Are Width, Length, Depth and Area of Submucosal Invasion Predictive for Lymph Nodes Metastasis in pT1 Colorectal Cancer?

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Research Article

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Abstract

Background and study aim: Colorectal carcinomas limited by submucosa (pT1) remain the challenge in terms of choice for treatment options from local excision to radical surgery. The aim of the study was to evaluate morphometric and morphologic risk factors of regional lymph nodes metastasis (LNM) for pT1 colorectal carcinoma (CRC).

Patients and methods: The histology of patients undergoing oncological resection from 2016 to 2022 was reviewed. Tumor grade (G), budding (Bd), poorly differentiated clusters (PDC), cancer gland rupture (CGR), lymphovascular invasion (LVI) and deep submucosal invasion (DSI) as well as width, length, total area and deep submucosal invasion (DSI) were evaluated as risk factors of LNM.

Results: Two hundred and sixty-four cases of colon and rectal carcinomas with invasion into the submucosal layer (pT1) were identified. Of them LNM was found in 46/264 (17.4%) cases. All morphometric parameters and DSI (p=0.33) failed to demonstrate association with LNM. High grade (G3) (p=0.05), Bd (p=0.056) and PDC (p<0.0001) were associated with LNM. In multivariate analysis LVI+ remains the only significant independent risk factor [OR 15.7; 95% CI 8.5 - 94.9] (p<0.0001).

Conclusion: The DSI invasion of T1 CRC as well as other morphometric parameters of submucosal tumor spread do not have any predictive value in terms of LNM. Poor differentiation of colorectal carcinoma, tumor budding (Bd), poorly differentiated clusters (PDC) and lymphovascular invasion (LVI) are the significant risk factors for LNM in T1 CRC. Among them LVI was the only independent risk factor.

Introduction

Colorectal cancer screening (CRC) programs and advances of medical imaging techniques in terms of tumor staging increased the proportion of patients with tumors limited to submucosal invasion. In turn, the development of endoscopic technologies: transanal endomicrosurgery (TEM) and endoscopic submucosal dissection (ESD) allowed to perform precise local excision of tumors with tumor-free resection margins (R0) in the vast majority of T1 tumors.

Local excision for T1 CRC is almost free from complications related to the radical surgery. On the other hand, this kind of surgery is a mere tumorectomy without control of regional lymphatics and therefore it has a higher risk of recurrence. An assessment of the risk of lymph nodes involvement and the selection of patients for salvage surgery are a major task. A deep invasion of the tumor into the submucosal layer (sm3) is considered as one of the most significant or at least well accepted in routine practice predictors of metastasis to the lymph nodes metastasis (LNM).

According to the guidelines of Japanese Society for Cancer of the Colon and Rectum (JSCCR), European Association for Endoscopic Surgery (EAES) and European Society of Gastrointestinal Endoscopy (ESGE), tumors with a depth invasion of more than 1000 µm have a high risk of metastasis [1–3]. This parameter is recommended to be measured either from the level of the mucosal muscularis propria or from the
tumor surface when it cannot be determined due to removal of the tumor within submucosal layer. Also, the European guidelines recommend assessing the depth of submucosal invasion of T1 CRC using the Kikuchi classification for sessile and flat tumors or Haggitt classification for pedunculated lesions [4–6]. Exceeding 1000 µm threshold or SM3 are current indication for radical surgery.

These recommendations are based on the concept that with deepening of the tumor invasion into submucosa, the risk of regional LNM increases from 2% for T1sm1 to 20–23% for T1sm3 [7–9], which can be explained by expanded lymphatic and vascular invasion, though the association between the depth of carcinoma invasion and lymph node involvement were not confirmed universally [10, 11].

In addition, the Kikuchi and Haggitt subclassifications of T1 colorectal carcinomas can be applied only to the surgical specimens obtained after full-thickness excision, and thus they have limitations in assessing malignant colon polyps/tumors removed within submucosa because of the lack of microanatomical landmarks. On the other hand, “1000 µm rule” is a controversial parameter due to the absence of conventional marks of measurement [12, 13]. In this regard, other morphometric parameters such as the width and area of carcinoma invasion have been proposed for the assessment of tumor metastatic potential [14, 15].

Along with submucosal tumor invasion, other histopathologic factors which determine the aggressiveness of CRC are included into pathologic report. Of them, low tumor differentiation (G3), lymphovascular invasion (LVI) and tumor budding of high score (Bd) are associated with a high risk of LNM [6, 16–18].

It should be noted that LVI is associated with the depth of submucosal invasion of the tumor and the risk of regional LNM increasing by 4–6 times with carcinoma penetration through bowel wall [7–9].

In addition to tumor differentiation the tumor budding (Bd) is now recognized as a high histological manifestation of the epithelial-mesenchymal transition (EMT) which determines a higher metastatic potential of the tumor. In 2016, the method for determining and calculating Bd score was standardized and validated by the International Tumor Budding Consensus Conference (ITBCC), which allowed to use this parameter in routine practice [19, 20].

A less studied feature - tumor aggressiveness was described by Ueno, H. et al. as poorly differentiated clusters (PDC) consisting of 5 or more tumor cells that do not form glandular structures and are located mainly along the invasive edge of the tumor. The author reported PDC as more significant indicator of the malignant potential of CRC than the degree of glandular differentiation. Presence and degree of PDC can be used as a risk factor for LNM like the tumor budding [21–23].

Another possible predictor of regional LNM in T1 CRC is a recently proposed histological sign of cancer gland rupture (CGR), which was defined as focal or partial disappearance of neoplastic epithelial cells constituting the cancer gland at the invasive tumor front. This histological feature has been proposed to
improve the selection of patients with a high risk of lymph node metastasis after endoscopic removal of early CRC [24].

It should be noted that prognostic value of proposed risk factors is controversial. Thus, a number of studies have shown the absence of a prognostic value of the depth of submucosal invasion in T1 CRC and conflicting data on the prognostic value of tumor budding. A further study of established characteristics of tumor aggressiveness as well as a search of new ones are still an important task [10, 18, 23]. This retrospective study was aimed to compare quantitative morphometric parameters of tumor with qualitative risk factors.

Patients And Methods

The data for patients who underwent surgery from 2016 to 2022 for histologically confirmed colorectal adenocarcinoma with invasion limited by a submucosal layer (pT1) were retrospectively collected from the archive of the Ryzhikh National Medical Research Center of Coloproctology, Moscow, Russia. The exclusion criteria were distant metastasis, neo-adjuvant chemotherapy and/or radiotherapy. The study protocol complied with the requirements of the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Ethic Committee.

Sample Preparation and Analysis

The surgical specimens obtained after resection of the large intestine with the tumor were fixed in 10% neutral formalin solution for 24–48 hours, and then the tumor was examined totally on the serial sections. The specimens obtained after ESD or TEM were stretched on a plastic plate and fixed in formalin solution for 12-24 hours. The tissue preparation was in accordance with the standard protocol and performed using Tissue Processor Leica ASP 6025 and then embedded in paraffin blocks. Three-micrometer sections were taken and stained by hematoxylin and eosin.

Morphometric studies were performed on digital images of the tumor sections obtained using an Aperio T2 Leica scanning microscope (magnification, ×20) using a software package for digital image analysis (Aperio Imagescope V12.4.0, Aperio Technologies).

Morphometry of the tumors was performed using a software package for digital image analysis (Imagescope v12.4.0 Aperio Technologies, San Diego, USA) on selected digital images of the tumor sections with the most pronounced tumor invasion. The following parameters were measured according to the method of Toh, E. et al. [14]:

- Maximum width of the tumor, including carcinoma and adenoma component (Fig. 1);
- Maximum width of adenocarcinoma in the tumor (Fig. 1)
- Total area of adenocarcinoma, including intra- and submucosal components in sections with the most pronounced tumor invasion (Fig. 2)
- Length of the invasive adenocarcinoma front (Fig. 3)
The depth of invasion into the submucosal layer in non-polypoid tumors was assessed using the Kikuchi classification [4]:

- sm1 – invasion into upper 1/3 (0.2-0.3 mm) of submucosa
- sm2 – invasion into upper 2/3 of submucosa
- sm3 – invasion into the entire thickness of submucosal layer

The Haggitt classification [5] was used for pedunculated polypoid tumors:

- Level 0 – Carcinoma in situ or intramucosal carcinoma; non-invasive.
- Level 1 – Carcinoma invades muscularis mucosa to submucosa; limited to head of polyp.
- Level 2 – Carcinoma invades neck of polyps.
- Level 3 – Carcinoma invades any part of the stalk.
- Level 4 – Carcinoma invades submucosa of bowel wall, below polyp stalk but above muscularis propria.

Tumor differentiation and grade (G) were determined according to the WHO classification criteria for gastrointestinal tumors (5th edition, 2019) [25]. Tumor staging was performed according to the TNM classification (7th edition) [26].

Tumor budding was defined in accordance with ITBCC as a single tumor cell or a cell cluster of up to 4 tumor cells at the invasive margin of colorectal cancer [19]. The severity of Bd was assessed according to the three-stage JSCCR system [20]. Tumors were classified into 3 grades on the basis of the number of poorly differentiated clusters detected under ×20 objective lens in a field of 0.785 mm$^2$ where they appeared most intensively (hotspot method).

- 0–4 buds—low budding (Bd 1).
- 5–9 buds—intermediate budding (Bd 2).
- 10 or more buds—high budding (Bd 3).

Poorly differentiated clusters (PDC) were defined as cancer clusters in the stroma composed of ≥5 cancer cells which lost an ability to form gland-like structures. PDC were counted along the invasive edge of the tumor after determining the area with the highest number of PDCs (hotspot method) under ×20 objective lens. PDC were classified in accordance with Ueno, H. et al [21]: tumors with <5, 5 to 9 and ≥10 clusters were classified as G1, G2 and G3, respectively.

Lymphovascular invasion was determined if the tumor cells were present in the lumen of small vessels limited by the endothelial layer [27].

With the aim to enhance detection of LVI, Bd and PDC, selected sections of tumors were additionally stained in accordance with the recommended protocols using Ventana BenchMark Ultra.
immunohistostainer and the UltraView Universal DAB Detection Kit imaging system (Ventana - Roche Diagnostics) with antibodies to CK8/18 (clone B22.1 & B23.1, Roche Diagnostics), CD31 (clone JC70, Cell Marque, 1:100 dilution).

Statistics

Clinical and morphological characteristics of selected cases were entered into a database of the EXEL for Windows platform. Comparison of medians and means was performed using the Mann-Whitney U-test and unpaired Student's t-test, respectively. Categorical variables were compared using the Chi-square test (for more than two degrees of freedom) or Fisher's exact test for binary data.

Odds ratio (OR) at 95% confidence interval (95%CI) was calculated for risk factors in the univariate analysis. For continuous values, the ROC analysis was used to determine the cut-off point and area under the curve (AUC). A p<0.05 value was considered statistically significant. Significant risk factors were included in a logistic regression to identify an independent predictor of lymph node metastasis. The statistical analysis was performed using SPSS 22.0 software (Chicago, Ill.) and GraphPad Prism 6.0 (La Jolla, CA).

Results

Two hundred and sixty-four cases of colon and rectal carcinomas with invasion into the submucosal layer (T1) were selected from the Institutional database. Of them, 24 patients had radical operation as a salvage surgery within 4 to 6 weeks after local excision of the colonic tumor. A primary surgery included ESD (n = 11) and polypectomy (n = 1) or TEM for rectal lesions (n = 12). The indications for salvage surgery were the presence of unfavorable prognostic factors (T1sm3 tumor, high grade G, LVI+, R1 resection). Characteristics of patients and surgical interventions are presented in Table 1.
Table 1
Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>264 (100.0%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>115 (43.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>149 (56.4%)</td>
</tr>
<tr>
<td>Mean age ± SD, years</td>
<td>63.0 ± 12.4</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
</tr>
<tr>
<td>Right colon including 2/3 of transverse</td>
<td>59 (22.63%)</td>
</tr>
<tr>
<td>Left colon</td>
<td>122 (46.2%)</td>
</tr>
<tr>
<td>Rectum</td>
<td>83 (31.4%)</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
</tr>
<tr>
<td>Right colectomy</td>
<td>53 (20.1%)</td>
</tr>
<tr>
<td>Left colectomy</td>
<td>118 (44.7%)</td>
</tr>
<tr>
<td>Subtotal colectomy</td>
<td>11 (4.2%)</td>
</tr>
<tr>
<td>Colproctectomy</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>Anterior resection</td>
<td>40 (15.2%)</td>
</tr>
<tr>
<td>Low anterior resection</td>
<td>41 (15.5%)</td>
</tr>
</tbody>
</table>

Metastasis in regional lymph nodes was found in 46 (17.4%) out of 264 surgical specimens. The median number of harvested lymph nodes was 20 (14:30 quartiles). Depending on the number of affected lymph nodes, the following N stage was found: N1a 26 (9.8%), N1b 14 (5.3%), N2a 5 (1.9%) and N2b – 1 (0.4%).

Surprisingly, deep submucosal invasion (DSI) had no association (Table 2) with lymphatic tumor involvement [OR 1.2; 95% CI 0.6–2.3] (p = 0.33).

High grade adenocarcinoma (G3) was found just in 9 (3.4%) cases. Almost half of poorly differentiated tumors (44.4%) were associated with lymph node metastasis [OR 4.1; 95% CI 1–15.7] (p = 0.05).
Table 2
Univariate analysis of categorical risk factors of lymph node involvement T1

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>N0</th>
<th>N1-2</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>female</td>
<td>149 (100)</td>
<td>119 (79.9)</td>
<td>30 (20.1)</td>
<td>0.64</td>
<td>0.3–1.2</td>
<td>0.12</td>
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<tr>
<td>male</td>
<td>115 (100)</td>
<td>99 (86.1)</td>
<td>16 (13.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site (I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>181 (100)</td>
<td>154 (85.1)</td>
<td>27 (14.9)</td>
<td>1.7</td>
<td>0.9–3.3</td>
<td><strong>0.1</strong></td>
</tr>
<tr>
<td>Rectum</td>
<td>83 (100)</td>
<td>64 (77.1)</td>
<td>19 (22.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td>60 (100)</td>
<td>57 (95.0)</td>
<td>3 (5.0)</td>
<td>4.7</td>
<td>1.4–16.3</td>
<td><strong>0.008</strong></td>
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<td>Left colon</td>
<td>121 (100)</td>
<td>97 (80.2)</td>
<td>24 (19.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>sm1-2</td>
<td>131 (100)</td>
<td>110 (84.0)</td>
<td>21 (16.0)</td>
<td>1.2</td>
<td>0.6–2.3</td>
<td>0.33</td>
</tr>
<tr>
<td>sm3</td>
<td>133 (100)</td>
<td>108 (81.2)</td>
<td>25 (18.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation G</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>255 (100)</td>
<td>213 (83.5)</td>
<td>42 (15.5)</td>
<td>4.1</td>
<td>1.0–15.7</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>9 (100)</td>
<td>5 (55.6)</td>
<td>4 (44.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape of lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exophitic</td>
<td>172 (100)</td>
<td>141 (82.0)</td>
<td>31 (18.1)</td>
<td>0.9</td>
<td>0.5–1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>sessile</td>
<td>92 (100)</td>
<td>77 (83.7)</td>
<td>15 (16.3)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LVI –</td>
<td>148 (100)</td>
<td>145 (98.0)</td>
<td>3 (2.0)</td>
<td>28.5</td>
<td>8.5–94.9</td>
<td>&lt;<strong>0.0001</strong></td>
</tr>
<tr>
<td>LVI +</td>
<td>16 (100)</td>
<td>73 (62.9)</td>
<td>43 (37.1)</td>
<td></td>
<td></td>
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<tr>
<td>Budding, grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>82 (100)</td>
<td>72 (87.8)</td>
<td>10 (12.2)</td>
<td>–</td>
<td></td>
<td>0.056</td>
</tr>
<tr>
<td>2</td>
<td>69 (100)</td>
<td>60 (87.0)</td>
<td>9 (13.0)</td>
<td></td>
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<tr>
<td>3</td>
<td>113 (100)</td>
<td>86 (76.1)</td>
<td>27 (23.9)</td>
<td></td>
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<tr>
<td>Characteristic</td>
<td>Total</td>
<td>N0</td>
<td>N1-2</td>
<td>OR</td>
<td>95%CI</td>
<td>P</td>
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<td>----------------------------------------</td>
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<td>Poor differentiated clusters, grades</td>
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<td></td>
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<tr>
<td>1</td>
<td>127 (100)</td>
<td>115 (90.6)</td>
<td>12 (9.4)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>72 (100)</td>
<td>59 (81.9)</td>
<td>13 (18.1)</td>
<td></td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>3</td>
<td>65 (100)</td>
<td>44 (67.7)</td>
<td>21 (32.3)</td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Cancer gland rupture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>114 (100)</td>
<td>99 (86.8)</td>
<td>15 (13.2)</td>
<td>1.7</td>
<td>0.9–3.4</td>
<td>0.14</td>
</tr>
<tr>
<td>Yes</td>
<td>150 (100)</td>
<td>119 (79.3)</td>
<td>31 (20.7)</td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
</tbody>
</table>

High grades of tumor budding (p = 0.056) and poor differentiated clusters (p < 0.0001) correlated to LNM. The phenomenon of tumor glands rupture was found in most cases (33 out of 49) with metastasis to the lymph nodes, though obtained difference did not reach statistical significance (p = 0.11).

The highest odds ratio of lymph nodes metastasis was detected for lymphovascular invasion [OR 28.5; 95% CI 8.5–94.9] (p < 0.0001). In the multivariate analysis LVI + remains the only significant independent risk factor [OR 15.7; 95% CI 8.5–94.9] (p < 0.0001). As a diagnostic test LVI had following Sensitivity 0.93 [95% CI 0.82–0.99], Specificity 0.67 [95% CI 0.60–0.73], Positive Predictive Value 0.37 [95% CI 0.28–0.47] and Negative Predictive Value 0.98 [95% CI 0.94–1.0]

Comparisons of morphometric characteristics of tumors with and without lymph nodes metastasis in terms of median width of lesion, width of carcinoma, length of carcinoma invasive front and median area of carcinoma demonstrated no difference (Table 3).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lymph nodes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median width of Lesion</td>
<td>20.5 (14.3–31.4)</td>
<td>18.6 (11.7–26.8)</td>
</tr>
<tr>
<td>Median width of carcinoma (25%-75%), mm</td>
<td>10.9 (8.3–15.0)</td>
<td>10.6 (7.5–14.7)</td>
</tr>
<tr>
<td>Median length of invasive front (25%-75%), mm</td>
<td>7.9 (5.8–11.3)</td>
<td>8.1 (5.1–11.0)</td>
</tr>
<tr>
<td>Median area of carcinoma (25%-75%), mm²</td>
<td>35.6 (21.8–60.8)</td>
<td>39.8 (23.5–69.0)</td>
</tr>
</tbody>
</table>

The ROC analysis of association between morphometric characteristics and pT1 LNM showed absence of predictive value of these tumor parameters (Fig. 4).
Discussion

Local excision of T1 colorectal cancer using endoscopic methods results in organ-saving treatment and avoids many complications inherent in radical surgery, especially for rectal cancer. The main problem of this approach is the absence of the opportunity to evaluate the regional lymph nodes status. An indication for salvage resection is based on a final pathologic assessment of several unfavorable morphological tumor features which allow to divide tumors into high and low risk regional LNM. Currently, the risk factors of LNM of T1 CRC included into the treatment guidelines are deep tumor invasion into the submucosal layer (sm3, DSI ≥ 1000 µm), high grade adenocarcinoma, presence of lymphovascular invasion (LVI) and high degree of tumor budding (Bd2-3) [1–4, 28].

Depth of invasion as a risk factor is most commonly used and recommended for assessment nowadays. At the same time, the assessment of DSI of locally removed CRC is associated with obstacles for practical use due to the lack of universal measurement techniques. It is especially cumbersome for tumors removed within submucosa (ESD) because at least three methods of DSI measurement are suggested, i.e. from the surface of the tumor, from the lamina propria mucosae and from the baseline when the lamina propria is completely destroyed by tumor. The obtained results obviously depend on the method and the macroscopic type of tumor growth (exophyte, elevated or depressed lesions) [11, 12, 13]. The choice of baseline is also very complicated and arbitrary.

Metastasis of CRC in regional lymph nodes was found in 46 (17.4%) cases in the presented group of 264 cases of pT1, which is comparable to the frequency of metastasis reported in previous studies [6, 16, 18]. For deep submucosal invasion (DSI) of sm3 (or ≥ 1000 µm) a rate of regional metastasis was 18.8%, which did not have a statistically significant difference from 16.0% (p = 0.33) for sm1-2 tumors.

Our results correspond to several other studies which showed the absence of a predictive value of the depth of invasion into the submucosal layer in relations to LNM for T1 CRC. A large meta-analysis by Ichimasa K et al. [11] found out that among all risk factors (DSI, high grade adenocarcinoma, LVI, Bd) the depth of submucosal invasion had the lowest prognostic value.

Another meta-analysis [10] failed to prove DSI as an independent risk factor for LNM. Eight studies (1,146 patients) analyzed DSI as a solitary risk factor. It was found that the absolute risk of LNM was 2.6% and pooled incidence rate was 2.83 (95% CI 1.66–4.78).

Width of invasion as well as an area of submucosal invasion of carcinoma were suggested as more objective and reproducible parameters in the histological examination of a locally removed T1 tumor [9, 14, 15]. It was established that most lymphatic vessels are located in the upper third of the submucosal layer and their density does not increase in the deeper parts of the submucosal layer [29, 30]. In accordance with this concept the probability of vascular invasion and LMN are rather determined by the length and the area of submucosal invasive front than the depth of tumor invasion.
However, though the prognostic value of these parameters for LNM in T1 CRC was shown, the authors identified a significant fluctuation in the obtained cut-off points of the width and the area of submucosal tumor invasion, which do not currently allow to use these parameters in practice [14, 15, 16].

In our study the ROC analysis failed to reveal a prognostic value of width, length or area of tumor invasion as the AUC was about 0.5 for all of them.

Histological signs such as Bd and PDC showed independent prognostic value as risk factors for LNM in the study group (p = 0.05 and p < 0.0001, respectively). In contrast to our previous study [31], the prognostic value of these signs was determined by the Chi-square test regardless of the severity (Bd1-3/PDC G1-3).

Poorly differentiated clusters [21, 22, 23] showing even a more pronounced association with LNM demonstrated a statistically significant correlation with lymph node metastasis, regardless of the quantitative value (Grade) compared to tumor budding where only high degree of Bd (2–3) increased the risk of LNM [20]. In this context the assumption that PDC better reflects the biological aggressiveness of the tumor than the differentiation sounds reasonable. The obtained results suggest that PDC can serve as an additional or alternative to Bd sign in cases where the assessment of the last one is complicated.

The Grade of tumor differentiation has also demonstrated an association with LNM. However, it should be noted that the practical use of high grade adenocarcinoma differentiation as a risk factor for LNM is limited by the low incidence in CRC [25] and the reproducibility of this feature among pathologists [11, 21, 33, 34].

In the present study, we attempted to elucidate the role of cancer gland rupture (CGR) phenomenon as a potential risk factor for lymph node metastasis in T1 CRC. CGR was twice as common in the group with lymph node metastasis; however, as in the previous study [31], we failed to show a statistically significant association with LNM in the univariate analysis (p = 0.14).

The study also confirmed that lymphovascular invasion (LVI) is the most significant risk factor for metastasis to regional lymph nodes in the univariate analysis: OR 28.5; 95% CI 8.5–94.9 (p < 0.0001). In the logistic regression model, LVI remained the only independent risk factor for metastasis to regional lymph nodes, p < 0.0001. The high sensitivity (93%) and moderate specificity (67%) of LVI, along with a high negative predictive value of 98%, which was determined in our study, allow to interpret T1 tumors without LVI as tumors with a very low risk of LNM.

The assessment of LVI is a matter of the significant variability among pathologists, which, according to the studies, demonstrates low values of the kappa coefficient of 0.16–0.44, i.e. low interobserver agreement. Some studies have shown that the use of immunohistochemical staining using D2-40 resulted in more accurate detection of the LVI and increased k-coefficient up to 0.56 [18, 32, 35].

In summary, the results of our study showed that lymphovascular invasion, tumor budding, poorly differentiated clusters and low tumor differentiation are the most significant predictors of LNM in T1 CRC,
which corresponds to the data obtained from a number of other studies where morphological characteristics of the tumor demonstrated a superior predictive value compared to invasion of submucosal layer. The role of the DSI as an independent predictor of LNM is revised, though it remains one of the most mentioned risk factors in current guidelines. Obviously, tumor morphology (Grade, LVI, Bd, PDC) is more reliable predictor of LNM than morphometry. On the other hand, results of the studies and meta-analysis of the main LNM predictors in T1 CRC showed that none of the currently used morphological signs can be used independently since it does not have sufficient sensitivity and specificity [10, 11, 16, 18, 20, 32].

The obvious limitations of our study are a single center and a relatively small group of patients with T1 CRC which included only 46 cases (18.5%) with LMN. Morphometry of tumor included such parameters as width of adenocarcinoma, length of the invasive front and total area of adenocarcinoma, and was carried out in accordance with the previously proposed methods [13, 21, 24], but all assessment was conducted by one pathologist.

**Conclusion**

The DSI invasion of T1 CRC as well as other morphometric parameters of submucosal tumor spread do not have any predictive value in terms of LNM. Low differentiation of adenocarcinoma, tumor budding (Bd), poorly differentiated clusters (PDC) and lymphovascular invasion (LVI) are the significant risk factors for LNM in T1 CRC. Among them LVI was the only independent risk factor.

**Declarations**

**Statements and Declarations**

Authors declare no conflict of interest.

The study had no funding

**Authors’ Contributions:**

Olga Maynovskaia and Evgeny Rybakov wrote manuscript

Olga Maynovskaia examined all specimens and prepared histology pictures

Evgeniy Khomyakov and Evgeny Rybakov provided statistical analysis

Stanislav Chernyshov worked up clinical data collection

Sergey Achkasov provided revision of the article and prepared final edition of manuscript

**References**


Figures
Figure 1

A digital slide showing the Imagescope measurements of the maximal width of lesion (blue bar) and width of carcinoma (red bar).

Figure 2
A digital slide showing the Imagescope measurements (contained within the red area) for the estimated maximal total (intramucosal and submucosal) area of carcinoma invasion (19.6 mm²)

**Figure 3**

A digital slide showing the Imagescope measurements for the maximal length of invasive front of 10.07 mm
Figure 4

ROC analysis of morphometric characteristics of tumors and lymph nodes metastases