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New Biomarkers for T1 Colorectal Tumors Management: Contribution of Immune Component Assessment and Immunoscore Testing

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Abstract

Endoscopic treatment for T1 Colorectal Cancers (CRC) generates a risk of metastasis in the draining lymph nodes that have been left in place, and of recurrence after Local Resection (LR). Depending on the risk of Lymph Node Metastasis (LNM) determined by the pathologic assessment, approximately 70% of patients with T1 CRC are classified as high risk, whereas postsurgical pathologic results demonstrate that around 20% of these patients actually have LNM. This highlights the limited diagnostic ability of post-endoscopic pathological examination to predict LNM. There is a need for additional biomarkers to allow robust detection of high-risk patients with T1 tumors, minimizing secondary radical surgery, reducing patient complications, physical burdens, and associated health care costs. The objective of this review is to provide an update on the latest advances in predictive biomarkers for LNM of T1 colorectal tumors. A particular focus will be made on the immune component assessment contribution and Immunoscore test.

Keywords: Colorectal neoplasms; Lymph nodes; Neoplasm metastasis; Risk factors; Endoscopic submucosal dissection; Immunoscore

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Introduction

Colorectal Cancer (CRC) is the third most common cancer diagnosed in both men and women. It was the second cause of cancer-related death worldwide in 2020 [1], with colon cancer accounting for approximately 70% of CRC. Colonoscopy screening and surveillance programs has increased the early CRC detection rate, improving prognosis of CRC patients. Therapeutic decision in colorectal cancer is mostly based on the UICC/AJCC - Tumor-Node-Metastasis (TNM) staging system. Focusing particularly on recent studies, this review aimed to investigate current CRC T1 tumors management and new predicting factors of Lymph Node Metastasis (LNM). It further considers whether the treatment and follow-up strategy for T1 CRC should be selected according to intratumor immune infiltrate assessment.

Current management of T1 colorectal tumors

A T1 colorectal cancer is defined as a tumor invading the submucosa layer but do not spread beyond it [2]. The frequency of T1 tumors among all CRC is approximately 15%, this percentage is increasing due to ongoing nationwide screening programs [3]. The progress made over the past two decades in therapeutic endoscopy now makes it possible to locally resect these T1 colorectal cancers, either by Endoscopic Mucosal Resection (EMR) or by Endoscopic Submucosal Dissection (ESD) [4-6].

T1 colorectal tumors are heterogeneous in clinical presentation and prognosis. Endoscopically removed sessile malignant polyps have a significantly greater incidence of adverse outcomes than

pedunculated malignant polyps [5,6]. According to the ESGE guidelines (https://doi.org/10.1055/a-1811-7025), for a pedunculated polyp with a pT1 carcinoma confined to the head, neck, and stalk (Haggitt 1-3) or a sessile/flat polyp with a pT1 carcinoma with submucosal invasion below 1000 μm, endoscopic resection with proper follow-up is enough, provided that no other unfavorable factors are present. Conversely, the presence of any post-endoscopic pathological factor in a pT1 carcinoma requires an additional surgical resection with lymph node dissection in patients with average operative risk [4]. Regarding the risk of Lymph Node Metastasis (LNM), T1 CRC tumors are divided into high- or low-risk tumors. The high-risk factors for LNM, based on the post-endoscopic pathological examination, includes: Positive vertical margins, poor tumor differentiation, presence of vascular or lymphatic invasion, deep submucosal invasion (>1000 μm) and high-grade tumor budding [7,8]. Using these criteria, approximately 50% to 70% of patients with T1 CRC are classified as high risk, however, post-surgery pathology results suggest that only 8% to 16% of these patients have LNM and hence, many patients without LNM undergo surgery. This unnecessary use of surgery has become a major issue [9]. Finding positive horizontal resection margins constitutes only a risk for local recurrence and can be managed by excision repetition or local surveillance.

Endoscopic treatment methods consist of polypectomy, endoscopic mucosal resection, or endoscopic submucosal dissection. Endoscopic treatment makes it possible to avoid surgery, which has much higher morbidity and mortality than endoscopic techniques, with equally satisfactory oncological results whereas the benefit of secondary oncological surgery in terms of disease-free survival is not well established [10]. Despite technical advances, surgery for colorectal cancer is still marked by a mortality of 2% to 5%. Incidence of anastomotic leakage ranges from 1% to 4%, and cardiorespiratory complications are particularly common [11]. An overall rate of morbidity approaching 30% is observed with digestive, urological, and sexual functional sequelae. In addition, the risk of a permanent stoma in rectal surgery significantly influences the patient's quality of life [12-14].

Organ preservation (no secondary surgery) could be offered to a larger number of patients with T1 CRC presenting pejorative histological criteria if a biomarker would demonstrate the possibility to predict the absence of concomitant LNM and tumor recurrence. New classification systems are required to determine the risk of LNM in T1 CRC patients to reduce the current likelihood of overtreatment, while not hampering the oncological safety.

New classification systems; from bench to beside

Current studies using different approaches have investigated predictors of LNM in T1 CRC beyond the classical histopathologic parameters.

Assessment of the histologic features using Artificial Intelligence (AI)

In recent years, AI has been introduced into health care. Kudo et al. [15] created an artificial neural network model which is a nonlinear adaptive dynamic system that has many processing units that can simulate biological nerve structure. This predictive model was developed using 8 factors: Patients' age, sex, tumor size, location, morphology, lymphatic invasion, vascular invasion, and histologic grade. The algorithm showed higher discriminating power than the current US or Japanese guidelines to predict LNM in patients with T1 CRCs. Song et al. [16] developed an AI program by applying a deep learning technique on hematoxylin and eosin -stained endoscopic resection specimens without manual-pixel-level annotation for predicting LNM in T1 CRC. They compared the predictive performance of their model with that of JSCCR guidelines using a test set (n=80). The model safely avoided 15.1% of unnecessary surgeries than prediction using the current Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines. In patients with SM invasion depth of 1000 μm to 2000 μm, the AI model avoided 16.1% of unnecessary surgeries than using JSCCR guidelines. Takamatsu et al. [17], trained Convolutional Neural Networks (CNN) to extract cancer tile images from whole-slide images, then re-labeled them with LNM status for re-training. Statistical parameters of the tile images based on the probability of primary endpoints were assembled to predict LNM in 43 cases with random forest algorithm and defined its predictive value as random forest score. They evaluated the performance for both training and validation datasets with area under the receiver operating characteristic curves (AUC). The accuracy for classifying cancer tiles was 0.980, and the accuracy for classifying tiles that were LNM-positive or LNM-negative was 0.740. The AUCs of the prediction models in the training and validation sets were 0.971 and 0.760, respectively. Overall, the application of machine-learning techniques including neural networks has expanded in the field of Medicine. Most of these models utilize CNN for classifying histologic images because of their strong and stable tissue classification ability. One concern, however, is the lack of clarity regarding the relationship between histologic features and the decision process of CNN.

Liquid biopsy: mRNAs, miRNA, and ctDNA

In recent years, exosomes and extracellular vesicles have received increasing attention as promising cancer biomarkers in liquid biopsy settings. Circulating tumor DNA (ctDNA) is a noninvasive biomarker for molecular residual disease and relapse detection after treatments including surgical and endoscopic resection of solid tumors. Wada et al. [18] identified their previously reported transcriptomic biomarkers (miRNAs and mRNAs) with the use of liquid biopsy methods and evaluated the predictive value of this transcriptomic panel for the noninvasive identification of LNM in patients with high-risk T1 CRC. They established a new risk model of transcriptome profiles based on a panel of 4 miRNAs (miR-181b, miR-193b-3p, miR-195-5p, and miR-411-5p) and 5 mRNAs (AMT, FOXA1, MMP1, MMP9, and PIGR) from blood liquid biopsy, that can reliably predict LNM in patients with T1 CRC. Identification of LNM was notably superior when they used all 4 miRNAs and 5 mRNAs to establish a combined transcriptomic panel (AUC= 0.86; 95% CI, 0.72-0.94). The DENEB study is a prospective study for patients with pT1 CRC who underwent complete local resection and were scheduled for additional intestinal resection with lymph node dissection based on the standard pathologic risk stratification criteria for LNM [19]. Based on the CIRCULATE-Japan platform, the DENEB study assessed the ability of ctDNA to help predict the risk of LNM in patients diagnosed with pT1 CRC after complete local resection, compared with the standard pathological criteria. The study is still in progress. Using The Cancer Genome Atlas dataset as the discovery cohort, Ozawa et al. established a panel of 5 miRNAs (MIR32, MIR181b-1, MIR193b, MIR195, and MIR411) which can be successfully applied to even tiny biopsy samples [20]. The miRNA signature significantly detected LNM in the training cohort (AUC= 0.83) and, by applying the same model and the cutoff thresholds in a large, independent validation cohort, they validated 5-miRNA signature with AUC= 0.74. In addition to this 5-miRNA signature, they added another two

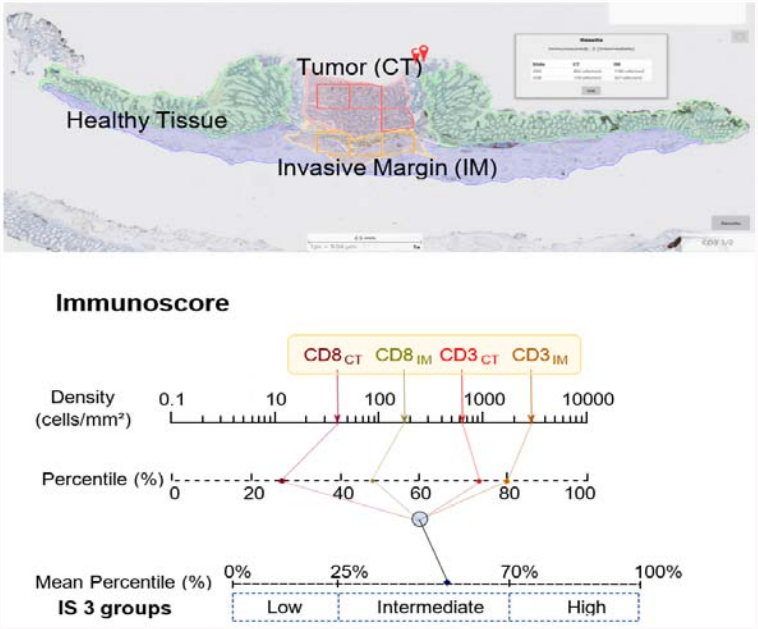

Figure 1: Immunoscore (IS) determination.
Top: Automatic detection of the tumor (red), invasive margin (yellow) and healthy tissue (blue) by a digital pathology software in a T1 colonic cancer. Down: Chart illustrating the IS calculation method: Densities of CD3+ and CD8+ T cells in the tumor and invasive margin are converted into percentile values. The mean percentile of the densities is then calculated to generate IS percentile value, where ISB low, ISB intermediate, and ISB high subgroups are reflected by 0%-25%, >25%-70%, and >70%-100% percentile, respectively.

other risk factors - the degree of lymphatic invasion and tumor depth - for the development of a risk-classification model. This new model, consisting of these 3 factors, was even more superior in detecting LNM (AUC= 0.86). The miRNA-based LNM detective signature, which is superior to currently used clinicopathologic criteria in patients with T1 CRC and the MicroRNA signature can make the discrimination between patients that are true candidates for endoscopic treatment and patients' candidates for radical surgery, and reduce unnecessary treatment and health care burden [20]. Similar results were obtained by Miyazaki et al. with a risk stratification model which includes a panel of 4 miRNAs (miR-181b, miR-193b, miR-195, and miR-411), within the exosomal component with promising biomarker potential in preoperative serum for the prediction of Lymph Node Metastasis (LNM) among these patients. Subsequently, they demonstrate that exosomal miRNAs (AUC= 0.86) are superior cancer biomarkers compared to cell-free miRNAs (AUC= 0.82), and a combination of cell-free serum miRNAs and exosomal miRNAs are superior to individual biomarker panels [21].

Molecular and genetic components

For the past twenty years, we have been witnessing the development of personalized medicine in oncology, based on the genetic and molecular study of tumors. Today, several mutations associated with colorectal cancer are systematically screened for, due to their prognostic and therapeutic impact. Thus, as recommended by the European Group on Tumor Markers [22], the following biomarkers should be studied: The KRAS/NRAS genes, which, in the event of a mutation, are predictive of non-response to anti-EGFR treatment in the metastatic colorectal cancer; the BRAF gene, which, in the event of a mutation, is a factor of poor prognosis; the genes of the MMR system, which, in the event of a mutation, will be responsible for Microsatellite Instability (MSI), a good prognosis factor. Recently, an international consortium published a classification of colorectal cancers based on gene expression data from tumors, called Consensus Molecular Subtypes (CMS) [23]. The work has made it possible to form a consensus on the existence of 4 molecular subtypes. The "MSI immune" type (CMS1; 14% of cases) is defined by "hypermutated" tumors, the majority of which present Microsatellite Instability (MSI) and a strong immune reaction. The "Canonical" type (CMS2; 37% of cases) defined by tumors presenting chromosomal instability and mutations of the APC and TP53 genes. The "Metabolic" type (CMS3; 13% of cases) characterized by tumors, the vast majority of which have a mutation for the KRAS oncogene and an overexpression of metabolic pathways. Finally, the "Mesenchymal" type (CMS4; 23% of cases) categorized as a group with poor prognosis. The tumors show strong angiogenesis, strong stromal infiltration, and activation of the growth factor TGF-β. Expression of mesenchymal-like markers has been shown to correlate with LNM in T1 CRC [24]. More recently, it was reported that CMS4 in T1 tumors is associated with an increased risk for adverse outcome (HR: 3.56, 95% CI, 1.02-12.4, p=0.046), in line with CMS4 prognostic impact observed in patients with advanced CRC [25]. However, the prevalence of CMS4 in T1 CRC is very low, indicating a limited utility in clinical decision-making.

In addition, Kandimalla et al. [26] demonstrated from RNA extraction of T1 colorectal cancers a molecular signature of 8 genes (AMT, MMP9, FOXA1, LYZ, MMP1, C2CD4A, PIGR, RCC1) with a very good performance for detecting LNM with an Area under the Curve (AUC) value of 0.88, a specificity of 0.86 and a sensitivity of 0.79. The robustness of mRNA assay was quite comparable to the miRNA biomarkers that they reported previously [20,26]. Independent prospective study will determine the robustness of this signature for translation into clinical practice. All these elements suggest that the genomic approach could help to predict lymph node risk, whether by looking for mutations from DNA extraction from tumors or by studying gene expression profiles and CMS classification from tumor RNA.

Intratumor immune infiltrate assessment and the immunoscore test

Colorectal cancer presents a polymorphic immune infiltrate, which varies in diversity and intensity from one patient to another. We demonstrated that the immune density of T lymphocytes (CD3+), memory T lymphocytes (CD45RO+) and cytotoxic T lymphocytes (CD8+) in the tumor (CT) and its Invasive Margin (IM) is strongly associated with the prognosis of patients with colorectal cancer [27,28]. The prognostic value of these immunological parameters grouped under the term of "immune contexture", has even proven to be superior to that provided by the AJCC-UICC TNM classification [4]. Given the importance of the immune contexture as major determinant of clinical outcome, a new test called "Immunoscore" has been developed by Pagès and Galon at the European Hospital Georges Pompidou, Paris to facilitate the translation of this investigation in clinical practice. The Immunoscore (IS) is based on the quantification of total CD3+ T lymphocytes and cytotoxic CD8+ T lymphocyte subsets in the tumor (CT) and its Invasion Margin (IM). A specific image analysis module, called Immunoscore Analyzer, has been developed using Developer XD software (Definiens) for the quantification of CD3+ and CD8+ immune populations throughout tumor regions. A scoring system of the IS in three categories has been established ranging from: Immunoscore Low, characterized by a low density of CD3+ and of CD8+ cells in the two tumor regions, Immunoscore Intermediate, and Immunoscore High for tumors presenting a high density of the both cell populations in tumor regions (Figure 1). An analysis of IS analytical performance characteristics showed that the immune assay was robust, reproducible, and repeatable [29]. An international validation study conducted by Pagès et al. [30] on more than 3,500 patients in 13 countries confirmed the major prognostic impact of the Immunoscore in stage I to III colorectal cancers. The IS, is now the first validated digital-pathology-based assay recommended by academic institutions (the 2020 ESMO and Pan-Asian Adapted ESMO Clinical Practice Guidelines) for a prognostic purpose in patients with localized colon cancer [4,31]. A recent sub-analysis [32] from the international validation study of early-stage I proficient Mismatch Repair (pMMR) colon cancer patients (T1-2, N0M0; n=206), revealed that patients with High (33.5%), Int. (51.9%), and Low (14.6%) IS presented with recurrence rates at 5 years of 1.7%, 6.5%, 14%, respectively (unadjusted HR Hi *vs.* Lo=l; 95% CI, 0.01-0.61 P=0.0167). In multivariable analysis, the IS was the parameter with the most important relative contribution to the risk (Chi^2) of recurrence. This indicates that even in stage I patients, considered as very low-risk patients for recurrence, the IS could be useful to predict high-risk Stage I patients. This observation could be extended to the subgroup of T1 colorectal tumors treated by endoscopic resection, to predict lymph node risk and later recurrence. This hypothesis is in accordance with recent publications. In a test cohort of 221 T1 colorectal tumors, intra-tumoral CD8+ cell density was identified as a significant parameter for predicting LNM [33]. Further, a LASSO model incorporating histopathologic parameters and CD3+ and CD8+ densities in the tumor and the invasive margin, showed superior performance compared to conventional Japanese criteria in predicting LNM [33]. Another publication investigated a case-cohort study of 212 patients with non-pedunculated T1 CRC treated by surgical resection. Immune filtration was assessed by tissue microarrays using cores punched in the tumor and the invasive margin. Using a dichotomization of the cohort into two groups with an immunoscore-like method (Low/High: 25e/75e percentile), patient's group with a low score showed a trend toward an increased risk for an adverse outcome (HR: 1.58, 95% CI, 0.88-2.83, p=0.13). This scoring method expressed as a continuous variable did show an increased risk for lower scores (HR: 0.67, 95% CI, 0.63-0.96, p=0.03) [25].

Conclusion, Perspectives

The diagnosis and treatment of CRC have evolved dramatically over the past several decades. Incidence of early CRCs detected is growing. The improvement of endoscopic techniques has raised the possibility of local resection as curative strategy enabling organ preservation in selected patients. The identification of high-risk tumors characteristics for LNM is awaited, because of the limited prognostic ability and the low interobserver agreement in histologic assessment of the risk factors. Novel biomarkers covering the fields of the tumor and its microenvironment have emerged, with varying degrees of accuracy to predict LNM. Strikingly, failure of the in situ immune response, as evaluated with the Immunoscore test, could be a major determinant of tumor progression even in the early stage T1 cancers. Current limitations of the studies are mainly a lack of validation in independent cohorts and in prospective studies for a translation into the clinical practice. Shared decision-making in cancer treatment, especially for organ preservation, is likely to play an increasing role. Novel biomarkers are highly awaited to help the physician and patients to make an informed choice hence improving decision quality.

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