https://doi.org/10.33878/2073-7556-2021-20-4-22-33

New morphological risk factors for metastasis to regional lymph nodes in rectal cancer with invasion into the submucosa

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ABSTRACT *AIM: to assess prognostic significance of pathologic features of T1 rectal carcinoma in relation to regional lymph nodes involvement (N+).*

MATERIAL AND METHODS: removed specimens (n = 66) after rectal resection for carcinoma pT1 were studied. Following prognosticators were evaluated: depth of submucosal invasion, grade of differentiation, lymphovascular invasion (LVI), tumor budding (Bd), poorly differentiated clusters (PDC) of tumor and rupture of cancer glands (CGR).

RESULTS: lymph nodes metastases were found in 13 (19.7%) specimens. LVI was associated lymphatic spread in great possibility OR 38.0 95% CI 2.1-670 (p < 0.0001). Tumor budding of high grade (Bd3) OR 6.2 95% CI 1.2-31 (p < 0.0001) and poorly differentiated clusters (p = 0,03) also increased risk of lymph node metastases. Depth of submucosal invasion, grade of differentiation, and rupture of cancer glands failed to demonstrate significant association with N+. Logistic regression analysis allowed to determine LVI as independent prognostic factor of lymph node tumor involvement.

CONCLUSION: lymphovascular invasion, tumor budding and poorly differentiated clusters of tumor are the risk factors of T1 rectal carcinoma lymph node metastases.

KEYWORDS: *rectal adenocarcinoma T1, lymph node metastases, morphological predictors of metastasis (lymphovascular invasion, tumor budding, poorly differentiated clusters, rupture of tumor glands)*

CONFLICT OF INTEREST: *The authors declare no conflict of interest*

FOR CITATION: Maynovskaya O.A., Rybakov E.G., Chernyshov S.V., Achkasov S.I. New morphological risk factors for metastasis to regional lymph nodes in rectal cancer with invasion into the submucosa. *Koloproktologia.* 2021;20(4):22–33. (in Russ.). https://doi.org/10.33878/2073-7556-2021-20-4-22-33

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Received — 09.09.2021 *Revised — 11.10.2021* *Accepted for publication — 30.11.2021*

INTRODUCTION

Surgery for rectal adenocarcinoma with invasion limited by the submucosal layer (T1) currently varies from local excision to radical surgery.

The potential risk of metastases to the lymph nodes in T1 tumors, according to various trials, ranges from 6.3% [1] to 17% [2], which is confirmed by the worst results of local excision in relation to local recurrence (~10%), compared with curative surgery (~3%) [3]. Preoperative detection of rectal cancer metastases in regional lymph nodes is not accurate enough: sensitivity 53% and specificity 77% with endorectal ultrasound [4], and 54% and 59% with pelvic MRI, respectively [5].

Recently, the main predictors of lymph nodes involvement in CRC T1, used in scientific research and practical work, are the morphological features of the tumor, determined after its local excision.

According to the guidelines of the European Association for Endoscopic Surgery (EAES) for local excision of early rectal cancer, histological features associated with a high risk of metastases to lymph nodes are: poor tumor differentiation, lymphovascular and venous invasion and foci of dedifferentiation [6]. In the latest version of the NCCN recommendations [7], a high

risk of lymph node lesion is due to a tumor size exceeding 3 cm, deep invasion of the submucosa (sm3), lymphovascular invasion (LVI) and poor tumor differentiation (G3).

The most unequivocal opinion on this risk factor has developed in relation to poor differentiation of tumor and mucous forms of colorectal cancer (CRC), which are currently a contraindication for local excision of the tumor [2,8–10].

The depth of tumor invasion into the submucosal considered as one of the main predictors of metastatic disease in CRC T1. For practical use the Kikuchi classifications [11] for sessile and flat neoplasms and the Haggitt classification, for neoplasms on the pedicle are recommended [12]. According to these classifications, the depth of invasion into the submucosal layer is determined. According to most authors, it directly affects the incidence of lymph node involvement, up to 20% when the tumor involves the submucosal layer [1–4,8,9]. A lymphovascular invasion is associated with the depth of tumor invasion into the submucosal layer (Fig.1), which increases the chances of regional lymph nodes lesion by 4–6 times [8,9].

Since the use of existing morphological features of the tumor and their use in practice has limitations, in recent decades, new predictors of the tumor metastatic potential have been worked out, allowing more accurately selecting patients with a low risk of lymph node involvement, who can avoid major radical surgery and possible complications. The existing practice of selecting patients for subsequent salvage surgery, in accordance with the JSCCR criteria included in a number of clinical guidelines, is not always justified, and, apparently, it is necessary to create a certain algorithm for using morphological risk factors taking into account clinical data for the selection of patients with high and low risk of metastases.

Several histological features of the tumor proposed as additional predictors, which are believed to reflect its biological aggressiveness and metastatic potential.

One of these most well-studied features is tumor budding (Bd), which is a phenomenon of single tumor cells or small (⩽ 4 cells) clusters in the area of the invasive tumor front (Fig. 2), which is recognized as an independent unfavorable prognostic marker for any (T1–4) primary tumor [13]. This phenomenon is considered as a histological manifestation of impaired adhesion and differentiation of epithelial cells, as well as epithelial-mesenchymal transition (EMT). Currently, the methodology for determining and calculating Bd has been standardized and validated by the International Tumor Budding Consensus Conference, which makes it possible to widely apply this parameter in routine practice and scientific research [14].

It should be noted that the determination of the degree of differentiation of the tumor, in accordance with the accepted criteria (WHO), is carried out without taking into account less differentiated tumor structures, mainly located in the area of tumor invasion. Ueno, H. et al. described such structures as poorly differentiated clusters (PDC) consisting of 5 or more tumor cells that do not form glandular structures (Fig. 3).

According to the results of the studies, PDC, largely than the degree of glandular differentiation, is an indicator of the biological aggressiveness of the tumor in CRC [15].

Another potential predictor of metastases to regional lymph nodes in T1 CRC is a recently proposed histological feature in the form of rupture of tumor glands (cancer gland rupture — CGR), which is a violation of the integrity and continuity of the epithelial lining of tumor glands located along the invasive tumor front (Fig. 4). This histological feature is proposed to improve the selection of patients with a high

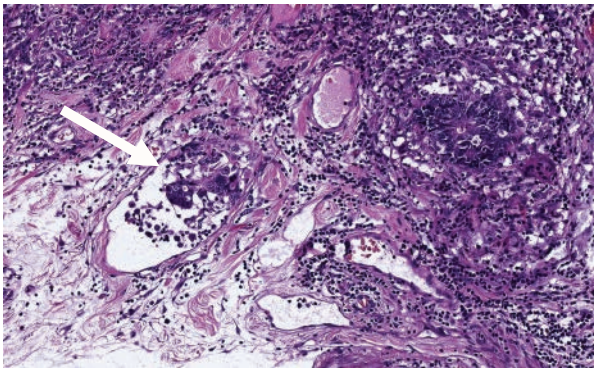

Figure 1. *Lymphovascular invasion: tumor cells in the lumen of the vessel (arrow). Staining with hematoxylin and eosin ×100*

risk of lymph node metastases after endoscopic removal of early CRC [16].

Despite the fact that there is a group of validated and recommended predictors for practical use to assess the risk of metastases in CRC T1, the results of their use are quite contradictory and ambiguous. Many researchers emphasize the need for further investigation of existing features and the search for new objective predictors of metastatic disease.

In this regard, the aim of the study was to evaluate the prognostic value of the main unfavorable predictors and their correlation with to regional lymph nodes metastases in rectal cancer pT1.

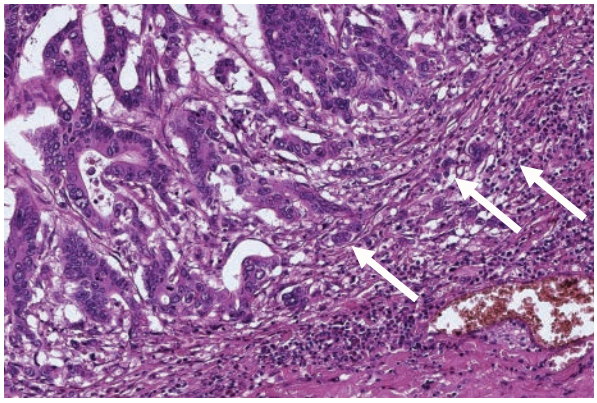

Figure 2A. *Tumor "budding": A — Bd1 (single isolated cells along the invasive edge of the tumor — arrows). Staining with hematoxylin and eosin. ×200*

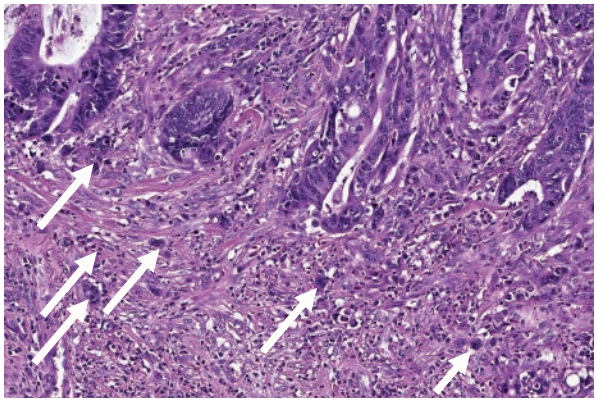

Figure 2Б. *Tumor "budding": Б — Bd2 (isolated cells along the invasive tumor edge — arrows). Staining with hematoxylin and eosin. ×200*

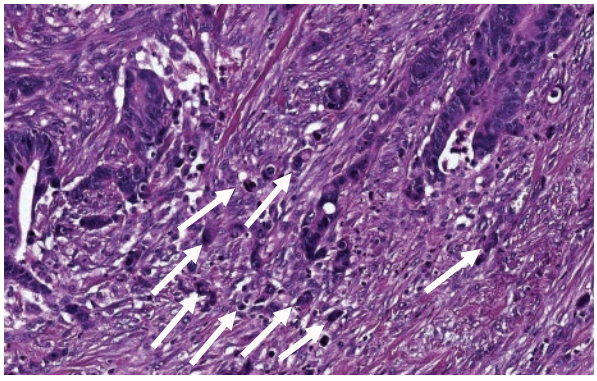

Figure 2B. *Tumor "budding": B — Bd3 (multiple isolated cells along the invasive edge of the tumor — arrows). Staining with hematoxylin and eosin. ×200*

MATERIAL AND METHODS

The material for the single-center study was rectal specimens removed during radical procedures for cancer in 2016–2020. The selection criterion was the presence of rectal adenocarcinoma with invasion limited to the submucosal layer. It should be noted that in 10 cases, radical surgery was performed after local excision of the rectal tumor: in 9 patients with transanal endomicrosurgery and in one case by endoscopic submucosal dissection. The decision to perform radical 'salvage surgery' in these patients was made after an oncological MDT based on the available clinical guidelines [18] and the

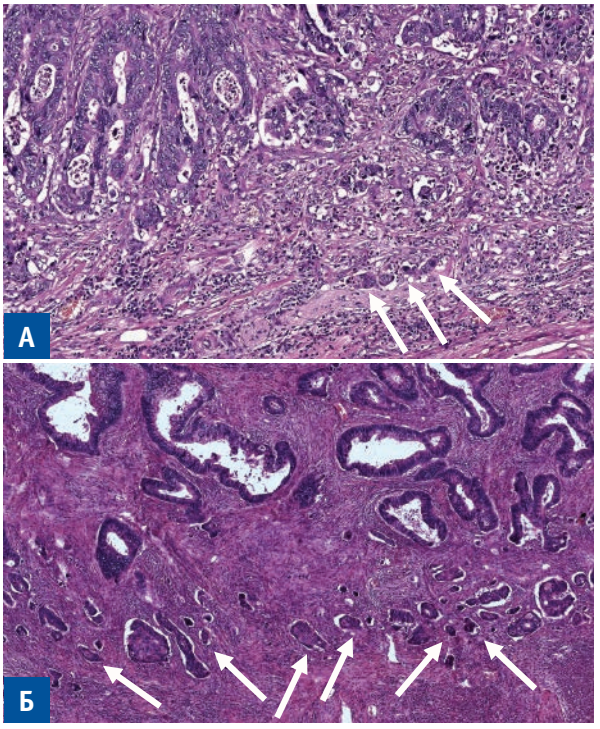

Figure 3. *Poorly differentiated clusters along the invasive edge of the tumor (arrows): A — PDC1 (single); Б — PDC3 (more than 10). Staining with hematoxylin and eosin. ×100*

patients' preferences. In the other cases, the surgery with total or partial mesorectal excision was the primary method of treatment. The study did not include patients who underwent neoadjuvant therapy, patients with distant metastases and other histological variants of the tumor. The clinical and morphological characteristics of the study material are presented in Table 1.

The rectal specimens were fixed in a 10% solution of neutral formalin for 48 hours, after which they were examined on serial cross-sections. After local excision, the specimens were stretched on a plate and fixed in a 10% neutral formalin solution for 12 hours, after which they were cut into parallel slices of 3 mm thick with the margins of the resection marked. All the removed tumors were studied totally. The histological processing of the tumor tissue was carried out according to a generally accepted technique in a Leica ASP 6025 histoprocessor; then it was poured into a paraplast; 3 microns thick slices were cut, which were stained with hematoxylin and eosin.

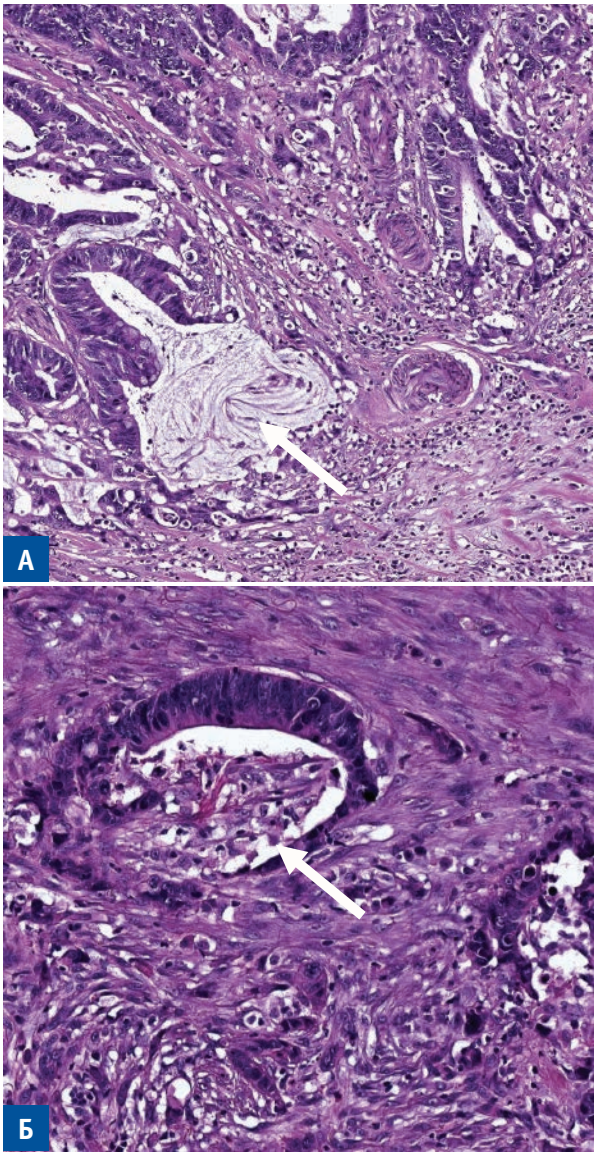

Figure 4. *Rupture of tumor glands — CGR1: А — rupture of the gland with accumulation of mucus; Б — rupture of the gland with accumulation of detritus (arrows). Staining with hematoxylin and eosin. ×200*

The obtained tumor slices were examined in a light microscope to assess the main histological parameters. The morphometric studies were performed on the digital images of the tumor slices obtained by scanning with an x20 magnification.

For more accurate determination of lymphovascular invasion, Bd and PDC, the selected tumor slices were additionally stained by immunohistochemical method in the Ventana Bench Mark Ultra immunohistosteiner, using the Ultra View Universal DAB Detection Kit (Ventana — Roche Diagnostics) with antibodies to SC8/18 (clone B22.1&B23.1, Roche Diagnostics), CD31 (clone JC70, Cell Marque, dilution 1:100), in accordance with the recommended protocols.

To assess the depth of tumor invasion into the submucosal layer, the Kikuchi subclassification was used (sm1 — invasion to a depth of 0.2–0.3 mm, sm2 — invasion to 2/3 of the submucosal layer and sm3 — invasion to the entire thickness of the submucosal layer) for flat neoplasms [11] and the Haggitt classification for polypoid tumors on the pedicle (level 1–4: level 1 — invasion into the 'head' of the polyp; level 2 — tumor germination to the border with unchanged mucosa; level 3 — invasion into the 'leg' of the polyp, level 4 — invasion into the submucosal layer of the intestinal wall) [12].

The differentiation and the degree of malignancy of the tumor (G) were determined in accordance with the criteria of the WHO classification of gastrointestinal tumors (5th ed., 2019) [19]. Tumor staging was carried out in accordance with the TNM classification (7th ed.) [20].

Tumor budding (Bd) was assessed by the invasive edge of the tumor in accordance with the

Table 1. *Clinical and pathomorphological characteristics of patients and the frequency of lesions of regional lymph nodes*

	***n* (%)**	**N1–2 (%)**	***P***
N patients	**66 (100.0%)**	**13 (19.7%)**	**–**
Number of patients with tumor T1N1-2	13 (19.7%)	–	–
Gender			
Male	28 (42.4%)	5 (7.6%)	1.0
Female	38 (57.6%)	8 (12.1%)	
Age (years)	62.0 (22–80)		
< 62 years	32 (51.5%)	6 (9.1%)	1.0
⩾ 62 years	34 (54.5%)	7 (10.6%)	
Distance from the anal verge, cm			
0–6	18 (27.3%)	4 (6.1%)	0.68
7–12	26 (39.4%)	6 (9.1%)	
13–15	22 (33.3%)	3 (4.5%)	
Surgery			
Anterior rectal resection	21 (31.8%)	4 (6.1%)	0.8
Anterior rectal resection with total mesorectal excision	32 (48.5%)	6 (9.1%)	
Intrasphincteric resection	6 (9.1%)	2 (3.0%)	
Abdominoperineal resection	1 (1.5%)	1 (1.5%)	
Coloprotectomy + TME*	6 (9.1%)	–	
pT1			
sm1	15 (22.7%)	3 (4.5%)	1.0
sm2-3	51 (77.3%)	10 (15.2%)	
Lymph nodes in the specimen 23 ± 9.8			
< 23	34 (54.5%)	5 (7.6%)	0.36
⩾ 23	32 (51.5%)	8 (12.1%)	
Tumor differentiation (G)			
G1-2	63 (95.5%)	11 (16.7%)	0.09
G3	3 (4.5%)	2 (3.0%)	
Macroscopic form			
plaque-shaped or flat-raised	20 (30.3%)	3 (4.5%)	0.73
exophytic	46 (69.7%)	10 (15.2%)	
Lymphovascular invasion (LVI)			
LVI+	35 (53.0%)	13 (19.7%)	< 0.0001
LVI–	31 (47.0%)	0	
Tumor Budding (Bd)			
Bd1 (0–4)	16 (24.2%)	1 (1.5%)	0.03
Bd2 (5–9)	14 (21.2%)	1 (1.5%)	
Bd3 (10 or more)	36 (54.5%)	11 (16.7%)	
Poorly differentiated tumor clusters (PDCG)			
PDC1	28 (42.4%)	1 (1.5%)	0.01
PDC2	16 (24.2%)	4 (6.1%)	
PDC3	22 (33.3%)	8 (12.1%)	
Rupture of cancer glands (CGR)			
CGR0	18 (27.3%)	3 (4.5%)	1.0
CGR1	48 (72.7%)	10 (15.2%)	

**in 4 cases, colproctectomy was performed for rectal cancer with inflammatory bowel diseases, in 2cases — against the background of adenomatous polyposis syndrome.*

 # the specimens removed after colproctectomy are excluded from the analysis

recommendations of the ITBCC (2016): the presence of single cells or groups/clusters of cells (up to four cells) along the invasive edge of the tumor at the site of their greatest accumulation (hotspot method) on an area of 0.785 mm^2 (lens ×20). The severity of Bd was assessed using the three-stage JSCCR system [17] included in the recommendations (ITBCC) [14]: 0–4 'budding' — low degree of budding (Bd 1); 5–9 'budding' — medium degree of budding (Bd 2); 10 or more 'budding' — high degree of budding (Bd 3).

Poorly differentiated clusters (PDC) were determined in accordance with the criteria of Ueno, H.: clusters of ⩾ 5 tumor cells without glandular structures. To assess the presence of PDC, the entire tumor, including the invasive margin, was examined on the slices stained with hematoxylin and eosin at low magnification of the microscope. After determining the area with the largest number of PDC (hotspot method), a quantitative calculation was performed with an ×20 magnification. Tumors with the number of clusters < 5, from 5 to 9 and > 10 were classified as G1, G2 and G3, respectively [15].

Tumor gland ruptures (cancer gland rupture — CGR) were evaluated by histology of the slices stained with hematoxylin and eosin (H&E), including tumor sites with the greatest depth of invasion.

The presence of CGR was defined as the focal or partial absence of epithelial cells that make up the cancerous gland located along the invasive edge of the tumor (with C-shaped structures with flattening and dissociation of cells), regardless of the concomitant inflammatory or stromal reaction, as well as mucus accumulation or the presence of an abscess. A case with the presence of at least one glandular structure corresponding to these criteria was considered CGR-positive [16].

Lymphovascular invasion was determined in the presence of tumor cells in the lumen of small vessels limited by the endothelial layer [21].

Statistical Analysis

The clinical and morphological characteristics of the patients and removed specimens were entered into the database on the EXCEL for Windows platform. The normality of the distribution was checked using the Kolmogorov method. Continuous variables with non-Gaussian distribution were described by median and amplitude.

The medians were compared using the Mann-Whitney test. The variation series with Gaussian distribution was characterized by the mean and standard deviation. The mean values were compared using an unpaired t-test. Categorical variables were compared using the χ^2 test (more than two degrees of freedom), binary variables were compared using the Fisher's exact test. The odds ratio (OR) at 95% coincidence interval (95% CI) was calculated for risk factors in a univariate analysis. The value of $p < 0.05$ was considered significant. The significant risk factors were included in the logistic regression in order to identify an independent predictor of lymph node metastases. The statistical analysis was performed using software SPSS 22.0 (Chicago, Ill.) and GraphPadPrism 6.0 (LaJolla, CA).

RESULTS

The study included 66 rectal specimens removed during radical procedures for cancer with morphologically verified adenocarcinoma pT1 (Table 1). The mean number of examined lymph nodes relevant to the rectum was 23.0±9.8. During the morphology, metastases in pararectal lymph nodes were detected in 13 (19.7%) cases. At the same time, there was one affected node (N1a) in 5 (7.5%) specimens: T1sm1N1a $n = 2$, T1sm3N1a $n = 3$; in 4 (6.1%) specimens, 2–3 metastases in lymph nodes (N1b) were detected at the depth of invasion of T1sm3. In two (3.0%) cases, 5 affected lymph nodes (N2a) were found — pT1sm2N2a and pT1sm3N2a. In two cases, with the depth of invasion of the tumor pT1sm2 and pT1sm3, metastases were found in 7 and 13 lymph nodes (N2a), respectively. Despite the fact that metastases to mesorectal lymph nodes were detected with deep invasion of the tumor into the submucosal layer 3 times more often (4.5% at T1sm1 vs 15.2% at T1sm2-3), these differences are not significant ($p = 1.0$). Also, the differences ($p = 0.73$) in the rate of lymph node

lesions in high grade adenocarcinoma (G3 — 2 cases 3%) and low grade (G1-G2 — 11 cases 16.7%) are not significant.

With a high degree of reliability in the presence of lymphovascular invasion (Fig. 1), were detected metastases in the lymph nodes of mesorectum: OR 38.0 95%, CI 2.1–670 ($p < 0.0001$).

The tumor budding of high degree — Bd3 (Fig. 2) was significantly more often detected in the tumors with metastases in the mesorectal lymph nodes: OR 6.2, CI 1.2–31 ($p < 0.0001$).

Poorly differentiated tumor clusters (PDC) (Fig. 3) were also significantly associated with metastases in mesorectal lymph nodes ($p = 0.03$). Noteworthy is the fact that when combining the degrees of PDC, the differences, unlike tumor budding, were identical: PDC G1 vs. PDC G2-3, OR 4.5 CI 1.2–16 ($p = 0.02$), PDC G1-2 vs. PDC G3, OR 4.5 CI 1.2–16 ($p = 0.02$) (Fig. 3).

The presence of the cancer glands rupture CGR (Fig. 4) was detected in most of the cases (10 of 13) with metastases in the lymph nodes, but no reliable correlation with the rate of metastases of rectal cancer T1 in the lymph nodes of mesorectum was obtained ($p = 1.0$).

In a logistic regression model, the only independent risk factor for metastasis to regional lymph nodes was lymphovascular invasion (LVI) — $p < 0.0001$.

DISCUSSION

The development of endoscopic technologies allows for organ-preserving treatment in patients with CRC T1. For rectal tumors, the problem of organ-preserving treatment is particularly relevant, which is associated with the inevitable negative consequences of radical procedures: low anterior resection syndrome, genitourinary disorders, temporary or permanent colostomy.

The main problem after local excision of the tumor remains the assessment of the condition and probability of regional lymph nodes lesions to determine the indications for additional surgery in a particular patient. To solve this problem, an active search is being done for morphological risk factors for metastases, which make it possible to identify tumors with a high and low risk of metastatic disease.

Recently, the main predictors of lymph nodes metastases recommended for practical use are the depth of tumor invasion into the submucosal layer, the degree of differentiation (including special forms — signet-ring cell and mucinous adenocarcinoma), the presence of lymphovascular invasion, and tumor budding Bd. Additional surgery is recommended in the presence of one or more unfavorable morphological predictors of a high risk of metastatic disease, detected during histology of a tumor removed locally [6,7,22].

However, despite a large number of studies on these morphological predictors, their prognostic value is still ambiguous, due to the problem of reproducibility and the assessment methods used, as well as the level of sensitivity and specificity of each. In addition, most studies are devoted to the search of these predictors in groups of patients with CRC, while studies on the risk factors for metastases in early rectal cancer have been done much less.

In this study, an assessment of the main predictors of metastatic disease used and new poorly studied morphological features for the risk of metastases in rectal adenocarcinoma T1 was carried out.

Of the selected 66 cases of rectal adenocarcinoma pT1, metastases were detected in 13 (19.7%) cases, which is comparable with the rate of metastatic disease according to the previous studies [23,27,29]. It should be noted that lymph node metastases were detected in most cases with deep invasion into the submucosal layer of sm2-3 — 10 (15.2%) versus sm1-3 (4.5%). However, this difference had no significance ($p = 1.0$). The depth of invasion into the submucosal layer remains one of the main practice parameters, determining the risk of lymph node metastases. However, in a number of studies, the prognostic value of this feature is interpreted ambiguously [8,17,26–28]. Moreover, the exact threshold value of the depth of invasion, the so-called 'N0 Threshold' for early rectal cancer, which determines the risk of metastases, has not been determined now. The values given in a wide range from 200 to 1500 microns

[6]. The most commonly used threshold value of the depth of invasion is 1,000 microns (1 mm), exceeding which significantly increases the risk of metastases (relative risk 5.2 at 95% CI 1.8–15.4). This parameter showed high sensitivity (96.7%), but low specificity (24.1%), which can lead to a large number of patients with exceeded indications for salvage surgery after local excision of the primary tumor [23].

The degree of tumor differentiation was not significantly associated with the rate of metastatic disease (p = 0.73), which may be due to a small number of cases of G3 adenocarcinoma (n = 2) in the group with metastases. A small number of adenocarcinomas G3 has the following explanation: the routine practice in the Center includes pre-op histological confirmation of a rectal tumor. Cases of suspected malignancy in the polyp, which, with the development of special endoscopic methods (high-resolution endoscopy, chromoendoscopy, examination in the spectrum close to infrared), has been the subject of discussion in recent years. The presence of histology after biopsy leads to the exclusion of patients with low-grade and mucus-producing tumors, which led to the selection of mainly G1-2 adenocarcinoma for the study.

It should be noted that in most cases, CRC has the structure of a high or moderate differentiated adenocarcinoma, and only in 5–10% of cases there is a low differentiation of adenocarcinoma or undifferentiated cancer [6]. The unfavorable prognostic value of high grade adenocarcinoma, including mucinous and cricoid cell cancers, is well known, especially when the tumor is localized in the rectum. However, given the rate of their occurrence, this feature can be used only in a small number of cases of CRC T1.

In addition, when analyzing and comparing the data of the studies, one should take into account the fact that there are different approaches to determining high grade adenocarcinoma: by the least differentiated component of the tumor, regardless of its volume, which is recommended for assessing local excision, and by the predominant component in the tumor after radical surgery [26,27]. There are also differences between the WHO classification and the JSCCR: according to the JSCCR criteria, G2-G3 adenocarcinomas are included in the high grade category, i.e. moderate and low-grade adenocarcinoma, and according to the WHO criteria, adenocarcinoma G2 refers to a low grade tumor [19,22]. Despite the fact that low tumor differentiation is cited in many studies as a significant risk factor for metastases, at the same time, there is a low reproducibility of this feature among pathologists and the need to develop more objective criteria for its assessment [15,23,26,27].

Such features like Bd and PDC showed a significant correlation with metastases to mesorectal lymph nodes (p = 0.03 and p = 0.01, respectively). According to the data obtained, Bd2 and Bd3 are associated with a high risk of lymph node metastases in early CRC [6,8,9,13,14,30]. In the study, we obtained a significant prognostic value only for the Bd3 (high grade), which was determined in the majority of cases — 11 (16.7%), with metastases to lymph nodes, which does not contradict the available published data. The absence of a prognostic value of Bd2 in the studied group may be due to a small number of cases.

For PDC, a significant correlation with lymph node metastases was observed both when using a three-stage PDC1, PDC2, PDC3 assessment, and regardless of the quantitative value/Grade. Such results are consistent with the data of the previous studies. Despite the fact that the determination of the number of PDC in the proposed by Ueno H. system, by analogy with Bd, should be carried out according to the three-stage PDC1-3 system, in a large number of the studies devoted to the study of this feature, a binary (yes/no) evaluation system was used (especially for local excision), which also showed that the presence of PDC, regardless of their number, is a predictor of metastases to lymph nodes [12,25,26]. It should be noted that the morphological assessment of the PDC demonstrated a fairly high rate of coincidences and low variability between pathologists with the values of the coefficient k (interobserver variability /agreement — kappa statistics) equal to 0.51 (Ueno, 2014) — 0.82 (Konishi, 2018) [25].

The phenomenon of rupture of tumor glands — CGR is a new histological feature proposed as a potential risk factor for lymph nodes metastases in CRC T1.

The first study (Oishi et al., 2020) showed that CGR has a predictive value with high sensitivity (100%), but low specificity (25%) and is closely related to the depth of tumor invasion into the submucosal layer ($p < 0.001$). The method proposed by the authors for assessing this is simple and well reproducible in tumor slices stained with hematoxylin and eosin (coefficient k with values 0.61–0.80) [16].

In the study, we found this feature in 10 out of 13 cases with lymph node metastases (76.9%), but we did not get a significant association of CGR with the rate of metastatic disease ($p = 1.0$), which is most likely due to the insufficient statistical power of the study. Nevertheless, the differences obtained, albeit statistically unreliable, indicate the need for further study.

Lymphovascular invasion is a universal unfavorable prognostic feature for cancer of any site and prevalence. Recently, a vascular (lymphovascular and venous) invasion is considered an important prognostic feature that affects the determination of treatment approach in patients with CRC stage I-II.

The results of the study showed a high degree of reliability in detecting metastases in regional lymph nodes in the presence of lymphovascular invasion (LVI): OR 38.0 95% CI 2.1–670 ($p < 0.0001$).

In the logistic regression model, lymphovascular invasion (LVI) turned out to be the only independent risk factor for metastases to regional lymph nodes — $p < 0.0001$. It can be stated that in the absence of LVI, metastatic lymph node lesion was not detected (pN0). The strong prognostic value of LVI has been determined in a large number of studies that have shown that invasion of lymphatic vessels is the most significant predictor of metastases to lymph nodes [8–10,21–23,27]. At the same time, it is noted that the diagnosis of lymphovascular invasion is associated with a large variability of results among pathologists, demonstrating low values of the coefficient $k = 0.28–0.30$, the value of which improved somewhat when using an additional immunohistochemical method for detecting vessels with a panendothelic marker CD31 and a marker of lymphatic vessels D2-40 [23,27].

Thus, according to the results of the study, lymphovascular invasion, tumor budding, and the presence of poorly differentiated clusters turned out to be the most significant predictors of metastases in rectal adenocarcinoma T1. Detecting lymphovascular invasion is mandatory in the histology of CRC, especially in early T1 cancer, and reflects the quality of morphology. It should be noted that LVI, despite the difficulties in detecting, was an independent predictor of metastatic lymph node lesion in almost all the previous studies, while the prognostic value of the remaining features was ambiguous.

The ITBCC recommendations (2016) emphasize that Bd is an independent predictor of metastases to regional lymph nodes in CRC T1, the prognostic value of which is equivalent to the degree of differentiation of the tumor, vascular and perineural invasion. Its definition should be included in the overall assessment of the clinical and morphological characteristics of the tumor that determine the treatment approach of patients [14]. However, the introduction of the definition of this feature into practice has identified a number of problems, such as its reproducibility and accuracy of assessment, the role of the immunohistochemical method in the detection of Bd, the study of the biological nature and relationship with PDC. It is assumed that Bd and PDC are associated with EMT and have a similar biology. Therefore, a number of studies have attempted to jointly calculate these parameters as a manifestation of one phenomenon, since the separation of these features by a threshold value of 5 tumor cells (Bd ⩽ 4 cells; PDC ⩾ 5 cells) is quite arbitrary [28,29].

At the same time, taking into account the data of the other studies on the prognostic value of PDC and the results obtained by us, it is necessary to further study this and standardize the assessment methodology. Perhaps, PDC can serve as an additional or alternative Bd feature,

in cases where its assessment is difficult or the 'budding' of the tumor is not detected. Apparently, tumor budding, PDC, and, probably, cancer glands rupture, represent the structures of the so-called poorly differentiated component of the tumor, which is detected mainly by the invasive front, and, in accordance with the existing concept, reflects the process of tumor dedifferentiation and epithelial-mesenchymal transition, being an indicator of its biological aggressiveness.

The results of this and the previous studies indicate a large variability in the prognostic value of the main morphological risk factors for metastases used today. Moreover, the results of meta-analyses and reviews of the main used predictors of lymph node metastasis in CRC T1 have shown that none of the currently used morphological features has sufficient sensitivity and specificity to accurately determine the risk of metastasis and cannot be used independently [23,27–29].

Apparently, it is necessary to search for a set of the most significant predictors of metastases and create an algorithm for its application, which will allow more accurate selection of patients with a high risk of metastases in rectal cancer T1 for subsequent additional treatment.

CONCLUSION

According to the results of the study, lymphovascular invasion (LVI), tumor budding (Bd) and poorly differentiated clusters (PDC) were the most significant predictors of metastases in the rectal adenocarcinoma T1. The obtained results indicate the expediency of including the Bd assessment in the protocol of pathomorphological examination as an additional predictor. It is necessary to further study the prognostic value of PDC in order to standardize the methodology for practical use, as well as to study the prognostic value of the depth of invasion and differentiation of adenocarcinoma in order to create a histological model for more accurate selection of patients with a high risk of metastases of rectal adenocarcinoma T1, who need additional surgery.

AUTHORS CONTRIBUTION

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