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Variation in the Thoroughness of Pathologic Assessment and Response Rates of Locally Advanced Rectal Cancers After Chemoradiation

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Abstract

Background Pathologic complete response (pCR) is associated with better prognosis and guides management for patients with advanced rectal cancer. Response rates vary between series for unclear reasons. We examine whether the thoroughness of pathologic assessment explains differences in pCR rates.

Methods We retrospectively reviewed pathology reports from patients with stage II/III rectal cancer who underwent chemoradiation and resection in a prospective, multicenter trial. We utilized a novel measure for the thoroughness of pathologic assessment by dividing residual tumor size by the number of cassettes evaluated (tumor size to cassette ratio, TSCR), and evaluated whether TSCR is associated with pCR. We validated our findings using a separate cohort.

Results From the trial cohort, 71 of 247 (29%) patients achieved pCR. The pCR rate ranged from 0 to 45% and mean TSCR ranged 0.29 to 0.87 across 12 institutions. Within each institution, a lower TSCR was associated with pCR, demonstrating a higher degree of thoroughness used for tumors that achieved pCR. Moreover, across all samples, low TSCR was independently associated with pCR on multivariable analysis. This finding was corroborated in a separate cohort of 201 tumors evaluated by five pathologists; each pathologist had a lower mean TSCR for pCR calls compared with non-pCR calls. However, the mean TSCR for an institution was not associated with its overall pCR rate.

Conclusions Pathologists assess rectal cancers that have responded significantly to neoadjuvant therapy more thoroughly. Thoroughness does not appear to explain differences in pCR rates between institutions. Our results suggest pCR is not a sampling artifact.

Keywords Rectal cancer · Chemoradiotherapy · RECIST · Residual tumor · Surgical pathology

Introduction

The standard of care for locally advanced rectal cancer is neoadjuvant chemoradiation followed by total mesorectal excision.[1] A subset of around 15–40% of patients with these cancers have tumors that achieve pathologic complete response (pCR), where no viable tumor cells are identified in the surgical specimen.[2–5] These patients go on to have better long-term outcomes compared to those with tumors that do not achieve pCR.[6, 7]

Our understanding of what contributes to pCR remains limited. Most clinicians believe that response to neoadjuvant therapy is determined primarily by tumor biology, which is supported by reports that certain mutational profiles have been associated with pCR.[8–11] Numerous trials and studies have also demonstrated that variations in neoadjuvant therapy—either by intensifying the neoadjuvant regimen with agents traditionally reserved for the adjuvant setting or by increasing the duration between chemoradiation and surgery—can increase the pCR rate.[12–15]

Overall, it remains unclear why pCR rate can vary dramatically between institutions.[16] In a recently completed multicenter clinical trial evaluating the impact of sequentially

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intensifying neoadjuvant therapy, we observed pCR rates ranging from 0 to 45% across different institutions. This wide variation did not appear to be explained from the differences in the neoadjuvant therapy provided alone, and so we hypothesized that pCR calls could in part be due to differences in the sampling of surgical specimens. To date, no studies exist evaluating whether the thoroughness of pathologic assessment contributes to the finding of pCR. We therefore decided to investigate whether the thoroughness of pathologic assessment contributes to the rate of pCR.

The clinical relevance of whether pCR can be attributed to the thoroughness of pathologic assessment is important, because the finding of pCR can determine whether or not a patient undergoes adjuvant therapy and also has a bearing on long-term surveillance. Additionally, pCR has been used as a surrogate for long-term oncologic outcome,[6, 7] and it would be crucial to know whether it is impacted by variations in sampling.

Because there are no validated measurements of the thoroughness of pathologic assessment, we devised and utilized a novel measure by taking the ratio of the maximum residual tumor size (cm) to the number of cassettes cut from the residual tumor, the tumor size to cassette ratio (TSCR). A high TSCR would therefore represent a lower degree of thoroughness, and a low TSCR corresponds to a higher degree of thoroughness. If we observed an association between high TSCR and pCR, it would suggest that pCR calls might be due to a lower degree of thoroughness in pathologic assessment. In this study, we find that the TSCR varies considerably between institutions and between pathologists, and that there is consistently a lower TSCR in specimens that are called pCR than those that are called non-pCR. This finding suggests that pathologists intuitively evaluate tumors that are highly responsive to neoadjuvant treatment with more scrutiny than those that show less response, and that pCR is not a sampling artifact.

Methods

Study Populations

Pathology reports were reviewed from patients accrued to the "Timing trial" ($n = 259$), a multicenter, prospective, phase 2 clinical trial (ClinicalTrials.gov, number NCT00335816) exploring the impact of increasing the duration between chemoradiation and surgery through the delivery of cycles of FOLFOX in the neoadjuvant setting.[17] In the "Timing trial," 17 institutions accrued patients with clinical stage II (T3–4, N0) or III (any T, N1–2) invasive rectal adenocarcinoma. All patients underwent chemoradiation prior to total mesorectal excision, and four separate study groups received 0, 2, 4, or 6 cycles of chemotherapy between chemoradiation and surgical excision, respectively. The primary outcome measured was the rate of pCR, and patient demographics, clinical staging, and details of the neoadjuvant treatment regimen were included in that assessment. The prescription of the clinical trial design was that there should be at least one cassette cut per centimeter of tumor specimen being analyzed by the pathologist. For inclusion into this study exploring the thoroughness of pathologic assessment, all sites that accrued 10 or more patients into the trial were included.

In addition, pathology reports from a separate cohort of patients ($n = 201$) treated at Memorial Sloan Kettering Cancer Center (MSK) were evaluated, whose surgical specimens were evaluated by five different attending pathologists. This cohort was used to validate and further characterize our findings.

Measurement of Tumor Size to Cassette Ratio

Maximum residual tumor size was obtained from pathology reports and was measured in centimeters. When pathologic assessment revealed only a residual scar, the dimensions of the scar were taken to represent the residual tumor size. The number of cassettes that were taken from the residual tumor was also taken the pathologic reports. Cassettes that had been cut to assess margins, normal surrounding mucosa, or lymph nodes from the surgical specimen were not included.

Treatment Received and Pathologic Assessment

All included patients had received neoadjuvant radiotherapy with a fluoropyrimidine-based chemosensitizing agent. The patients from the clinical trial were divided into four groups corresponding to the trial protocol, with patients receiving CRT followed by 0, 2, 4, or 6 cycles (SG 1–4) of FOLFOX before surgery. Patients at MSK received FOLFOX prior to CRT and surgery. Following neoadjuvant therapy, surgical resection was performed using the principles of sharp total mesorectal excision. Pathologic complete response was defined as the complete absence of tumor cells in the surgical specimen at the primary tumor site and regional lymph nodes.

Statistical Considerations

The testing of patient characteristics comparing those with pCR vs. non-pCR were performed using Fisher's test for categorical variables and Wilcoxon rank sum test for continuous variables. A generalized estimating equation model was used to adjust for site variability, which tested specifically for a relationship between TSCR and pCR.

Results

Primary Study Cohort Patient, Treatment, and Tumor Characteristics

There were 259 eligible patients accrued across 17 different institutions in the "Timing trial." Twelve institutions accrued 10 or more patients to the study, providing 247 patients to be included in the current analysis. These patients received 0–6 cycles of FOLFOX after CRT and had surgery between 5.4 and 61.4 weeks after CRT completion. Residual tumor size ranged from 0 to 10.1 cm. Of the 247 included patients, 71 (29%) achieved pCR.

Pathologic Assessment and its Association with pCR

Across the 12 sites, the pCR rate ranged from 0 to 45%. The proportion of patients in different study groups was not associated with the pCR rate within each institution (Fig. 1). The number of cassettes prepared from the residual tumors or scars ranged from 1 to 25. The mean TSCR ranged from 0.29 to 0.87. This corresponds to approximately 4 cassettes per centimeter of tumor in some sites to 1 cassette per centimeter of tumor in others.

We found no significant association between the mean TSCR for an institution and its pCR rate (Fig. 2). When taking all samples across the 12 institutions as a single cohort, however, TSCR was associated with pCR on univariable analysis ($p = 0.004$) and remained independently associated with pCR on multivariable analysis after adjusting for radiation dose, clinical stage, tumor size, distance from anal verge, and number of neoadjuvant FOLFOX cycles received, with an odds ratio of 0.05 (95% CI 0.008–0.302, Table 1).

Mean TSCR Is Lower (More Thorough) in pCR Than in Non-pCR Calls

There were seven sites that had 20 or more patients accrued. We evaluated each of these sites individually and noted that the mean TSCR for each sites' pCR calls was consistently lower than their non-pCR calls; that is, the tumors ultimately determined as having achieved pCR were consistently evaluated with a higher degree of thoroughness than those that did not achieve pCR (Fig. 3).

Validation of Finding Lower Mean TSCR in pCR Calls Than in Non-pCR Calls Across Five Different Pathologists at a Large Comprehensive Cancer Center

Across five pathologists at MSK, the pCR rate ranged from 27 to 39%. The mean TSCR for each pathologist's pCR calls was consistently lower than for their non-pCR calls, just as had been observed between institutions in the multicenter trial (Fig. 4).

Discussion

In this study, we set out to determine whether the institutional variation in pCR could be explained by differences in the thoroughness of pathologic assessment. It seemed plausible that extremely thorough pathologic assessments might decrease the likelihood of declaring pCR, and conversely, cursory evaluations might falsely label some tumors as having achieved pCR when residual malignancy was actually present. As there are no existing measures of pathologic thoroughness, we introduced a novel measure by taking the ratio of the

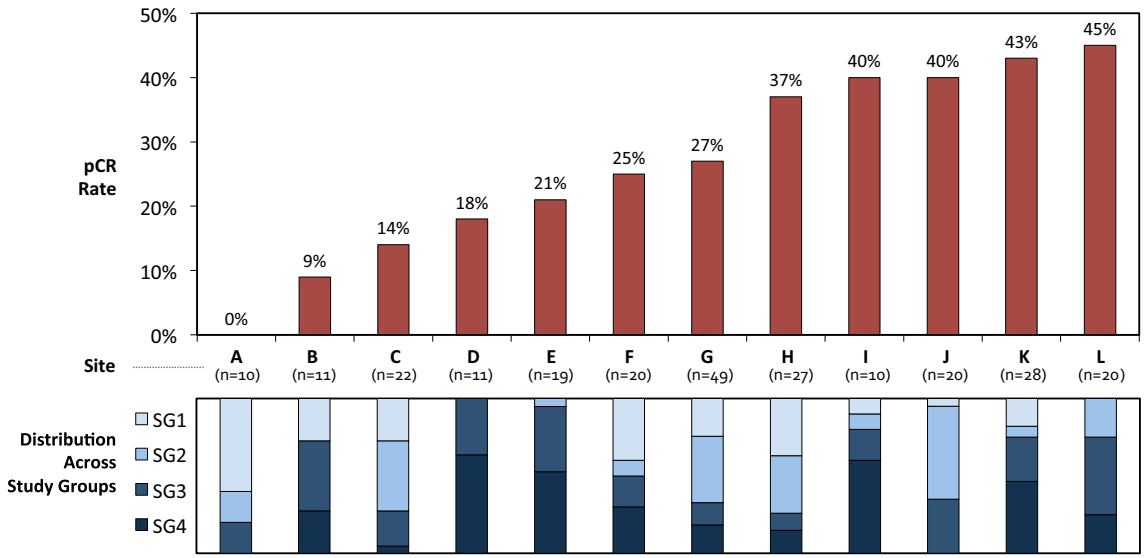

Fig. 1 Variability of pCR rate across sites with ≥ 10 patients accrued. In the upper panel, the pCR rate at each institution. In parentheses, the number of patients treated at the site. In the lower panel, the proportion of patients treated according to study group (SG1–4)

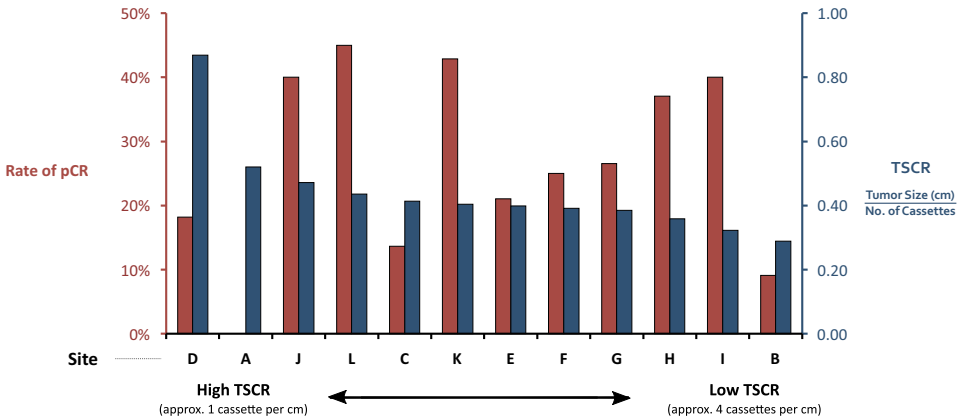

Fig. 2 The rate of pCR (red bars) across sites, ordered by the mean TSCR (tumor size/number of cassettes) for each site (blue bars)

maximum residual tumor size to the number of cassettes taken from the residual tumor. We found that TSCR varies widely across institutions in prospectively collected data from a multicenter trial. We further showed that the variation in pCR rates across different institutions was not explained by variation in TSCR. However, we did find that across different institutions, tumors that achieved pCR consistently had a lower mean TSCR than those that did not achieve pCR. This suggests that tumors that were eventually determined to have achieved a pCR were consistently evaluated with more scrutiny (lower TSCR) than tumors that were found to have residual cancer. We further investigated this finding within a single large-institution and found that across five different pathologists, the mean TSCR for their pCR calls was lower than that for non-pCR calls.

Our findings suggest that pathologic complete response is not a sampling artifact, and that pCR calls are made despite efforts on the pathologists' part to prove otherwise. Pathologists evaluate tumors that have achieved significant response to neoadjuvant treatment with more scrutiny than those that have clear residual disease.

Our measure of pathologic thoroughness is a novel, but arbitrary measure. The number of cassettes taken per centimeter of residual tumor may not necessarily translate into greater thoroughness in pathologic assessment; the number of slides or sections obtained from each cassette, and the time spent evaluating each section may also be important. Moreover, the number of cassettes taken from the gross specimen at many institutions is determined by grossing technicians and trainees who, like the pathologists, undoubtedly have a highly variable practice. This highlights the lack of standardized criteria for this important diagnostic step. Some histopathologic guidelines—and our clinical trial protocol—mandate at least one cassette to be taken per centimeter of a specimen, with other guidelines recommending slices at 2–3-mm intervals.[18] Our study shows that significant variation exists between institutions and pathologists even with these suggested guidelines. While our study cannot suggest an ideal TSCR, it suggests that variation in TSCR does not correspond to changes in pCR rate.

Our data suggests that pCR is a determination that is made in spite of increased efforts to find residual disease on the part

Table 1 Multivariable analysis evaluating association with pCR. A generalized estimating equation model was used. *TSCR* tumor size to cassette ratio

Variable	Odds ratio	95% Wald confidence limits	
TSCR	0.050	0.008	0.302
Radiation dose	0.999	0.997	1.000
cSTAGE 2 vs 3	1.367	0.623	3.001
Tumor size	0.954	0.832	1.094
Distance from anal verge	0.971	0.874	1.078
No. FOLFOX cycles	1.154	0.956	1.392

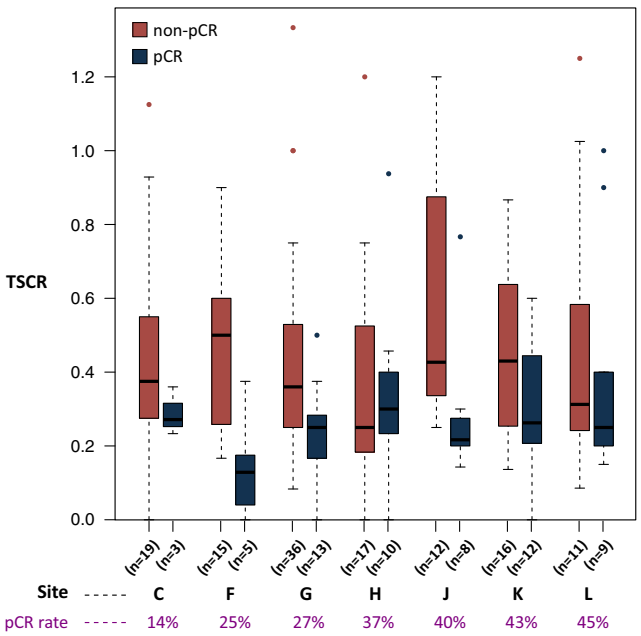

Fig. 3 TSCR (tumor size/number of cassettes obtained) for non-pCR (red) and pCR (blue) specimens across all sites with ≥ 20 patients, ordered from left to right based on increasing pCR rate

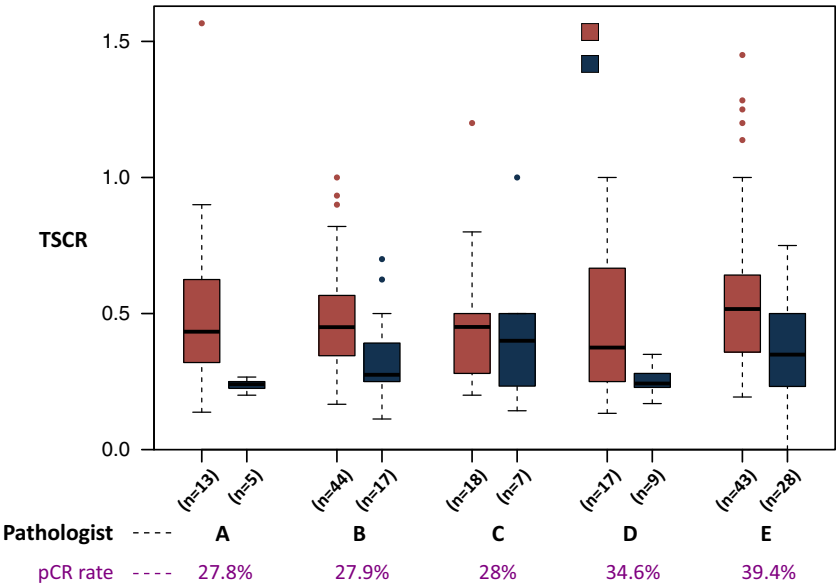

Fig. 4 TSCR (maximum residual tumor size/number of cassettes obtained) for non-pCR (red) and pCR (blue) specimens, within a single institution across five pathologists, ordered from left to right based on increasing pCR rate

of the pathologist. This argues against pCR being a sampling artifact. Increased standardization for the thoroughness of pathologic assessment would be desirable, especially as the response to neoadjuvant chemoradiation becomes more important in determining clinical management strategies for patients with locally advanced rectal cancer.

Statement of Author Contribution Each of the authors included had a substantial contribution to the design, data acquisition, analysis, interpretation, and drafting and review of this manuscript and have agreed to the accuracy and the integrity of the work.

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