Original paper

Poorly differentiated clusters: prognostic significance in colorectal carcinomas

Aura Jurescu[1], Adrian Văduva[1], Sorina Tăban[1,2], Adelina Gheju[1], Gh. Emilian Olteanu[1], Ioana Mihai[1], Codruța Lăzureanu[1,2], Marioara Cornianu[1,2], Fulger Lazăr[3,4], Alis Dema[1,2]

[1]Department of Pathology, "Victor Babeş" University of Medicine and Pharmacy, Timisoara, Romania
[2]Department of Pathology, Emergency County Hospital "Pius Brînzeu" Timisoara, Romania
[3]Department of Surgery II, "Victor Babeş" University of Medicine and Pharmacy, Timisoara, Romania
[4]Department of Surgery II, Emergency County Hospital "Pius Brînzeu" Timisoara, Romania

Colorectal cancer (CRC) remains a major public health burden worldwide, despite increased knowledge on its pathogenesis and advances in therapy. We aimed to evaluate a new histological grading system based on poorly differentiated clusters (PDCs) counting – the PDCs grade (PDCs-G), and its clinicopathological and prognostic significance, compared to the World Health Organisation (WHO) grading system (WHO grade).

We reviewed 71 surgical resection specimens for CRC from the Emergency County Hospital "Pius Brînzeu" Timisoara. The cases were graded using the WHO grade and the PDCs-G, with further analysis of their association with the other recognised prognostic parameters.

Using the WHO grade, 9% of the analysed cases were G1, 80% G2, 11% G3, and none of the tumours was graded G4, while in the PDCs-G 16% were G1, 45% G2, and 39% G3. In multivariate analysis PDCs-G was significantly associated with the American Joint Committee on Cancer stage of the disease (AJCC stage) ($p = 0.0003$), depth of invasion (pT) ($p = 0.0084$), nodal status (LNM) ($p < 0.0001$), lymphovascular invasion (LVI) ($p < 0.0001$), perineural invasion (PNI) ($p < 0.0052$), and tumour border configuration ($p < 0.0001$).

The novel grading system based on PDCs counting is an additional histological tool in the evaluation of CRC and a promising new prognostic factor for these patients.

Key words: colorectal carcinoma, WHO tumour grade, poorly differentiated clusters, prognostic.

Introduction

Colorectal carcinoma (CRC) is one of the most prevalent cancers worldwide, statistically ranked as the third most common malignancy in males, the second most common in females, and the fourth among all cancer-related deaths [1]. Colorectal carcinoma appears to be on an ascending slope regarding its incidence, with high rates of morbidity and mortality, although much progress has been made in early detection, identification of prognostic markers, and therapeutic management [2].

The American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) tumour staging system (TNM), based on the evaluation of local extension of the tumour, nodal status, and distant metastases, represents the fundamental standard for predicting the evolution, and for

guiding the therapy of CRC [3, 4]. Different clinicopathological factors such as the depth of tumour invasion (pT), the degree of tumour differentiation (G), the status of resection margin, the presence/absence of lymphovascular (LVI) and perineural invasion (PNI), the lymph node status including the presence of nodal micrometastases, tumour border configuration, and tumour budding (TB) are useful criteria for stratifying CRC patients [5, 6]. However, an appreciable variability in clinical outcome for the CRC patients with the same disease stage was reported in several studies [7, 8]. Therefore, additional parameters are required to predict the outcome and to identify patient subgroups for which personalised approaches to therapy could be helpful.

In addition to the TNM stage, the histological grade is an important prognostic parameter in CRC [5, 6]. Unfortunately, there is significant inter-observer variability in grading CRC, incurred by the use of several grading systems and the lack of explicit diagnostic criteria and rule-based classifications [8]. According to the WHO criteria, based on the quantification of glandular structures (percentage method), CRCs are graded as well-differentiated (G1), moderately differentiated (G2), poorly differentiated (G3) adenocarcinomas, and undifferentiated carcinomas (G4) [6, 7, 8]. Although this method is widely used, this grading system is subjective because it is difficult to appreciate the formation of glands [9, 10, 11, 12]. In addition, controversies persist regarding which factor is to be primarily considered – the least differentiated area (highest grade) or overall impression (the predominant grade) [13, 14]. Furthermore, there are some histological subtypes, like mucinous, signet-ring cells, or micropapillary carcinomas, for which the tumour grade has an uncertain prognostic value [10, 11, 12, 13]. Nonetheless, medullary carcinoma must be recognised and not graded [10]. According to the WHO 2010 classification of tumours of the digestive system, the assessment based on glandular differentiation of CRC applies only to "adenocarcinoma, NOS" (NOS – not otherwise specified) excluding the special variants [6]. Consequently, the utility of this system is questionable.

A new grading system for CRC, based on the number of poorly differentiated clusters (PDCs), was introduced in 2012 by Ueno *et al*. [15]. According to Ueno's definition, PDCs are groups of five or more cancer cells, with no gland formation, quantifiable in their highest density area (hotspot), at the invasion front of the tumour [15]. PDCs-G promises to be a more reliable and accurate system of grading compared to the WHO grade, the lymphovascular invasion, or the tumour border configuration, to predict the metastatic potential of CRC [7, 8, 9, 11, 12, 15, 16]. This new grading system seems to provide prognostic information as well as many advantages regarding reproducibility. Being a fairly recently described histological tool for the evaluation of CRC, it requires further validation and standardisation [9, 11, 15, 16, 17, 18].

We aimed to compare the two grading systems (WHO grade and PDCs-G) for CRC and to correlate them with other clinicopathological parameters, in order to evaluate and demonstrate the feasibility of PDCs-G, and its potential value as a prognostic factor in CRC.

Material and methods

This study represents a retrospective analysis of a group of 50 consecutive surgical resection specimens from patients with CRC, diagnosed in 2014, and 21 cases of robotic surgery CRC specimens (da Vinci Xi® Surgical System), diagnosed between 07/2015 and 07/2016 in the II Surgery Clinic of the Emergency County Hospital "Pius Brînzeu" Timisoara, Romania.

All the data were collected from the patients' records of histopathological results registered in the Pathology Department database. Carcinomas treated by chemotherapy or radiotherapy before surgeries were excluded.

The surgical specimens were processed according to the standard histopathological protocols. Briefly, the tumour sections, previously fixed in 10% buffered formalin and embedded in paraffin, were cut at 3-5 microns thickness and subsequently stained with haematoxylin and eosin (HE). For each case, we selected one representative slide that included the deepest tumour infiltration into the intestinal wall, being at the same time part of the invasion front of the tumour.

The following parameters were collected and analysed: patient's gender, age, site of the tumour, histological type, histological grade, pT parameter, lymphovascular invasion (LVI), perineural invasion (PNI), tumour border configuration, peritumoral inflammatory infiltrate (TILs), tumour necrosis, tumour ulceration, lymph nodes metastases (LNM), clinically documented distant metastases (cM), and the stage of the disease (AJCC stage).

The patients were distributed into the following age groups: 41-50, 51-60, 61-70, 71-80, and 81-90 years. We evaluated two subgroups of age < 65 years and ≥ 65 years. A different analysis was performed with respect to the site of the tumour: right-sided (caecum, ascending, and proximal transverse colon), left-sided tumours (the distal transverse, descending, sigmoid colon), and the rectum (rectosigmoidian junction and rectum).

We assessed the tumours, according to the WHO 2010 classification, as conventional adenocarcinomas (NOS) and as mucinous adenocarcinomas when more

than 50% of the tumour mass was composed of pools of extracellular mucin. The tumours containing mucinous areas of less than 50% are classified as having a mucinous component [6].

The pT parameter was established according to the eighth edition of the AJCC Cancer Staging Manual [4]. The cases were further classified into early invasive (pT1-2) and deeply invasive (pT3-4), to differentiate the incipient from the advanced tumour extension into the intestinal wall.

The histological grade of CRC was assessed for conventional adenocarcinomas, according to the WHO 2010 classification, based on the extent of glandular appearance: G1 – well differentiated (> 95% gland formation), G2 – moderately differentiated (50-95% gland formation), G3 – poorly differentiated (0-49% gland formation), and G4 – undifferentiated carcinomas (no gland formation or mucin, no squamous or neuroendocrine differentiation) [6].

Each case of conventional adenocarcinomas was further evaluated by applying the new histological grading system (PDCs-G), based on PDCs counting, according to Ueno's method [15]. In brief, our PDCs study protocol comprised several steps. All the representative slides (one slide/case), containing the deepest part of the tumour, were scanned on a Leica Aperio AT2 machine. The slides were examined at low magnification (4×) in order to identify the area with the highest density of PDCs along the invasion front. This area was considered as the hotspot and was further evaluated at an intermediate power objective (20×), with a field size of 0.785 mm^2. We classified the CRC cases in a three-tier system: tumours with < 5 PDCs (PDCs-G1), 5 to 9 PDCs (PDCs-G2), and ≥ 10 clusters of PDCs (PDCs-G3) in the analysed area. For the cases of mucinous adenocarcinomas, we did not perform the WHO grade or the PDCs-G.

We quantified the lymphocytic infiltrate at the invasion front of the tumour and classified the cases in a two-tier system: TILs- for absent or minimal ≤ 5% lymphocytes per high-powered field and TILs+ for the cases with an increased number of inflammatory cells at the invasive margin, forming a band-like infiltrate (with the destruction of adjacent cancer cell groups). We also evaluated the tumour necrosis, due to the rapid growth of the tumour and consecutive hypoxia, as absent (< 10% of tumour mass) or present (≥ 10% of tumour mass). The presence of ulceration in the tumour surface and the perineural invasion (PNI) were parameters also quantified as absent/present. The pattern of tumour invasion, the tumour border configuration, was considered as pushing type when we noticed a smooth expanding border, or as infiltrating type when an irregular pattern of growth was observed. For the cases with both characteristics, we considered the predominant component and we classified them accordingly.

Statistical analysis

The collected parameters were statistically analysed using Graph Pad Prism v8 and IBM SPSS v25 software. We used the χ^2 test to compare the two grading systems with the other clinicopathological factors. The resulting p-value was considered of statistical significance if it was lower than 0.05 (Table I). Spearman's correlation coefficient (Rs) was used to highlight the associations between the analysed parameters.

Ethics statements

All the procedures included in this study were carried out according to the principles of the Declaration of Helsinki of good clinical practice. Each patient signed an informed consent form, allowing the use of the tissue fragments in scientific studies.

Results

The study group included 27 females (38%) and 44 males (62%), aged between 42 and 85 years at initial diagnosis, with a mean age of 66.47 years. Thirty (42%) CRC cases were patients < 65 years old and 41 (58%) were patients ≥ 65 years old. We noticed the highest incidence of CRC in the seventh decade of life in men 17/44 (39%). In women, the highest incidence was noted in the seventh and in the eighth decades of life 9/27 (33.5%).

Tumours were located in the caecum in five patients (7%), in the ascending colon and the hepatic flexure in seven (10%), in the transverse colon in three (4%), at the splenic flexure and in the descending colon in two (3%), in the sigmoid colon in 21 (30%), at the rectosigmoid junction in 13 (18%), and in the rectum in 20 patients (28%). According to our definition related to the tumour site, in 15 (21%) cases the right colon was involved, 23 (32%) cases were left-sided colon cancers, and 33 (47%) adenocarcinomas were identified in the rectum or in the rectosigmoid junction.

Tumour necrosis was present in 47 cases (66%) and tumour ulceration in 61 (86%) cases. The analysis of the tumour border configuration showed that 26/71 (37%) cases were pushing type and 45/71 (63%) cases were infiltrative type.

Regarding the depth of tumour invasion into the intestinal wall (pT), three (4%) cases were pT1, eight (11%) cases pT2, 38 (54%) cases pT3, and 22 (31%) cases pT4. According to our classification, 11 (15%) cases were early invasive (pT1-2), and 60 (85%) cases were deeply invasive (pT3-4) tumours. 34/71 (48%) patients presented lymph node metastases (LNM+) and 6/71 (8%) patients presented distant metastases (M+). The cases were staged

Table I. The statistical correlations between the clinicopathological parameters and the WHO grade vs. PDCs investigated through the χ^2 test and the Spearman's correlation coefficient

Parameters	WHO grade			P value	Rs value	PDCs grade			P value	Rs value
	G1	G2	G3			PDCs-G1	PDCs-G2	PDCs-G3		
Total CRC cases										
69	6	55	8			11	31	27		
Gender										
Male	3	32	7	0.2409	–0.185	9	16	17	0.2027	0.052
Female	3	23	1			2	15	10		
Age (years)										
< 65	3	21	3	0.849	0.097	4	15	8	0.3373	0.07
≥ 65	3	34	5			7	16	19		
Site of tumour										
Right colon	0	11	2	0.2296	–0.095	1	6	6	0.1579	–0.165
Left colon	1	21	1			1	13	9		
Rectum	5	23	5			9	12	12		
Depth of invasion										
pT1-2	4	7	0	0.0012	0.377	4	7	0	0.0084	0.372
pT3-4	2	48	8			7	24	27		
Nodal status										
LNM–	5	30	1	0.0235	0.326	9	22	5	< 0.0001	0.526
LNM+	1	25	7			2	9	22		
Distant metastases										
M–	6	50	7	0.6949	0.094	11	29	23	0.284	0.191
M+	0	5	1			0	2	4		
AJCC stage										
I	4	6	0	0.0028	0.397	4	6	0	0.0003	0.570
II	1	25	1			5	17	5		
III	1	20	6			2	7	18		
IV	0	4	1			0	1	4		
Lymphovascular invasion										
LVI–	5	36	1	0.0082	0.347	10	24	8	< 0.0001	0.509
LVI+	1	19	7			1	7	19		
Perineural invasion										
PNI–	5	39	2	0.0242	0.298	10	24	12	0.0052	0.386
PNI+	1	16	6			1	7	15		
Lymphocytic infiltration										
TILs–	1	8	2	0.7512	–0.061	1	4	6	0.4982	–0.141
TILs+	5	47	6			10	27	21		
Tumour necrosis										
absent	3	19	2	0.6215	0.114	5	12	7	0.4281	0.156
present	3	36	6			6	19	20		
Tumour ulceration										
absent	2	5	2	0.1392	0.022	2	5	2	0.5293	0.132
present	4	50	6			9	26	25		

Table I. Cont.

Parameters	WHO grade			P value	Rs value	PDCs grade			P value	Rs value
	G1	G2	G3			PDCs-G1	PDCs-G2	PDCs-G3		
Tumour border configuration										
Pushing	5	21	0	0.0062	0.382	10	16	0	< 0.0001	0.683
Infiltrating	1	34	8			1	15	27		

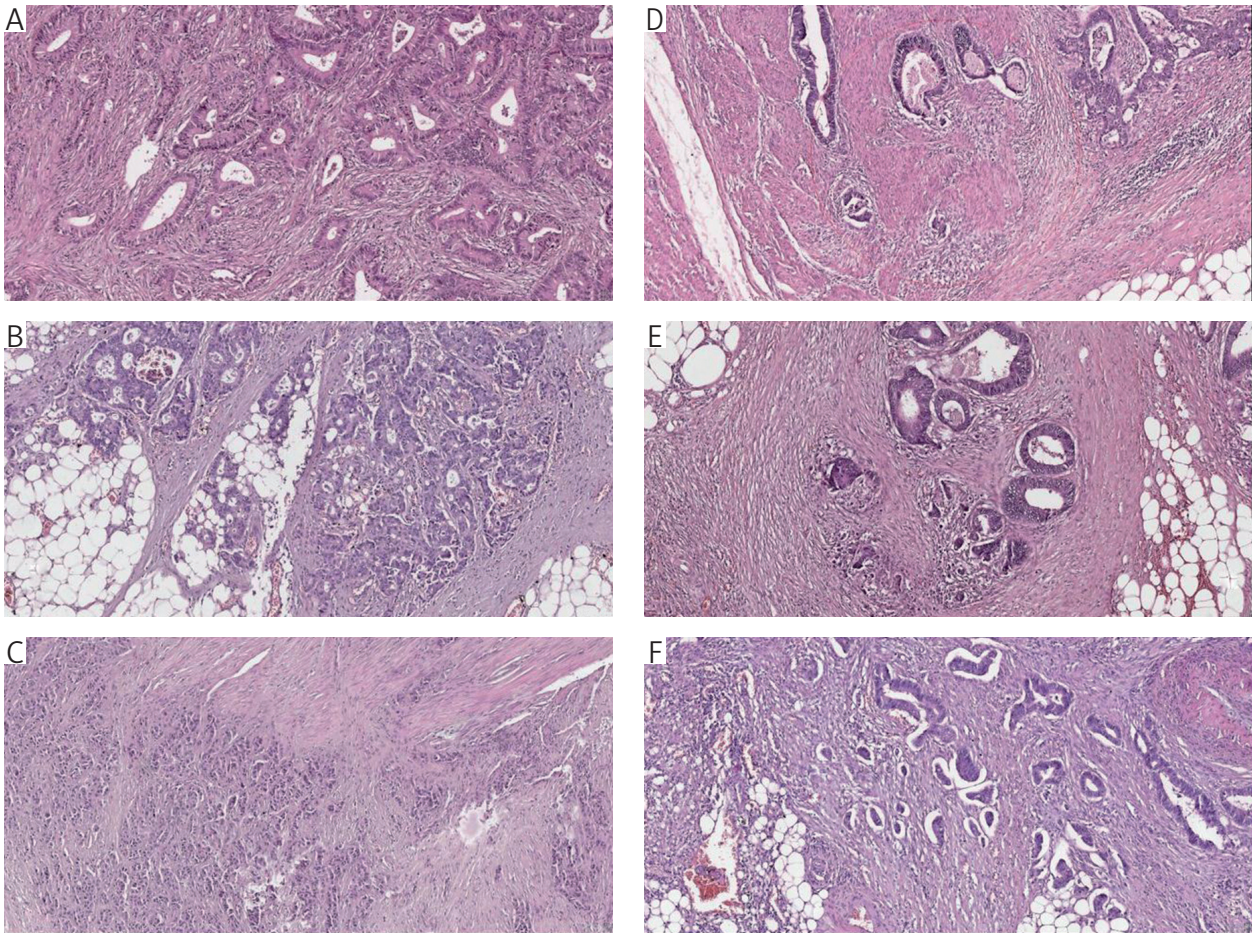

Fig. 1. A) WHO grade 1 CRC, HE×100. **B)** WHO grade 2 CRC, HE×100. **C)** WHO grade 1 CRC, HE×100. **D)** PDCs-G1 CRC, HE×100. **E)** PDCs-G2 CRC, HE×100. **F)** PDCs-G3 CRC, HE×100

as follows: 10 (14%) stage I, 28 (39.5%) stage II, 28 (39.5%) stage III, and 5 (7%) stage IV.

With respect to the peritumoral lymphocytic infiltrate, 60 (85%) cases were TILs+. Lymphovascular invasion (LVI+) was observed in 28/71 (39%) cases, and 23/71 (32%) patients presented perineural invasion (PNI+). We found that all the 23 cases with perineural invasion belonged to the pT3-4 group, and 19 (83%) of the cases presenting perineural invasion were also LVI+ and LNM+. Moreover, all of the 28 LVI+ cases were classified as pT3-4, LNM+, and 27/28 (96%) of the LVI+ cases presented also infiltrating type tumour borders configuration.

Histologically, 69/71 (97%) cases were diagnosed as NOS adenocarcinomas and 2/71 (3%) as mucinous adenocarcinomas. Sixteen (23%) out of the 69 cases of conventional adenocarcinomas presented mucinous differentiation in less than 50% of the tumour mass.

Using a histological grading system (WHO grade) for the evaluation of the 69 cases of conventional adenocarcinomas, we found six (9%) well differentiated tumours (G1) (Fig. 1A), 55 (80%) moderately differentiated tumours (G2) (Fig. 1B), and eight (11%) poorly differentiated (G3) adenocarcinomas (Fig. 1C). Based on PDCs-G3 system, 11/69 cases (16%) were

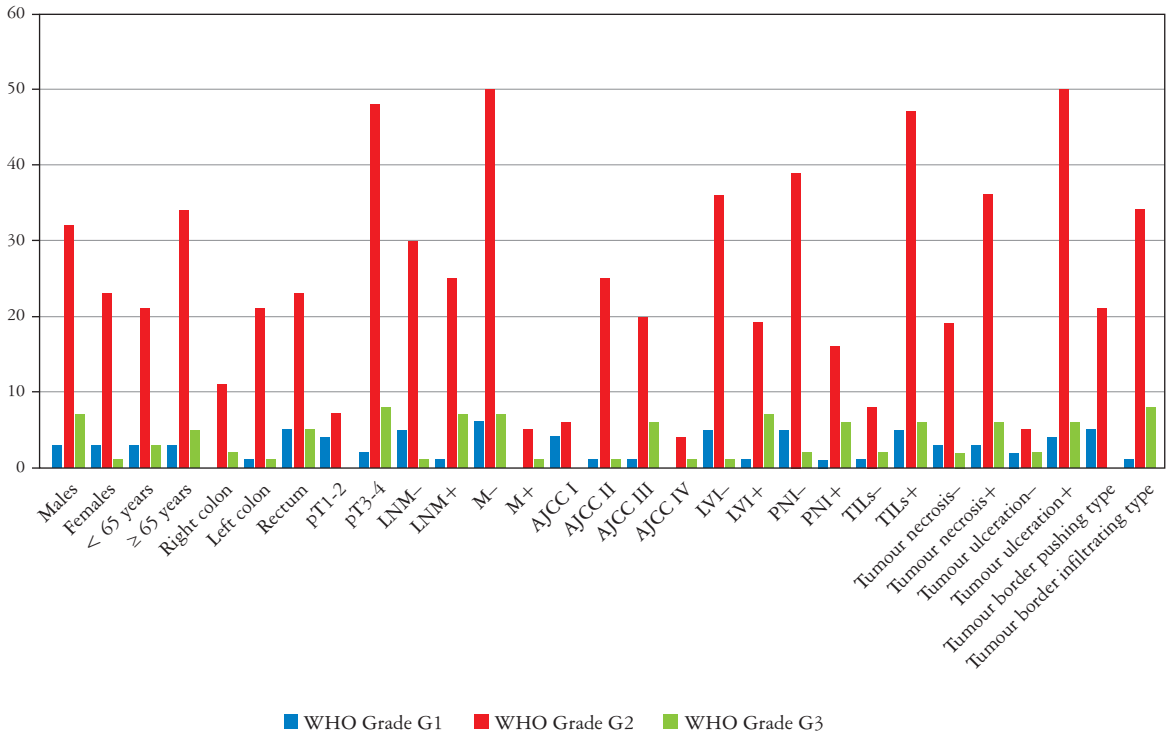

Fig. 2. Correlation between the clinicopathological parameters and the WHO grade

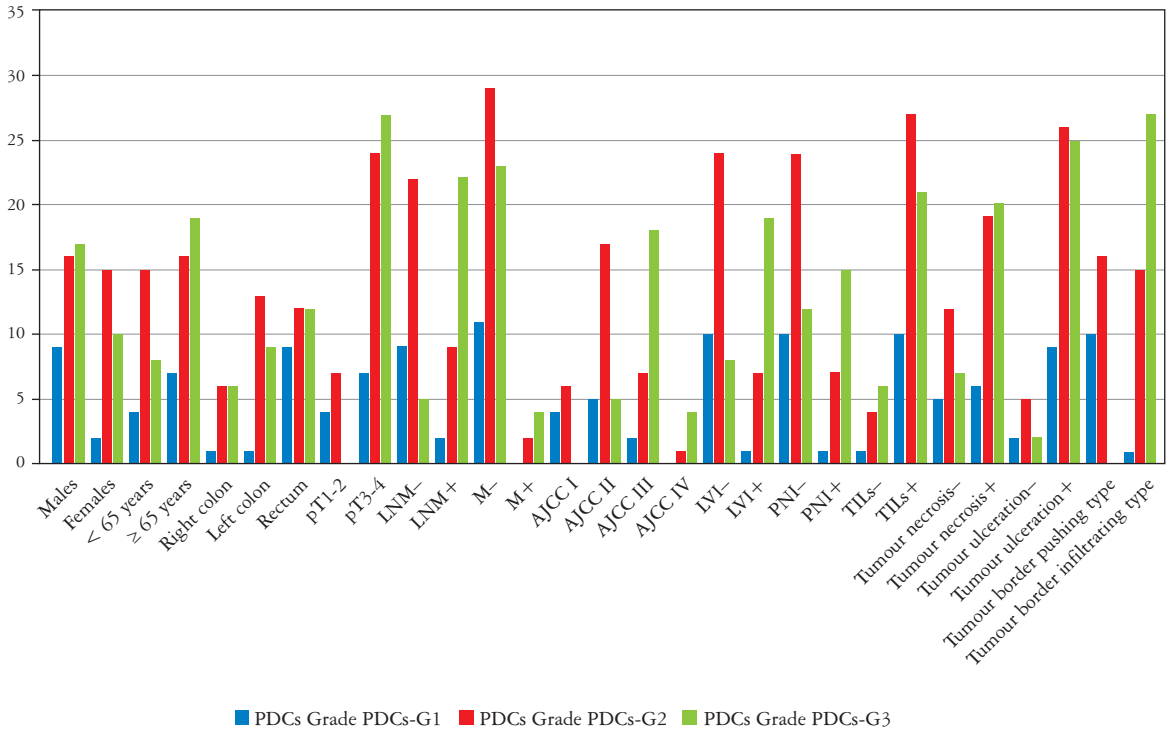

Fig. 3. Correlation between the clinicopathological parameters and the PDCs-G

PDCs-G1 (Fig. 1D), 31/69 (45%) PDCs-G2 (Fig. 1E), and 27/69 (39%) PDCs-G3 (Fig. 1F). For the two cases of mucinous adenocarcinomas, we did not perform the WHO grade or the PDCs-G.

Statistical analysis of the correlations between the clinicopathological parameters and the WHO grade showed significant p-value correlations with the pT, LNM, AJCC stage, LVI, PNI, and tumour border configuration parameters and did not significantly correlate with the others analysed parameters (Table I, Fig. 2). In multivariate analysis, PDCs-G showed a more significant association with LNM ($p < 0.0001$), AJCC stage ($p = 0.0003$), LVI ($p < 0.0001$), PNI ($p = 0.0052$), and tumour border configuration ($p < 0.0001$) (Table I, Fig. 3).

Discussion

The degree of tumour differentiation (the grade of the tumour) is an important prognostic factor for many malignant proliferations and should be included in the pathological report. Similarly, for CRC, the tumour grade is consistently reported and recognised as one of the most important parameters correlated with CRC aggressiveness [6]. However, there is no consensus on grading methodology, with different systems based on two-, three-, or four-tiered classification. Thus, the need for a single grading method is still present; one that is widely accepted as a standard for the classification of the malignant epithelial tumours of the colon [10, 13, 15, 16, 19, 20]. Over the years, a variety of grading schemes have proven to be questionable in practice, due to their lack of clear and well-established criteria for evaluation. Therefore, architecture, glandular formation, cytological criteria, a single microscopic feature, or a large number of characteristics have been considered, and the evaluation method varies widely in general practice [21, 22, 23, 24].

However, the most commonly used method for grading CRC is based on the percentage of the tumour showing glandular or tubular formation (the architectural model). According to the WHO 2010 classification of tumours of the digestive system [6], the four-tier histologic grading system is used for grading CRCs: gland-like structures > 95% of the tumour (Grade 1), between 50-95% (Grade 2), and between 0-49% of the tumour (Grade 3). Grade 4, undifferentiated carcinoma, is considered a diagnosis of exclusion for carcinomatous proliferations, with no evidence of differentiation (glandular, mucinous, squamous, neuroendocrine, or sarcomatoid) [5, 6, 10, 25] but with epithelial differentiation proven by immunohistochemistry (IHC) staining [9].

In addition, to eliminate discordances between the three- and four-class systems, and to mitigate the inter-observer variation in grading of the well differentiated and moderately differentiated adenocarcinomas, the third edition of WHO classification recommended a two-tiered system [24]. This revised classification is based on evidence that some similarities exist in the evolution of patients with well and moderately differentiated adenocarcinomas [5, 10]. Thus, low-grade CRC is established when > 50% of the tumour forms gland-like structures (grades 1 and 2), and high-grade (CRC) when < 50% of the tumour presents glandular structures (grades 3 and 4) [6]. Again, a significant inter-observer variation and a decline in the prognostic importance of the WHO grade were noted [7, 8].

In their study, Kuijpers *et al*. [26] reported a significant inter-laboratory and intra-laboratory variation in CRC assessment, regarding the grade, with consequential high impact on the patient's treatment. Furthermore, in the pathologists' methodology, several guidelines and/or books are used as references, and the guidelines are not consistent regarding the area that should be graded: the predominant grade or the area of the poorest differentiated component. Another source of variability is the evaluation of the heterogeneous tumours. In this situation, grading should be performed taking into account the least differentiated component, not considering the invasive front, where tumours generally present the worst pattern [5, 6, 10, 19, 24, 25]. Moreover, when the least differentiated component is analysed, the extension of the area to be considered is not yet defined, thus increasing the amount of the observer bias [25, 27, 28].

Considering the above-mentioned controversies, better standardisation of the grading criteria is surely needed. Recently, Ueno *et al*. [15] introduced a new grading system for CRC, based on PDCs counting (PDCs-G), which seems to reduce the inter-observer variability and to correlate better with CRC patients prognosis. Using this new grading system, Barresi *et al*. [9, 11, 17, 18] confirm and strengthen the idea that PDCs-G guarantees a less biased interpretation, being a more informative parameter regarding the outcome of CRC patients, compared to the conventional histological grading system (WHO grade) or even the TNM stage.

In addition, PDCs-G can be evaluated on HE-stained slides without using IHC, thus making them easier to evaluate as well as cheaper [29]. In contrast to the PDCs evaluation, for the accurate assessment of other significant morphological prognostic factors, such as lymphovascular invasion (LVI), tumour budding (TB), nodal micrometastases, or host response against tumour growth (tumour inflammatory infiltrate), IHC stains or multilevel cuts are required, thus being time-consuming and limiting their utility in daily practice [25, 27, 28].

In this study, using only HE-stained slides, we assessed the conventional adenocarcinomas according to the WHO grade, on one hand, and to the new grading system based on PDCs counting, on the other. We found poor concordance between the two systems of grading. By WHO grade, we noticed that most tumours, 55 cases (80%), were classified as moderately differentiated adenocarcinomas (G2), six cases (9%) as well differentiated CRC (G1), eight cases (11%) as poorly differentiated CRC (G3), and none of the identified cases was graded G4. After the reclassification based on the new grading scheme, we noticed a more uniform distribution of the cases: 11 cases (16%) PDCs-G1, 31 cases (45%) PDCs-G2, and 27 cases (39%) PDCs-G3.

Our results show that PDCs-G presented more important correlations with other clinicopathological parameters, as compared to WHO grade, with evidence that the PDCs-G represents an independent prognostic indicator in CRC patients, associated with other unfavourable parameters such as advanced stages of the disease, LNM+, LVI+, PNI+, and infiltrative tumour border configuration.

Among the morphological factors investigated lately, in terms of potential value for the improvement of the diagnosis of CRC, there is also TB. Although PDCs and TB present similar morphology, they are two different entities [17]. PDCs must be distinguished from TB, which is defined as a single isolated malignant cell or a small group of fewer than five cells [17, 18, 27, 28]. TB is classified as intratumoural when identified within the tumour stroma, and peritumoral when seen at the invasion front of a tumour [27, 29]. The International Tumour Budding Consensus Conference (ITBCC) established that TB is a prognostic factor that is necessary in the histopathological report [30]. Tumour budding as an aggressiveness marker has been associated with poor outcomes in CRC patients [31, 32, 33]. Based on their similar morphology, on the presence of TB and PDCs in the same tumour, and on the evidence of epithelial-to-mesenchymal transition (EMT) in both, it was suggested that PDCs are the possible result of the sequential transformation of TB [27, 29, 31, 32]. TB are difficult to identify on HE-stained slides when a dense inflammatory infiltrate is present in the peritumoral stroma, which could hide the buds, or when reactive mesenchymal cells and peritumoral desmoplastic tissue surrounds isolated cancer cells [28, 29, 31]. Therefore, the use of immunostains (CK) is mandatory for their correct identification in these situations [29, 34, 35, 36]. By contrast, the identification of PDCs does not require the use of immunostaining, PDCs being larger groups of cells, more easily recognisable on HE-stained slides [11, 15, 16, 17, 18, 28].

The presence of PDCs is fairly well correlated with the depth of invasion in the submucosa in early invasive CRC (pT1), particularly when the sub-mucosal invasion is more than 1 mm [7, 11, 37]. Moreover, the PDCs-G has proven to be a strong parameter for stratifying the risk of LNM in early invasive CRC [7, 37]. The prediction of lymph node involvement was more important in the PDCs grading system compared to conventional grading, and this was highlighted in several studies [9, 17, 18, 28, 37, 38, 39]. In a study on 3243 cases of CRC, Ueno *et al*. showed that the incidence of LNM is higher in PDCs-positive tumours compared with PDCs-negative tumours [16]. The most significant risk factors for the presence of LNM mentioned in the literature are the LVI, followed by PDCs-G [9, 11, 16].

Although it would be very useful, few published studies have focused on the PDC grading system on preoperative biopsies [40, 41]. However, it has been shown that the numbers of PDCs on preoperative biopsies are directly correlated with LNM and the depth of tumour invasion when the pathologic stage was established on the whole surgical resection specimen [40]. In addition, high numbers of PDCs in preoperative biopsy specimens are correlated with other parameters associated with an unfavourable outcome in resection specimens, like the configuration of tumour borders, lymphovascular invasion, and TB [41]. The inter observer agreement in the assessment of PDCs-G on biopsy is higher than using the conventional grading system [41]. However, PDCs assessment is more difficult in preoperative biopsies due to the difficulties of PDCs evaluation in the presence of ulceration, necrosis, inflammation, high tissue fragmentation, and tangentially sectioned glands [29, 37]. In addition, the number of PDCs on biopsies could be underestimated because samples are taken from the superficial part of the tumour, which does not contain the invasion front, where PDCs are more numerous [40, 41].

A possible shortcoming of PDCs-G is represented by the unclear definition of the PDCs in mucinous adenocarcinomas. In this study, we identified two cases that were mucinous adenocarcinomas, and another 16 cases that presented a mucinous component. Due to the divergences in grading the mucinous adenocarcinoma of the colon, we did not classify our cases with any of the two grading systems (WHO grade and PDCs-G). The histological grading of mucinous adenocarcinomas is still an unresolved problem. In the WHO 2000 classification of tumours of the digestive system, mucinous adenocarcinomas, by convention, were considered poorly differentiated (G3) tumours. According to the WHO 2010 criteria, mucinous adenocarcinomas should not be graded; for such cases, microsatellite instability status is more relevant as a prognostic

factor, with evidence that many mucinous adenocarcinomas which were MSI-H behaved as low-grade lesions [6]. Importantly, PDCs-G could be assessed in this type of carcinoma only if the PDCs are identified in the areas with minimal extracellular mucin, at the invasive front of the tumour [9, 42, 43, 44, 45]. This problem of grading mucinous adenocarcinomas using PDCs-G occurs because there is no clear definition for clusters in the presence of mucin in the initial paper [15], which proposed this classification system for CRC.

The major problem for adequate grading using PDCs counting is the distinction of PDCs from tangentially cut glands or fragmented tumour glands within necrosis and the identification of PDCs when the inflammatory infiltrate surrounds the glands [29, 46, 47]. These circumstances determine discordances in PDCs counting and increase the variation in PDCs assessment [48], but the criteria for PDCs identification would be their marked cytological atypia as compared to normal glands. Another source of error in PDCs assessment is the presence of mucin secretion, which could be misinterpreted as glandular lumina and, as a consequence, the clusters in the assessed area would not be counted [9, 11]. On the other hand, the clusters could be erroneously interpreted as intravascular tumour emboli, when there are clear spaces surround them, or vice-versa [9]. To differentiate the intravascular emboli from PDCs, it is necessary to perform IHC staining (CD31, CD34, D2-40) [35, 36, 45, 46]. Similar issues are found in the micropapillary variant of CRC, as a result of reversed cell polarity of the cancer cells, which present secretory activity at the cluster-stroma interface [49, 50, 51]. Barresi *et al*. [51] considered that the micropapillary pattern and PDCs may represent the same phenomenon in CRC. To strengthen this hypothesis, they demonstrated a reversed pattern of MUC1 expression in PDCs, similar to the micropapillary CRC.

Study limitations

The study was performed on a small number of cases, and we do not have enough follow-up data on the patients. A greater number of cases would bring confirmation of our results and their follow-up would favour the identification of possible correlations with short- and long-term survival outcomes.

Conclusions

We have shown that tumour grading based on poorly differentiated clusters counting (PDCs-G) significantly correlates with clinicomorphological parameters with known unfavourable prognostic value, especially with the depth of tumour invasion (pT3-4), advanced stages of the disease, nodal metastases (LNM), lymphatic vessel invasion (LVI), perineural invasion (PNI), and infiltrative tumour border configuration. We clearly showed that the novel grading system of CRC, based on PDCs counting, represents an independent predictor for LVI and LNM positivity, factors known to be associated with poor prognosis.

PDCs-G may be considered, along with other clinicopathological parameters, a promising prognostic factor for the management of patients with CRC and should be included in pathological reports, but it still needs standardisation and further validation.

Acknowledgments

This paper is supported by the project Multicentre study on the use of a robotic approach in order to correlate a decrease of circulating adipokine level in obesity with the risk of pelvic cancer POS CCE – ID 1846/Code SMIS 48478 – No. 669/01.09.2014.

The authors declare no conflict of interest.

References

1. Fitzmaurice C, Allen C, Barber RM, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study Global Burden. JAMA Oncol 2017; 3: 524-548.
2. Arnold M, Sierra MS, Laversanne M, et al. Global patterns and trends in colorectal cancer incidence and mortality. Gut 2017; 66: 683-691.
3. Brierley JD, Gospodarowicz MK, Wittekind C, et al. Digestive System Tumors. Colon and Rectum. TNM Classification of Malignant Tumors. Wiley Blackwell (8th ed.). Oxford, UK 2017; 73-77.
4. Amin MB, Edge SB, Greene FL, et al. AJCC Cancer Staging Manual. 8th ed. Springer, New York 2017; 252-254.
5. Loughrey M, Quirke P, Shepherd N, et al. Standards and datasets for reporting cancers Dataset for histopathological reporting of colorectal cancer. The Royal College of Pathologists, London 2018; G049: 19-20.
6. Hamilton SR, Bosman FT, Boffetta P, et al. Carcinoma of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, Theise ND (eds.). WHO Classification of Tumors of the Digestive System (4th ed.). IARC Press, Lyon 2010; 137-138.
7. Ueno H, Hase K, Hashiguchi Y, et al. Novel risk factors for lymph node metastasis in early invasive colorectal cancer: a multi-institution pathology review. J Gastroenterol 2014; 49: 1314-1323.
8. Ueno H, Hashiguchi Y, Kajiwara Y, et al. Proposed objective criteria for 'grade 3' in early invasive colorectal cancer. Am J Clin Pathol 2010; 134: 312-322.
9. Barresi V, Bonetti LR, Ieni A, et al. Histological grading in colorectal cancer: new insights and perspectives. Histol Histopathol 2015; 30: 1059-1067.
10. RCPA (Royal College of Pathologists of Australasia) Colorectal Cancer Structured Reporting Protocol (3rd ed.). RCPA, Surry Hills, NSW 2016; 23-31.
11. Barresi V, Reggiani Bonetti L, Branca G, et al. Colorectal carcinoma grading by quantifying poorly differentiated cell clus-

ters is more reproducible and provides more robust prognostic information than conventional grading. Virchows Arch 2012; 461: 621-628.
12. Reggiani Bonetti L, Lionti S, Domati F, et al. Histological grading based on poorly differentiated clusters is predictive of tumor response and clinical outcome in rectal carcinoma treated with neoadjuvant chemoradiotherapy. Histopathology 2017; 71: 393-405.
13. Chandler I, Houlston RS. Interobserver agreement in grading of colorectal cancers-findings from a nationwide web-based survey of histopathologists. Histopathology 2008; 52: 494-499.
14. Jass JR, O'Brien MJ, Riddell RH, et al. Recommendations for the reporting of surgically resected specimens of colorectal carcinoma. Virchows Arch 2007; 450: 1-13.
15. Ueno H, Kajiwara Y, Shimazaki H, et al. New criteria for histologic grading of colorectal cancer. Am J Surg Pathol 2012; 36: 193-201.
16. Ueno H, Hase K, Hashiguchi Y, et al. Site-specific tumor grading system in colorectal cancer: Multicenter pathologic review of the value of quantifying poorly differentiated clusters. Am J Surg Pathol 2014; 38: 197-204.
17. Reggiani Bonetti L, Barresi V, Bettelli S, et al. Poorly differentiated clusters (PDC) in colorectal cancer: What is and ought to be known. Diagn Pathol 2016; 22: 11-31.
18. Barresi V, Reggiani Bonetti L, Ieni A, et al. Poorly differentiated clusters: clinical impact in colorectal cancer. Clin Colorectal Cancer 2017; 16: 9-15.
19. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology 2007; 50: 113-130.
20. Sagaert X. Prognostic biomarkers in colorectal cancer: Where do we stand? Virchows Archiv 2014; 464: 379-391.
21. Morson B, Dawson I, Spriggs A, Jones F. Gastrointestinal pathology. 2nd ed. Oxford, Blackwell Scientific Publications 1979; 551-610.
22. Morson B, Sobin L. Histological typing of intestinal tumors. Geneva, World Health Organization 1976; 13-38.
23. Shepherd NA, Saraga EP, Love SB, et al. Prognostic factors in colonic cancer. Histopathology 1989; 14: 613-620.
24. Hamilton SR, Vogelstein B, Kudo S, et al. Carcinoma of the colon and rectum. In: Hamilton SR, Aaltonen LA (eds.). Pathology and Genetics of Tumors of the Digestive System. IARC. Lyon, France 2000; 105-119.
25. College of American Pathologists (CAP). Protocol for Examination of Specimens From Patients With Primary Carcinoma of the Colon and Rectum. Version: ColoRectum 4.0.1.0. CAP 2017; 12-16.
26. Kuijpers CJ, Sluijter CE, Von der Thüsen JH, et al. Interlaboratory variability in the histologic grading of colorectal adenocarcinomas in a nationwide cohort. Am J Surg Pathol 2016; 40: 1100-1108.
27. Zlobec I, Lugli A. Prognostic and predictive factors in colorectal cancer. Postgrad Med J 2008; 8: 403-411.
28. Kim JW, Shin MK, Kim BC. Clinicopathologic impacts of poorly differentiated cluster-based grading system in colorectal carcinoma. J Korean Med Sci 2015; 30: 16-23.
29. Dema A, Taban S, Jurescu A, et al. Paving the way to tumor budding assessment using digital pathology: a pilot study in Timisoara City (Romania). Rom J Morphol Embryol 2018; 59: 703-715.
30. Lugli A, Kirsch R, Ajioka Y, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol 2017; 30: 1299-1311.
31. Zlobec I, Lugli A. Epithelial mesenchymal transition and tumor budding in aggressive colorectal cancer: tumor budding as oncotarget. Oncotarget 2010; 1: 651-661.
32. Ueno H, Shinto E, Kajiwara Y, et al. Prognostic impact of histological categorization of epithelial-mesenchymal transition in colorectal cancer. Br J Cancer 2014; 111: 2082-2090.
33. Compton CC, Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000; 124: 979-994.
34. Karamitopoulou E, Zlobec I, Kölzer V, et al. Proposal for a 10-high-power-fields scoring method for the assessment of tumor budding in colorectal cancer. Mod Pathol 2013; 26: 295-301.
35. Satoh K, Nimura S, Aoki M, et al. Tumor budding in colorectal carcinoma assessed by cytokeratin immunostaining and budding areas: Possible involvement of c-Met. Cancer Sci 2014; 105: 1487-1495.
36. Kai K, Aishima S, Aoki S, et al. Cytokeratin immunohistochemistry improves interobserver variability between unskilled pathologists in the evaluation of tumor budding in T1 colorectal cancer. Pathol Int 2016; 66: 75-82.
37. Barresi V, Branca G, Ieni A, et al. Poorly differentiated clusters (PDCs) as a novel histological predictor of nodal metastases in pT1 colorectal cancer. Virchows Arch 2014; 464: 655-662.
38. Kinoshita O, Kishimoto M, Murayama Y, et al. The number of metastatic lymph nodes exhibiting poorly differentiated clusters predicts survival in patients with pStage III colorectal cancer. Int J Colorectal Dis 2016; 31: 283-290.
39. Song BR, Xiao CC, Wu ZK. Predictors of lymph node metastasis and prognosis in pT1 colorectal cancer patients with signet-ring cell and mucinous adenocarcinomas. Cell Physiol Biochem 2017; 41: 1753-1765.
40. Barresi V, Bonetti LR, Ieni A, et al. Histologic grading based on counting poorly differentiated clusters in preoperative biopsy predicts nodal involvement and pTNM stage in colorectal cancer patients. Hum Pathol 2014; 45: 268-275.
41. Barresi V, Tuccari G. Colorectal carcinoma grading quantified by counting poorly differentiated clusters: is it feasible on endoscopic biopsies? Am J Surg Pathol 2013; 37: 943-945.
42. Park JS, Huh JW, Park YA, et al. Prognostic comparison between mucinous and nonmucinous adenocarcinoma in colorectal cancer. Medicine (Baltimore) 2015; 94: e658.
43. Kinoshita O, Kishimoto M, Murayama Y, et al. 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 2015; 13: 140.
44. Marzouk O, Schofield J. Review of histopathological and molecular prognostic features in colorectal cancer. Cancers (Basel) 2011; 3: 2767-2810.
45. Barresi V, Reggiani Bonetti L, Ieni A, et al. Prognostic significance of grading based on the counting of poorly differentiated clusters in colorectal mucinous adenocarcinoma. Hum Pathol 2015; 46: 1722-1729.
46. Barresi V, Reggiani Bonetti L, Ieni A, et al. Histologic prognostic markers in stage IIA colorectal cancer: a comparative study. Scand J Gastroenterol 2016; 51: 314-320.
47. Yang M, Rehman AU, Zuo C, et al. A novel histologic grading scheme based on poorly differentiated clusters is applicable to treated rectal cancer and is associated with established histopathological prognosticators. Cancer Med 2016; 5: 1510-1518.
48. Maguire A, Sheahan K. Controversies in the pathological assessment of colorectal cancer. World J Gastroenterol 2014; 20: 9850-9861.
49. Hong M, Kim JW, Shin MK, et al. Poorly Differentiated clusters in colorectal adenocarcinomas share biological similarities with micropapillary patterns as well as tumor buds. J Korean Med Sci 2017; 32: 1595-1602.
50. Zhang S, Zhang D, Yang Z, et al. Tumor budding, micropapillary pattern, and polyploidy giant cancer cells in colorectal

cancer: current status and future prospects. Stem Cells Int 2016; 2016: 4810734.
51. Barresi V, Branca G, Vitarelli E, et al. Micropapillary pattern and poorly differentiated clusters represent the same biological phenomenon in colorectal cancer: a proposal for a change in terminology. Am J Clin Pathol 2014; 142: 375-383.

Address for correspondence

Sorina Tăban MD, PhD
Associate Professor, Department of Microscopic Morphology – Pathology
"Victor Babeş" University of Medicine and Pharmacy
2 Eftimie Murgu Square, 300041
Timisoara, Romania
phone/fax: +40256-204 476, int. 1446
e-mail: sorinataban@yahoo.com