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Original contribution

Histologic grading based on counting poorly differentiated clusters in preoperative biopsy predicts nodal involvement and pTNM stage in colorectal cancer patients☆

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Summary Histologic grading is commonly assessed in colorectal cancer preoperative biopsies. Nevertheless, its clinical impact is limited by low interobserver reproducibility and poor concordance with grading found in the final resection specimen. In the present study, we aimed to investigate the reproducibility, accuracy, and predictive value on lymph node status or pTNM stage of a novel grading system based on the number of poorly differentiated clusters in colorectal cancer preoperative endoscopic biopsies. Grading based on counting poorly differentiated clusters was assessed in 163 colorectal cancer endoscopic biopsies and corresponding surgical specimens. With this system, 152 biopsies could be graded with good interobserver agreement ($\kappa = 0.735$). In comparison with the surgical specimens, 75% of colorectal cancers were correctly graded in the biopsy, and 81% of poorly differentiated colorectal cancers were identified at initial biopsy. High poorly differentiated clusters grade in the biopsy was significantly associated with nodal metastasis, high pTNM stage ($P < .0001$), or histologic features suggestive of more aggressive behavior (tumor budding, perineural invasion, vascular invasion, and infiltrating tumor border) in the surgical specimen. Furthermore, this system identified colorectal cancer with nodal involvement or high pTNM stage with a 78% positive predictive value and 71% and 69% negative predictive values, respectively. Our findings suggest that a grading system based on the quantification of poorly differentiated clusters is feasible in most colorectal cancer endoscopic biopsies. In view of its good reproducibility, accuracy, and predictive value on the anatomical extent of the disease, it may be taken into account for decision-making in colorectal cancer treatment.

1. Introduction

Colorectal carcinoma (CRC) is one of the most common malignancies in Western countries. At present, the pathologic (p) TNM stage, established in accordance to the International Union Against Cancer [1] and the American

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Joint Committee on Cancer [2] staging classifications, is regarded as the main predictor of outcome of this tumor [3] and is taken into account as a basis for therapeutic management. Nonetheless, several other histologic features have received attention as prognostic factors for this neoplasia [4]. Among these, tumor histologic grading is commonly described in the pathologic report of surgically resected tumors, due to its supposed prognostic value on the clinical course of CRC [5-7].

According to the World Health Organization (WHO) classification of tumors of the digestive system [8,9], histologic grade of CRC is defined by considering the percentage of the tumor showing formation of gland-like structures: well-differentiated (grade 1) CRCs exhibit glandular structures in more than 95% of the tumor; moderately differentiated (grade 2), in 50% to 95%; poorly differentiated (grade 3), in 5% to 50%; and undifferentiated (grade 4), in less than 5% [8]. In addition, in the fourth edition of the WHO classification [9], it has been proposed to group grades 1 and 2 as "low-grade" and grades 3 and 4 as "high-grade" CRCs. Although widely used, this grading system suffers from a significant degree of interobserver variability [10-12], which limits its utility for prognostic purposes [12], due to the lack an objective method to assess the amount of glandular component.

With the need to standardize the criteria for histologic grading of CRC, a novel grading system based on the count of cancer clusters composed of greater than or equal to 5 cancer cells and lacking a gland-like structure (poorly differentiated clusters) was recently proposed [13]. There is evidence that grading based on poorly differentiated clusters (PDCs) is more reproducible and provides more significant prognostic information than grading assessed by the percentage of glandular component in CRC surgical specimens [13,14].

In routine practice, histologic grading is also assessed on preoperative endoscopic biopsies of CRC. Nevertheless, serious concerns exist about the clinical relevance of presurgical grading [11,15,16], due to its low interobserver reproducibility and poor concordance with grading found in the final resection specimen [11,17]. In a recent Letter to the Editor [18], we suggested that the histologic grading based on the PDC count may also be performed on CRC endoscopic biopsy. Hence, in the present study, we aimed to investigate the interobserver reproducibility of this grading system in preoperative endoscopic biopsy and its agreement with grading assessed in the final resection specimen as well as its clinical relevance in terms of predictive value on lymph node status or pTNM stage of the tumor.

2. Materials and methods

All procedures were performed in compliance with relevant laws and institutional guideline and approved by the local institutional committee of Policlinic G. Martino, Messina, Italy.

The design and main findings of the study are illustrated in Fig. 1. In detail, 163 consecutive endoscopic preoperative biopsies and corresponding surgical specimens of CRC (74 male and 89 female patients; age range, 43-90 years; mean age, 71.42 years) were initially selected from the files of our institutions and included in the study. Selection was based on the review of the histopathologic records to have a comparable number of cases with presence or absence of nodal or metastatic disease. In addition, only those CRCs with 12 or more regional lymph nodes retrieved from the perivisceral adipose tissue of the surgical specimens were considered. According to their localization, the tumors were subdivided into 3 groups: CRCs located in the right colon, including cecum, ascending, and transverse colon; in the left colon, including descending and sigmoid colon; and in the rectum. None of the patients had received neoadjuvant therapy for their tumor.

During the assessment of grading on preoperative biopsies, 11 cases were excluded as it was impossible to distinguish and count PDCs due to excessive fragmentation of the specimens, presence of extensive necrosis, or thermal electrocoagulation–induced cytological artifacts. Hence, the final cohort in the study comprised 152 CRCs (68 female and 84 male patients; age range, 43-90 years; mean age, 70.82 years). Of these, 42 cases were localized in the right colon, 48 in the left colon, and 60 in the rectum, whereas the number of retrieved and analyzed nodes ranged between 12 and 79 (median, 22).

All cases had been formalin fixed and paraffin embedded for the histologic evaluation with hematoxylin and eosin (H&E) stain. In the surgical specimens, pathologic staging had been performed according to the TNM classification system [9]. The number of metastatic nodes had been recorded in all the N1-N2 CRCs. In addition, for each case, the histologic grade according to the WHO 2010 criteria [9], cancer growth (expanding versus infiltrative), lymphovascular invasion, perineural invasion, and tumor budding were assessed. *Tumor budding* was defined as isolated single cancer cells or clusters of cells composed of less than 5 elements in the stroma of the actively invasive margin of the tumor. After choosing 1 field where the budding was the most intensive, a budding count was made under magnification ×200, with a count of less than 5 foci considered negative, and a count of 5 or more as positive [19].

2.1. Histologic grading

For each case, histologic grading based on PDC counting was performed on H&E-stained sections of the preoperative biopsy and corresponding surgical specimen. At least 3 biopsies had been taken for each patient (range, 3-6). In addition, most biopsy specimens had been sectioned at 3 levels, and all levels were examined for PDC count. Then the