Real-World Predictors of Pathologic Response to FLOT in Patients with Resectable Gastric Cancer: A Retrospective Analysis

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ABSTRACT

Pathologic response to neoadjuvant FLOT chemotherapy is a key prognostic indicator in gastric cancer, yet reliable predictors remain unclear. This study aimed to identify clinicopathologic and biologic factors associated with response and to develop a preliminary predictive model. A single-center, retrospective cohort of 75 patients with resectable gastric cancer treated with perioperative FLOT between October 2022 and September 2025 was analyzed. Clinicopathologic and laboratory parameters were compared between good responders (Ryan 0–1) and poor responders (Ryan 2–3) using Mann–Whitney U and Chi-square tests. A random-forest classifier incorporating pre-treatment variables was built to explore multivariate interactions and feature importance. The median age was 65 years, and 84% were male. Stage III disease was observed in 81% of patients. Good pathologic response occurred in 45 patients (60%). Poor response correlated significantly with stage III, T3–T4, and high-grade tumors (p= 0.001, 0.003, 0.002, respectively) and higher platelet-to-lymphocyte ratio (p= 0.04). In multivariate analysis, advanced T stage (OR 4.39, p= 0.018), high tumor grade (OR 5.24, p= 0.005), and proximal tumor location (OR 0.30, p= 0.04) independently predicted poor response. The random-forest model achieved an AUC of 0.611 with 65% accuracy. Key predictive features were T stage, N stage, CEA level, and BMI. Tumor depth, histologic grade, and location significantly affect FLOT response. Non-proximal tumors showed more favorable outcomes. The modest machine-learning performance highlights the need to integrate molecular and radiomic markers to refine prediction and personalize perioperative therapy.

Keywords: Resectable gastric cancer, FLOT, Pathologic response, Predictors of response, Perioperative therapy

INTRODUCTION

Gastric adenocarcinoma (GAC) remains a major global health problem with an estimated 968,784 new cases and 660,175 deaths reported in 2022.[1] Perioperative chemotherapy is the standard of care for patients with resectable tumors.[2] The FLOT regimen (fluorouracil, leucovorin, oxaliplatin, and docetaxel) was established as the optimal perioperative treatment following the FLOT4-AIO trial.[3,4] Compared with epirubicin and cisplatin combined with either fluorouracil or capecitabine (ECF/ECX), FLOT demonstrated superior pathologic response rates, disease-free survival and overall survival. Despite these encouraging outcomes, recurrence rates remain high. Numerous studies were designed to reduce recurrence, including those evaluating the addition of immunotherapy to FLOT[5-8] or the use of total neoadjuvant FLOT[9,10] instead of perioperative approach. However, these strategies have not yet achieved a meaningful improvement in recurrence outcomes.

Pathologic response is recognized as the most important predictor of survival in patients receiving perioperative chemotherapy for resectable gastric cancer.[11,12] However, the wide variation in pathological response rates reported across different studies studies[3,13-15] using the same FLOT regimen underscores the need to identify real-world predictors and to in different studies[3,13-15] make it mandatory to evaluate real-world predictors and to in different studies[3,13-15] make it mandatory to evaluate real-world predictors and to tailor the perioperative treatment according to clinical, pathological and molecular characteristics.

In this study we evaluate the real-world predictors of perioperative FLOT with random forest model which will help to guide future therapies for better pathologic response rates and survival outcomes.

PATIENTS AND METHODS

Patients and Variables

A total of 75 patients with gastric adenocarcinoma who underwent D2 gastrectomy after receiving at least four cycles of FLOT chemotherapy between October 2022 and September 2025 were included in this study. Clinical data were extracted from institutional databases. The analyzed variables included age, sex, smoking history, body mass index (BMI), stage at diagnosis, primary tumor location, baseline T and N stage, tumor grade, carcinoembryonic antigen (CEA) level, carbohydrate antigen 19-9 (CA 19-9) level, neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR).

The study was approved by the institutional ethics committee (approval no. 2024-691). It was conducted in accordance with the principles of the Declaration of Helsinki. As this was a retrospective analysis, the requirement for informed consent was waived.

Staging, Treatment, and Follow-up

All patients underwent baseline imaging with computed tomography (CT) or positron emission tomography (PET)/CT and endoscopic evaluation. Staging was retrospectively reviewed and classified according to the 9th edition of the American Joint Committee on Cancer (AJCC) staging system.

FLOT chemotherapy was administered intravenously every 2 weeks. Prior to surgery, all patients were re-evaluated with CT or PET/CT imaging. The decision to proceed with surgery was based on the most recent staging results, comorbidities, and performance status.

Pathological Assessment

Surgical pathology specimens were reviewed by a pathologist to confirm the achievement of R0 resection. Tumor regression was assessed according to the modified Ryan criteria 16. Patients were stratified into two groups based on pathologic response:

• Good responders: Ryan scores 0 or 1, indicating complete or near-complete response (absent or minimal residual tumor cells).

• Poor responders: Ryan scores 2 or 3, indicating partial or poor/no response (substantial residual viable tumor with limited or absent regression).

Ethical Approval: This sttudy is approved by the Ethics Committee of Ankara Etlik City Hospital (AESH-BADEK-2024-69; July 31, 2024).

Statistical Analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY) and R Studio version 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were summarized as medians with interquartile ranges (IQRs), and categorical variables as frequencies and percentages. The normality of continuous variables was assessed with the Shapiro–Wilk test. Between-group comparisons were conducted using the Mann–Whitney U test for non-normally distributed continuous variables and the Pearson chi-square test for categorical variables.

Machine-learning analyses were performed to complement conventional regression models. Random forest classifiers (1.000 trees) were trained using stratified training/test splits. Data preprocessing included median imputation for numerical variables, mode imputation and one-hot encoding for categorical variables. The positive class was defined as responders, and class weights proportional to inverse class frequency were applied. Predictive performance was evaluated using the area under the receiver operating characteristic curve (ROC-AUC) with 95% confidence intervals and confusion matrix metrics at the Youden index–derived threshold (accuracy, sensitivity, specificity). Precision–recall AUC (PR-AUC) and the Brier scores

Table 1. Clinicopathological characteristics of 75 patients treated with neoadjuvant FLOT

Clinical feature	n (%)
Age, median (IQR)	65 (59-72)
Sex	
Male	63 (84)
Female	12 (16)
Stage at diagnosis	
Stage 1-2	14 (18.7)
Stage 3	61 (81.3)
Primary tumor location	
Proximal (cardia/fundus/ GEJ)	37 (49.3)
Body (corpus)	13 (17.3)
Distal (antrum/pylorus)	25 (33.3)
T stage	
T1-T2	54 (72)
T3-T4	21 (28)
N stage	
N positive	69 (92)
N negative	6 (8)
Grade	
Grade 1-2 (low)	43 (57.3)
Grade 3 (high)	31 (41.3)
Median FLOT cycles, IQR	6 (4-8)
CEA level, ng/dl, median IQR	3.73 (1.57-9.22)
CA 19-9 level, U/ml, median IQR	16.4 (8.05-95.5)
NLR, median IQR	2.66 (1.97-3.32)
PLR, median IQR	163.37 (129.4-208.6)

were also reported. Feature contributions were assessed by permutation importance (ROC-AUC metric, event level= "second", 200 permutations), excluding post-treatment or follow-up variables to prevent data leakage. In sensitivity analyses, five-fold cross-validation was used to summarize model AUC and permutation importance.

RESULTS

Patient Characteristics

A total of 75 patients with GAC who received at least four cycles of preoperative FLOT and had technically resectable disease at both diagnosis and restaging were included in the study. The demographic, clinical and pathologic characteristics of the cohort are summarized in Table 1. The median age at diagnosis was 65 years (range; 59-72), and 84% of patients were male. The majority presented with stage III disease (81.3%), and half of the tumors were proximally located. Baseline T stage was T1-T2 in 72%, while N status was positive in 92% of all patients. Most tumors (57.3%) were moderately differentiated.

The median number of FLOT cycles administered was 6 (range; 4-8). All patients underwent D2 gastrectomy 6-8 weeks after completion of preoperative chemotherapy. Among the entire cohort, 60% were classified as good responders, whereas 40% were poor responders according to the modified Ryan criteria.

Clinical and Pathological Variables Associated with Good Pathologic Outcomes

When clinical and pathological features were compared between good and poor responders, significant differences were observed in stage at diagnosis, tumor grade, and median PLR (Table 2). Among patients with a good response, 31.1% had clinical stage I-II disease and 68.9% had stage III disease (p= 0.001). The majority of good responders (71.1%) had well- or moderately-differentiated tumors (p= 0.002). Poor responders, who comprised 40% of the cohort, exhibited a significantly higher median PLR compared with good responders (p= 0.04).

In univariate analysis, lower clinical T stage and better tumor differentiation (Table 3) were significantly associated with good pathologic response. In multivariate analysis, independent predictors of favorable pathologic response included non-proximal tumor location (OR: 0.30, 95% CI: 0.09-0.96, p= 0.04), early clinical T stage (OR: 4.39, 95% CI: 1.28-14.99, p= 0.018) and well/moderate tumor grade (OR: 5.24, 95% CI: 1.63-16.7, p= 0.005).

The machine learning model integrating baseline clinicopathologic variables achieved an AUC of 0.611 (95% CI: 0.351-0.871) for distinguishing good from poor pathologic responders (Figure 1). Although the performance exceeded random classification, its overall discriminative ability was modest. Permutation-based feature-importance analysis identified clinical T stage, N stage, and baseline CEA level as the strongest predictors of good pathologic response to FLOT therapy (Figure 2). These were followed by BMI, tumor grade,

Table 2. Comparison of patients with good vs poor response to neoadjuvant therapy

Clinical features	Good Responders n: 45, %	Poor Responders n: 30, %	p
Age, median (IQR)	65 (56.5-72)	66 (59-72)	0.42
Sex			0.44
Male	39 (86.6)	24 (80)	
Female	6 (13.4)	6 (20)	
Smoking history			0.18
Yes	23 (51.1)	20 (66.6)	
No	22 (48.9)	10 (33.4)	
BMI, median (IQR)	22.6 (20.2-25)	24.6 (21.2-28.0)	0.17
Stage at diagnosis			0.001
Stage 1-2	14 (31.1)	0 (0)	
Stage 3	31 (68.9)	30 (100)	
Primary tumor location			0.09
Proximal (cardia/fundus/ GEJ)	18 (40)	19 (63.3)	
Body (corpus)	8 (17.7)	5 (16.7)	
Distal (antrum/pylorus)	19 (42.3)	6 (20)	
T stage			0.003
T1-T2	38 (84.4)	16 (53.3)	
T3-T4	7 (15.6)	14 (46.7)	
N stage			0.004
Positive	41 (91.1)	28 (93.3)	
Negative	4 (8.9)	2 (6.7)	
Grade			0.002
Grade 1-2 (low)	32 (71.1)	11 (36.6)	
Grade 3 (high)	12 (28.9)	19 (63.4)	
CEA level, ng/dl, median IQR	3.16 (1.33-5.89)	4.54 (2.09-10.05)	0.37
CA 19-9 level, U/ml, median IQR	16.3 (6.85-61.25)	22 (9.9-180.5)	0.39
NLR, median IQR	2.55 (1.86-3.20)	2.79 (2.20-3.66)	0.12
PLR, median IQR	159.89 (111.98-192.17)	181.07 (142.45-241.0)	0.04

and baseline CA 19-9 level, suggesting that both tumor burden and host characteristics influence chemosensitivity. In contrast, systemic inflammatory indices (NLR, PLR) and routine biomedical parameters contributed minimally to do the model's predictive accuracy.

DISCUSSION

Our findings demonstrate that baseline tumor burden (T stage), histologic grade, and anatomic location significantly influence pathologic response to FLOT chemotherapy. The modest predictive performance of the random forest model (AUC 0.61) suggests that traditional clinicopathologic variables alone are insufficient to accurately identify responders. Incorporation of molecular, genomic, and radiomic parameters may enhance predictive accuracy in future models. Furthermore, the correlation between elevated PLR and poor response underscores a potential association between systemic inflammation and chemoresistance.

Pathologic response is widely recognized as the most important predictor of survival in patients undergoing perioperative chemotherapy for resectable gastric cancer.[11,12] Numerous studies have sought to increase pathologic response rate to FLOT therapy. In the pivotal FLOT-AIO trial, FLOT achieved a significantly higher pathologic complete response rate compared with ECF/ECX (16% vs. 6%; p=

Table 3. Univariate and multivariate logistic regression analyses of factors associated with pathological response

Clinical parameters	Univariate Analyses OR	95 % CI	p	Multivariate Analyses OR	95 % CI	p
Age, years (≤ 65; > 65)	1.25	0.49-3.16	0.63			
Sex (Male, Female)	1.65	0.47-5.61	0.44			
Smoking history (Yes, No)	1.91	0.73-4.98	0.18	2.67	0.79-9.0	0.110
BMI (< 25; ≥ 25)	2.25	0.5-10.0	0.28			
Primary tumor location (proximal; non-proximal)	0.40	0.15-1.04	0.06	0.30	0.09-0.96	0.040
T stage (T1-2; T3-4)	4.75	1.61-13.9	0.05	4.39	1.28-14.99	0.018
N stage (positive; negative)	1.36	0.23-7.97	0.72			
Grade (low; high)	4.60	1.70-12.4	0.003	5.24	1.63-16.7	0.005
CEA, ng/dl (≤ 5; > 5)	1.09	0.42-2.83	0.84			
CA 19-9, U/ml (normal; ULN)	1.04	0.40-2.74	0.92			
NLR (< 2.5; ≥ 2.5)	1.43	0.55-3.72	0.45			
PLR (< 165; ≥ 165)	1.78	0.70-4.55	0.22			

0.02) 3. In the phase III component, FLOT also significantly improved survival outcomes compared with ECF/ECX 4. Despite these advances, recurrence rates remain high, prompting ongoing studies into enhanced neoadjuvant strategies capable of improving both pathologic response and survival outcomes.

One of the most extensively studied strategies involves adding immunotherapy to FLOT. Although the KEYNOTE-585 trial, which combined pembrolizumab with perioperative chemotherapy, yielded significantly negative results[8,17], it was criticized for using fluorouracil-cisplatin regimen rather than standard FLOT in most patients.[13] In contrast, the MATTERHORN trial, which incorporated durvalumab into the FLOT backbone, demonstrated a positive outcome, with a 12% increase in pathologic response rate among patients receiv-

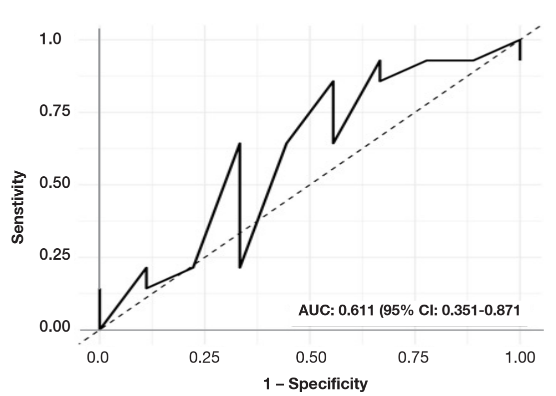

Figure 1. Receiver Operating Characteristics (ROC) curve of the Random Forest Model for treatment response prediction.

The Random Forest model built with pre-treatment variables achieved an AUC of 0.611 (95% CI 0.351–0.871), indicating a modest discriminatory ability. The dashed diagonal line represents the line of no discrimination (AUC= 0.5).

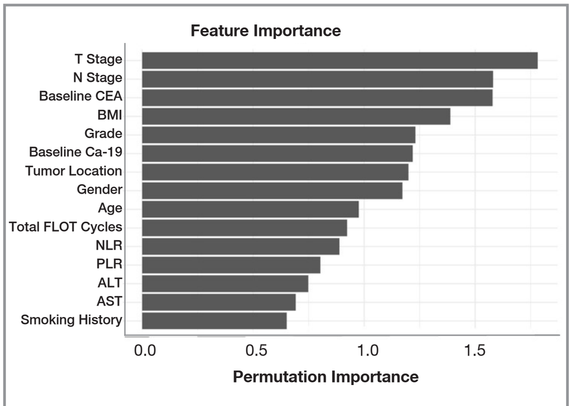

Figure 2. Random Forest model for treatment response prediction: feature importance.

ing immunotherapy.[7] Collectively, these findings indicate even when statistical significance is not reached, augmenting the immune response within the neoadjuvant setting can favorably influence key endpoints, supporting the continued development of immune-enhancing strategies.

A a second approach-adding radiotherapy to chemotherapy-has not shown a consistent benefit in terms of pathologic response or survival.[14,15,18] Similarly, total neoadjuvant therapy, in which all eight cycles of FLOT are administered preoperatively instead of using a perioperative regimen, has not produced significant differences in terms of pathologic response and survival outcomes.[9,10]

These mixed and often negative findings likely reflect the biological heterogeneity of gastric cancer. Consequently, identifying predictive markers of pathologic response is essential for developing more personalized and effective treatment strategies.

In our study, tumor depth, histologic grade, and anatomical location were confirmed as key determinants of FLOT response, with non-proximal tumors exhibiting more favorable outcomes. Although our machine-learning model showed limited discriminative ability, it underscores the potential value of integrating molecular and radiomic features to refine prediction and individualize perioperative treatment for resectable gastric cancer. Notably, Agnes et al., demonstrated that radiomic-based models can predict pathologic response to various neoadjuvant regimens, with particularly robust performance in patients treated with FLOT, showing a low probability of misclassifying non-responders.[19]

The present study has several limitations, including its retrospective design and modest cohort size, which may limit generalizability. Future prospective, multi-parameter studies integrating clinicopathologic, biochemical, molecular and radiomic data are warranted to better stratify patients likely to benefit from FLOT and guide personalized perioperative treatment strategies aimed at improving both pathologic response and survival.

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