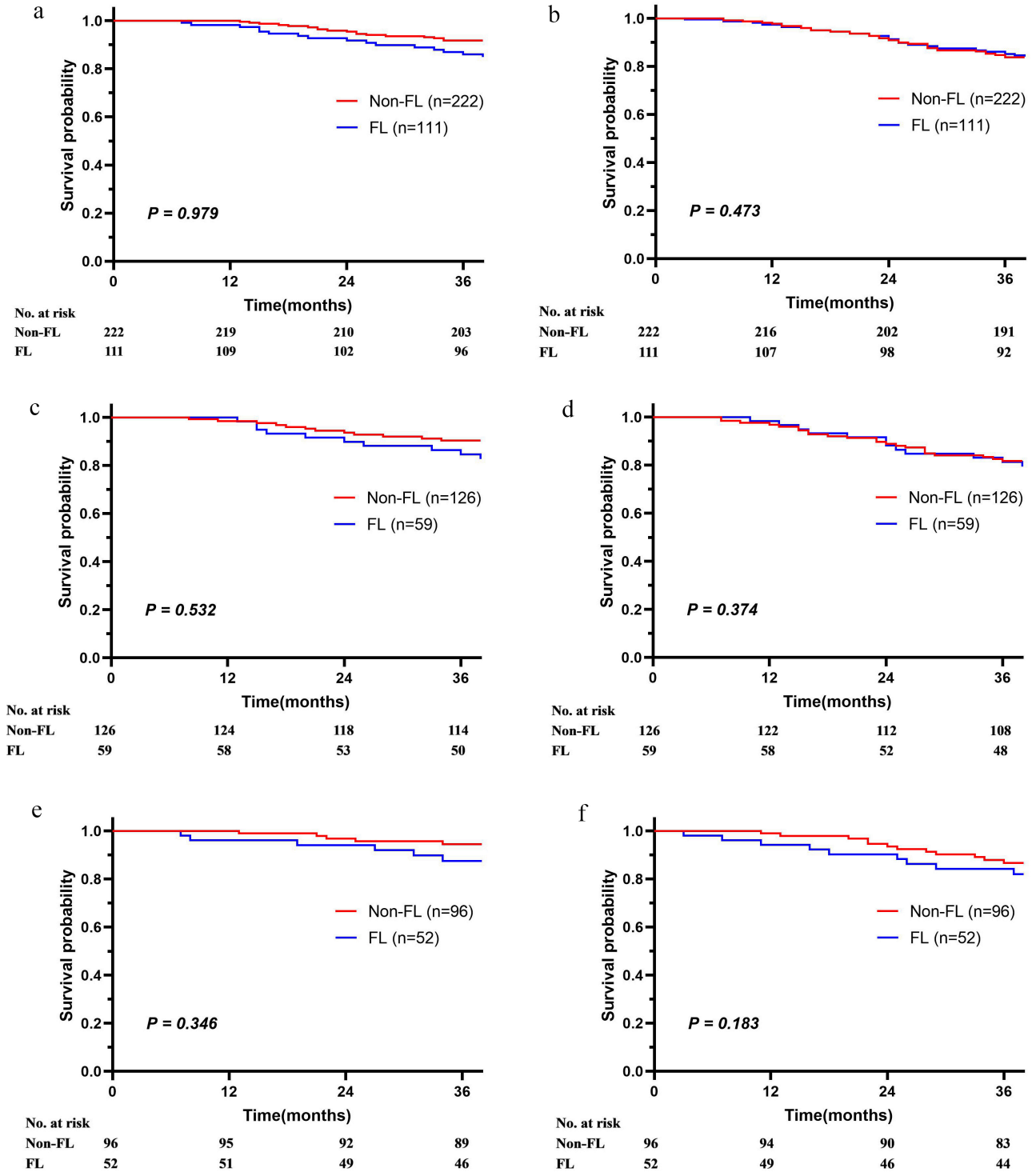

Figure 2

Kaplan–Meier survival curves were generated to compare overall survival between the fluorescent lymphography (FL) group and non-fluorescent lymphography (non-FL) group. These comparisons were made for:

a. All patients,

b. Patients who received neoadjuvant chemoradiotherapy (nCRT),

c. Patients who did not receive neoadjuvant chemoradiotherapy (non-nCRT group),

d. Relapse-free survival for all patients,

e. Relapse-free survival for patients who received neoadjuvant chemoradiotherapy (nCRT),

f. Relapse-free survival for patients who didn’t receive nCRT (non-nCRT group).