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LYMPH NODE YIELD AFTER COLECTOMY FOR CANCER: IS ABSENCE OF MMR A FACTOR?

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Abstract

Background—Nodal staging is crucial in determining use of adjuvant chemotherapy for colon cancer. Number of metastatic lymph nodes has been positively correlated with number of lymph nodes examined. Current guidelines recommend that at minimum 12–14 lymph nodes be assessed. In some studies, mismatch-repair-deficiency has been associated with lymph node yield.

Objective—To determine whether mismatch-repair-deficient colorectal tumors are associated with increased lymph node yield.

Design—We queried an institutional database to analyze colectomy specimens with immunohistochemistry for mismatch-repair genes in patients treated for colorectal cancer 1999–2012. Before 2006, immunohistochemistry was done at the request of an oncologist or surgeon. After 2006, it was routinely performed for patients younger than 50. We measured association of clinical and pathological features with lymph node quantity. Fourteen predictors and confounders were jointly analyzed in a multivariable linear regression model.

Setting—A single tertiary care institution.

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Patients—Tissue specimens from 256 patients.

Main Outcome Measures—Correlation of tumor, patient, operative variables to yield of mesenteric lymph nodes.

Results—Of 256 colectomy specimens reviewed, 94 had mismatch-repair-deficiency. On univariate analysis, mismatch-repair-deficiency was associated with lower lymph node yield, older patient age, right-sided tumors, poor differentiation. Linear regression model identified 5 variables with independent relationships to lymph node yield: patient age, specimen length, lymph node ratio, perineural invasion, tumor size. Positive correlation was observed with tumor size, specimen length, perineural invasion. Tumor location had a more complex, nonlinear, quadratic relationship with lymph node yield; proximal tumors were associated with higher yield than more distal lesions. Mismatch-repair-deficiency was not independently associated with lymph node yield.

Limitations—Mismatch-repair immunohistochemistry based on patient age, family history, pathologic features may reduce the generalizability of these results. Our sample size is too small to identify variables with small measures of effect. The study's retrospective nature did not permit true assessment of extent of mesenteric resection.

Conclusions—Patient age, length of bowel resected, lymph node ratio, perineural invasion, tumor size, tumor location were significant predictors of lymph node yield. However, when controlling for surgical and pathological factors, mismatch-repair protein expression did not predict lymph node yield.

Keywords

Colorectal cancer; MMR; Lymph node yield; Colectomy

INTRODUCTION

Accurate staging of colorectal cancer (CRC) is critical in determining patient prognosis and the need for adjuvant therapy. Multiple studies show a positive association between the number of lymph nodes (LNs) removed and survival.^{1–3} The National Comprehensive Cancer Center, National Cancer Institute, and other organizations recommend removal of at least 12 LNs for adequate staging.^{3–5}

Multiple factors influence the number of LNs identified in a colon resection. A previous study from our institution found that tumor size, location, number of resected pedicles, and use of endoscopic tattoos were related to LN retrieval.⁶ Other studies found LN yield to be significantly associated with the pathologist's training,^{5–7} patient age,^{8–10} American Society of Anesthesiologists status,⁹ tumor size,^{11–14} stage,⁹ differentiation,^{9, 13, 15} biology, and proximal location.^{13, 16} Mismatch repair deficiency (MMR) has also been associated with LN yield.^{13, 16} Patients with MMR-deficient tumors have a decreased risk of recurrence compared to patients with MMR-intact tumors.^{17, 18}

Some theorize that MMR-deficient tumors portend less aggressive disease due to their dense lymphocytic infiltration, amplified rate of apoptosis, and less frequent p53, DCC, and KRAS mutations.^{19–22} The cytotoxic lymphocytes of the immune system play a role in anti-tumor immunity, resulting in superior clinical outcome.^{20, 23–30} Existing research suggests that

MMR deficiency and its association with tumor-infiltrating lymphocytes may lead to detection of more LNs in surgical specimens.^{31–33} In this study, we analyze correlation of various factors to number of LNs retrieved, and test the hypothesis that colorectal cancers arising in MMR-deficient tumors are associated with increased LN yield.

MATERIALS AND METHODS

Patients

In 2006, using immunohistochemistry (IHC) for MMR protein expression, Memorial Sloan Kettering (MSK) began to prospectively analyze CRC specimens from every patient aged 50 years undergoing colectomy or local excision. IHC testing was performed selectively in patients aged >50 years who had family history of CRC and/or clinical features suspicious for MSI.

Following approval by the MSK Institutional Review Board, clinical and histopathologic data were collected for patients undergoing partial colectomy with IHC testing from 1999–2012. We measured association of clinical and pathological features with LN quantity. Fourteen predictors and confounders were identified *a priori* and jointly analyzed in a multivariable linear regression model.

Patients undergoing total colectomy, subtotal colectomy, colectomy at another institution, or previous treatment with chemotherapy and/or radiation were excluded. Patients with familial adenomatous polyposis, metastatic colon cancer, rectal or rectosigmoid adenocarcinoma <20cm from the anal verge were also excluded.

Pathology

In the MSK Pathology Department, the specimen was grossed in standard fashion. All mesenteric tissue was manually dissected and examined for lymph nodes. Fat-clearing techniques were not used. If fewer than 12 LNs were identified on first examination, a second attempt to locate LNs was made. Routine microscopy was performed by 1 of 5 specialized gastrointestinal pathologists. IHC was performed by the MSK Department of Pathology, using the standard streptavidin-biotin-peroxidase procedure. Primary MoAbs against MLH1 (clone G168-728, diluted 1:250 (PharMingen)), MSH2 (clone FE11, diluted 1:50 (Oncogene Research Products)) MSH6 (clone GRBP.P1/2.D4, diluted 1:200 (Serotec Inc)), and PMS2 (clone A16-4, diluted 1:200 (BD PharMingen)) were used. Tumors deficient in MLH1, MSH2, MSH6, or PMS2 were used as external controls. MMR deficiency was not routinely done on preoperative tissue biopsy. Tumors showing a total absence of nuclear staining while adjacent benign tissue showed nuclear staining were scored “negative” for expression of that protein. Specialized pathologists interpreted all histopathology and IHC results. This protocol for lymph node assessment remained constant over the 13-year period.

Statistics

The mean LN yields by surgeon and pathologist were compared, using analysis of variants (ANOVA). Fourteen potential confounding and predictive variables were identified *a priori*

and included in the linear regression model. Two potential confounders were body mass index (BMI) and LN ratio (LNR). Potential predictors were age, gender, tumor stage (Tis, T1, T2, T3, T4) and size, location from proximal to distal (cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon), differentiation (well, well-to-moderate, moderate, moderate-to-poor, poor), lymphovascular invasion (LVI), perineural invasion (PNI), mucinous histology (>50%), and preoperative endoscopic tattoo. When appropriate, continuous variables were compared with t-test and categorical variables with Chi-square or Fisher's exact test. Simple and multiple linear regression analysis of predictors and confounders of LN quantity were performed. All significant tests were conducted at the $\alpha=0.05$ level.

In preliminary analyses, the degree of linear relationship between clinically related variables, such as tumor stage and size, was calculated with the Pearson correlation coefficient. The main analysis was based on multivariable linear regression analysis of LN yield that controlled for potential confounding variables. Goodness-of-fit of the regression model was assessed using influence diagnostics based on leverage, studentized residuals and Cook's distance. The model was re-run excluding individual outliers (identified by graphing leverage against studentized residuals), and Cook's distance. Comparisons to the model were made with all 256 patients. Variables such as age, tumor size, and specimen length may have a non-linear relationship to LN yields; these were individually tested for non-linearity by adding a quadratic term to the model. Collinearity among predictor variables was assessed by examining tolerance statistics for the model.

RESULTS

Descriptive statistics

Two hundred and fifty six patients fulfilled our study criteria. The majority of the patients (n=244) in this study were accrued after 2006, when it became routine to perform IHC on patients who were under 50 years of age. MIS was utilized in 55% of cases. Fifty percent had endoscopic tattooing of lesions prior to resection. Distribution of the outcome variable, LN count, was approximately symmetric and acceptably close to normal; residuals from the regression analysis had approximately normal distribution. Population characteristics are shown in Table 1. The study population selected for MMR testing was not typical of the general population of colon cancer patients. Mean age was lower; the population was enriched for right-sided lesions, with a majority AJCC stage II. In separate univariable analyses, MMR deficiency was associated with lower malignant LN yield, older patient age, right-sided tumors, larger tumors, and poor differentiation. Correlations among independent variables were low. Using simple univariable linear regression, absence of MMR protein expression was not associated with higher LN yield (mean 24.8 vs. 26.9 LN, $P=0.12$). There was no association between LN yield and the operating surgeon, operative technique (open vs. laparoscopic) or the pathologist analyzing the specimen (data not shown).

Linear regression model

The 14 predictors and confounders were jointly analyzed in a multivariable linear regression model. When controlling for other variables, MMR-deficient tumors were estimated to have

LN yield similar to that of MMR-intact tumors ($P=0.43$, Table 2). Five variables demonstrated an independent linear relationship with LN yield: patient age, specimen length, LN ratio, PNI, tumor size (Table 2). A negative relationship was expected between patient age and LN yield, based on previous studies within older populations. Our data showed one less LN for each six-year increase in patient age. A positive relationship existed between specimen length and LN yield, with one additional LN for each 5cm of additional length. Tumor size was positively associated with LN yield; there were 1.2 more LNs for each additional centimeter in size (consistent with our previous model based on a different dataset).⁶ We found a negative relationship between malignant LN ratio and yield, with two fewer LNs identified for each 0.1 increase in malignant LN ratio. A novel relationship was noted between PNI and LN yield, with 3.6 more LNs identified in the presence of PNI.

Tumor location had a more complex, non-linear, quadratic relationship with LN yield. Proximal tumors were associated with higher yield than distal lesions. Using the adjusted R^2 , 27% of the variation in LN count was explained by the 14 variables (Table 2). Removing the six significant variables resulted in an adjusted R^2 of 0.1%. Patient age, specimen length, LN ratio, PNI, tumor size and location explained 27% of the variation in LN count.

Influence diagnostics

Using studentized residuals and Cook's distance, three outliers were identified. The model was re-run, excluding these. No significant changes in measures of effect, standard errors, or P-value were noted, indicating that the model was not disproportionately influenced by the outliers in our dataset. Therefore all 256 patients, including outliers, were retained in the final model.

DISCUSSION

This study identified significant relationships between LN yield and patient age, LN ratio, length of colon resected, and tumor size and location. However, unlike previously reported series,^{13, 16, 32} our study did not identify an independent association between MMR deficiency and higher LN yield, when controlling for other variables. We believe these other studies were limited by 1) confounding by poorly controlled variables, 2) small sample size, 3) relatively low proportions of MMR deficient tumors, and 4) low mean LN yields. In a recent study by Belt, et al¹⁶ patients with stage II disease and high LN yield had a lower rate of recurrence than patients with low yield or with MSS tumors. They concluded that the MSI phenotype was independently associated with LN yield, and that high LN yield might be a result of the biological behavior of MSI tumors. However, they did not describe how they had conducted the multivariable analysis, and did not report the results of it. Without this, it is impossible to know how they controlled for several confounding variables. Furthermore, their statistical analysis did not use LN yield as a continuous variable. Similarly, a study by Søreide, et al¹³ examined tumor-related factors in relation to number of harvested LNs in 121 colon cancer patients. They concluded that LN retrieval was an independent prognostic factor, and that proximal tumor location and MSI were associated with higher number of harvested LNs, possibly indicating underlying immunological and

genetic mechanisms. However, the authors did not describe how the multivariable model in their study was constructed, nor did they show the full model in their results. We infer from the report that they excluded stage I patients from the LN yield model, as MSI was not significant when it was included (which suggests over-fitting of the data). In a small study including 82 colon cancer patients with stage I or II disease Eveno, et al.³² also reported an association between LN yield and MSI. They concluded that the good prognosis associated with high LN yield might reflect the high prevalence of MSI tumors. Unfortunately, there were too few patients in the study for a controlled analysis, and the authors appropriately urged caution in using quantity of evaluated LNs as a criterion for quality. If LN yield and MMR deficiency were related it may have explained why lower LN yields are associated with poor prognosis and would have cast doubt on the value of LN yields for prognostication. However, based on our data, both MMR deficiency and lymph node yield should be considered separately when interpreting pathology findings and selecting patients for adjuvant therapy.³⁴ Unfortunately, we were not able to perform subgroup analysis stratified by germline vs. sporadic mutation, as our sample size was insufficient for a meaningful statistical analysis. As a result, we may have missed subtle differences due to biology.

The inverse relationship between LN yield and patient age has been well documented and analyzed for its effect on cancer-specific survival.³⁴ Our data corroborate an inverse relationship between LN yield and patient age. Numerous hypotheses attempt to explain this, including those based on MSI; however, our data does not support MSI as the mechanism. Notably, patients with MMR-deficient tumors were significantly older than those with intact MMR. We believe this is a sampling bias; reflex MMR IHC testing has been performed in patients aged ≥ 50 years at MSKCC since 2006; IHC is performed in older patients only in cases of clinical suspicion for MMR deficiency. To support the validity of our findings, we performed additional analysis of the younger cohort ($n=111$). This produced similar results (data not shown); MMR deficiency was not associated with LN yield ($P=0.87$) in younger patients.

Total number of harvested nodes was inversely correlated with the ratio of positive-to-total LNs, with two fewer LNs for every 0.1 increase in the ratio ($p=0.0004$). This significant inverse relationship was noted in one other study.¹³ In our study, length of resected bowel was directly related to number of LNs in the specimen. In a recent predictive model of LN yield we demonstrated that extent of mesenteric resection (measured by number of vascular pedicles) was a significant predictor of LN yield, independent of length of resected bowel.⁶ We also found that PNI was a significant predictor of LN yield. Liebig et al identified PNI as an independent prognosticator of outcome in CRC patients, hypothesizing that PNI is an important indicator of tumor-stromal interaction.³⁵ The association between PNI and LN supports this.

Our data demonstrated a linear relationship between LN yield and tumor size. Some have proposed that larger tumors elicit an intense antigenic immune response within the regional LN basin, making them more visible to pathologic examination and resulting in increased yields.¹¹ A recent study used the SEER database to assess mean number of LNs examined in 153,483 colon and rectal cancer patients; location of the primary tumor was associated

with LN yield.⁸ Many investigators have reported that right-sided colon cancers yield more evaluable LNs.^{36–38} This appears to be independent of other factors, including tumor size, specimen length, and MSI. We used an *a priori* approach to tumor location. Rather than discriminate based on right versus left, we divided the colon into six segments: cecum, ascending colon, hepatic flexure, transverse colon, descending colon, sigmoid colon. Location was analyzed as a continuous variable, giving more power to the analysis and demonstrating a negative quadratic relationship with LN yield. The more distant the location of tumor, the greater the impact of location on LN yield. For example, the difference in LN yield between descending and sigmoid colon tumors was much greater (6 LNs) than the difference between cecum and ascending colon tumors (<2 LNs).

Our study is limited by several factors. Selective use of MMR IHC based on clinical criteria including patient age, family history of CRC, and pathologic features suggestive of MSI, may reduce the generalizability of the results. Although our sample size is the largest to date, it is still too small to identify variables with small measures of effect, such as use of endoscopic tattoo. Furthermore, the retrospective nature of this study did not allow assessment of extent of mesenteric resection; specimen length is a crude substitute.

Although we included 14 variables in our analysis, the model explained only 27% of variability in LN yield. In our attempt to determine the drivers behind LN yield, we have only scratched the surface. Our observations do not exclude enhanced immune response as the potential underlying mechanism for improved prognosis in MSI tumors; however, such a response may be uncoupled from LN yield. High mean LN yields (26 LNs) and lack of variability of yield among surgeons and pathologists strongly support our hypothesis that MMR protein expression is not a biological predictor of LN yield in colon cancer resection specimens, even when controlling for surgical and pathologic factors, including thorough grossing of the specimen. Significant predictors of LN yield were patient age, length of bowel resected, LN ratio, PNI, size and location of tumor. Factors driving LN yield seem intrinsic to the biology of tumor and patient. MMR protein expression appears to be a confounding variable, rather than a predictor of LN yield. Application of this model to a novel dataset could confirm these findings. Any such analysis would have to control for other important variables associated with MMR status and LN yield: tumor size, stage, location, patient age.

We believe these data undermine the use of LN quantity as an indicator of quality of care for an individual patient. In our study, 99% of specimens yielded >10 LNs. This may be due to the wide mesenteric resection performed by our surgeons, and careful gross examination by our pathologists and LN yields are achievable in a majority of colon cancer resections; however, some patients will have lower yields. Surgeons and pathologists should strive for higher median yields as this will allow for accurate staging of groups of patients. Nevertheless, the clinical significance of lower yields in an *individual* patient remains unknown, and biological predictors of LN yield have yet to be identified.

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REFERENCES

1. Wong JH, Bowles BJ, Bueno R, Shimizu D. Impact of the number of negative nodes on disease-free survival in colorectal cancer patients. *Dis Colon Rectum*. 2002; 45:1341–1348. [PubMed: 12394433]
2. Johnson PM, Porter GA, Ricciardi R, Baxter NN. Increasing negative lymph node count is independently associated with improved long-term survival in stage IIIB and IIIC colon cancer. *J Clin Oncol*. 2006; 24:3570–3575. [PubMed: 16877723]
3. Compton CC, Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med*. 2000; 124:979–994. [PubMed: 10888773]
4. Nelson H, Petrelli N, Carlin A, et al. Guidelines 2000 for colon and rectal cancer surgery. *J Natl Cancer Inst*. 2001; 93:583–596. [PubMed: 11309435]
5. Bamboat ZM, Deperalta D, Dursun A, Berger DL, Bordeianou L. Factors affecting lymph node yield from patients undergoing colectomy for cancer. *Intl J Colorectal Dis*. 2011; 26:1163–1168.
6. Nash GM, Row D, Weiss A, et al. A predictive model for lymph node yield in colon cancer resection specimens. *Ann Surg*. 2011; 253:318–322. [PubMed: 21169808]
7. Reese JA, Hall C, Bowles K, Moesinger RC. Colorectal surgical specimen lymph node harvest: improvement of lymph node yield with a pathology assistant. *J Gastrointest Surg*. 2009; 13:1459–1463. [PubMed: 19459019]
8. Chou JF, Row D, Gonen M, Liu YH, Schrag D, Weiser MR. Clinical and pathologic factors that predict lymph node yield from surgical specimens in colorectal cancer: a population-based study. *Cancer*. 2010; 116:2560–2570. [PubMed: 20499400]
9. Tekkis PP, Smith JJ, Heriot AG, et al. A national study on lymph node retrieval in resectional surgery for colorectal cancer. *Dis Colon Rectum*. 2006; 49:1673–1683. [PubMed: 17019656]
10. Steele SR, Chen SL, Stojadinovic A, et al. The impact of age on quality measure adherence in colon cancer. *J Am Coll Surg*. 2011; 213(1):95–105. [PubMed: 21601492]
11. Wright FC, Law CH, Last L, et al. Lymph node retrieval and assessment in stage II colorectal cancer: a population-based study. *Ann Surg Oncol*. 2003; 10:903–909. [PubMed: 14527909]
12. Chou CL, Lin JK, Wang HS, Yang SH, Li AF, Chang SC. Microsatellite instability screening should be done for right-sided colon cancer patients less than 60 years of age. *Intl J Colorectal Dis*. 2010; 25:47–52.
13. Soreide K, Nedrebo BS, Soreide JA, Slewa A, Korner H. Lymph node harvest in colon cancer: influence of microsatellite instability and proximal tumor location. *World J Surg*. 2009; 33:2695–2703. [PubMed: 19823901]
14. Rullier A, Laurent C, Capdepon M, et al. Lymph nodes after preoperative chemoradiotherapy for rectal carcinoma: number, status, and impact on survival. *Am J Surg Pathol*. 2008; 32:45–50. [PubMed: 18162769]
15. Rajput A, Romanus D, Weiser MR, et al. Meeting the 12 lymph node (LN) benchmark in colon cancer. *J Surg Oncol*. 2010; 102:3–9. [PubMed: 20578172]
16. Belt EJ, te Velde EA, Krijgsman O, et al. High lymph node yield is related to microsatellite instability in colon cancer. *Ann Surg Oncol*. 2012; 19:1222–1230. [PubMed: 21989661]
17. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based chemotherapy for colon cancer. *N Engl J Med*. 2003; 349:247–257. [PubMed: 12867608]
18. Sinicrope F, Foster NR, Thibodeau SN, et al. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst*. 2011; 103:863–865. [PubMed: 21597022]
19. Guidoboni M, Gafa R, Viel A, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. *Am J Pathol*. 2001; 159:297–304. [PubMed: 11438476]
20. Michael-Robinson JM, Biemer-Huttmann A, Purdie DM, et al. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. *Gut*. 2001; 48:360–366. [PubMed: 11171826]

21. Michael-Robinson JM, Reid LE, Purdie DM, et al. Proliferation, apoptosis, and survival in high-level microsatellite instability sporadic colorectal cancer. *Clin Cancer Res.* 2001; 7:2347–2356. [PubMed: 11489812]
22. Nash GM, Gimbel M, Cohen AM, et al. KRAS mutation and microsatellite instability: two genetic markers of early tumor development that influence the prognosis of colorectal cancer. *Ann Surgical Oncol.* 2010; 17:416–424.
23. Dolcetti R, Viel A, Doglioni C, et al. High prevalence of activated intraepithelial cytotoxic T lymphocytes and increased neoplastic cell apoptosis in colorectal carcinomas with microsatellite instability. *Am J Pathol.* 1999; 154:1805–1813. [PubMed: 10362805]
24. Prall F, Duhrop T, Weirich V, et al. Prognostic role of CD8+ tumor-infiltrating lymphocytes in stage III colorectal cancer with and without microsatellite instability. *Hum Pathol.* 2004; 35:808–816. [PubMed: 15257543]
25. Vermorken JB, Claessen AM, van Tinteren H, et al. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. *Lancet.* 1999; 353:345–350. [PubMed: 9950438]
26. van den Eertwegh AJ. Active specific immunotherapy in colon cancer. Recent results in cancer research Fortschritte der Krebsforschung Progres dans les recherches sur le cancer. 2005; 165:260–267. [PubMed: 15865042]
27. Naito Y, Saito K, Shiiba K, et al. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res.* 1998; 58:3491–3494. [PubMed: 9721846]
28. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. *Cancer.* 2001; 91:2417–2422. [PubMed: 11413533]
29. Takemoto N, Konishi F, Yamashita K, et al. The correlation of microsatellite instability and tumor-infiltrating lymphocytes in hereditary non-polyposis colorectal cancer (HNPCC) and sporadic colorectal cancers: the significance of different types of lymphocyte infiltration. *Japanese J Clin Oncol.* 2004; 34:90–98.
30. Phillips SM, Banerjee A, Feakins R, Li SR, Bustin SA, Dorudi S. Tumour-infiltrating lymphocytes in colorectal cancer with microsatellite instability are activated and cytotoxic. *Br J Surg.* 2004; 91:469–475. [PubMed: 15048750]
31. Tikidzhieva A, Benner A, Michel S, et al. Microsatellite instability and Beta2-Microglobulin mutations as prognostic markers in colon cancer: results of the FOGT-4 trial. *Br J Cancer.* 2012; 106:1239–1245. [PubMed: 22353804]
32. Eveno C, Nemeth J, Soliman H, et al. Association between a high number of isolated lymph nodes in T1 to T4 N0M0 colorectal cancer and the microsatellite instability phenotype. *Arch Surg (Chicago, Ill : 1960).* 2010; 145:12–17.
33. Steinhagen E, Shia J, Markowitz AJ, et al. Systematic immunohistochemistry screening for Lynch syndrome in early age-of-onset colorectal cancer patients undergoing surgical resection. *J Am Coll Surg.* 2012; 214:61–67. [PubMed: 22192923]
34. Wang L, Hollenbeak CS, Stewart DB. Node yield and node involvement in young colon cancer patients: is there a difference in cancer survival based on age? *J Gastrointest Surg.* 2010; 14:1355–1361. [PubMed: 20585992]
35. Liebig C, Ayala G, Wilks J, et al. Perineural invasion is an independent predictor of outcome in colorectal cancer. *J Clin Oncol.* 2009; 27:5131–5137. [PubMed: 19738119]
36. Prandi M, Lionetto R, Bini A, et al. Prognostic evaluation of stage B colon cancer patients is improved by an adequate lymphadenectomy: results of a secondary analysis of a large scale adjuvant trial. *Ann Surg.* 2002; 235:458–463. [PubMed: 11923600]
37. Johnson PM, Malatjalian D, Porter GA. Adequacy of nodal harvest in colorectal cancer: a consecutive cohort study. *J Gastrointest Surg.* 2002; 6:883–890. [PubMed: 12504228]
38. Hernanz F, Revuelta S, Redondo C, Madrazo C, Castillo J, Gomez-Fleitas M. Colorectal adenocarcinoma: quality of the assessment of lymph node metastases. *Dis Colon Rectum.* 1994; 37(4):373–377. [PubMed: 8168417]

Table 1

Comparison of patients with intact or deficient MMR expression

Variables	MMR intact, n = 162	MMR deficient, n = 94	P-value
Mean LN yield	24.8 (10.3)	26.9 (10.1)	0.12
Mean malignant LN yield	1.6 (2.5)	0.4 (1.0)	<0.0001
Mean age (years)	53 (12)	61 (16)	<0.0001
Female	50%	59%	0.19
Right-sided tumor	49%	89%	<0.0001
Mean tumor size (cm)	4.3 (2.1)	5.5 (2.7)	<0.0001
T3/4	76%	80%	0.48
N0	54%	80%	<0.0001
Poor differentiation	16%	38%	<0.0001
Lymphovascular invasion	49%	41%	0.26
Perineural invasion	30%	18%	0.04
Mucinous type	7%	4%	0.24

LN = lymph node, MMR=mismatch repair, Standard deviation in parentheses

Table 2

Linear Regression Model of Lymph Node Yield

Variables of Interest	Measure of Effect *	Standard Error	P
Tumor Factors			
MMR deficient	−1.08	1.36	0.43
Tumor size (cm)	1.19	0.28	<0.0001
Tumor location **	4.12	1.58	0.01
Tumor location squared**	−0.56	0.19	0.004
T-stage	−0.66	0.83	0.43
Lymph node ratio (per 0.1)	−1.97	0.54	0.0003
Mucinous type	−0.19	2.57	0.94
Lymphovascular invasion	1.52	1.30	0.19
Perineural invasion	3.59	1.49	0.02
Differentiation	−0.40	0.80	0.62
Patient Factors			
Age (years)	−0.16	−0.04	0.0005
Female	1.22	1.15	0.29
BMI (per 1.0)	0.03	0.04	0.51
Operative Factors			
Total specimen length (cm)	0.20	0.05	0.0002
Preoperative tattoo	1.49	1.16	0.2

* Additional lymph nodes per unit of variable, e.g. 1.19 additional LNs per 1cm increase in size and 0.16 fewer lymph nodes per increase year in age

** Tumor location has a non-linear relationship in model

MMR = mismatch repair, BMI = body mass index