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Clinical implications of perineural invasion in patients with colorectal cancer

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Abstract

Perineural invasion (PNI) is a prominent characteristic of multiple solid tumors and indicates poor prognosis. Previous data concerning the impact of PNI on prognosis of patients with colorectal cancer (CRC) are conflicting, and little is known about risk factors of PNI. The aim of our study was to reveal the clinical implication of PNI on survival outcome and identify risk factors for the poor prognosis in patients with CRC.

We retrospectively reviewed 627 patients who were diagnosed with CRC and underwent curative surgical resection. The differences in several clinicopathologic characteristics were compared between PNI positive and PNI negative groups. Multivariate logistic regression analysis was performed to identify predictors of CRC with PNI. Five-year overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan–Meier method, and the difference in survival rate was assessed by the log-rank test. The variables that had prognostic potential, as indicated by univariate analyses, were subjected to multivariate analyses with the Cox proportional hazards regression model.

PNIs were identified in 79 patients (12.6%). Age, T classification, N classification, M classification, UICC classification, and lymphovascular invasion were significantly associated with PNI. Multivariate logistic regression analysis demonstrated that only lymphovascular invasion was a predictor of PNI. Pathologic evidence of PNI was not associated with survival outcome (the 5-year OS [$P = .560$] and DFS [$P = .083$]). Cox proportional hazards regression model revealed that age and N2/3 classification were independent prognostic factors for poorer OS and DFS. M1 stage (95% confidence interval [CI] = 0.228–0.585, $P = .000$), III/IV stage (95% CI = 0.335–0.920, $P = .022$), and number of sampled lymph nodes (95% CI = 0.951–0.987, $P = .001$) were independently prognostic for poorer OS, while history of other malignancy (95% CI = 1.133–2.813, $P = .012$) was identified as an independent prognostic factor for poorer DFS.

Our study indicates that PNI is not an independent poor prognostic factor in patients with CRC and those patients with PNI may not benefit from postoperative adjuvant chemotherapy.

Abbreviations: BMI = body mass index, CRC = colorectal cancer, DFS = disease-free survival, OS = overall survival, PNI = perineural invasion, UICC = International Union Against Cancer.

Keywords: colorectal cancer, perineural invasion, prognosis

1. Introduction

Perineural invasion (PNI) is a prominent characteristic of multiple solid tumors (including pancreatic ductal adenocarcinoma, cutaneous squamous cell carcinoma of the head and neck, and prostate cancer) and indicates poor prognosis.[1–3] In the majority of cases, PNI is not associated with neurologic symptoms, however, local or referred pain is a warning sign of malignancy. The invasion of the surrounding nerves by cancer cells not only provides route for metastasis but also contributes to neural remodeling and changes in the neuronal milieu that can profoundly influenced the microenvironment of tumor.[4]

The vast majority of colorectal cancer (CRC) cases are readily curable with complete surgical resection with and without adjuvant chemotherapy unless high-risk features are present. Recently, as a new biologic feature, PNI has attracted more and more attention in CRC. Previous data concerning the impact of PNI on prognosis of patients with CRC are conflicting, and little is known about risk factors of PNI.[5,6] To overcome limitations of previous studies on the relationship between PNI and CRC, which arise from small cohorts of patients and short follow-up periods, we collected detailed histopathologic and clinical data from our prospective follow-up database. This study was aimed to reveal the clinical implication of PNI on outcome and identify risk factors for the poor prognosis in patients with CRC.

2. Materials and methods

2.1. Patients

This study retrospectively reviewed 1429 patients who were diagnosed with CRC and underwent curative surgical resection at

Editor: Surinder Kumar.

The authors report no conflicts of interest

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How to cite this article: Hu G, Li L, Hu K. Clinical implications of perineural invasion in patients with colorectal cancer. Medicine 2020;99:17(e19860).

Received: 11 September 2019 / Received in final form: 28 January 2020 / Accepted: 10 March 2020

http://dx.doi.org/10.1097/MD.0000000000019860

First Affiliated Hospital of Anhui Medical University from January 11, 2012, through February 15, 2019. The patients were enrolled according to the following criteria: pathologically diagnosed with primary CRC; available test results for PNI status; available and complete clinical, pathologic and follow-up data. A total of 627 patients met these criteria and were included in this study. Age, gender, body mass index (BMI), diabetes mellitus, medical history of other malignancy, neoadjuvant chemotherapy, primary tumor site, histology, T classification, N classification, M classification, UICC classification, lymphovascular invasion, PNI, and number of sampled lymph nodes were extracted for statistical analysis. This study protocol was approved by the ethics committee of our college. All patients signed an informed consent regarding their understanding of the procedure and its potential complications as well as their approval of participation in the research.

2.2. Determination of PNI status

About 4-μm thick 10% formalin-fixed, paraffin-embedded tissue sections of CRC were analyzed for PNI and other pathologic parameters were carried out by 1 institutional gastrointestinal pathologist. Questionable cases were reexamined by 2 pathologists. PNI was defined as tumor cells found within the perineural space or the infiltration of cancer cells into the endoneurium[7] (Fig. 1). The pathologic staging was grouped according to the American Joint Committee on Cancer Staging Manual, 8th edition.[8]

2.3. Statistical analysis

All measurement data were represented as ($\bar{x} \pm s$) and the count data were expressed in percentage. Patient characteristics were compared using t tests for continuous variables and Chi-squared or Fisher exact tests for categorical variables. To select final predictors of PNI, all candidate predictors with a $P < .2$ in univariate analysis were included in a multivariate logistic regression model. Variates with $P < .05$ in the multivariate analysis were deemed independent predictors. Five-year overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan–Meier method, and the difference in survival rate was assessed by the log-rank test. OS was measured from the date of diagnosis to the date of death or last follow-up. DFS was defined as the time from diagnosis to the first occurrence of any of the following events: recurrence of ACC at any site or death from any cause without documentation of a cancer-related event. The variables that had prognostic potential, as indicated by univariate analyses, were subjected to multivariate analyses with the Cox proportional hazards regression model. All analyses were performed on statistical package SPSS version 23.0. All *P* values were 2-sided, and a value of <.05 indicated statistical significance. Variables used to stratify survival included age, gender, BMI, diabetes mellitus, medical history of other malignancy, neoadjuvant chemotherapy, primary tumor site, histology, T classification, N classification, M classification, UICC classification, number of sampled lymph nodes, and lymphovascular invasion.

3. Results

3.1. Patient and tumor characteristics

Total of 627 patients with CRC met inclusive criteria and were enrolled our study. The clinical and pathologic characteristics of the patients are shown in Table 1. All patients underwent surgical resection of the primary tumor and the diagnosis of adenocarcinoma was made pathologically. Of the 627 CRCs, 79 (12.6%) and 548 (87.4%) tumors were classified as PNI positive (PNI(+)) and PNI negative (PNI(−)), respectively. Significant differences between CRC with and without PNI were observed with respect to age ($P = .038$), T classification ($P = .015$), N classification ($P = .000$), M classification ($P = .000$), UICC classification ($P = .000$), and lymphovascular invasion ($P = .000$); whereas, there were no significant differences with respect to gender ($P = .984$), BMI ($P = .770$), type 2 diabetes mellitus (T2DM; $P = .578$), history of other malignancy ($P = .322$), neoadjuvant chemotherapy ($P = .765$), primary tumor site ($P = .746$), histology ($P = .180$), and number of sampled lymph nodes ($P = .507$). A total of 151 (24.1%) patients had T2DM and 77 (12.3%) patients had history of other malignancy. Neoadjuvant chemotherapy such as FOLFOX (infusional fluorouracil, leucovorin plus oxaliplatin) or CAPOX (capecitabine plus oxaliplatin) was observed in 27 (4.3%) patients. According to logistic regression analysis (Table 2), only lymphovascular invasion was an independent factor in CRC with PNI.

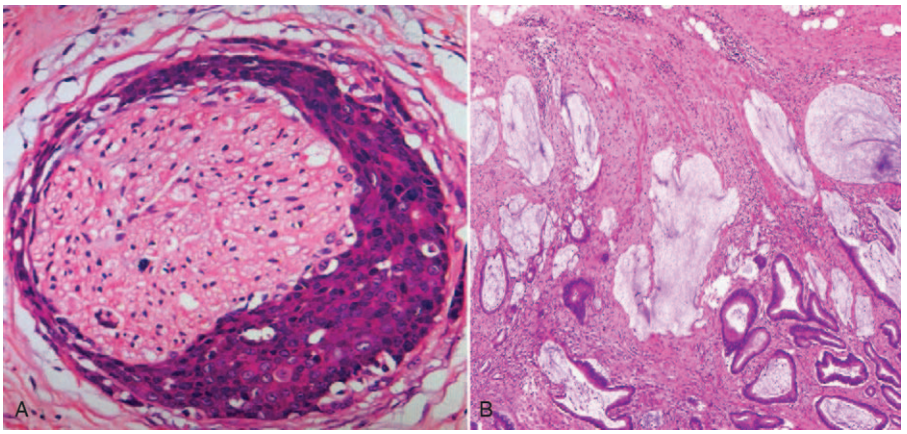

Figure 1. (A) Colorectal cancer (CRC) with perineural invasion and (B) CRC without perineural invasion.

Table 1

Clinicopathologic characteristics of the patients.

Features	PNI (+)	PNI (−)	*P* value
Total	79 (12.6%)	548 (87.4%)	
Age, yr	65.19 ± 14.56	66.43 ± 12.56	.038
Gender, n (%)			.984
Male	42 (53.2%)	292 (53.3%)	
Female	37 (46.8%)	256 (46.7%)	
BMI, kg/m^2	25.75 ± 4.66	24.89 ± 4.48	.770
Type 2 diabetes mellitus, n (%)			.578
Yes	21 (26.6)	130 (23.7)	
No	58 (73.4)	418 (76.3)	
Other malignancy, n (%)			.322
Yes	7 (8.9)	70 (12.8)	
No	72 (91.1)	478 (87.2)	
Neoadjuvant chemotherapy, n (%)			.765
Yes	4 (5.1)	23 (4.2)	
No	75 (94.9)	525 (95.8)	
Location, n (%)			.746
Right*	23 (29.1)	150 (27.4)	
Left†	56 (70.9)	398 (72.6)	
Histology, n (%)			.180
Wel/mod	57 (72.2)	432 (78.8)	
Por	22 (27.8)	116 (21.2)	
T classification, n (%)			.015
T1/2	8 (10.1)	120 (21.9)	
T3/4	71 (89.9)	428 (78.1)	
N classification, n (%)			.000
N0/1	51 (64.6)	458 (83.6)	
N2/3	28 (35.4)	90 (16.4)	
M classification, n (%)			.000
M0	56 (70.9)	482 (88.0)	
M1	23 (29.1)	66 (12.0)	
UICC classification, n (%)			.000
I/II	27 (34.2)	319 (58.2)	
III/IV	52 (65.8)	229 (41.8)	
Lymphovascular invasion, n (%)			.000
Yes	60 (75.9)	172 (31.4)	
No	19 (24.1)	376 (68.6)	
Number of sampled lymph nodes, n (%)	21.92 ± 15.51	21.49 ± 12.00	.507

BMI = body mass index, mod = moderately differentiated adenocarcinoma, por = poorly differentiated adenocarcinoma, PNI = perineural invasion, wel = well-differentiated adenocarcinoma.

* Right = cecum, ascending colon, and transverse colon.

† Left = descending colon, sigmoid colon, rectosigmoid colon, and rectum.

3.2. Prognostic value of PNI and factors affecting survival outcomes

With a median follow-up duration of 22 (6–82) months, the 5-year OS rates were 25.2% for patients with PNI and 51.9% for those without PNI, and the 5-year DFS rates 27.3% and 57.0% in CRC with and without PNI, respectively. During the analyses, a total of 139 (22.2%) patients experienced disease recurrence and 130 (20.7%) died. First of all, we performed a univariate analysis to investigate the impact of clinicopathologic parameters on patients' outcome (Table 3). A significant difference was identified for OS ($P = .007$) and DFS ($P = .001$) according to the PNI status in CRC. In addition, age, pT classification, pN classification, M classification, UICC classification, and lymphovascular invasion were also found to be significant predictors of 5-year OS and DFS.

The impact of the parameters that were found to be significant on univariate analysis was then evaluated by a multivariate model (Table 4). Pathologic evidence of PNI was not associated with outcome (the 5-year OS [$P = .560$] and DFS [$P = .083$]) (Fig. 2). Age and N2/3 classification were independent prognostic factors for poorer OS and DFS. M1 stage (95% confidence interval [CI] = 0.228–0.585, $P = .000$), III/IV stage (95% CI = 0.335–0.920, $P = .022$), number of sampled lymph nodes (95% CI = 0.951–0.987, $P = .001$) were independently prognostic for poorer OS, while history of other malignancy was identified as an independent prognostic factor for poorer DFS.

Table 2

Multivariate analysis of factors predicting colorectal cancer with PNI.

	Odds ratio	95% CI	*P* value
Age	1.008	0.988–1.028	.442
Histology (por:wel/mod)	0.820	0.465–1.445	.493
T classification (T1/2:T3/4)	1.620	0.712–3.684	.250
N classification (N0/1:N2/3)	1.234	0.648–2.352	.522
M classification (M0:M1)	1.691	0.882–3.242	.114
UICC classification (I/II:III/IV)	0.889	0.446–1.773	.738
Lymphovascular invasion (yes:no)	0.169	0.093–0.307	.000

CI = confidence interval, mod = moderately differentiated adenocarcinoma, PNI = perineural invasion, por = poorly differentiated adenocarcinoma, wel = well-differentiated adenocarcinoma.

4. Discussion

Recently, as a new biologic feature, PNI has attracted more and more attention in CRC. At present, to explore the prognostic role of PNI in CRC, the main objectives are as follows: to improve the clinical staging standards and to evaluate the necessity of postoperative adjuvant chemotherapy.

The PNI was found in 12.6% of our patients, which is similar to the results of previous studies. The reported incidence of PNI in patients with CRC ranges between 9% and 30%, but its prognostic significance is still controversial.[5,6,9] Quah and colleagues claimed that PNI status can be used to facilitate the selection of patients with stage II CRC for adjuvant chemotherapy.[5] Fujita and coworkers also reported that PNI significantly reduced the survival rates in patients with stages II and III CRC and might be useful for characterizing patients who might benefit from adjuvant system therapy.[10] On the contrary, Burdy et al suggested that PNI was a significant prognostic factor in univariate analysis but was not significant in multivariate analysis in patients undergoing surgery for T3/4N0 colon cancer.[9] Moreover, in Di Fabio and coworkers' study, the study group showed that only T4 stage and age over 70 were independent factors associated with significantly poor cancer-related survival in patients with stage II CRC.[11] We concur with this and confirmed PNI is not a prognostic factor for the survival of patients with CRC.

Zhou et al suggested that PNI status could be used as a complementary factor for TNM staging.[12] Their results[5,13] indicated that PNI significantly influenced the survival outcomes of TNM stages II and III patients, which was consistent with the findings of some studies. By combining TNM staging and PNI status, patient prognosis could be stratified into 3 levels: stage II PNI negative; stage II PNI positive/stage III PNI negative; and stage III PNI positive. Patients at stages II and III could be further divided according to their PNI status, thereby providing a basis for individualized auxiliary treatment.[13,14] Liebig et al reported that stage II PNI-positive patients have poorer prognosis than stage III patients.[15] Some study indicated that the value of cancer

Table 3

Univariate and multivariate analyses for overall survival.

		Overall survival			
		Univariate analysis		Multivariate analysis	
Variable	Comparator vs reference	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age		1.012–1.043	.000	1.018–1.050	.000
Gender	Male vs female	0.726–1.448	.886	0.641–1.229	.612
BMI		0.940–1.014	.210	0.940–1.020	.308
Type 2 diabetes mellitus	Yes vs no	0.745–1.620	.636	0.665–1.498	.994
Other malignancy	Yes vs no	0.795–2.062	.310	0.596–1.618	.943
Neoadjuvant chemotherapy	Yes vs no	1.767–6.187	.000	0.849–3.624	.129
Location	Right* vs left†	0.873–1.831	.215	0.948–2.065	.091
Histology	Por vs wel/mod	1.025–2.214	.037	0.828–1.806	.312
T classification	T1/2 vs T3/4	0.220–0.760	.005	0.366–1.360	.297
N classification	N0/1 vs N2/3	0.218–0.443	.000	0.372–0.953	.031
M classification	M0 vs M1	0.192–0.402	.000	0.228–0.585	.000
TNM classification	I/II vs III/IV	0.226–0.472	.000	0.335–0.920	.022
Lymphovascular invasion	Yes vs no	1.312–2.633	.000	0.755–1.774	.502
Perineural invasion	Yes vs no	1.184–2.880	.007	0.706–1.900	.560
Number of sampled lymph nodes		0.962–0.994	.009	0.951–0.987	.001

BMI = body mass index, CI = confidence interval, HR = hazard ratio, mod = moderately differentiated adenocarcinoma, por = poorly differentiated adenocarcinoma, wel = well-differentiated adenocarcinoma.
* Right: cecum, ascending colon, and transverse colon.
† Left: descending colon, sigmoid colon, rectosigmoid colon, and rectum.

staging could be enhanced by PNI assessment using a grading system based on PNI location within the bowel.[16,17] Previous reports indicated that PNI can be detected in 10% to 35% of the resected tumor samples of CRC, and it increases with higher tumor grade and stage.[18,19] However, the current cancer evaluation system of PNI lacks uniform standards and concrete guidelines.

Several studies have shown that the number of lymph nodes dissected during surgery is an important prognostic factor in patients with stage II CRC.[6,20] Harvesting more lymph nodes is associated with a better prognosis, and this arises from more accurate staging or a prominent host immunologic response.[7,21,22] Caplin and coworkers suggested that stage II patients with 6 or fewer examined lymph nodes have poorer outcomes than those with a higher number examined due to under staging.[20] They also observed that recovering more lymph nodes is associated with a better OS in patients with stage II CRC. Huh et al suggested that PNI did not impact OS at the completion of their analysis.[13] Therefore, further large-scale studies with long-term follow-up periods are necessary to determine the prognostic role of PNI for OS in patients with stage II CRC.

This study's limitations deserve commentary. First, this was a nonrandomized retrospective analysis from a single center, and as such, there were potential biases for comparison. Second, we did not evaluate the circumferential resection margin using the resection specimen and the effect of adjuvant chemotherapy on

Table 4

Univariate and multivariate analyses for disease-free survival.

		Disease-free survival			
		Univariate analysis		Multivariate analysis	
Variable	Comparator vs reference	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age		0.972–0.998	.029	0.966–0.994	.005
Gender	Male vs female	0.914–1.795	.150	0.987–1.975	.059
BMI		0.978–1.053	.447	0.974–1.050	.561
Type 2 diabetes mellitus	Yes vs no	0.662–1.436	.897	0.612–1.359	.651
Other malignancy	Yes vs no	1.046–2.523	.031	1.133–2.813	.012
Neoadjuvant chemotherapy	Yes vs no	1.000–3.624	.050	0.811–3.681	.156
Location	Right* vs left†	0.675–1.434	.932	0.736–1.591	.688
Histology	Por vs wel/mod	0.854–1.837	.249	0.758–1.652	.571
T classification	T1/2 vs T3/4	0.223–0.729	.003	0.298–1.050	.071
N classification	N0/1 vs N2/3	0.290–0.583	.000	0.448–1.113	.134
M classification	M0 vs M1	0.266–0.559	.000	0.383–0.952	.030
TNM classification	I/II vs III/IV	0.307–0.612	.000	0.520–1.368	.491
Lymphovascular invasion	Yes vs no	1.267–2.469	.001	0.771–1.687	.511
Perineural invasion	Yes vs no	1.294–2.934	.001	0.948–2.375	.083
Number of sampled lymph nodes		0.990–1.016	.651	0.981–1.009	.489

BMI = body mass index, CI = confidence interval, HR = hazard ratio, mod = moderately differentiated adenocarcinoma, por = poorly differentiated adenocarcinoma, wel = well-differentiated adenocarcinoma.
* Right: cecum, ascending colon, and transverse colon.
† Left: descending colon, sigmoid colon, rectosigmoid colon, and rectum.

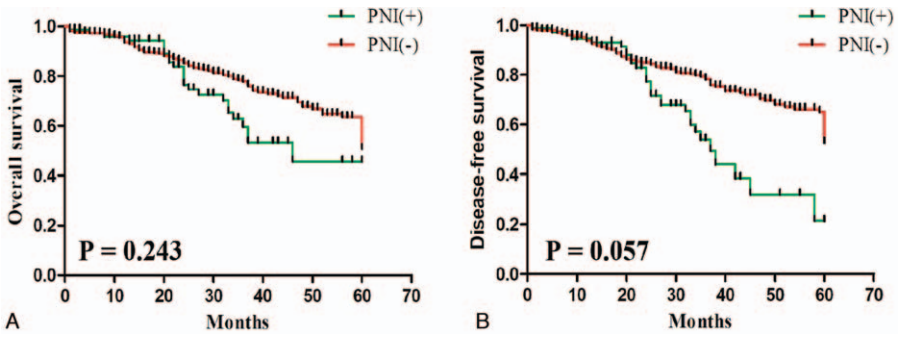

Figure 2. Five-year (A) overall survival and (B) disease-free survival rates calculated by the Kaplan–Meier method. PNI = perineural invasion.

outcomes. However, our data reflect that PNI is not an independent poor prognostic factor in patients with CRC and could not guide therapeutic regimen. M1 stage, III/IV stage, and the number of sampled lymph nodes were independently prognostic for poorer OS, while history of other malignancy was identified as an independent prognostic factor for poorer DFS. Both age and N2/3 classification were significantly correlated with poorer OS and DFS. The results of the present analysis will hopefully lead to a prospective randomized study with the ultimate goal of a centralized national program for prognostic value of PNI in CRC.

Author contributions

Data curation: Gang Hu, Kaibing Hu.
Formal analysis: Gang Hu, Kaibing Hu.
Supervision: Gang Hu, Liang Li.
Validation: Gang Hu.
Visualization: Gang Hu, Kaibing Hu.
Writing – original draft: Gang Hu, Liang Li.

References

[1] Yang YH, Liu JB, Gui Y, et al. Relationship between autophagy and perineural invasion, clinicopathological features, and prognosis in pancreatic cancer. World J Gastroenterol 2017;23:7232–41.
[2] Zareba P, Flavin R, Isikbay M, et al. Perineural invasion and risk of lethal prostate cancer. Cancer Epidemiol Biomarkers Prev 2017;26:719–26.
[3] Amit M, Binenbaum Y, Trejo-Leider L, et al. International collaborative validation of intraneural invasion as a prognostic marker in adenoid cystic carcinoma of the head and neck. Head Neck 2015;37:1038–45.
[4] Huh JW, Kim HR, Kim YJ. Lymphovascular or perineural invasion may predict lymph node metastasis in patients with T1 and T2 colorectal cancer. J Gastrointest Surg 2010;14:1074–80.
[5] Quah HM, Chou JF, Gonen M, et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. Dis Colon Rectum 2008;51:503–7.
[6] Tsai HL, Cheng KI, Lu CY, et al. Prognostic significance of depth of invasion, vascular invasion and numbers of lymph node retrievals in combination for patients with stage II colorectal cancer undergoing radical resection. J Surg Oncol 2008;97:383–7.
[7] Batsakis JG. Nerves and neurotropic carcinomas. Ann Otol Rhinol Laryngol 1985;94:426–7.
[8] Weiser MR. AJCC 8th edition: colorectal cancer. Ann Surg Oncol 2018;25:1454–5.
[9] Burdy G, Panis Y, Alves A, et al. Identifying patients with T3-T4 node-negative colon cancer at high risk of recurrence. Dis Colon Rectum 2001;44:1682–8.
[10] Fujita S, Nakanisi Y, Taniguchi H, et al. Cancer invasion to Auerbach's plexus is an important prognostic factor in patients with pT3-pT4 colorectal cancer. Dis Colon Rectum 2007;50:1860–6.
[11] Di Fabio F, Nascimbeni R, Villanacci V, et al. Prognostic variables for cancer-related survival in node-negative colorectal carcinomas. Dig Surg 2004;21:128–33.
[12] Zhou Y, Wang H, Gong H, et al. Clinical significance of perineural invasion in stages II and III colorectal cancer. Pathol Res Pract 2015;211:839–44.
[13] Huh JW, Kim HR, Kim YJ. Prognostic value of perineural invasion in patients with stage II colorectal cancer. Ann Surg Oncol 2010;17:2066–72.
[14] De Dosso S, Sessa C, Saletti P. Adjuvant therapy for colon cancer: present and perspectives. Cancer Treat Rev 2009;35:160–6.
[15] Liebig C, Ayala G, Wilks J, et al. Perineural invasion is an independent predictor of outcome in colorectal cancer. J Clin Oncol 2009;27:5131–7.
[16] Ueno H, Shirouzu K, Eishi Y, et al. Characterization of perineural invasion as a component of colorectal cancer staging[J]. Am J Surg Pathol 2013;37:1542–9.
[17] Ceyhan GO, Liebl F, Maak M, et al. The severity of neural invasion is a crucial prognostic factor in rectal cancer independent of neoadjuvant radiochemotherapy. Ann Surg 2010;252:797–804.
[18] Knudsen JB, Nilsson T, Sprechler M, et al. Venous and nerve invasion as prognostic factors in postoperative survival of patients with resectable cancer of the rectum. Dis Colon Rectum 1983;26:613–7.
[19] Bellis D, Marci V, Monga G. Light microscopic and immunohistochemical evaluation of vascular and neural invasion in colorectal cancer. Pathol Res Pract 1993;189:443–7.
[20] Caplin S, Cerottini JP, Bosman FT, et al. For patients with Dukes' B (TNM stage II) colorectal carcinoma, examination of six or fewer lymph nodes is related to poor prognosis. Cancer 1998;83:666–72.
[21] Wong JH, Bowles BJ, Bueno R, et al. Impact of the number of negative nodes on disease-free survival in colorectal cancer patients. Dis Colon Rectum 2002;45:1341–8.
[22] Murphy J, Pocard M, Jass JR, et al. Number and size of lymph nodes recovered from dukes B rectal cancers: correlation with prognosis and histologic antitumor immune response. Dis Colon Rectum 2007; 50:1526–34.