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ORIGINAL ARTICLE

Study of histopathologic parameters to define the prognosis of stage II colon cancer

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Abstract

Purpose Stage II colon cancer (CC) represents a challenging scenario for the choice of adjuvant chemotherapy; here, histologic factors need to be weighed up to establish the risk of recurrence. Tumor budding (TB) has recently been indicated as a confident predictor of clinical outcome in CC. Likewise, the presence of poorly differentiated clusters (PDCs) in a tumor has been pointed out as a leading criterion of a tumor grading system. Our aim was to evaluate in patients with stage II CC the relationship between these features and clinical outcome.

Patients and methods The study included 174 cases of stage II CC; histopathologic parameters such as TB, PDCs, microsatellite instability (MSI), and CDX2 expression were analyzed.

Results There were 107 (70.9%), 32 (21.2%), and 12 (7.9%) TB scored 1, 2, and 3 respectively; 113 (72.9%), 30 (19.4%), and 12 (7.7%) tumors showed grade 1, 2, and 3 PDCs respectively. A high-MSI was detected in 32 cases (18.4%) while CDX2 was negative in 20 (11.5%) tumor samples. In the whole study population, only the TB was found to be associated with disease-specific survival ($P = 0.01$). No parameter apart from age ($P = 0.04$) was a significant prognostic factor for overall survival ($P < 0.05$). Other commonly reported variables, including tumor size, degree of tumor differentiation, lymphovascular invasion, number of lymph nodes harvested ≥ 12, MSI, and PDCs, were not shown to have significant results.

Conclusions Although confirmatory studies are awaited, our work supports the role of the TB in defining risk groups of the stage II CC.

Keywords Colon cancer · Stage II · Tumor budding · Poorly differentiated cluster · CDX2 · Microsatellite instability

Introduction

Stage II colon cancer (CC) is characterized by a good prognosis. Indeed, the 5-year overall survival (OS) is as high as 80% after surgery alone. The adjunct of a 5-fluorouracil-based chemotherapy elicits an 18% relative risk reduction of death that translates in an absolute improvement in survival of 3.6% [1]. Thus, on account of both the good prognosis and little benefit of adjuvant chemotherapy, prognostic and predictive factors ought to be weighed up to establish the subjective risk of disease recurrence. Moreover, a discussion with the patient concerning risks and benefits of the treatment is broadly suggested [2, 3].

The presence of lymphovascular and perineural invasion, bowel obstruction/perforation, and < 12 lymph nodes examined as well as a poorly differentiated histology is a clinicopathologic factor generally used to categorize the risk of recurrence in stage II CC. However, because a significant degree of interobserver variability in the histological grading has been reported [1, 4, 5], additional prognostic parameters have been proposed. Tumor budding (TB), defined as single cells or clusters of up to four cells at the invasive margin of CC [6], has been indicated as a confident predictor of clinical outcome, because of its relevance to prognosis and its reproducibility [7]. Likewise, the presence

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of poorly differentiated clusters (PDCs) in a tumor (composed of ≥ 5 cancer cells and lacking a gland-like structure) has been pointed out as a leading criterion of a tumor grading system [8]. Furthermore, both the microsatellite instability (MSI) [4, 9–11] and the expression of the homedomain transcription factor CDX (CDX2) [12–14] can be considered to prognosticate in the stage II CC.

We examined in patients with stage II CC several clinicopathologic features, including TB, PDCs, MSI, and CDX2 to evaluate the relationship between their expression and clinical outcome.

Patients and methods

Patients

One hundred seventy-four stage II CC cases, which were subject to histological examination at the Sant'Andrea Hospital in Rome, from 2009 to 2015, were collected consecutively and analyzed retrospectively. For each patient, demographics data (sex and age at surgery), tumor location, and pathological features were retrieved. Pathological data included grading, T, and the number of nodes retrieved in surgery.

The study was conducted in accordance with the Declaration of Helsinki and the protocol approved by the institutional (Sapienza University) ethical committee (N.3874_2015/22.10.2015).

Histological evaluation

All cancers were evaluated for histological and immunophenotype features by two pathologists (E.P and A.D.C). Cancers were graded according to WHO guidelines as well (G1), moderately (G2), and poorly (G3) differentiated, based on the percentage of glands formation (> 95%; 50 to 95%; 1 to 49% respectively). Moreover, each tumor sample was scored for TB and PDC formation.

Tumor budding

Tumor budding is defined as a single neoplastic cell or a cell cluster up to 4 cells on invasive front of the tumor. In this study, we evaluated TB according to "International Tumour Budding Consensus Conference 2016" [15]. Specifically, the cancer slides were scanned at × 10 objective and the hotspot field was chosen. In this field, buds, as previously defined, were counted at × 20 objective. Then, TB was scored as 1-low (< 5 buds), 2-intermediate (5–9 buds), and 3-high (≥ 10 buds) respectively. Tumors were divided into two groups: score 1 and 2 were considered low-grade budding and score 3 was considered high-grade budding [6, 15, 16]. Tumor budding was not assessed in mucinous, medullary, micropapillary cancers and in those specimens with heavy granulocytic infiltration at the edge front causing glandular fragmentation.

Table 1 Clinicopathologic features (valid cases and percentages)

	Number	Percentage
Total	174	100
Age		
< 70 years	75	43.1
≥ 70 years	99	56.9
Sex		
Male	97	55.7
Female	77	44.3
Tumor location		
Proximal	91	52.3
Distal	83	47.7
Tumor size		
T3	148	85.1
T4	26	14.9
Tumor differentiation		
Well to moderate	124	72.9
Poor	46	27.1
Lymphovascular/perineural		
Not	105	61.8
Yes	65	38.2
Node retrieval		
≥ 12	157	
< 12	15	
Microsatellite instability		
MSS	142	81.6
MSI-H	32	18.4
CDX2		
Positive	154	88.5
Negative	20	11.5
Tumor budding		
Score 1 (< 5)	107	70.9
Score 2 (5–9)	32	21.2
Score 3 (≥ 10)	12	7.9
Poorly differentiated cluster		
Grade 1 (< 5)	113	72.9
Grade 2 (5–9)	30	19.4
Grade 3 (≥ 10)	12	7.7
Adjuvant chemotherapy		
Not	92	54.8
Yes	76	45.2

Poorly differentiated clusters

Poorly differentiated clusters are defined as cancer cell clusters (≥ 5 cancer cells) infiltrating the stroma without gland formation [8]. PDCs were scored at × 20 objective after

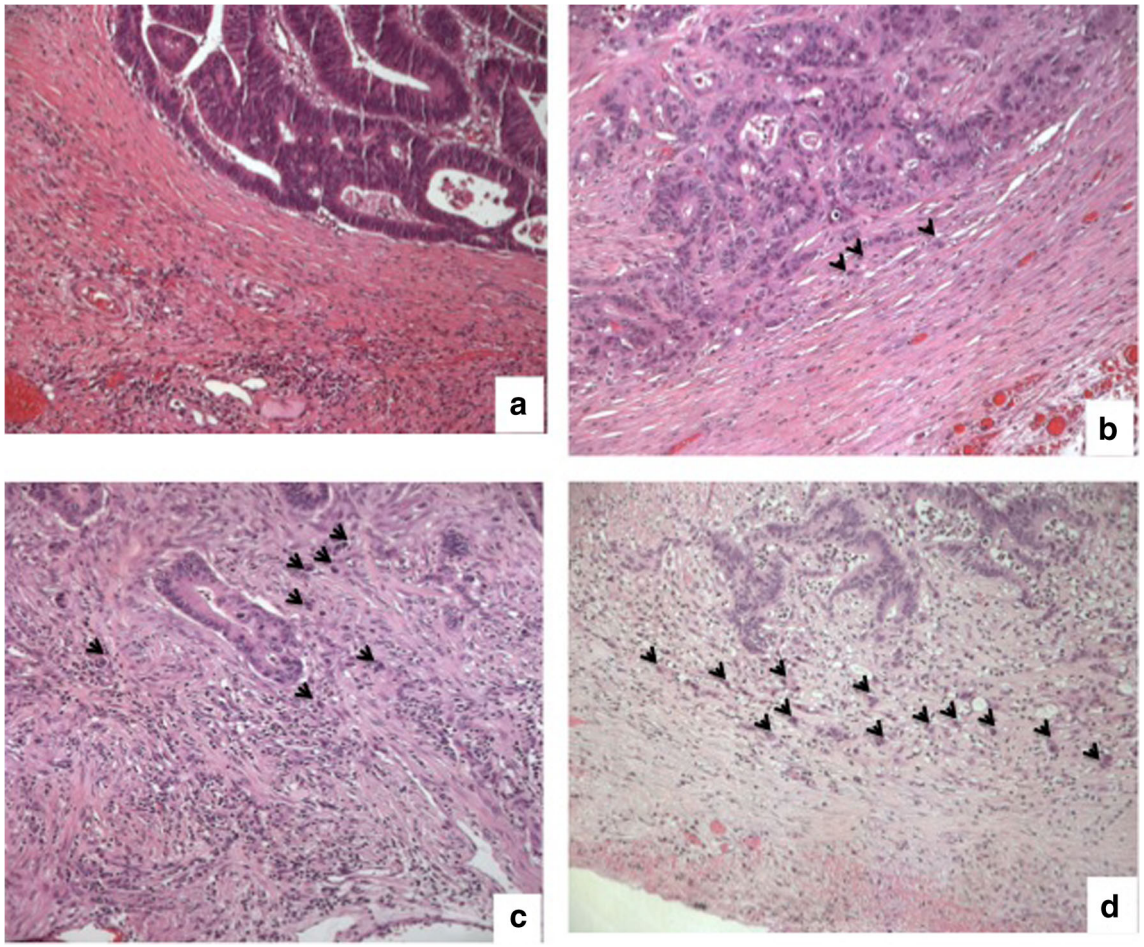

Fig. 1 **a**–**d** Tumor Budding scores according to "International Consensus Conference on Tumor Budding 2016" are represented. Arrows outline buds at the invasive tumor edge. **a** and **b** Budding G1 or low: absence of tumor buds (**a**) or < 5 tumor buds (**b**). **c** Budding G2 or intermediate (5–9 buds). **d** Budding G3 or high (≥ 10). (Hematoxylin/eosin × 10 objective lens)

scanning the sections at low-power magnification to identify the area with the greatest number of PDCs. Tumors with < 5, 5 to 9, and ≥ 10 clusters were classified as grade G1, G2, and G3 respectively.

Because, in stage II CC, tumors with G3 PDCs showed the worst prognosis compared with both G1 and G2 and any significant difference was between G1 and G2 tumors [8] and also according to other studies [17, 18], tumors were divided into two groups: < 10 PDCs were considered negative; ≥ 10 PDCs were considered positive.

Immunohistochemistry

Three-micrometer-thick sections were immunostained with CDX2 antibody (clone 88, Biogenex) and visualized by Envision-Flex (Dako) in a Dako Autostainer instrument. In each section was represented neoplastic tissue and normal mucosa, and the latter was evaluated as the internal control. Immunostained slides were evaluated by the two pathologists (ADC and EP). Samples that in neoplastic cells showed either complete lack of CDX2 expression or a faint expression in a minority of neoplastic cell (< 20%) were scored as negative. All other samples were regarded as CDX2 positive.

DNA extraction and microsatellite instability evaluation

A fragment of cancer tissue was collected for each of 174 selected patients. For each sample of paraffin embedded tissue, 3 sections of 7 μm were cut and put on slides, de-waxed, rehydrated, and stained with hematoxylin. A pathologist performed micro-dissection of neoplastic tissue under microscope using a needle. Genomic DNA extraction was carried out using QIAmp DNA mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. At molecular level, all samples were evaluated for MSI through PCR amplification of 2 quasimonomorphic mononucleotide loci (BAT25 and BAT26) as previously described [19]. Presence in both loci of extra alleles in neoplastic tissue was defined as high-MSI (MSI-H).

Table 2 Association between tumor budding and poorly differentiated cluster with clinical-pathological parameters

Characteristic	Tumor budding				Poorly differentiated cluster			
	Score 1 (< 5) Number (%)	Score 2 (5–9)	Score 3 (≥ 10)	*P*	Grade 1 (< 5)	Grade 2 (5–9) Number (%)	Grade 3 (≥ 10)	*P*
Age								
< 70 years	45 (69.2)	15 (23.1)	5 (7.7)	0.88	49 (74.2)	13 (19.7)	4 (6.1)	0.79
≥ 70 years	62 (72.1)	17 (19.8)	7 (8.1)		64 (71.9)	17 (19.1)	8 (9.0)	
Sex								
Male	53 (64.6)	19 (23.2)	10 (12.2)	0.05	60 (71.4)	17 (20.2)	7 (8.3)	0.90
Female	54 (78.3)	13 (18.8)	2 (2.9)		53 (74.6)	13 (18.3)	5 (7.0)	
Tumor location								
Proximal	60 (75.9)	13 (16.5)	6 (7.6)	0.30	60 (73.2)	12 (14.6)	10 (12.2)	0.03
Distal	47 (65.3)	19 (26.4)	6 (8.3)		53 (72.6)	18 (24.7)	2 (2.7)	
Tumor size								
T3	92 (73.6)	23 (18.4)	10 (8.0)	0.21	96 (74.4)	26 (20.2)	7 (5.4)	0.05
T4	15 (57.7)	9 (34.6)	2 (7.7)		17 (65.4)	4 (15.4)	5 (19.2)	
Tumor differentiation								
Well to moderate	81 (73.0)	22 (19.8)	8 (7.2)	0.63	90 (76.9)	22 (18.8)	5 (4.3)	0.01
Poor	26 (65.0)	10 (25.0)	4 (10.0)		23 (60.5)	8 (21.1)	7 (18.4)	
Node retrieval								
< 12	96 (70.6)	29 (21.3)	11 (8.1)	0.99	103 (73.6)	25 (17.9)	12(8.6)	0.14
≥ 12	9 (69.2)	3 (23.1)	1 (7.7)		8 (61.5)	5 (38.5)	0 (0.0)	
Lymphovascular/perineural invasion								
Not	73 (81.8)	11 (12.2)	6 (6.7)	0.002	84 (88.4)	9 (9.5)	31 (51.7)	< 0.001
Yes	34 (55.7)	21 (34.4)	6 (9.8)		29 (48.3)	21 (35.0)	10 (16.7)	
CDX2								
Positive	94 (69.6)	30 (22.2)	11 (8.1)	0.61	101 (72.1)	29 (20.7)	10 (7.1)	0.34
Negative	13 (81.3)	2 (12.5)	1 (6.3)		12 (80.0)	1 (6.7)	2 (13.3)	
MSI								
MSS	85 (68.0)	30 (24.0)	10 (8.0)	0.12	93 (72.1)	27 (20.9)	9 (7.0)	0.44
MSI-H	22 (84.6)	2 (7.7)	2 (7.7)		20 (76.9)	3 (11.5)	3 (11.5)	
Poorly differentiated cluster								
Grade 1 (< 5)	88 (81.5)	14 (13.3)	6 (5.6)	< 0.001				
Grade 2 (5–9)	12 (41.4)	12 (41.4)	5 (17.2)					
Grade 3 ((≥ 10)	6 (50.0)	5 (41.7)	1 (8.3)					

Statistical analysis

Outcome variables were disease-specific survival (DSS) and overall survival (OS) [20]. SPSS statistical software, Version 24 (SPSS Inc. Chicago, Illinois, USA) was used. The χ^2 test and *t* test for unpaired data were applied to compare frequencies and means, respectively. The interaction among clinicopathologic parameters was first analyzed using univariate logistic regression. Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used for the difference assessment.

A multivariate Cox proportional hazard model was used to identify independent prognostic factors for survival.

Results

Frequency and associations of clinicopathologic parameters

The study included 174 consecutive stage II colon adenocarcinoma; patients' mean age was 70 years (range 35–92) (Table 1). The tumors were located in the right-sided colon in 91 cases (52.3%) and were pT3 in 85.1% of the cases. The majority (97%) were conventional adenocarcinoma, while 5 (3%) of them were mucinous. According to WHO classification, 124 (72.9%) and 46 (27.1%) tumors were classified as well to moderate and poorly differentiated respectively. Sixty-

Table 3 Survival analysis according to clinicopathologic parameters

	5-year disease-specific survival				5-year overall survival			
	All patients		No chemotherapy		All Patients		No chemotherapy	
	%	*P*	%	*P*	%	*P*	%	*P*
Age (< 70 v ≥ 70 years)	94 v 96	0.93	98 v 88	0.65	88 v 74	0.04	76 v 72	0.26
Sex (female v male)	98 v 93	0.15	100 v 90	0.19	86 v 76	0.23	77 v 62	0.28
Tumor location (proximal v distal)	95 v 95	0.37	97 v 90	0.84	77 v 84	0.23	65 v 71	0.36
Tumor size (T3 v T4)	95 v 84	0.69	95 v 87	*0.02*	82 v 71	0.49	74 v 30	*0.007*
Tumor differentiation (G1–2 v G3–4)	96 v 91	0.89	96 v 80	0.99	81 v 77	0.35	68 v 63	0.60
Node retrieval (≥ 12 v < 12)	95 v 93	0.86	97 v 100	0.84	81 v 84	0.53	70 v 100	0.99
LV/PnI (negative v positive)	94 v 96	0.39	94 v 96	0.41	84 v 75	0.21	78 v 55	*0.004*
Stage risk (low v high)	98 v 93	*0.05*	100 v 89	*0.04*	87 v 75	*0.05*	84 v 56	*0.02*
MSI status (MSS v MSI-H)	96 v 90	0.58	97 v 80	0.81	80 v 81	0.72	67 v 70	0.34
CDX2 (positive v negative)	95 v 94	0.80	94 v 100	0.51	82 v 65	*0.08*	71 v 48	0.34
Tumor budding (low v high grade)	96 v 92	*0.01*	98 v 80	*0.008*	80 v 76	0.39	69 vs 53	0.25
Poorly differentiated cluster (negative v positive)	96 v 100	0.35	97 v 100	0.78	80 v 83	0.82	70 v 50	0.15

LV lymphovascular, *PnI* perineural invasion, significant values are presented as italized data

five (38.2%) tumors showed lymphovascular/perineural invasion. There were 107 (70.9%), 32 (21.2%), and 12 (7.9%) tumor budding scored 1, 2, and 3 respectively (range 0–15 of buds). Cases with different degrees of TB are shown in Fig. 1a–d. There were 113 (72.9%), 30 (19.4%), and 12 (7.7%) tumors showing grade 1, 2, and 3 PDCs respectively. A MSI-H was detected in 32 cases (18.4%) while CDX2 was negative in 20 (11.5%) tumor samples. Adjuvant chemotherapy was given in 76 (45.2%) patients; in 37% of these cases, it was oxaliplatin-based. TB grade was associated with sex ($P = 0.05$), lymphovascular/perineural invasion ($P = 0.002$), and PDCs ($P < 0.001$)(Table 2).

PDC grade was associated with tumor location ($P = 0.03$), tumor size ($P = 0.05$), tumor differentiation ($P = 0.01$), and lymphovascular/perineural invasion ($P < 0.001$).

MSI-H was associated with younger age ($P = 0.01$), localization of the tumor to a site proximal to the splenic flexure ($P < 0.001$), tumor differentiation ($P < 0.001$), and a CDX2-negative status ($P < 0.001$) (Supplementary Table 1).

CDX-2 negative expression was associated with tumor differentiation ($P = 0.001$) and MSI status ($P < 0.001$) (Supplementary Table 1).

Survival analysis

The follow-up data for all but two patients (99%), who were lost to follow-up, were available. The median follow-up was 48 months (range 4–95). There were 42 recurrence or death events. Thirty-four patients died for any cause, and nine patients died of the disease. The 5-year DSS and OS were 93% and 80% respectively.

In the whole study population, only the TB was found to be associated with DSS ($P = 0.01$) (Table 3). In a multivariate model including risk factors for the stage II (tumor size, degree of tumor differentiation, lymphovascular invasion, number of lymph node harvested ≥ 12), TB was an independent predictor of DSS ($P = 0.03$) (Fig. 2a).

No parameter but for age ($P = 0.04$) was a significant prognostic factor for OS ($P < 0.05$). However, CDX2-negative cases showed the worst, despite not statistically significant, OS ($P = 0.09$) (Fig. 2b). Other commonly reported variables including tumor size, degree of tumor differentiation, lymphovascular invasion, number of lymph node harvested ≥ 12, MSI, and PDCs were not shown to have significant results.

When the group of patients (92 cases), who had not been treated with adjuvant chemotherapy, were considered, tumor size ($P = 0.02$) and TB ($P < 0.01$) were prognostic for DSS. In a multivariate model including risk factors for the stage II (tumor size, degree of tumor differentiation, lymphovascular invasion, number of lymph node harvested ≥ 12), both pT4 ($P = 0.02$) and TB ($P < 0.01$) were independent predictors of DSS.

Tumor size ($P < 0.01$) and lymphovascular/perineural invasion ($P < 0.01$) were prognostic for OS. In the multivariate model, tumor size ($P = 0.02$) retained statistical significance.

Discussion

Although the role of adjuvant chemotherapy in reducing recurrence and deaths in stage III CRC is unquestionable, the

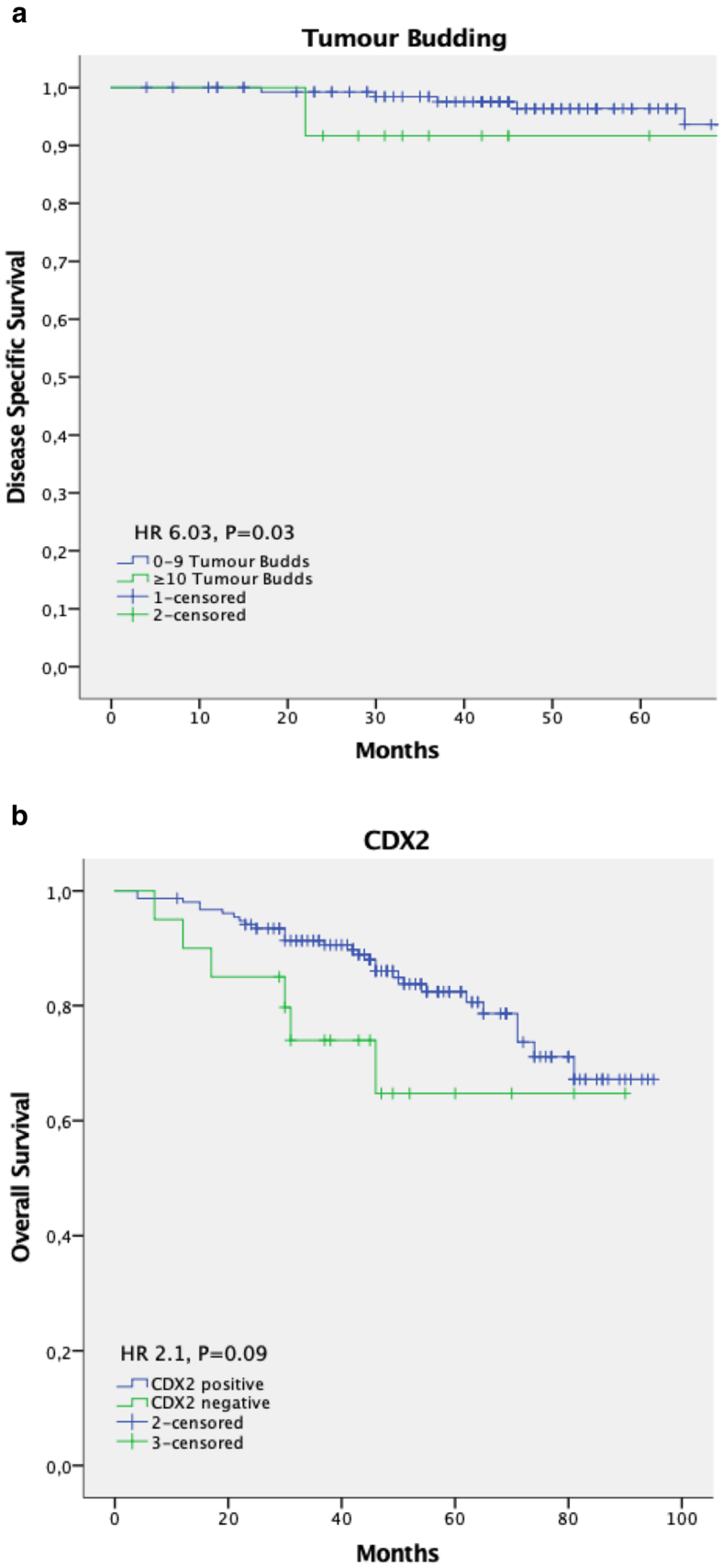

Fig. 2 **a** Disease-specific survival (DSS) according to tumor budding. **b** Overall survival (OS) according to CDX2 expression

magnitude of its benefit in stage II is still debatable. In this stage, pathological factors as T4, lymphovascular and perineural invasion, bowel obstruction/perforation, < 12 lymph nodes examined, and a poorly differentiated histology need to be weighed up to establish the subjective risk of disease recurrence even though their value in some case is so far disputed. For instance, tumor grade has not been consistently associated with increased risk of recurrence in stage II disease [12, 21–23] while an interobserver variability was demonstrated [24]. Moreover, it is known that a poor differentiation is positively correlated with MSI and it does not negatively impact prognosis in this molecularly defined subset of patients [25]. Furthermore, the methodological approach in assessing and reporting blood or lymphatic vessel involvement by tumor may present considerable heterogeneity [24].

Tumor budding, a histopathologic approach to assess tumor aggressiveness, has recently been proposed to define prognosis of the stage II CC [16, 26, 27]. Moreover, an international consensus conference suggested that stage II colorectal cancer with high-grade TB might be considered for adjuvant therapy [15].

Current studies also reported the histologic feature PDCs, defined as solid cancer nests including ≥ 5 cancer cells, as a parameter reflecting CC metastatic potential [28, 29]. It showed a robust prognostic power in a multi-institution pathologic review [30].

In our analysis, previous data that pointed out TB as a prognostic factor for the stage II CCs were confirmed [27, 31, 32]. Indeed, patients with low to intermediate grade had a better survival compared with those with a high grade of TB in both univariate and multivariate analysis.

We also observed that tumor size and lymphovascular and perineural invasion were prognostic in untreated patients. However, no other pathologic parameters including histological grading were significantly correlated with the clinical outcome. According to a latest study [33], we observed a strong association between TB and PDCs; nonetheless, no association of this histologic feature with clinical outcome has been detected. However, there are also results consistent with ours [26] in which TB, but none of the other classical histologic features, was an indicator of risk recurrence in stage II CC.

Moreover, the number and the type of patients included in our research as well as the low incidence of disease recurrences and deaths observed may explain differences from previous reports.

Indeed, our cases showed a low proportion of tumor located in the left side of the colon and a majority of pT3. Furthermore, the prevalence of G3 PDCs was just 7%, which is lower than that reported in other studies for the stage II (15.3%) while is near to the percentage observed in stage I (4.1%) [30]. These tumors' features could explain the high DSS observed and the lack of a significant association between PDCs and survival. The work undertaken in our institution by a multidisciplinary management team for the early diagnosis of colorectal cancer as well as the considerable proportion of patients treated with adjuvant chemotherapy could have contributed to such results.

In keeping with recent investigations [12, 13], by using an immunohistochemical analysis, we found the lack of expression of CDX2 in a minority of patients with stage II CC and observed a strong association between this feature and MSI-H status. CDX2-negative compared with CDX2-positive cases showed a worse 5-year OS though the difference was not statistically significant. MSI-H status, whose frequency was comparable to that previously reported for stage II [9, 34–36], did not apparently reduce the risk of recurrence and death in our study.

In conclusion, to date, we know that, in stage I CC, nothing is expected but for the tumor's resection; in stage III, adjuvant therapy should always follow surgery and, in stage II, pathological factors should be considered to establish the subjective risk of disease recurrence and decide further treatments. Nevertheless, so far, we cannot point with certainty to the choice of these factors to be made, particularly whether the use of chemotherapy should be just based on morphological pathologic parameters or on further immunohistochemical features. In this context, our work, despite the limited sample size, supports the role of TB in defining risk groups of the stage II CC. However, confirmatory studies as well as prospective trials drawn with new selection criteria to deliver adjuvant chemotherapy in this particular setting of patients are awaited.

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Compliance with ethical standards

The study was conducted in accordance with the Declaration of Helsinki and the protocol approved by the institutional (Sapienza University) ethical committee (N.3874_2015/22.10.2015).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, Kerr DJ (2007) QUASAR adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 370(9604):2020–2029
2. Benson AB, Robert CH, Venook AP, Cederquist L, Chan E, V.-I. Cancer Center Yi-Jen Chen, H.S. Cooper, A. Fichera, J.L. Grem, P. Buffett Cancer Center, A. Grothey, H.S. Hochster, S. Hunt, A. Kamel, S. Krishnamurthi, M.F. Mulcahy, J.D. Murphy, S. Nurkin,

L. Saltz, D. Shibata, J.M. Skibber, C.T. Sofocleous, E.M. Stoffel, E. Stotsky-Himelfarb, C.G. Willett, C.S. Wu, N. Deborah Freedman-Cass, K.M. Gregory (2017) NCCN Guidelines Version 2.2017 Panel Members Colon Cancer MD/Vice-Chair UCSF Helen Diller Family Comprehensive Cancer Center

3. Labianca R, Nordlinger B, Beretta GD, Mosconi S, Mandala M, Cervantes A, Arnold D (2013) Early colon cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 24(suppl 6):vi64–vi72
4. Hutchins G, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, Richman S, Chambers P, Seymour M, Kerr D, Gray R, Quirke P (2011) Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol 29(10):1261–1270
5. Thomas GD, Dixon MF, Smeeton NC, Williams NS (1983) Observer variation in the histological grading of rectal carcinoma. J Clin Pathol 36(4):385–391
6. Ueno H, Murphy J, Jass JR, Mochizuki H, Talbot IC (2002) Tumour 'budding' as an index to estimate the potential of aggressiveness in rectal cancer. Histopathology. 40:127–132
7. Ueno H, Mochizuki H, Hashiguchi Y, Shimazaki H, Aida S, Hase K, Matsukuma S, Kanai T, Kurihara H, Ozawa K, Yoshimura K, Bekku S (2004) Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology. 127:385–394
8. Ueno H, Kajiwara Y, Shimazaki H, Shinto E, Hashiguchi Y, Nakanishi K, Maekawa K, Katsurada Y, Nakamura T, Mochizuki H, Yamamoto J, Hase K (2012) New criteria for histologic grading of colorectal cancer. Am J Surg Pathol 36:193–201
9. Klingbiel D, Saridaki Z, Roth AD, Bosman FT, Delorenzi M, Tejpar S (2015) Prognosis of stage II and III colon cancer treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: results of the PETACC-3 trial. Ann Oncol 26(1):126–132
10. Bertagnolli MM, Redston M, Compton CC, Niedzwiecki D, Mayer RJ, Goldberg RM, Colacchio TA, Saltz LB, Warren RS (2011) Microsatellite instability and loss of heterozygosity at chromosomal location 18q: prospective evaluation of biomarkers for stages II and III colon cancer—a study of CALGB 9581 and 89803. J Clin Oncol 29(23):3153–3162
11. Romiti A, Rulli E, Pilozzi E, Gerardi C, Roberto M, Legramandi L, Falcone R, Pacchetti I, Marchetti P, Floriani I (2016) Exploring the prognostic role of microsatellite instability in patients with stage II colorectal cancer: a systematic review and meta-analysis. Clin Colorectal Cancer
12. Dalerba P, Sahoo D, Paik S, Guo X, Yothers G, Song N, Wilcox-Fogel N, Forgó E, Rajendran PS, Miranda SP, Hisamori S, Hutchison J, Kalisky T, Qian D, Wolmark N, Fisher GA, van de Rijn M, Clarke MF (2016) CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med 374(3):211–222
13. Pilati C, Taieb J, Balogoun R, Marisa L, de Reyniès A, Laurent-Puig P (2017) CDX2 prognostic value in stage II/III resected colon cancer is related to CMS classification. Ann Oncol 28(5):1032–1035
14. Tomasello G, Barni, S, Turati L, Ghidini M, Pezzica E, Passalacqua R, Petrelli F (2018) Association of CDX2 expression with survival in early colorectal cancer: a systematic review and meta-analysis
15. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, Fléjou JF, Hansen TP, Hartmann A, Kakar S, Langner C, Nagtegaal I, Puppa G, Riddell R, Ristimäki A, Sheahan K, Smyrk T, Sugihara K, Terris B, Ueno H, Vieth M, Zlobec I, Quirke P (2017) Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol 30:1299–1311
16. Betge J, Kornprat P, Pollheimer MJ, Lindtner RA, Schlemmer A, Rehak P, Vieth M, Langner C (2012) Tumor budding is an independent predictor of outcome in AJCC/UICC stage II colorectal cancer. Ann Surg Oncol 19(12):3706–3712
17. Kinoshita O, Kishimoto M, Murayama Y, Yasukawa S, Konishi E, Otsuji E, Yanagisawa A (2015) Poorly differentiated clusters with larger extents have a greater impact on survival: a semi-quantitative pathological evaluation for 239 patients with non-mucinous pT2-3 colorectal carcinoma. World J Surg Oncol 13:140
18. Kinoshita O, Kishimoto M, Murayama Y, Kuriu Y, Nakanishi M, Sakakura C, Otsuji E, Yanagisawa A (2016) The number of metastatic lymph nodes exhibiting poorly differentiated clusters predicts survival in patients with pStage III colorectal cancer. Int J Color Dis 31:283–290
19. Pilozzi E, Maresca C, Duranti E, Giustiniani MC, Catalanotto C, Lucarelli M, Cogoni C, Ferri M, Ruco L, Zardo G (2015) Left-sided early-onset vs late-onset colorectal carcinoma: histologic, clinical, and molecular differences. Am J Clin Pathol 143(3):374–384
20. Punt CJA, Buyse M, Kohne C-H, Hohenberger P, Labianca R, Schmoll HJ, Pahlman L, Sobrero A, Douillard J-Y (2007) Endpoints in adjuvant treatment trials: a systematic review of the literature in colon cancer and proposed definitions for future trials. JNCI J Natl Cancer Inst 99(13):998–1003
21. Gray RG, Quirke P, Handley K, Lopatin M, Magill L, Baehner FL, Beaumont C, Clark-Langone KM, Yoshizawa CN, Lee M, Watson D, Shak S, Kerr DJ (2011) Validation study of a quantitative multigene reverse transcriptase-polymerase chain reaction assay for assessment of recurrence risk in patients with stage II colon cancer. J Clin Oncol 29(35):4611–4619
22. Merkel S, Wein K, Günther T, Papadopoulos, Hohenberger W, Hermanek P (2001) High-risk groups of patients with stage II colon carcinoma. Cancer. 92:1435–1443
23. Morris M, Platell C, de Boer B, McCaul K, Iacopetta B (2006) Population-based study of prognostic factors in stage II colonic cancer. Br J Surg 93(7):866–871
24. Compton CC, Fielding LP, Burgart LJ, Conley B, Cooper HS, Hamilton SR, Hammond ME, Henson DE, Hutter RV, Nagle RB, Nielsen ML, Sargent DJ, Taylor CR, Welton M, Willett C (2000) Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 124(7):979–994
25. Rosty C, Williamson EJ, Clendenning M, Walters RJ, Win AK, Jenkins MA, Hopper JL, Winship IM, Southey MC, Giles GG, English DR, Buchanan DD (2014) Should the grading of colorectal adenocarcinoma include microsatellite instability status? Hum Pathol 45(10):2077–2084
26. Tanaka M, Hashiguchi Y, Ueno H, Hase K, Mochizuki H (2003) Tumor budding at the invasive margin can predict patients at high risk of recurrence after curative surgery for stage II, T3 colon cancer. Dis Colon Rectum 46:1054–1059
27. De Smedt L, Palmans S, Sagaert X (2016) Tumour budding in colorectal cancer: what do we know and what can we do? Virchows Arch 468(4):397–408
28. Ueno H, Hashiguchi Y, Kajiwara Y, Shinto E, Shimazaki H, Kurihara H, Mochizuki H, Hase K (2010) Proposed objective criteria for "grade 3" in early invasive colorectal cancer. Am J Clin Pathol 134(2):312–322
29. Barresi V, Reggiani Bonetti L, Ieni A, Caruso RA, Tuccari G (2017) Poorly differentiated clusters: clinical impact in colorectal cancer. Clin Colorectal Cancer 16(1):9–15
30. Ueno H, Konishi T, Ishikawa Y, Shimazaki H, Ueno M, Aosasa S, Saiura A, Hase K, Yamamoto J (2014) Histologic categorization of fibrotic cancer stroma in the primary tumor is an independent prognostic index in resectable colorectal liver metastasis. Am J Surg Pathol 38(10):1380–1386
31. van Wyk HC, Park J, Roxburgh C, Horgan P, Foulis A, McMillan DC (2015) The role of tumour budding in predicting survival in patients with primary operable colorectal cancer: a systematic review

32. Wang LM, Kevans D, Mulcahy H, O'Sullivan J, Fennelly D, Hyland J, O'Donoghue D, Sheahan K (2009) Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. Am J Surg Pathol 33:134–141
33. Lee VWK, Chan KF (2018) Tumor budding and poorly-differentiated cluster in prognostication in stage II colon cancer. Pathol Res Pract 214(3):402–407
34. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, Gallinger S (2003) Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 349(3): 247–257
35. Sinicrope FA, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, Yothers G, Allegra C, Moore MJ, Gallinger S, Sargent DJ (2011) DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. J Natl Cancer Inst 103(11):863–875
36. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, French AJ, Kabat B, Foster NR, Torri V, Ribic C, Grothey A, Moore M, Zaniboni A, Seitz J-F, Sinicrope F, Gallinger S (2010) Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 28(20):3219–3226