

Prognostic Significance of Perineural Invasion in Patients with Rectal Cancer using R Environment for Statistical Computing and Graphics

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Abstract

Purpose: In recent studies perineural invasion (PNI) is associated with poor survival rates in rectal cancer, but the impact of PNI it's still controversial. We assessed PNI as a potential prognostic factor in rectal cancer. *Patients and Methods:* We analyzed 317 patients with rectal cancer resected at The Oncology Institute “Prof. Dr. Ion Chiricuță” Cluj-Napoca, between January 2000 and December 2008. Tumors were reviewed for PNI by a pathologist. Patients data were reviewed and entered into a comprehensive database. The statistical analysis in our study was carried out in R environment for statistical computing and graphics, version 1.15.1. Overall and disease-free survivals were determined using the Kaplan-Meier method, and multivariate analysis using the Cox multiple hazards model. Results were compared using the log-rank test. *Results:* In our study PNI was identified in 19% of tumors. The 5-year disease-free survival rate was higher for patients with PNI-negative tumors versus those with PNI-positive tumors (57.31% vs. 36.99%, $p=0.009$). The 5-year overall survival rate was 59.15% for PNI-negative tumors versus 39.19% for PNI-positive tumors ($p=0.014$). On multivariate analysis, PNI was an independent prognostic factor for overall survival (Hazard Ratio = 0.6; 95% CI = 0.41 to 0.87; $p = 0.0082$). *Conclusions:* PNI can be considered an independent prognostic factor of outcomes in patients with rectal cancer. PNI should be taken into account when selecting patients for adjuvant treatment. R environment for statistical computing and graphics is complex yet easy to use software that has proven to be efficient in our clinical study.

Keywords: Rectal cancer; Prognostic factors; Perineural invasion; R environment for statistical computing and graphics.

Introduction

Colorectal cancer (CRC) remains the second principal cause of cancer-related death in the Western world, after lung cancer [1]. Determining the optimal treatment of rectal cancer is a complex process. The availability and use of various antitumoral treatment modalities has led to the

research and understanding of different prognostic markers that could improve outcome through patient classification and selection for specific therapies. Currently, the invasive status of lymphatic nodes is the only proven prognostic marker to aid in the identification of patients with aggressive disease [2-7]. Thus, there is an ongoing need of defining new predictive markers that can help guide the opportunity of adjuvant therapy for patients with rectal cancer.

One of the most widely studied factors in malignant tumors is perineural invasion (PNI). It has been proven in many series to be a frequent pathologic feature of colorectal cancer and it registers a reported incidence of up to 33% of these tumors at the time of resection [8,9]. We assessed the value of PNI as a potential prognostic indicator in rectal cancer, focusing on its impact on the outcome of node-negative patients.

The statistical analysis in our study was carried out in R environment for statistical computing and graphics, version 1.15.1 [10]. R is an integrated set of software facilities for data manipulation, calculation and graphical display [11]. R delivers a broad variety of statistical (linear and nonlinear modelling, classical statistical tests, time-series analysis, classification, clustering, etc) and graphical techniques. One of its main advantages is represented by the simplicity with which well-designed publication-quality plots can be created. It is also highly extensible and the user retains full control.

Material and Method

Patients and Database

The study included a total of 317 patients with rectal cancer treated in the Oncology Institute "Prof. Dr. Ion Chiricuța" Cluj-Napoca during an 8-year period (January 2000 to December 2008). Patients data (demographics, staging, pathology, and outcomes) were reviewed and entered into a comprehensive database. The observation time was the interval between start of the treatment (surgical resection or neoadjuvant treatment date) and the last contact (death or last follow-up). The mean duration of follow-up was 5.5 years.

Assessment of Tumor Stage

Tumor stage was established postoperatively by a pathologist and stages 0 to IV were defined according to the American Joint Commission on Cancer (AJCC) TNM staging system.

Evaluation of PNI

For each patient included in the study, original hematoxylin and eosin - stained slides from the tumor resection were collected from the pathology department. All slides containing tumor were rereviewed for PNI by a pathologist. PNI was assessed as positive if cancer cell infiltration into the perineurium or neural fasciculus was detected at the leading point.

Statistical Analysis

Survival data was presented with Kaplan-Meier method, probability of survival at fixed time points, and survival time for the third quartile (where possible) with 95% confidence intervals. Overall a group comparison for survival data was performed with logrank test. Cox proportional hazard regression was performed to check for differences between the different groups, offering hazard ratios with associated statistical significance test and 95% confidence intervals. Proportional hazard assumption was checked graphically and with a proportional hazard test. For all statistical tests used, the significance level alpha was 0.05, and the two tailed p value was computed. The statistical analysis was carried out in R environment for statistical computing and graphics, version 1.15.1.

Results

A total of 317 patients, with rectal adenocarcinoma, were studied. Surgery was performed in all patients: abdominoperineal resection Milles in 142 (44.8%) patients, anterior resection of rectum Dixon in 161 (50.7%) patients and Hartmann procedure in 14 patients (4.5%), in 236 (74.4%) patients surgery was the first line treatment. The patients characteristics are presented in Table 1.

Table 1. Patients characteristics

| | No. | % | | No. | % |
|-----------------------|-----|------|-------------------------|-----|------|
| Age, years (mean, 54) | | | Distant metastasis | | |
| < 54 | 75 | 23.6 | M0 | 309 | 97.4 |
| ≥ 54 | 242 | 76.4 | M1 | 8 | 2.6 |
| Sex | | | Differentiation grading | | |
| Male | 201 | 63.4 | Undifferentiated | 1 | 0.3 |
| Female | 116 | 36.6 | Poorly | 30 | 9.4 |
| Tumor rectum location | | | Moderately | 217 | 68.4 |
| Upper 1/3 | 42 | 13.4 | Well | 69 | 21.9 |
| Middle 1/3 | 134 | 42.2 | Perineural invasion | | |
| Lower 1/3 | 141 | 44.4 | Yes | 60 | 19 |
| Tumor stage | | | No | 257 | 81 |
| pT1 | 12 | 3.7 | Recurrences | | |
| pT2 | 65 | 20.5 | Local | 32 | 10.1 |
| pT3 | 185 | 58.3 | Distant | 33 | 10.4 |
| pT4 | 55 | 17.5 | Neoadjuvant therapy | | |
| Nodal status | | | Radiotherapy | 26 | 8.2 |
| Negative | 164 | 51.7 | Chemotherapy | 2 | 0.6 |
| Positive | 153 | 48.3 | Radio-Chemotherapy | 53 | 16.7 |
| TNM stage | | | Adjuvant therapy | | |
| I | 56 | 17.6 | Radiotherapy | 32 | 10.2 |
| II | 75 | 23.6 | Chemotherapy | 61 | 19.2 |
| III | 178 | 56.3 | Radio-Chemotherapy | 143 | 45.2 |
| IV | 8 | 2.5 | | | |

PNI and Other Prognostic Factors

The incidence of PNI was studied with different pathologic features (TNM stage, positive nodal status, differentiation grading, local and distant recurrences, and distant metastasis). 10.7% of stage I tumors exhibited PNI, whereas 12% of stage II, 18.7% of stage III, and 50% of stage IV tumors were PNI positive ($p < 0.001$). Likewise 40% of poorly differentiated tumors exhibited PNI, whereas only 5.8% of well differentiated tumors were PNI positive ($p < 0.001$). We identified only one patient with undifferentiated adenocarcinoma who was excluded from the study. This study shows that PNI is also associated with positive nodal status (a mean of 2.5 positive lymph nodes), local and distant recurrences and distant metastasis (Table 2).

Prognostic Significance of PNI in Disease-Free Survival (DFS) and Overall Survival (OS)

Overall survival analyses were performed in 236 patients who underwent primary surgical resection. The median overall survival of the PNI-positive patients was significantly shorter than that of the PNI-negative patients (42 versus 93 months, $p = 0.014$, Figure 1). Similar results were found for the median disease-free survival (42 versus 86 month, $p = 0.009$, Figure 2). The 5-year overall survival rate and disease-free survival rate were higher for patients with PNI-negative

tumors versus patients with PNI-positive tumors (59.15% vs. 39.19%, $p=0.014$, Figure 1, respectively 57.31% vs. 36.99%, $p=0.009$, Figure 2).

Table 2. Associations between PNI and other prognostic factors

| Perineural invasion (PNI) | Yes | % | No | % | P |
|---------------------------|-----|-----------|-----|-----------|--------|
| Differentiation grading | | | | | <0.001 |
| Well | 4 | 5.8 | 65 | 94.2 | |
| Moderately | 44 | 20 | 173 | 80 | |
| Poorly | 12 | 40 | 18 | 60 | |
| TNM stage | | | | | <0.001 |
| I | 6 | 10.7 | 50 | 89.3 | |
| II | 9 | 12 | 66 | 88 | |
| III | 41 | 18.7 | 137 | 81.3 | |
| IV | 4 | 50 | 4 | 50 | |
| Recurrences | | | | | |
| Local | 19 | 37.2 | 32 | 62.8 | 0.007 |
| Distant | 11 | 25 | 33 | 75 | 0.027 |
| Positive nodal status | | 2.5 [0-5] | | 0 [0-2.5] | <0.001 |
| Distant metastasis | | | | | 0.003 |
| M0 | 56 | 18.1 | 253 | 81.9 | |
| M1 | 4 | 50 | 4 | 50 | |

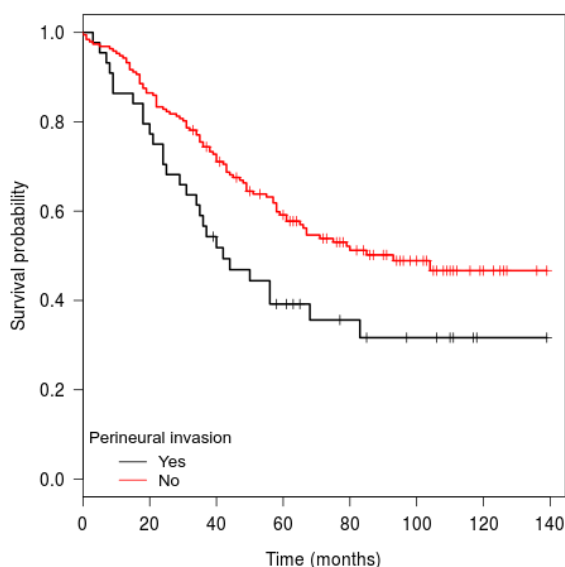


Figure 1. Kaplan-Meier curves depicting overall survival based on perineural invasion (PNI) status among 236 patients who underwent primary surgical resection of their rectal cancer

On Cox multivariate regression models using age, sex, TNM stage, lymph nodes invasion, differentiation grading, PNI was an independent prognostic factor for overall survival (Hazard Ratio = 0.6; 95% CI = 0.41 to 0.87; $p = 0.0082$).

Impact of PNI on survival in node-negative rectal cancer patients

The 5-year overall survival rate for node-negative patients with PNI-positive tumors was lower than those PNI-negative (68.42% vs. 69.92%, $p = 0.82$).

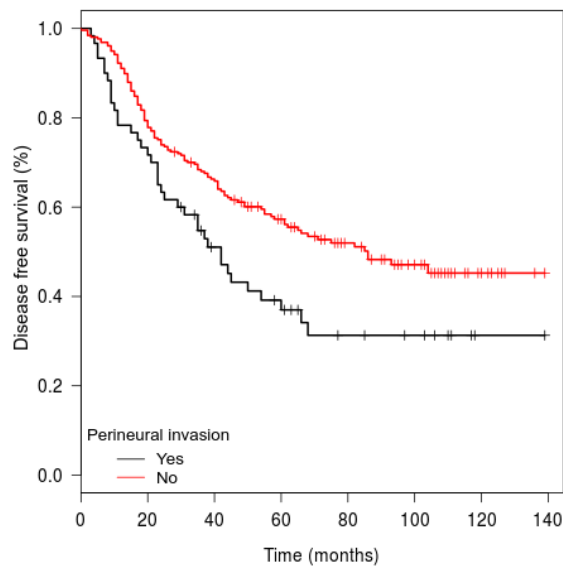


Figure 2. Kaplan-Meier curves depicting disease-free survival based on perineural invasion (PNI) status among 236 patients who underwent primary surgical resection of their rectal cancer

Discussion

Despite many progresses recorded in the understanding of CRC, especially in the molecular field, it still remains a major health issue worldwide. Although there are differences in the etiologies and epidemiology of colon and rectal cancer, most of the published studies chose to examine colon and rectum cancers combined. However, a better understanding of these diseases shows that this difference have an important influence in their approaches.

Perineural invasion (PNI) is a pathologic process of malignant infiltration along the neural fascicles and it represents an under-recognized route of metastatic spread. It is increasingly acknowledged as an important pathologic characteristic of several malignancies, including those of the pancreas, prostate, head and neck, biliary tract, colon and rectum and stomach. PNI is usually associated with locoregional recurrence, decreased overall survival and, in general, with a poor prognosis. It is a distinct pathologic entity that can be observed in the absence of lymphatic or vascular invasion. Despite increasing recognition of this metastatic process, there has been little progress in the understanding of molecular mechanisms behind PNI and, to date, no targeted treatment modalities aimed at this pathologic entity. It is also known that PNI is more frequent in rectal than colon cancer due to the rich nervous plexus that surround the pelvic rectum. For this reasons we've chosen to evaluate the role of PNI as a prognostic marker in rectal cancer alone.

PNI recognizes a variable incidence in CRC, reported in various studies between 10-33%. We found an incidence of 19% (60 patients) at the time of resection, which is consistent with previous reviews. PNI status was routinely reported by our pathologists throughout the study, using the same criteria for PNI definition. However our results might be slightly underreported due to the technical difficulties encountered by pathologist in identifying PNI (like the presence of inflammatory cells or large, mucinous pools that may obscure the presence of tumor cells around nerves [12]). Careful examination and re-review of slides by pathologists specified in this area may improve the detection rate. Although, according to the College of American Pathologists, PNI status is currently not a required feature of the pathology report for colon and rectal tumors [13,14] (because of the variable definitions of PNI and technical difficulties), we consider that PNI status should be specifically reported in pathology reports for rectal tumors.

Prior studies detected PNI related with distant and lymph node metastases, advanced stage and high grading [15,16]. In the present study we similarly demonstrated that PNI positivity correlates with already established histopathologic markers known to indicate aggressive behavior in rectal

cancer. Patients with positive PNI rectal cancer presented significant higher rates of advanced tumoral stages (III,IV), high tumoral grading (poorly differentiated tumors), distant metastases, lymph node invasion and local and distant recurrence. Although metastatic risk is higher in patients PNI-positive in concordance with other studies, our study included only 8 patients with distant metastases at the time of tumoral diagnosis. Nevertheless these findings sustain the role of PNI in the process of malignant progression and spread.

Regarding the impact of PNI on overall survival and disease-free survival, most studies combined colon and rectal cancer together [15-17] ore have suggested it's importance in univariate analysis [18,19]. We found that there was a statistical different outcome with respect to overall survival and disease-free survival between PNI-positive and negative patients, in favor of PNI-positive patients. The median overall survival for patients with PNI-positive and negative patients was 42 and 93 months ($p=0.014$) respectively, and the median disease-free survival was 42 and 86 month ($p=0.009$). At multivariate analysis, the presence of PNI appeared as an independent prognostic factor on survival. We concluded that PNI is an independent prognostic factor in rectal cancer which is not influenced by tumor stage, lymph node metastases and other classical factors. In our study PNI-negative patients had two fold higher overall survival and disease-free survival than PNI-positive ones. The last could represent a distinct subgroup of patients with rectal cancer that could benefit from a more aggressive therapeutic approach.

A particular situation is that of node-negative PNI-positive patients. Lymph node positivity is an established indication for adjuvant therapy in colorectal cancer, while node-negative patients do not currently receive such treatment. However there is a subset of patients who have worse outcome, with more recent locoregional recurrence and reduced overall survival. Liebig et al. [15] have suggested, in a recent study regarding colorectal cancer, that the poorer prognosis in this subgroup of node-negative patients might be explained by the presence of PNI. However, in our study, we didn't find a significant difference in survival between PNI-positive and PNI-negative node-negative patients with rectal cancer alone. Further studies are necessities to clarify this issue.

The use of R environment for the statistical analysis of this study was useful, efficient and flexible. R has extensive and powerful graphics abilities that are tightly linked with its analytic abilities. R is a free interactive computing environment which is rapidly developing. Simple and complex data analysis, data visualization, data management, simple or complex programming and connection to other software, as well as being a programming language in its own right, are specific properties for R environment.

Conclusions

In conclusion, our study demonstrated a high incidence of PNI in rectal cancer. PNI positivity was related to advanced tumoral stage, lymph node involvement, high tumoral grading and higher rates of malignant recurrence. The presence of PNI appeared as an independent prognostic factor on survival at multivariate analysis. It can be an independent prognostic factor which is not influenced by tumor stage, lymph node metastases and other classical factors. A better understanding of PNI in rectal cancer may lend insight at tumor recurrence and metastasis and may be improved staging strategies and new treatment modalities.

R environment for statistical computing and graphics is a complex yet easy to use software that has proven to be efficient in our clinical study

List of abbreviations

- CRC = Colorectal cancer.
- OS = Overall survival
- DFS = Disease-free survival
- PNI = Perineural invasion

Conflict of Interest

The authors declare that they have no conflict of interest.

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