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Gastrointestinal Cancer

Sidedness Matters: Surrogate Biomarkers Prognosticate Colorectal Cancer upon Anatomic Location

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Oncotype Recurrence Score assay • CDX2 • Tumor location • Stage II colorectal cancer • Prognostic biomarkers

Abstract

Background. Anatomic location of primary tumors across the colon correlate with survival in the metastatic setting, whereas left-sided tumors may exhibit superior survival compared with right-sided tumors. The Oncotype Recurrence Score (RS) assay is a clinically validated predictor of recurrence risk in patients with stage II colorectal cancer (CRC). Previous studies had indicated that without adjuvant chemotherapy, CDX2-negative stage II CRC tumors are associated with a lower rate of disease-free survival than CDX2-positive stage II CRC tumors. We aimed to evaluate whether these two validated prognostic biomarkers may correlate with primary tumor location, and whether tumor location may reflect differential prognosis in stage II CRC.

Materials and Methods. We retrospectively analyzed patients with T3 mismatch repair-proficient (MMR-P) stage II CRC for whom RS assay was performed. Pathological report was reviewed for exact primary tumor location and CDX2 immunostaining. RS and CDX2 expression were correlated with primary tumor location.

Results. The analysis included 1,147 patients with MMR-P stage II CRC (median age 69 years [range 29–93]). Tumor distribution across the colon was as follows: 46% (n = 551) were right-sided and 54% (n = 596) were left-sided. RS was higher in right-sided tumors (p = .01). The RS results gradually decreased across the colon (cecum, highest score; sigmoid, lowest score; p = .04). Right-sided tumors exhibited more CDX2-negative tumors (p = .07).

Conclusion. Our study indicates that right-sided colorectal tumors may display worse prognosis compared with left-sided tumors in MMR-P stage II CRC. Primary tumor location may serve as a prognostic factor that should be taken into account for recurrence risk assessment and consideration of adjuvant treatment. ***The Oncologist*** 2019;24:e696–e701

Implications for Practice: Sidedness matters, even in stage II colorectal cancer (CRC). Using two previously established prognostic tools, the Oncotype DX assay and CDX2 expression, this study found that right-sided tumors may display worse prognosis compared with left-sided tumors in mismatch repair-proficient stage II CRC. Therefore, primary tumor location should be taken into account for recurrence risk assessment and consideration of adjuvant treatment.

Introduction

Colorectal cancer (CRC) is one of the leading cancers in the Western world, with high recurrence incidence and poor prognosis. Although the development of early diagnosis and comprehensive treatment is dramatic, the mortality rate of colorectal cancer is still very advanced in both genders [1, 2]. At presentation, 25% of colon cancer cases are

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diagnosed as stage II, for which surgical resection remains the mainstay of treatment. Although surgery alone can be curative in most cases of localized colon cancer, 15%–20% of patients with stage II colon cancer will eventually experience disease recurrence [3]. However, the role of adjuvant chemotherapy remains controversial [4–9], and identifying the patients who will benefit from the treatment remains a challenge.

Recent evidence indicates that the anatomic location of primary tumors across the colon correlates with survival in the metastatic setting; left-sided tumors may exhibit superior survival compared with those that are right-sided. It has been shown in a retrospective post hoc analysis of two phase III studies that were designed to compare the addition of either bevacizumab or cetuximab (CALGB/SWOG 80405 and FIRE-3) in combination with chemotherapy as first-line therapy for RAS wild-type metastatic colorectal cancer that patients with left-sided tumors had markedly better overall survival and objective response rate than those with right-sided primary tumors. Furthermore, the benefit of anti-epidermal growth factor receptor (EGFR) agents was markedly superior in left sided tumors compared with right-sided tumors [10, 11]. Retrospective analysis of sidedness in other phase III and II studies evaluating the role of anti-EGFR therapy in metastatic colorectal cancer revealed the same trend of better response to anti-EGFR agents and better prognosis for left-sided tumors. We aimed to evaluate whether sidedness may also prognosticate in the setting of stage II colorectal cancer. We used two factors that have been previously established as prognostic tools for stage II colorectal cancer: Oncotype DX colon cancer assay (Genomic Health, Redwood City, CA) and CDX2 expression.

The Oncotype DX colon cancer assay is a 12-gene reverse transcriptase polymerase chain reaction-based colon cancer assay designed to predict recurrence risk in patients with stage II and III colon cancer [12]. The assay is based on three stromal genes (BGN, FAP, INHBA), three cell cycle-related genes (Ki-67, C-MYC, MYBL2), one early response gene (GADD45B), and five reference genes (ATP5E, GPX1, GPK1, UBB, VDAC2). It is a continuous variable ranging from 0 to 100, with low- (<30), intermediate- (31–40), and high-recurrence (>41) risk groups representing 8%, 11%, and 25% risk of recurrence at 3 years, respectively [13]. The validation studies of the assay used archived samples from four major prospectively designed clinical trials (the Quick and Simple and Reliable study, the Cancer and Leukemia Group B 9581 study, the National Surgical Adjuvant Breast and Bowel Project C-07 study, and the SUNRISE) [14], involving a total of 3,018 patients. These studies demonstrated that the Recurrence Score result is an independent predictor of recurrence and is able to predict the risk of recurrence beyond traditional clinical and pathological parameters. The greatest clinical benefit has been shown in average-risk patients—a large group of approximately 70% stage II patients with T3 mismatch repair-proficient (MMR-P) tumors, for whom conventional prognostic factors are not informative [13, 15–17]. An additional validation study was performed on 279 patients from the Dutch Total Mesorectal Excision (TME) trial with stage II and III rectal cancer who were randomized to TME surgery alone. This study demonstrated that the 12-gene Recurrence Score (RS) assay is a predictor of recurrence risk and cancer-specific survival in patients with rectal cancer as well, suggesting a similar underlying biology in colon and rectal cancers [18]. It should be noted that the validation studies failed to demonstrate prediction of benefit from chemotherapy treatment.

CDX2 is a homeobox transcription factor that is a master regulator of intestinal development and oncogenesis and had recently been identified as a biomarker of mature colon epithelial tissue. Tumors enriched in cells with an undifferentiated, stem-like phenotype might exhibit more aggressive clinical behavior. Previous studies found that tumors lacking CDX2 expression are often associated with several adverse prognostic variables such as high levels of ALCAM expression (characteristic of human colon cancer stem cell) and a high pathological grade. It has been shown that CDX2-negative colorectal tumors are associated with a higher risk of recurrence and seem to benefit from adjuvant chemotherapy compared with CDX2-positive colorectal tumors. This observation was noted not only in stage III but also in stage II disease [19, 20].

We aimed to evaluate whether these two prognostic biomarkers may correlate with primary tumor location, and whether tumor location may reflect differential prognosis in stage II colorectal cancer.

Materials and Methods

Patients and Study Design

This trial was a multicenter retrospective study that included Clalit Health Services (CHS) patients with stage II/III colorectal cancer who underwent the 12-gene Recurrence Score assay between January 2011 and August 2016. The analysis was restricted to patients with MMR-P tumors [21]. Pathological reports of the included patients were reviewed for exact primary tumor location and were correlated to the 12-gene Recurrence Score assay and CDX2 expression. Rectal tumors were analyzed separately. The study was conducted in accordance with Good Clinical Practice guidelines and was approved by the institutional review board of the CHS as well as the institutional review boards of the participating institutions (Davidoff Cancer Center, Hadassah-Hebrew University Medical Center, Kaplan Medical Center, Lin Medical Center, Rambam Healthcare Campus, Soroka University Medical Center, and Tel Aviv Sourasky Medical Center).

Recurrence Score Result Determination

The Recurrence Score results are derived from reference-normalized gene expression measurements made by quantitative real-time reverse transcriptase polymerase chain reaction using RNA extracted from a formalin-fixed paraffin embedded tumor block obtained by surgical resection. The gene panel used for the assay comprises 12 genes: 7 cancer-related genes, including 3 cell-cycle genes, 3 stromal genes, and the early response gene, *GADD45B*, and 5 reference genes [18]. Stromal group score and cell-cycle group score are calculated from reference-normalized individual gene expression measurements, and an unscaled Recurrence

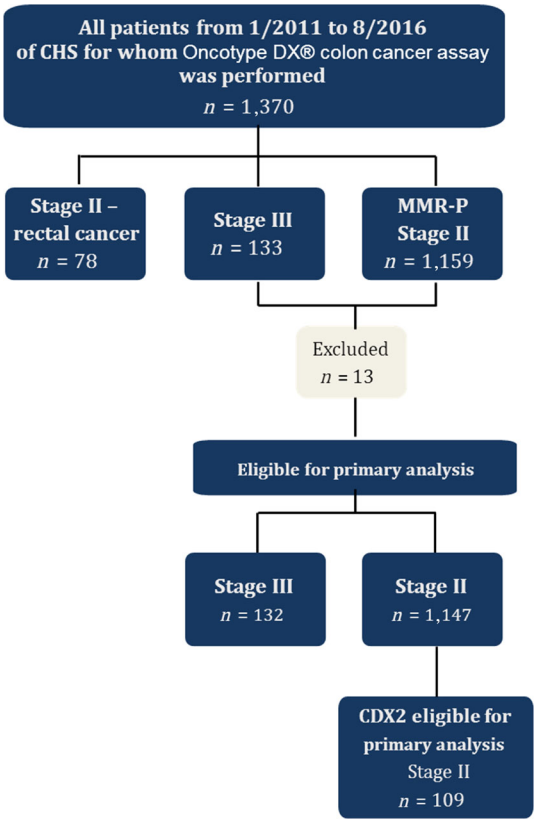


Figure 1. Study flow chart.
Abbreviations: CHS, Clalit Health Services; MMR-P, mismatch repair-proficient.

Score result is determined using the following calculation: RSu = (0.15 × Stromal group score) − (0.3 × Cell-cycle group score) + (0.15 × *GADD45B*). The Recurrence Score result is then rescaled from 0 to 100. Patients are categorized into three risk groups according to their Recurrence Score results: low (<30), intermediate (30–40), and high (≥41).

Immunohistochemical Analysis of CDX2 Expression

CDX2 staining was performed as a part of the routine pathological examination using a CDX2 antibody (clone EPR2764Y, CellMarque, AH-Diagnostics, 1:100). The average nuclear CDX2 expression was estimated across the whole section, and tumors were classified by either "high/normal" or "low/absent" expression. Normal epithelial cells were used as an internal control [20]. All staining was evaluated by a gastrointestinal pathologist.

Statistical Analysis

Correlation of Recurrence Score results to tumor location was done using *t* test analysis when compared with the two colon groups of tumor location (left/right) and using one-way analysis of variance test when compared with specific location. Fisher's exact test was used to analyze the correlation between CDX2 expression and tumor location. $p < .05$ was considered statistically significant. All analyses were conducted in SPSS software (IBM, Armonk, NY).

Table 1. Patient characteristics

Characteristics	Right-sided	Left-sided
Gender		
Male	276 (50)	333 (56)
Female	275 (50)	263 (44)
Age, years, median (range)	72 (40–90)	68 (31–86)
Stage		
II	551 (48)	596 (52)
III	60 (45.5)	72 (54.5)
II – Rectal cancer	—	78
Anatomic location		
Cecum	95 (17.2)	
Hepatic flexure	38 (6.8)	
Right side (not specified)	335 (60.7)	
Transverse	83 (15.3)	
Splenic flexure		53 (8.8)
Sigmoid		306 (51.3)
Rectosigma		103 (17.5)
Left side (not specified)		134 (22.4)
Rectum		78 (—)

Data are presented as *n* (%).
Abbreviation: —, not relevant.

Results

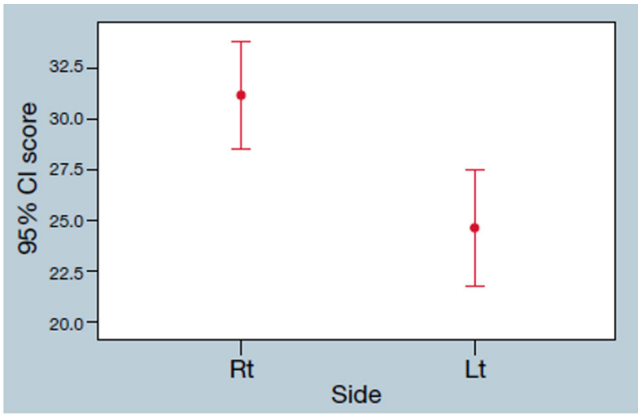
Patients

Of 1,370 patients of CHS for whom Oncotype DX colon cancer assay was performed, 1,357 were eligible for primary analysis; 13 patients were excluded because pathological review was not available. A total of 1,147 patients were diagnosed with stage II disease, and 132 were diagnosed with stage III disease; 78 patients with stage II rectal cancer were analyzed separately (Fig. 1). Median age was 69 years (range 30–90); left-sided tumors were associated with younger patients (median age 68 vs. 72 years) and a higher incidence in males (56% vs. 44% in right-sided tumors).

Tumor distribution across the colon in the stage II cohort was as follows: 48% (n = 551) were right-sided (cecum 17.2%, hepatic flexure 6.8%, transverse colon 15.3%, right-sided unspecified 60.7%) and 52% (n = 596) were left-sided (splenic flexure 8.8%, sigmoid colon 51.3%, rectosigmoid 17.5%, left-sided unspecified 22.4%; Table 1).

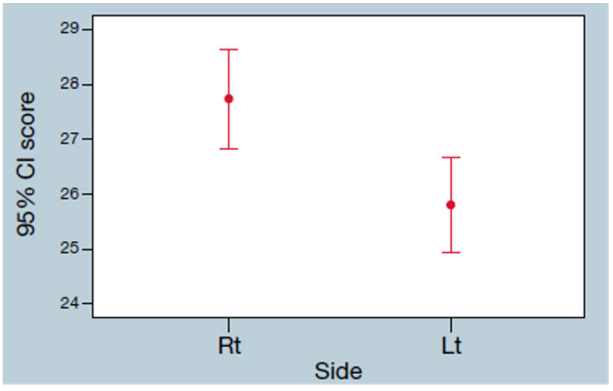
Recurrence Score Results According to Tumor Location

Stage II patients demonstrated a higher Recurrence Score in right-sided tumors compared with left-sided tumors, with a mean score of 27.72 (range 6–71) and 25.79 (range 6–54), respectively (p = .002; Fig. 2). Comparing the Recurrence Score in specific locations rather than left versus right revealed a gradual decrease across the colon, with the cecum-located tumors receiving the highest Recurrence Score (29.75, range 8–71), hepatic flexure-located tumors receiving a lower Recurrence Score (27.76, range 7–57), and the sigmoid-located tumors receiving the lowest RS (24.49, range 0–52; p = .014; Fig. 3).



	Right side	Left side	
n	551 (48.01%)	596 (51.97%)	
Mean score (range)	27.72 (6–71)	25.79 (6–54)	*p* = .002

Figure 2. Recurrence Score of stage II colon cancer: Mean Recurrence Score of left- and right-side stage II colon cancer samples is presented with a confidence interval of 95%. Abbreviations: CI, confidence interval; Lt, left; Rt, right.



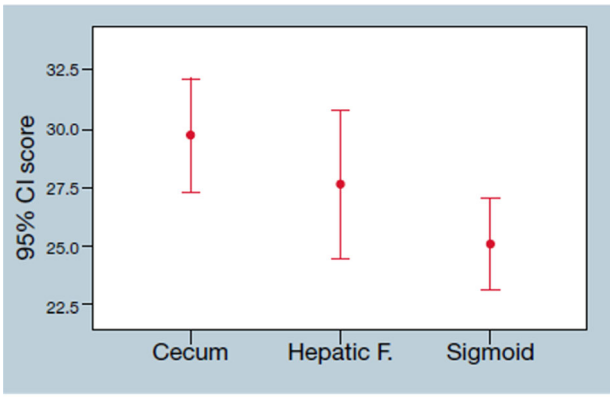
	Right side	Left side	
n	60 (45.4%)	72 (54.6%)	
Mean score (range)	31.15 (3–63)	24.6 (7–52)	*p* = .001

Figure 3. Recurrence Score of stage III colon cancer: Mean Recurrence Score of left- and right-side stage III colon cancer samples is presented with a confidence interval of 95%. Abbreviations: CI, confidence interval, Lt, left; Rt, right.

Similar results were demonstrated in stage III patients with mean Recurrence Score of 31.15 (range 3–63) in right-sided tumors and 24.6 (range 7–52) in left-sided tumors (p = .001; Fig. 4). Rectal tumors had a higher Oncotype DX colon cancer assay compared with left-sided colon tumors in both stage II tumors (RS 27.06 vs. 25.79, p = .04) and stage III tumors (RS 27.15 vs. 24.6, p = .05).

CDX2 Expression According to Tumor Location

CDX2 status was available for 109 stage II patients. Right-sided tumors exhibited more CDX2-negative tumors compared with left-sided tumors—35.8% (n = 19) and 16.1% (n = 9), respectively (p = .029; Table 2). CDX2-negative tumors in general (both left- and right-sided) had a higher RS: 32 versus 24.42 (p = .02; Fig. 5).



	n	Mean score (range)
Cecum	95	29.75 (8–71)
Hepatic flexure	38	27.76 (7–57)
Sigmoid	306	24.49 (0–52) *p* = .014

Figure 4. Gradient recurrence score of stage II colon cancer: Mean Recurrence Score across the colon (cecum, hepatic flexure, and sigmoid) in stage II colon cancer is significantly decreased (p = .014).

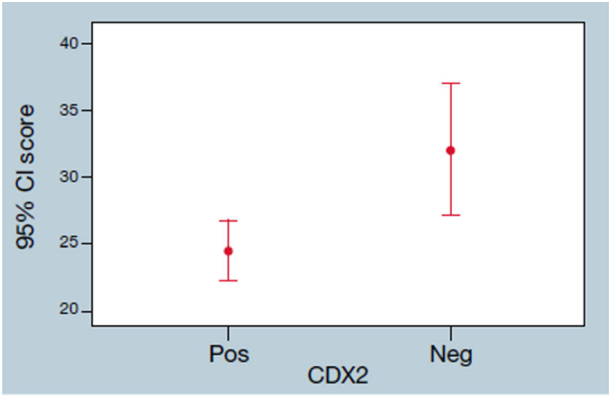
Table 2. CDX2 expression according to tumor location

CDX2 expression	Right side, *n* (%)	Left side, *n* (%)
CDX2(+)	34 (64.2)	47 (83.9)
CDX2(–)	19 (35.8)	9 (16.1)
Total	53	56 *p* = .029

Abbreviations: CDX2(–), CDX2 negative; CDX2(+), CDX2 positive.

Discussion

The clinical management of patients with stage II colon cancer remains controversial, and attempts are made to optimally define the patients who are at higher risk for recurrence and who may benefit from adjuvant chemotherapy. The aim of this large study was to evaluate whether tumor location may reflect differential prognosis in stage II colorectal cancer by examining two prognostic biomarkers: the Oncotype DX colon cancer assay and CDX2 expression. The results presented above, based upon more than 1,300 cases, indicate that right-sided tumors displayed worse biological features manifested by significantly higher Recurrence Score compared with left-sided tumors, as well as higher incidence of CDX2-negative tumors. Despite the fact that the median RS for both right- and left-sided tumors was below 30 and therefore considered low risk, the Recurrence Score is a continuous variable, and the difference between the groups was statistically significant and may indicate the differential biology of colon cancer across the colon. This observation is in correlation with recent data in the metastatic setting indicating worse prognosis for right-sided tumors [11]. Nevertheless, in the metastatic setting, the data used only distinguished between the right and left colon in general, without referring to the specific segments across the colon. Most studies that were focusing on primary



	Recurrence score (mean)	SD
CDX2(+)	24.42	10.30
CDX2(−)	32.00	12.69 p = .020

Figure 5. Recurrence Score and CDX2 expression: Stage II colon cancer Recurrence Score in correlation with CDX2-negative or -positive expression.
Abbreviations: CDX2(−), CDX2 negative; CDX2(+), CDX2 positive; CI, confidence interval; Neg, negative; Pos, positive.

tumor location used a dichotomized distinction between right colon and left colon, with the differences attributed at least in part to the different embryonal origin. In our large cohort, we observed a gradient across the colon with tumors of the hepatic flexure representing lower recurrent score than the cecum and higher than the sigmoid colon. The main limitation is the relatively small number of patients in the subgroups analyzed, but nevertheless, these results together with the results of Salem et al. [22] raise the question of whether there is a broader clinically relevant spectrum of tumor location than right versus left.

With regard to CDX2 expression, it should be noted that the CDX2-negative tumors represent a larger fraction in this cohort compared with the original Dalerba et al. cohort [19]. Our results are in concordance with recent studies that exhibited higher expression rates of CDX2 absence/loss in stage II tumors [23, 24]. Nevertheless, our results confirm the role of CDX2 expression as a prognostic factor, as has been previously demonstrated, and also display its correlation to tumor location. Although the biological correlation between the Oncotype DX colon cancer assay and CDX2 expression has not yet been established, we found that CDX2-negative tumors are associated with a higher Recurrence Score.

The association between CDX2-negative tumors and higher Recurrence Score might be attributed to the gene profile included in the assay. One example is the INHBA gene that encodes activin A, a ligand in the transforming growth factor β superfamily, which plays an important role in cell differentiation. Activin A expression has been implicated to be significantly increased in various types of cancer and correlates with cancer progression and metastasis [25, 26]. As described above, CDX2-negative tumors are associated with high levels of ALCAM expression, which is characteristic of human colon cancer stem cell. Therefore, a possible explanation for the association is that both prognostic tools identify different molecular signatures of the undifferentiated tumors associated with worse outcome.

Two subgroups of patients included in this study require separate discussion. The first is a group of patients with stage III CRC (n = 132), for whom the Oncotype DX colon cancer assay was conducted within the framework of the clinical trial. As expected, stage III CRC tumors displayed a higher RS as compared with stage II tumors. Notably, in this group, there was also a significantly higher RS in right-sided tumors compared with left-sided tumors. This observation alongside the mounting evidence regarding stage IV disease supports the hypothesis that tumor location has an impact on prognosis of CRC regardless of disease stage.

The second group comprised patients with rectal cancer. In this relatively small group of patients, we found a higher Recurrence Score in both stage II and stage III patients compared with same stage left-colon tumors. Because rectal tumors were not included in the large validation studies of the Oncotype DX colon cancer assay and were validated in a smaller study, these results represent a potential difference in prognosis, and further studies are required. Furthermore, recent studies demonstrate a different genomic profile of rectal tumors compared with those of the left colon, indicating that rectal cancer may represent a distinct entity that is not in continuum with colonic tumors [22, 27].

The main limitation of the study is the lack of long-term survival data to indicate whether tumor sidedness is indeed a prognostic factor, as reflected by the adverse biological features observed in our study. Because the median follow-up time is relatively short for a substantial fraction of the patients that were diagnosed in the last 2–3 years, survival analysis could not be performed for the entire cohort, and the data are being collected prospectively for future mature survival and recurrence analysis. Moreover, we could not evaluate the role of other potentially prognostic factors such as RAS and BRAF and their correlation to Oncotype RS or CDX2.

Conclusion

Right sidedness may reflect worse biological features in early colon cancer according to two validated prognostic tools. Future survival analysis may reveal whether primary tumor location may indeed serve as a prognostic factor that may be taken into account for recurrence risk assessment and consideration of adjuvant treatment. However, tumor location has not yet proved to be predictive.

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Disclosures

Ravit Geva: Bristol-Myers Squibb, Eli Lilly and Company, Medison, Roche, Novartis, Takeda, Merck Sharp & Dohme, Merck, Janssen (H), Bayer, Merck Sharp & Dohme, Novartis (SAB); **Lior Soussan-Gutman:** Teva Pharmaceutical (E), Genomic Health (representative). The other authors indicated no financial relationships.

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