Role of one-step nucleic acid amplification in colorectal cancer lymph node metastases detection

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Abstract
Current histopathological staging procedures in colorectal cancer (CRC) depend on midline division of the lymph nodes (LNs) with one section of hematoxylin and eosin staining. Cancer cells outside this transection line may be missed, which could lead to understaging of Union for International Cancer Control Stage II high-risk patients. The one-step nucleic acid amplification (OSNA) assay has emerged as a rapid molecular diagnostic tool for LN metastases detection. It is a molecular technique that can analyze the entire LN tissue using a reverse transcriptase loop-mediated isothermal amplification reaction to detect tumor-specific cytokeratin 19 mRNA. Our findings suggest that the OSNA assay has a high diagnostic accuracy in detecting metastatic LNs in CRC and a high negative predictive value. OSNA is a standardized, observer-independent technique, which may lead to more accurate staging. It has been suggested that in stage II CRC, the upstaging can reach 25% and these patients can access postoperative adjuvant chemotherapy. Moreover, intraoperative OSNA sentinel node evaluation may allow early CRC to be treated with organ-preserving surgery, while in more advanced-stage disease, a tailored lymphadenectomy can be performed considering the presence of aberrant lymphatic drainage and skip metastases.

Key Words: Colorectal malignancies; One-step nucleic acid amplification; Diagnostic accuracy; Negative predictive value; Upstaging; Organ-sparing surgery; Tailored lymphadenectomy
Core Tip: Our findings suggest that the one-step nucleic acid amplification (OSNA) assay has high diagnostic accuracy and negative predictive value in detecting metastatic lymph nodes in colorectal cancer (CRC). The short turnaround time renders OSNA an attractive intra-operative method. OSNA results in upstaging in about 25% of stage II CRC cases. Moreover, organ-sparing surgery in early CRC and tailored lymphadenectomy, in more advanced cases, can be performed.

INTRODUCTION

Of the gastrointestinal cancers, the colorectal cancer (CRC) is the most represented. Among the indication criteria for chemotherapy, lymph node (LN) positivity (stage III) is the most important[1]. The histopathological study of the LNs is performed on one or at most two sections of each LN with hematoxylin and eosin (HE). Therefore the conventional study presents the possibility of not detecting micro-metastases (MMs) or macro-metastases leading to an "understaging". The high relapse rates (20%–25%) in patients with negative LNs could be due to this "understaging"[2]. Multilevel LN sectioning combined with immunohistochemistry (IHC) can improve the detection rate of small nodal tumor infiltrates [i.e. isolated tumor cells (ITCs) and MMs], although it is a costly and protracted process [3-6].

Tsujimoto et al[7] were the first to describe the one-step nucleic acid amplification (OSNA) assay for detecting LN metastases (LNMs) in patients with breast cancer (BC). Numerous studies have followed which have confirmed the high sensitivity of OSNA in detecting LNMs of breast, gastric and CRCs[8-15]. Other studies[16-18] have underlined the usefulness of the OSNA assay as a complementary tool for diagnosing LNMs and upstaging in histologically node-negative stage II CRC.

The sentinel LN (SLN) is gaining more and more consensus because it allows to perform a more conservative surgery with considerable advantages, when applicable for the patient and for the operating times. Obviously in the early stages of CRCs this could play an important role, allowing to realize, in case of absence of lymph node metastases on SLNs, an organ preserving surgery.

In this review, we analyzed the use of OSNA in detecting LNMs in CRC.

LITERATURE SEARCH

Search strategy

After developing and piloting search terms, MEDLINE, SCOPUS, ClinicalTrials.gov, and Cochrane Database were used to conduct a comprehensive computerized literature search for articles pertaining to OSNA use in detecting LNMs in CRC. Medical subject headings terms and keywords were combined: colorectal malignant, cancer, colorectal tumor, colorectal neoplasm, carcinoma, lymph node metastasis, SLN, one-step nucleic acid amplification, OSNA, cytokeratin 19, CK-19, predictive value, upstaging, organ-sparing surgery, and tailored lymphadenectomy. The electronic search was supplemented by reviewing reference lists of included studies and previous systematic reviews. No time limitation was stipulated for the search, which was last updated December 20, 2021. In addition, we retrieved and cited high-quality references using the Reference Citation Analysis database (https://www.referencecitation-analysis.com/).

Study selection, data extraction, and quality assessment

The retrieval of articles was completed in three consecutive stages. Following reduplication of the sum of collected articles, their titles and abstracts underwent further screening and those deemed ineligible were removed. For duplicates, the most recent or complete publication was chosen. The remaining papers were evaluated in full text. Two reviewers (MB, SV) extracted data in duplicate using a standardized data extraction sheet.
LYMPHATIC DRAINAGE IN CRC

The American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) staging score divides the stages according to how many metastatic lymph nodes are present. Based on the location of the primary tumor, those with a course adjacent to the main vascular branches near the affected colon are considered regional lymph nodes. In particular, starting from the rectum up to the right colon, in addition to the peri-colic lymph nodes, regional lymph nodes are considered, those adjacent to the rectal arteries, the sigmoid arteries, the left colonic artery, the inferior mesenteric artery, the middle colic artery, the right colic artery, and the ileocolic artery[19].

In AJCC/UICC tumor-node-metastasis (TNM) staging system[20-22], patients with no metastatic LNs are N0, cases with one to three metastatic LNs are N1, and cases with more than three positive LNs are N2. Moreover, the N1 category is subdivided into N1a (1 metastatic LN), N1b (2-3 metastatic LNs), and N1c (no regional LNs are positive but there are tumor deposits in the subserosa, mesentery or non-peritonealized pericolic or perirectal/mesorectal tissues), whereas the N2 category is subdivided into N2a (4-6 metastatic LNs) and N2b (7 or more metastatic LNs). The minimum number of examined LNs needed for adequate staging should not be less than 12 to minimize the possibility of stage migration[19, 23-27].

The Japanese Society for Cancer of the Colon and Rectum (JSCCR) staging score classifies the involved LNs based on location and number. This system divides the regional LNs into three groups: main, intermediate and peri-colic. Regional LNs depend on their adjacency to the blood vessels following the primary tumor site. LNs adjacent to the marginal arcade are pericolic nodes, the LNs along the course of the main vessels of the colon are intermediate nodes (sigmoid arteries, the left colonic artery, the inferior mesenteric artery, the right and left middle colic artery, the right colic artery, the ileocolic artery). Lymph nodes located proximal to the origin of the main colonic vascular branches of the inferior and superior mesenteric artery are the main nodes. LNM is classified as N1 if up to 3 peri-colic or intermediate LNs are involved, N2 if they are ≥ 4, N3 when the main LNs are involved[28, 29].

ITCs and MMs

When single or few tumor cells smaller than 0.2 mm are found, these are called ITCs, if instead the deposits have a diameter between 0.2 and 2.0 mm these are called MMs. When ITCs or MMs with HE or IHC are found, they are classified as pN0 (i +), if instead the deposits are diagnosed only by reverse transcriptase polymerase chain reaction (RT-PCR), they are classified as pN0 (mol +)[19,20]. MMs, ITCs, and occult metastasis have been reported in 4.2%, 19.3%, and 5% of patients with stage I and II CRC, respectively, and attracted interest as prognostic factors[30-33].

Tumor deposits

In the literature, tumor deposits (TDs) are defined as foci of tumor separated from the main neoplasm and found in peri-rectal or peri-colonic adipose tissue or in mesocolon in the lymphatic drainage area, in the absence of identifiable LN tissue.

It is postulated that they are produced either by discontinuous dissemination of the tumor or by vascular/perineural dissemination. TDs can be found in 10.2%–22% of CRC cases and it has been suggested that TDs may represent a LN, a vascular structure, or a nerve completely replaced by carcinoma[34].

Several studies[35,36] have shown decreased disease-specific survival and overall survival (OS) in the presence of TDs. Moreover, the survival outcomes worsen when TDs occur concomitantly with LNM. Other studies confirmed this evidence in CRC. It has been suggested that TDs have negative prognostic value but are not sufficiently categorized in the current TNM staging and the number and/or presence of the TD should be added to the number of LNM to define the final N stage creating a specific category for TDs with LNM, which could be called category N2c or N3[37-41].

CLINICAL STAGE OF NODAL METASTASES

Diagnostic imaging

Diagnostic imaging assessment of lymphadenopathy in CRCs is challenging. Individual imaging modalities face specific intrinsic limitations, for example, transrectal ultrasound is operator-dependent, detrimentally affected by a small field of view, and cannot be employed in stenosing or rectal cancers. Computed tomography (CT) is hampered by its low soft tissue contrast resolution which, besides negatively impacting detection, precludes evaluation of fine LN details and therefore must rely only on LN size for assessing lymphadenopathy. Magnetic resonance imaging (MRI) requires long acquisition times and is prone to artifacts in the case of poor patient cooperation. Fluorodeoxyglucose (FDG)-positron emission tomography (PET)/CT and PET/MRI necessitate exposure to radiation, and its yields are influenced by the amount of metabolically avid cells in the affected LNs.
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Size

Lymphadenopathy is also intrinsically challenging. Despite malignant LNs tending to be larger than their benign counterparts, there is wide size superimposition between malignant and benign LNs. In one study that evaluated only LNs ≥ 5 mm, short axis range was 6-12 mm for malignant LNs and 5-13 mm for benign LNs[42,43]. Moreover, the size of metastatic LNs is often at the lower limits of imaging spatial resolution. In a study, median LN short axis was 3.2 mm for malignant LNs and 2.8 mm for benign LNs[44]. Additionally, 30%-94% of metastatic LNs from rectal cancer are < 5 mm in short axis [45-48]. To make things even more challenging, some benign etiologies, especially inflammation, might increase LNs size.

MRI

Due its superior anatomic layout and high soft tissue contrast resolution, MRI can explore LN nature trough size and morphologic criteria. LN size criteria have yielded different results in different studies. However, across studies, the bigger the short axis threshold, the higher the specificity and lower the sensitivity; a 3 mm short axis has been associated with 92% sensitivity, 3% specificity, and 40% accuracy; a short axis threshold of 9 mm has been associated with the opposite trend, giving 8% sensitivity, 100% specificity, and 62% accuracy[42]. Short axis thresholds of 7.2-7.5 mm have reached 32%-87% sensitivity and 70%-94% specificity, with 68% of accuracy[42,43]. However, the most accepted short axis cut off for lymphadenopathy in rectal cancer is 5 mm, yielding sensitivities of 50%-72%, specificities of 46%-60%, and an accuracy of 57%-65[42,46-50].

Beside size, several other MRI criteria can be used, with diffusion weighted imaging (DWI) believed to be one of the most promising tools. However, DWI has proven inadequate for this purpose so far. High b-value DWI is a powerful tool for LN detection. However, DWI and even apparent diffusion coefficient values[43,51,52] are unable to discriminate benign from malignant LNs (accuracy 40%). Therefore, in our practice, we use DWI just to detect all LNs, relegating the differentiation of benign from malignant ones to morphologic, size and/or metabolic criteria.

Chemical shift effect (CSE) refers to a black or bright border outlining organ contours, including LNs. In the case of neoplastic growth in the subcapsular sinus, the resonance frequencies of hydrogen protons in the subcapsular sinus and in the adjacent fat are similar, resulting in the loss of CSE.

Four patterns of LN CSE have been described: continuous and smooth, continuous, discontinuous, and irregular, or absent[44]. Once neoplastic cells have colonized the subcapsular sinus, they easily spread outside of the LN, likely more rapidly than toward the medulla. This results in irregular or obscure LN contours, reported in 60%-65% of malignant LNs and in 16%-20% of benign LNs, leading to sensitivity of 88%, specificity of 23%, and accuracy of 50% in one study. On the other hand, a smooth external contour has been described in 80%-84% of benign LNs and in 34%-40% of malignant LNs[42-44].

The internal structure of LNs may be heterogenous in the settings of metastases; this has been reported in 26%-52% of benign and 54%-91% of malignant LNs, with a sensitivity of 84%, specificity of 51%, and accuracy of 53%; on the other hand, homogeneous internal structure, has been observed in 48%-73% of benign and 8%-46% of malignant LNs[42-44]. The combination of inhomogeneous signal intensity and indistinct/irregular borders has been shown to yield sensitivity, specificity, and accuracy of 56%, 91%, and 77%, respectively[42].

Currently, LN size is the most used criterion to discriminate between malignant and benign LNs on MRI, but given the previously discussed inherent limitations, it is often integrated with the morphologic criteria described above. According to the European Society of Abdominal and Gastrointestinal Radiology (ESGAR) guidelines, a LN is considered metastatic in the case of[33]: Short axis diameter ≥ 9 mm, short axis 5-8 mm plus ≥ 2 morphologic criteria, short axis < 5 mm plus 3 morphologic criteria, or mucinous LN regardless of the size. Morphologic criteria chosen by ESGAR are round shape, irregular borders, and heterogeneous signal.

However, despite all of the above efforts, even MRI, the most promising imaging modality for LN evaluation is still inadequate for the scope. A recent study that explored the staging performance of MRI in rectal cancer, using surgical pathology as a standard of reference, showed that MRI LN status was correctly assigned in 68% of cases, overstaged in 28%, and understaged in 4%. Moreover, only 40% of MRI-positive LN cases were pathologically confirmed[54]. These results are in line with a FDG-PET/MRI study where N status was overstaged by MRI in 22.6% of patients and by PET/MRI in 8% of cases; correct N status was assigned by MRI in 58% of patients and by PET/MRI in 79% of patients[55].

PET

FDG-PET, in addition to structural information, includes the advantage of assessing the metabolic activity of colorectal patient LNs. However, even with metabolic information, sensitivity is limited. A recent meta-analysis including 13 studies published between 2007 and 2019 evaluated the pre-treatment ability of 18F-FDG PET/CT as a staging modality to detect metastatic LNs in CRC[15]. The pooled sensitivity, specificity, positive and negative likelihood ratios were 65%, 75%, 4.57, and 0.37, respectively. Prospective studies have demonstrated higher sensitivity and specificity compared to retrospective studies, and studies with sample sizes greater than 100 and that used a cut off value of
maximum standardized uptake value (SUV) ≤ 2.5 revealed better accuracy. An older meta-analysis of CRC patients found an even lower pooled sensitivity of 42.9% for detecting LN metastasis, but a higher specificity of 87.9%[56,57]. Differences in meta-analysis outcomes are thought to be due to the heterogeneity of baseline patient characteristics and included article methodologies. Regardless of the variances between the two meta-analyses, FDG-PET has limitations in sensitivity[58-61], likely due to a partial volume effect when assessing the SUV of small LNs (< 10 mm), as well as limitations in spatial resolution when differentiating between extension of primary tumor and adjacent positive LNs[62-64]. Specificity on the other hand is limited by false positives seen most often in reactive LNs.

Innovations
Advancements in the imaging evaluation of LNs in CRC are going to happen in the very near future due to innovative scanning technologies such as PET/MRI, which can investigate tumor biology, phenotypes, improve diagnosis, and impact the management of several solid organ malignancies including CRC[55,65-72], innovative radiopharmaceuticals such as fibroblast activation protein inhibitor (FAPI), which is already outperforming FDG in several settings[73,74], and due to the endless possibilities opened by artificial intelligence. Regarding FAPI, a study[74] comparing 68Ga-FAPI and 18F-FDG uptake in 35 patients with gastric, duodenal, and CRCs, showed a significantly higher sensitivity with 68Ga-FAPI PET/CT compared to 18F-FDG PET/CT (79% vs 54%) but an equivalent specificity (82% vs 89%). Artificial intelligence is going to play a major role in diagnostic imaging evaluation of LNs. A recently published metaanalysis, which focused on LN staging in CRC, showed that deep learning and radiomics outperform radiologists, with deep learning also being superior to radiomics. In rectal cancer, on a per patient basis, pooled area under receiver operator characteristic curve was 0.017 for deep learning, 0.808 for radiomics, and 0.727 for radiologists; and sensitivity and specificity were 89% and 94% for deep learning, 78% and 73% for radiomics, and 68% and 70% for radiologists respectively[75].

CONTROVERSIES IN LN DISSECTION IN CRC
Several studies have shown that in more than 80% of cases, the first metastatic LN in CRC is a paracolic LN located 5 cm or less from the tumor[76-81]. Besides this classic lymphatic drainage, aberrant drainage within the regional LNs can exist. Such drainage leads directly to main LN stations near the superior and inferior mesenteric vessels or to colic and paracolic LNs located a significant distance from the tumor. The prevalence of aberrant lymphatic drainage is reportedly up to 20%[82,83]. Drainage of this nature influences the scope of lymphadenectomy since “aberrant” LNs are potential locations for “skip metastases”[76,77,84-86].

In some individual studies, a higher rate of aberrant lymphatic drainage reaching up to 29% has been observed in patients undergoing lymphatic mapping[87]. There are some different points of view on the resection type between East and West. The Japanese concept is partial resection of the bowel according feeding artery (short bowel specimen, long lymph vascular pedicle), and the opposite European concept is wide resection of the bowel such as hemicolecotomy or extended hemicolecotomy.

European Society of Medical Oncology (ESMO) recommends that local excision could be considered in the early colon cancer (CC) Stage 0 (Tis) and in selected T1N0M0 (G1-2, N0). ESMO and National Comprehensive Cancer Network (NCCN) recommend wide surgical resection with a safe margin (ESMO suggests at least 5 cm from the tumor), and en bloc removal of LNs with the feeding arterial arcade (regional nodes). NCCN suggests removing only suspicious LNs that are not contained in the arcade[25,26,88,89].

The best results in terms of prognosis after the introduction of the TME concept in the treatment of rectal cancer led to the Hohenberger et al[90] hypothesis in which surgical dissection according to embryological planes could lead to a similar improvement also in surgery of the. On this hypothesis is based the complete mesocolic excision (CME) associated with the concept of central vascular ligation (CVL) in which the main blood vessels are tied at the origin after a dissection according to the embryological planes (i.e. removing the surgical trunk of Gillot in right-sided CC)[90].

JSCCR provides a detailed description of the extent of surgical lymphadenectomy, based on tumor stage. In brief, JSCCR advocates central (D3) lymphadenectomy in selected T2 and in all T3-T4 cancers, as well as in all N-positive patients.

In the study by West et al[91] the CME with CVL does not show significant differences with the Japanese D3 as regards the quality of the mesocolon surgical plan and the free margins. In the Japanese school, as indicated in the JSCCR guidelines, the longitudinal extension is less important; consequently the number of lymph nodes and the mesenteric surface were lower. Even if the operative pieces in western countries have a greater longitudinal extension than in the Asian ones, the TNM system does not include in its nomenclature the localization of regional lymph nodes. The indications to D3 Lymphadenectomy with CVL in the Western countries are still a subject of debate.
HYSTOPATHOLOGICAL DIAGNOSIS OF LNMs IN CRC

A relevant clinical finding is the fact that up to 30% of patients with CRC diagnosed as pN0 following surgery will die within 5 years due to regional recurrence or distant metastases[87,101-104]. A discussion to establish criteria for defining high-risk stage II patients who could benefit from adjuvant therapy was undertaken by the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer and National Surgical Adjuvant Breast and Bowel Project studies. Presently, the high-risk group, according to ESMO and NCCN treatment standards, comprises patients with T4 tumors (especially T4b), a high grade of histological malignancy, infiltration of vessels and perineural tissue, tumor budding (TB), a small number of removed LNs (< 12), and emergency surgery[101,105,106]. Several studies have identified prognostic genes that may select high-risk patients for adjuvant treatment[107-112], but only a few are routinely used in clinical practice.

Mismatch repair (MMR) genes act in DNA repair pathways. MMR deficiency results from the loss of function of their products (MMR-D), leading to microsatellite instability (MSI). MSI increases CRC risk by increasing tumor mutational burden and the number of tumor-infiltrating lymphocytes (TILs). There are two categories of CRC with MSI: MSI-high (MSI-H) and MSI-low (MSI-L). Instability in more than 30% of the markers as detected by PCR is defined as MSI-H, and alteration in 10%–30% of the markers is considered MSI-L. The MSI-H is associated with a high mutational burden in DNA.

Frameshift mutations can create antigenic epitopes that make MSI-H/MMR-D tumors more immunogenic compared with microsatellite-stable tumors. MSI-induced frameshift mutations produce a significant number of neoantigens. Accordingly, MSI-H/MMR-D tumors manifest a great number of TILs, many of which can be directed against tumor-related neoantigens[107].

Despite this, the most important risk factor is the presence of unidentified LN MMs and macrometastases. Rahbari et al[113] concluded that the presence of LN MMs is associated with poor OS and shorter disease-free survival (DFS) in stage II CRC patients. Therefore, the problem is to identify diagnostic methods that can improve selection based on this criterion in terms of both cost and effectiveness[101,114,115]. The relevant literature shows that examination of only one LN slide using HE staining leaves up to 33% of metastases unidentified. A single slide with HE staining through the center of a node 1 cm in diameter provides information on < 1% of its volume[114-118].

Additional HE histopathologic analyses of serial sections allows for the identification of micrometastatic disease in up to 20% of LNs determined to be negative by standard HE methods[119]. However, performing HE histopathologic analyses of sections can be technically challenging and time consuming, as well as entailing significantly greater cost. Other histopathologic methods utilized for more accurate assessment of the status of the regional LNs, such as IHC using antibodies against human cytokeratin (CK) or RT-PCR, require even more time and incur an even higher cost.

IDENTIFICATION OF AN OPTIMAL mRNA MARKER FOR THE OSNA ASSAY IN CRC

Malignant nodes identifies a number of markers for the OSNA assay[120]. In a study examining 98 candidate mRNA genetic markers, which were from a genome-wide database, comparing an expression frequency in CC. After four sequencing phases, CK19, carcinoembryonic antigen (CEA), and CK20 mRNAs were evaluated using
the OSNA assay. The expression of CK19 mRNA was observed in all pathologically positive LNs; however, CEA and CK20 mRNAs were not found in metastatic nodes.

DIAGNOSTIC PERFORMANCE OF THE OSNA ASSAY IN CRC

A novel technique for pathological examination, OSNA, uses the reverse transcription loop-mediated isothermal amplification method to amplify CK19 mRNA. In contrast to the current routine histopathological examination, it can examine whole LNs and detect metastases in a sufficiently short time (Table 1). A standard curve previously determined with three calibrators containing different CK19 mRNA copy numbers was used to calculate the amount of CK19 mRNA. Positive and negative control samples were used to ensure the quality of the assay.

A limit value of 250 copies/mL of CK19 mRNA copy had been chosen. A value less than 250 copies/mL was considered negative for metastasis, on the contrary, a value ≥ 250 copies/mL was considered positive. Previous studies defined this by the logarithmic midpoint between the maximum value of the CK19 mRNA copy number in non-metastatic patients and minus 2 or 3 standard deviations (SDs) from the average of CK19 mRNA copy number in node-positive patients. These studies also defined the MM threshold between 250 and 4999 CK19 mRNA copies/mL. LNs with 5000 or more mRNA copies/mL were considered macrometastases[7,120,121]. The utility of conventional OSNA as a molecular staging method has been demonstrated for various cancers[9,17,122-124].

Although most of the studies evaluated in this review were prospective in design, none was a randomized controlled trial (Table 1). The studies comparing the diagnostic performance between OSNA and pathological examination for the detection of LNMs in CRC are shown in Tables 2 and 3. Our review on OSNA and CRC shows high sensitivity, few false negatives results, and a concordance rate with pathological findings ranging from 61.8% to 98.7% (Table 2). Moreover, studies have shown that OSNA results in upstaging in about 25% of initially nodal-negative CRC patients after conventional HE analysis (Table 3). With the OSNA approach, the lymph node is homogenized without the need for other preparations and the results are ready in less than 40 min for 3 or 4 LNs, 20 min for a single LN. The stage of the tumor and the number of lymph nodes analyzed correlates with upstaging. Notably, the OSNA upstaging rate in Croner’s investigation for stage UICC I and II patients was 16.2% and 30.3%, respectively. Therefore, it was suggested that stage UICC I and II patients, who suffer from recurrent disease, were understaged by conventional HE analyses[9,124-126].

In a study by Yamamoto et al[13] OSNA-positive patients (2.0% of stage I CRC and 17.6% of stage II CRC) had more advanced features of CRC, such as deeper invasion to the colonic wall and severe invasion to lymphatic invasion compared with OSNA-negative cases. They found a 95% concordance rate between OSNA and classical histological analyses with HE and IHC. Yamamoto et al[13] concluded that OSNA is comparable to a 2-mm interval histopathological examination in its ability to detect LNMs.

In our previous published study[127], OSNA was superior to HE in identifying LNMs, with a false negative rate of 0% vs 44.4% and accuracy of 100% vs 76.4%, respectively (Table 2). As represented in Tables 2 and 3, few studies evaluated HE and IHC, few performed multi-sliced tissue sections using HE and the remaining single slice HE tissue section vs OSNA[11,128-131]. While the detection of small metastatic foci in LNs is influenced by the skill and experience of the pathologists, the advantage of the OSNA assay is the possibility to perform standard evaluations without being influenced by operator skill or experience. This explains the reason why the use of OSNA has recently garnered interest for detecting MMs[9,16,17,128].

Methods of LN division and pooled OSNA

Previous reports have detailed three major methods to compare LN status between pathological examination and the OSNA assay (Table 3). The first method[14,125] involves dividing LNs in half and sending each 50% portion for pathology and OSNA (half-division method). The second method[11,12,15] involves dividing LNs into four equal sections and sending two of these sections (50%) for pathology and OSNA (four-section method). In the third method[14,124], only 1 mm from the center of LNs are sent for pathological examination and the rest are used for OSNA measurement (center-cut method). The latter two methods described above are thought to be technically difficult for evaluating small LNs. By contrast, dividing in half and sending each 50% portion for pathology and OSNA is the simplest method.

In previous studies using classic OSNA (cOSNA), 50% of each LN was submitted for pathologic examination, followed by evaluation of each remaining half by OSNA. The obstacles for clinical applications of cOSNA include a need to simplify the procedure for halving the dissected LNs and reducing the operating costs associated with the equipment used for OSNA analyses.

Rakislova et al[125] conducted a study comparing two methods of LN evaluation by OSNA in CRC: an individual analysis of each LN (cOSNA) and a new approach involving pooling several LNs, known as the “pooling method.” The diagnostic performance of pooled OSNA (pOSNA) was comparable to that of cOSNA. In the pOSNA method, the LNs are pooled together in a test tube for OSNA analysis. The weight limitation for the LNs per tube was ≤ 600 mg, with those exceeding this limit placed in another
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| Ref.          | Nation         | Type of study                  | Patients number (sample number) | Tumor type | Purpose of the OSNA analysis |
|--------------|----------------|-------------------------------|--------------------------------|------------|------------------------------|
| Croner et al. | Germany        | Prospective study             | 184 (184)                      | Colorectal | Diagnosis of LN metastasis  |
| Yamamoto et al. | Japan      | Prospective multicenter study | 85 (385)                       | Colorectal | Diagnosis of LN metastasis  |
| Güller et al. | Switzerland    | Prospective study             | 22 (313)                       | Colon      | Diagnosis of LN metastasis  |
| Yamamoto et al. | Japan      | Not shown                     | 30 (66)                        | Colorectal | Identification of CK19      |
| Croner et al. | Germany        | Prospective multicenter study | 103 (1594)                     | Colon      | Pathologically nodenegative CC |
| Vogelaar et al. | Switzerland | Prospective multicenter study | 128 (325)                      | Colon      | Diagnosis of SLN metastasis|
| Yamamoto et al. | Japan      | Prospective multicenter study | 204 (1925)                     | Colorectal | Diagnosis of LN metastasis  |
| Aldecoa et al. | Spain         | Prospective multicenter study | 149 (1940)                     | Colorectal | Correlation between TTL and tumor’s characteristics |
| Rakislova et al. | Spain      | Observational study           | 188 (3206)                     | Colon      | Diagnosis of pooled LN metastasis |
| Miyake et al. | Japan          | Prospective study             | 25 (306)                       | Rectum     | Indication of LPLN dissection |
| Marhic et al. | France         | Prospective study             | 17                             | Colon      | Diagnosis of SLN metastasis |
| Colling et al. | United Kingdom | Prospective study             | 19 (82)                        | Colorectal | Diagnosis of LN metastasis  |
| Aldecoa et al. | Spain         | Prospective study             | 71 (936)                       | Colon      | OSNA with endoscopic tattooing |
| Yeung et al. | United Kingdom | Prospective study             | 16 (78)                        | Colorectal | OSNA with ICG detection     |
| Brito et al. | Portugal       | Prospective multicenter study | 59 (753)                       | Colon      | Pathologically node negative CRC |
| Esposito et al. | Italy        | Prospective study             | 34 (51)                        | Colorectal | Diagnosis of SLN metastasis |
| Diaz-Mercedes et al. | Spain | Prospective study             | 17 (980)                       | Colorectal | Budget impact analysis      |
| Itabashi et al. | Japan        | Prospective multicenter study | 195                            | Colorectal | Prognostic value of the OSNA assay for pStage ICRC patients |
| Archilla et al. | Spain        | Retrospective multicenter study | 342 (5931)                | Colorectal | Correlation between the TTL with patient outcome |
| Weixler et al. | Netherlands, Germany, Switzerland | Retrospective multicenter study | 87                             | Colon      | Prognostic value of OSNA |
| Tani et al. | Japan          | Prospective multicenter study | 92                             | Colon      | Diagnosis of pooled LN metastasis |
| Numata et al. | Japan          | Prospective study             | 34                             | Rectum     | Indication of LPLN dissection |

CRC: Colorectal cancer; ICG: Indocyanine green; LPLN: Lateral pelvic lymph node; OSNA: One-step nucleic acid amplification; SLN: Sentinel lymph node; TTL: Total tumor load.

In this study, the upstaging rate for early-stage CC patients was 9.1% (6/66). The upstaging rates of the study by Tani et al.[33] were slightly lower than those previously reported (Table 3).
Table 2 A comparison of the diagnostic accuracy of the one-step nucleic acid amplification assay in colorectal cancer patients

| Ref.          | Pathological evaluation | IHC             | LN number inspected by OSNA, mean | Sensitivity, % | Specificity, % | Concordance, % | PPV, % | NPV, % |
|--------------|------------------------|-----------------|----------------------------------|----------------|----------------|----------------|--------|--------|
| Croner et al[11] | Multi-slice            | CK19            | 1.0                              | 92.5           | 96.5           | 93.6           | 88.1   | 97.9   |
| Yamamoto et al[16] | Multi-slice            | CK19            | 4.5                              | 95.2           | 97.7           | 97.1           | 91.9   | 98.7   |
| Gülter et al[12]  | Multi-slice            | CK19            | 14.2                             | 94.5           | 97.6           | 97.1           | 89.7   | 98.8   |
| Yamamoto et al[17] | Single-slice           | None            | 9.4                              | 86.2           | 96.5           | 95.7           | 66.5   | 98.8   |
| Colling et al[129] | Single-slice           | None            | 4.3                              | 92.9           | 97.1           | 96.3           | 86.7   | 98.5   |
| Yeung et al[130]  | Single-slice           | None            | 4.9                              | 100            | 98.4           | 98.7           | 94.1   | 100    |
| Esposito et al[127] | Multi-slice           | None            | 1.5                              | 69.2           | 100            | 88.2           | 100    | 84.0   |
| Rakislova et al[125] | Not shown              | None            | 20.5 (pOSNA)                     | 88.9           | 79.2           | 80.2           | 33.3   | 98.4   |
| Vogelaar et al[15] | Multi-slice            | Anti pan-cytokeratin | 15.3                             | 51.6           | 84.1           | 67.7           | 76.7   | 63.1   |
| Miyake et al[193]  | Single-slice           | CEA             | 11                               | 100            | 86             | 88             | 57     | 100    |
| Marbic et al[184]  | Not shown              | None            | Not shown                        | 50             | 100            | 70.6           | 100    | 58     |
| Numata et al[188]  (predictive value for pathological LatLNM testing OSNA-MesLNM) | Not shown | None | 17 | 100 | 55 | 61.8 | 28 | 100 |
| Tani et al[33]    | Not shown              | CK19            | 6.9 (pOSNA)                      | 84.6           | 90.9           | 89.1%          | 78.6   | 93.7   |

CEA: Carcinoembryonic antigen; CK19: Cytokeratin 19; IHC: Immunohistochemistry; LatLNM: Lateral lymph node metastasis; MesLNM: Mesorectal lymph node metastasis; NPV: Negative predictive value; OSNA: One-step nucleic acid amplification; pOSNA: Pooled one-step nucleic acid amplification; PPV: Positive predictive value.

COMPARISON OF THE NUMBER OF POSITIVE NODES AND QUANTITATIVE OSNA RESULTS

The OSNA assay of retrieved LNs does not allow the number of involved LNs typically used for TNM staging, and therefore cannot be used for conventional cancer staging. Nevertheless, the OSNA assay can potentially be used to infer the size of metastatic foci based on the detected copy numbers[113,132]. Patient’s total tumor load (TTL) resulted from the sum of all CK19 mRNA tumor copies/μL of each positive LN from the colectomy specimen. Yamamoto et al[17] found that the sum of CK19 mRNA increased as the number of histologically positive LNs increased. Indeed, the median value of CK19 mRNA was significantly smaller in patients with < 3 regional LNs than in those with ≥ 4 regional LNs. The median TTL values of pN0, pN1 (1-3 positive LNs), and pN2 (4 or more positive LNs) were 1550 copies/mL (300-320000 copies/mL), 24050 copies/mL (250-890000 copies/mL), and 90600 copies/mL (7700-1635100 copies/mL), respectively. The TTL significantly increased as the node status increased.

In the study of Aldecoa et al[133] the TTL was related to pT stage (P = 0.01) and tumor size (P < 0.01) in low-grade tumors. In that study TTL, correlated with classical high-risk factors in stage I–II CC patients. These findings indicate that the sum of CK19 mRNA assessed by OSNA displays a trend compatible to the current pathological diagnosis system. These findings suggest the future possibility of novel molecular staging using OSNA, based on metastasis volume (amount of CK19 mRNA) rather than the number of LNMs. It has been suggested a correlation between CRC risk factors[11,12,16,18,122,126,134] such as pN, pT, tumor grade, male sex, tumor size, lymph vascular invasion (LVI), poor prognosis, worse DFS and TTL.

**Correlation of node TTL with TB and poorly differentiated clusters**

The physiological process that can lead epithelial cells to acquire mesenchymal properties and the potential for migration and stromal invasion, essential for the development of metastases, is the morphological manifestation of the epithelial-mesenchymal transition phenotype that can lead to the formation of TB and clusters poorly differentiated (PDCs). The presence of isolated tumor cells or cell clusters of ≤ 4 cells on the invasive front of the tumor is termed tuberculosis[134]. 5 or more neoplastic cells in the tumor stroma not organized into glandular structures constitute the PDCs. In stage II CC, both PDCs and TB are independent prognostic factors[134-136], associated with LNMs, distant
Table 3 Differences in lymph node processing methods and upstage rates of previous reports

| Ref.               | Subject (patients) | OSNA method | Harvested LN, n | Harvested LN, median | Dividing method of LN | Pathological staining | Measured LN by OSNA, n | Measured LN by OSNA, median | Upstage rate (pStage I and II) |
|--------------------|--------------------|-------------|----------------|----------------------|-----------------------|-----------------------|------------------------|----------------------------|-------------------------------|
| Yamamoto et al [16] | Stage 0, I (85)    | cOSNA       | 434            | N/A                  | Four; 4 mm over diameter of LN | HE and IHC               | 385                    | 4.5                        | 16.5% (2/16)                  |
| Güller et al [12]  | Stage I, II, III (22) | cOSNA     | 313            | 30 (16–60)           | Four; 3 mm over diameter of LN | HE and IHC               | 56                     | 13 (6–24)                  | 15.3% (2/13)                  |
| Croner et al [14]  | Stage I, II (105)  | cOSNA       | N/A            | N/A                  | Center; 6 mm over half; 4 mm to 6 mm diameter of LN | HE                     | 1594                   | 14 (1–46)                  | 25.2% (26/103)                |
| Vogelaar et al [15] | Stage I, II (128) | cOSNA      | N/A            | N/A                  | Four; 10 mm over half; 10 or less than 10 mm diameter of LN | HE and IHC               | 317                    | Mean 15.3 (4–40)           | 20.2% (20/90)                 |
| Yamamoto et al [17] | Stage I, II, III (204) | cOSNA   | 4324           | 19 (3–25)            | Half b | HE                     | 1925                   | 8 (2–25)                  | 17.6% (13/74)                |
| Aldcoca et al [133] | Stage I, II (149)  | cOSNA       | 2483           | 15                   | Center a | HE                     | 1940                   | 12                        | 51% (76/149)                 |
| Rakislova et al [125] | Stage I, II, III (188) | cOSNA, pOSNA | cOSNA 1828, pOSNA 1992, cOSNA 17 (13–22), pOSNA 20 (17–27) | Center b | HE                     | cOSNA 1757, pOSNA 1449 | cOSNA 13 (10–18), pOSNA 18 (13–25) | cOSNA 55.4% (51/92), pOSNA 20.7% (16/77) |
| Brito et al [126]  | Stage I, II (59)   | cOSNA       | 1046           | 13 (9–19)            | Center; 5 mm over half; 4 or less than 10 mm diameter of LN | HE                     | 753                    | 12 (7–16)                  | 28.8% (17/59)                |
| Itabashi et al [18] | Stage I, II, III (195) | cOSNA   | Not shown      | 19 (1–75)            | Half b; 4 mm over diameter of LN | HE                     | Not shown               | 8 (2–25)                  | 15.7% (11/70) in stage II patients |
| Tani et al [33]    | Stage II, IIIA (92) | pOSNA    | 2236           | 24.3 (5–66)          | Half b; 4 mm over diameter of LN | HE                     | 636                    | 6.9 (1–35)                  | 9.1% (6/66)                  |

aNot applicable.
bFour section method: dividing lymph nodes into four equal sections and sending two of these sections (50%) for pathology and one-step nucleic acid amplification (OSNA) measurement.
cCenter-cut method: 1 mm from the center of lymph nodes are sent for pathological examination and the rest are used for OSNA.
dHalf-division method divides lymph nodes in half and sends each 50% portion for pathology and OSNA.
HE: Hematoxylin and eosin; IHC: Immunohistochemistry; LN: Lymph node; OSNA: One-step nucleic acid amplification; pOSNA: Pooled one-step nucleic acid amplification.

metastases, extramural vascular invasion, LVI, perineural invasion (PNI), tumor grade and high pT stage [136-142].

International Consortium on TB Recommendations indicates how to count the number of TB at the invasive front of the tumor [135]. The classification for TB was as follows: Bd1/low (≤ 4 buds), Bd2/intermediate (5–9 buds), and Bd3/high (10 one more buds). The classification for PDCs evaluated [143] at the invasive front or in the center of the tumor was as follows: G1 (≤ 4 clusters), G2 (5–9 clusters), and G3 (10 or more clusters). Barresi et al [144] suggested not classifying tumor cells within mucin pools in mucinous carcinomas as TB, considering only tumor cells infiltrating the stroma with minimal extracellular mucin. While, the PDCs were evaluated within mucin lakes.

Recently Archilla et al [145] suggested the correlation of the TTL with patient outcome, TB, and PDC. The use of molecular methods to assess LN status, together with other pathological risk factors, could help improve risk stratification and management of patients with early-stage CRC.

Indeed, OSNA positivity was found in 38.3% of the cases (131/342) with a mean TTL of 36662 copies/mL, with no significant differences between both groups (P = 0.001). Low and intermediate TB had similar mean TTL (Bd1: 3292 copies/mL and Bd2: 18002 copies/mL), with no significant differences between both groups (P = 0.154). The mean TTL of high-Bd3...
TB was 45331 copies/mL, and it was significantly different from Bd1 and Bd2. Likewise, the mean TTL of PDC G1, with 4962 copies/mL and G2, with 13146 copies/mL did not show significant differences (P = 0.068), while PDC G3 had 61108 copies/mL, significantly different than low and intermediate grades. Thus, the authors grouped low and intermediate grades of TB and PDC into one category, obtaining two groups with significant differences for both TB and PDC (P < 0.001) as well. The authors also concluded that TTL can be used as an alternative method to better stage patients compared to the classic HE because it is able to identify real stage II or III patients, thereby selecting those who are candidates for adjuvant therapy[145].

SURVIVAL ANALYSES

In the meta-analysis of Wild et al.[146] it is emphasized that long-term outcomes and the use of adjuvant therapy in those upstaged by OSNA should be clarified before routine use of OSNA test.

Itabashi et al.[18] showed that pStage II patients with OSNA positive LN had a lower 3-year DFS than negative patients (55% vs 86%; P = 0.005), with no significant differences in 3-year OS (P = 0.914). In this study, the upstaging occurred for patients with pStage II, of whom 11 of 70 patients (15.7%) were OSNA-positive. Most of the OSNA positive LNs were located in the peri-colic or peri-rectal area (10 out of 11 OSNA-positive stage II CRC patients).

Weixler et al.[147] showed that the detection of positive LN by HE staining but not by OSNA as significant predictors of cancer-specific survival, cancer-specific and recurrence-free survival, and DFS. He concluded that in patients with CC, OSNA offers no prognostic advantage compared to conventional LN staging with HE contrasting findings in other cancers. It is important to highlight that the methodology of the histopathological evaluation for detection of the LNMs differed among studies. In Weixler’s multicenter study[147] all harvested LNs > 3 mm in greatest dimension or a short axis ≥ 10 mm was cut into four slices: two were stored for later OSNA analysis and two were allocated to conventional standard HE staining, multilevel HE staining, and IHC for CK19. Multilevel sectioning with IHC leads to relevant upstaging of 15.4%-26% of otherwise negatively classified patients[148,149]. In addition, stage I-III patients were included in this study, whereas most of the OSNA studies focused on stage I-II patients. Therefore this HE + IHC vs OSNA study including stage I-III patients, although well conducted and of great value and interest, is not amenable to a comparison with studies in which HE vs OSNA in stage I-II patients are evaluated.

ORGAN-PRESERVING SURGERY

CC

Early CRC can be treated by endoscopic mucosal resection or endoscopic submucosal dissection (ESD). In determining the indication for endoscopic treatment and the treatment method, information on the depth of invasion and morphology of the tumor is essential. Colorectal ESD is an “endoscopic resection technique, which enables en bloc resection of a tumor, regardless of size” and avoids piecemeal resection. It is of great importance to differentiate Tis and T1a cancers from T1b cancers (T1 cancer with ≥ 1000 μm submucosal invasion depth), as the former can be treated by endoscopy while the latter requires surgical operation with nodal status assessment[29,150-154]. Moreover, innovative organ-preserving procedures such as endoscopic full-thickness resection or transanal minimally invasive surgery have been proposed to perform high-quality resections with decreased incidence of specimen fragmentation without resorting to demolitive interventions[155]. Nevertheless, it is estimated that overall 10%–20% of patients in stage T1 will have LNs, and such patients subjected to a localized resection are undertreated[151].

Laparoscopic endoscopic cooperative surgery (LECS) can also lead to full thickness local resection by means of combined use of laparoscope and endoscope. The development of modified LECS procedures, such as non-exposed endoscopic wall-inversion surgery and closed LECS has almost resolved these drawbacks. This has led to a recent increase in the indication of modified LECS to include patients with gastric epithelial neoplasms. The LECS concept is also beginning to be applied in other organs such as the duodenum, colon and rectum. Further evolution of LECS procedures is expected in the future. SLN mapping could also be combined with LECS, resulting in a portion of early gastrointestinal cancers being treated by LECS with SLN mapping[156].

Rectal cancer

TEM, first described by Gerhard Buess[157-161], due to its ability to perform high-quality resections with decreased incidence of fragmentation, is superior to standard transanal excision for treating benign and malignant rectal lesions, most notably[162,163].

Transanal minimally invasive surgery (TAMIS) was initially born as a fusion between trans anal endoscopic microsurgery (TEM) and single-site laparoscopy. This technique was designed as a cost-
effective and easily reproducible alternative to TEM without specialized equipment.

The indications for TAMIS are similar to standard transanal resection for benign and malignant lesions determined by EUS or MRI[164,165]. TAMIS is also indicated for early malignant neoplasms confined to the submucosa[166]. T1 neoplasms of the rectum can still be divided into low-risk lesions [167] and at high risk for poor histopathological features (TB, poor differentiation, LVI or perineural invasion). Studies[155,168-170] identified a higher risk of LN metastasis in T1 sessile tumors with deeper submucosal invasion (sm3 or sm4).

THE OSNA ASSAY FOR THE DETECTION OF CRC METASTASIS IN SLN

LN status plays a crucial role in oncologic therapeutic strategies, and despite the use of increasingly sophisticated imaging techniques, pre-operative metastatic LN identification in patients with CRC is unsatisfactory[171,172].

The study of the SLN is gaining more and more popularity because it can avoid extensive lymphadenectomies, reduce operating times and morbidity. This can change the surgical strategy in patients with an apparent early stage of CRC, as patients with intraoperative positive SLN are submitted, during the same surgical procedure, to an adequate lymphadenectomy, whereas those with negative SLN can be treated with an organ-preserving surgery, avoiding unnecessary lymphadenectomy. The extemporaneous intraoperative examination performed on frozen specimen has a lower sensitivity than the classic postoperative analysis. The problem is mainly due to the low detection rate of MMs and ITC[173,174]. The disease detection rate increased with the technique of multi-step formalin-fixed tissue sections (FFTS) stained by HE with or without IHC[6,175-179]. Nonetheless, a significant number of MMs can still be overlooked, as during histological workup usually only small parts of LNs are screened.

Therefore, there are two methods for SLN mapping: staining and radioguided[180]. Three tracers have been used to detect SLNs: Dye, radioisotope, and indocyanine green (ICG). Each tracer has its respective disadvantages. The use of the ICG fluorescence method has officially been approved in Japan for LNs of BC and malignant melanoma; thus, it appears that ICG can be an acceptable tracer for the detection of LNs in gastric and CC.

ICG tattooing method is very useful for the marking of early gastric and CCs, especially when using a laparoscopic approach[181]. It has been suggested that SLN mapping with fluorescent dye can play an important role in the treatment of CC, particularly those at early stages, and can lead to ultraconservative surgery[182]. Because the results of OSNA are available in a relatively short time compared to the conventional technique, intraoperative OSNA analysis of SLNs may be employed easily in clinical practice.

In the study by Vogelaar et al[15] OSNA proved to be a promising method for the detection of SLN metastases in CC patients after ex vivo SLN mapping. OSNA appeared to outperform routine pathological examination with HE-stained slides with an upstaging rate of 20.2%. In the study by Yeung et al[130] OSNA was used intraoperatively, together with the technique of retrieving colorectal LNs by fluorescence imaging, to analyze the status of these specific LNs. In this study, OSNA is highly concordant with standard histology.

The results of the meta-analysis by Tranoulis et al[183] indicate that the use of OSNA can allow to identify the status of the LNs even when applied intraoperatively. Marhic et al[184] proposed that the OSNA technique may be a new method to reduce time to adjuvant chemotherapy after surgery for CC. In this study, SLN status was determined intraoperatively with the OSNA assay; when positive, a port-a-cath was placed during the procedure for upcoming adjuvant chemotherapy. In this study, there was no difference between the groups regarding cancer staging, duration of hospitalization, and major morbidities but the time interval between surgery and adjuvant chemotherapy was significantly shorter in the OSNA group at 35 ± 8 d vs 67 ± 36 d (P = 0.021).

In our previous study[127], we showed that SLN analysis with OSNA in combination with ICG-near infrared (NIR) lymphangiography is feasible and may allow intraoperative prediction of LN status in patients with CRC (Table 4). Patients with SLN positive by the OSNA method were considered pN-positive and subjected to adjuvant chemotherapy. The time to start chemotherapy was lower in OSNA (+) patients [39.1 ± 1.9 d vs 50.2 ± 4.1 d in the OSNA (+) group: P = 0.01].

Both ex vivo and in vivo ICG fluorescence imaging are feasible for the detection of SLNs in CRC. The submucosal injection technique and subserosal were both used. A NIR 30° laparoscope (Olympus, Tokyo, Japan) was used to inspect the mesocolon. The LNs found using fluorescence were considered SLNs and analyzed intraoperatively. More work needs to be done to define protocols, indications for its use, a standard number of LNs that need to be removed and to test its efficacy in larger patient populations.

Implementation SLN analysis with OSNA in combination with ICG-NIR lymphangiography could allow more precise staging, reducing the delay between surgery and the onset of adjuvant chemotherapy. SLN evaluation by intraoperative OSNA analysis combined with a LECS approach may allow, in case of OSNA-negative early CRC, to apply an organ-preserving surgery avoiding the complic-
LATERAL PELVIC LNs AND OSNA

There is disagreement in the international literature regarding the use of prophylactic lymphadenectomy in comparison with preoperative radiochemotherapy to improve prognosis in patients with locally advanced rectal cancer, due in part to the complex anatomy of the pelvic floor which makes diagnosis of lateral pelvic LNs (LPLNs) metastasis difficult. In Japan, the surgical oncology approach has been toward LN clearance and, as a result, LPLNs have been considered local-regional disease from the outset[185].

Nevertheless, the rate of pathological lateral (Lat) LNM (p-LatLNM) in patients without clinical LatLNM (c-LatLNM) remained low at 7%, and lateral LN dissection (LatLND) is generally considered technically demanding and can prolong the operative time[186-188]. As Sammour et al[189] proposed, patients who are candidates for curative-intent treatment should be stratified depending on their risk to have LPLN metastasis in high, moderate, and low risk in order to select the best option to manage the pelvic compartment. Nevertheless, with preoperative images or traditional criteria, it is difficult to predict LatLNM. Obtaining a preoperative or intraoperative diagnosis is essential to select patients with LatLNM.

If SLN navigation surgery could be applied in cases of middle and lower rectal cancer, unnecessary LatLND procedures could be avoided. SLN anlysis may be useful in deciding both the indication of LatLND and which side of the lateral pelvic wall should be dissected[190]. In the study of Noura et al[191] the existence of a lateral pelvic region SLN in 53 lower rectal cancer patients was investigated. The lateral pelvic region was observed using a NIR camera system (photodynamic eye) after the ICG has been injected into the submucosa along the dentate line. If SLNs were positive for metastasis a Bilateral LatLND was performed, if instead SLNs were negative for metastasis mesorectal excision only was performed. In 49 (92.5%) of the 53 patients the lateral SLNs were successfully identified, 4 of these patients (8.2%) had lymph node metastases; the mean number of lateral SLNs per patient was 2.0 (range, 1–4).

The results of Yasui et al[192] suggested the potential use of SLN with ICG strategy to identify cases with non-metastatic LPLN, and to omit LatLND in such cases, and thereby avoid both LatLND-related surgical complications and radiation-induced adverse events. Moreover, the author suggested that further studies are needed to shorten the required time and improve the accuracy of SLN biopsy by the intraoperative rapid diagnosis with different methods such as molecular biological diagnosis.

Miyake et al[193] attempted to perform an intraoperative OSNA assay to detect perirectal LNMs to predict LPLN metastasis in rectal cancer patients undergoing surgical resection plus LatLND. In their study, LPLN metastases were present in 16% of patients (4/25), and all of these patients were positive on an OSNA for perirectal LNMs. The sensitivity of OSNA was 100%, specificity 86%, positive predictive value 57%, and negative predictive value 100% for predicting LPLN metastasis, and the authors concluded that the OSNA of perirectal LNs might be useful for selecting candidates for omission of LatLND in rectal cancer surgery. OSNA can be associated with SLN to intraoperatively identify foci of metastasis in LPLNs.

With respect to risk factors for p-LatLNM, three previous studies reported that pathological mesorectal LN (MesLN) metastasis (p-MesLNM) is a consistent risk factor for p-LatLNM[194-196]. Furthermore, previous studies have shown that p-LatLNM rarely occurs without p-MesLNM[16,197].

Table 4 Studies analyzing colorectal cancer metastasis in sentinel lymph nodes with one-step nucleic acid amplification

| Author | Patients (samples), n | Injected dye | Intraoperative OSNA assay | Examined SLNs, n |
|--------|----------------------|--------------|--------------------------|-----------------|
| Vogelaar et al[15] | 128 (325) | Patent blue dye V or indocyanine green | No | 3.0 (median) |
| Marhic et al[184] | 17 | Blue dye | Yes | Not shown |
| Yeung et al[130] | 16 (78) | Indocyanine green | No | 4.9 (mean) |
| Esposito et al[127] | 34 (51) | Indocyanine green | Yes | 1.0 (median) |

OSNA: One-step nucleic acid amplification; SLN: Sentinel lymph node.
Negative OSNA diagnosis for mesorectal LNM (MesLNM) (OSNA-MesLNM) is highly correlated with negative p-LatLNM; hence, negative confirmation of OSNA-MesLNM may be useful in selecting patients in whom LatLND can be omitted [189].

In conclusion, the role of LatLND is still under discussion. Nevertheless, it has been suggested a selective and mono- or bilateral LatLND in advanced low and middle rectal cancer, based on OSNA positive mesorectal nodes or OSNA-positive ICG-stained sentinel LPLNs.

COSTS

A disadvantage of cOSNA is that it is more costly than pathologic examination. Depending on the number of samples analyzed in each lot, there is a variation in the cost of consumables and additional reagents. For example, a 12 LN analysis would have an indicative cost of single use products is £ 550–£ 590 per patient [excluding value added tax (VAT)]. If consumables are maximized and samples from more than 1 patient are tested in one batch, the cost could be as high as £ 33.50 per LN. The annual maintenance contract for the system (which would apply from the 2nd year after installation) is priced at £ 6628.48 (excluding VAT) with a 12 mo warranty.

The lifespan of the OSNA system declared by the manufacturer is at least 6 years. The duration of the analysis is approximately 90 min for 12 LNs, therefore it is possible to analyze samples of approximately 5 patients per day (7.5 h), and 1200 in 1 year (considering 240 annual working days). Average cost per patient (including capital, maintenance and disposable costs) ranges from £ 568 to £ 608, using the standard annuity method with a 3.5% discount rate [198]. Nonetheless, the OSNA use may reduce the reinterventions and allow earlier commencement of adjuvant treatment. The financial implications of OSNA have been previously investigated in BC, with an estimated saving between 400 and 700 £ per patient [199,200].

With pOSNA, only one measurement is required, at a cost of $ 225 (¥ 24000). In contrast, cOSNA requires three or more measurements, at a cost of $ 670 (¥ 72000) or more. In pOSNA, multiple LNs can be measured in one tube, taking into consideration the upper limit of the LN weight that can be assayed in one tube by the OSNA method is 600 mg. However, in cOSNA, only one LN can be measured in a single tube (max 50 mg). Using cOSNA to measure 12 or more halved LNs would require at least 12 tubes and three or more measurements per tube, whereas if 12 pericolic LNs are measured, pOSNA would require only one or two tubes for each case. The OSNA measuring device can measure up to four tubes at once; however, because pOSNA requires only one or two measurements per case, this allows the device to take measurements for two cases simultaneously depending on the number and weight of the removed LNs.

Diaz-Mercedes et al [109] analyzed the budget impact of introducing an OSNA assay in early-stage CRC patients and suggested that OSNA might have not only an economic benefit but also a clinical benefit in CRC patients, since it enabled more accurate staging, thereby avoiding unnecessary treatment. The results of Diaz-Mercedes et al [109] indicate that the Spanish National Health System would have saved over € 19 million from 2017 to 2019 if OSNA had been introduced in clinical practice for surgically treated CRC patients. In this study, HE-positive patients and OSNA-positive patients, both underwent adjuvant therapy. Savings are explained by the fact that OSNA ensures a more accurate diagnosis in CRC patients, allowing a reduction in treatment costs after initial surgery, as well as costs of adjuvant treatments and surgery after recurrence, compared with HE techniques. Although patients' LN staging is more expensive with OSNA than with HE, savings regarding treatment costs after surgery and treatment costs due to recurrence are high enough to justify the investment in OSNA analysis.

Although the costs of OSNA are high, the speed, simplicity, and reproducibility could allow a reduction in the hours of work of individual pathologists. Furthermore, two cases can be studied during a single procedure using the pOSNA method. Adding, as demonstrated by Diaz-Mercedes et al [109], the reduction in treatment costs after surgery and the reduction in costs relating to the treatment of recurrences, this method could be also attractive for developing countries.

ADVANTAGES AND DISADVANTAGES

Advantages

The innovative aspects of OSNA are that unlike standard histopathology, OSNA can analyze the whole LN as well as partial LNs. This may improve cancer staging accuracy. It can detect metastatic foci regardless of their size or location. It is seemingly superior to conventional FFITS in detecting MMs and ITC, as it can identify metastatic foci as small as 0.35 mm [201]. Yamamoto et al [17] found that the sum of CK19 mRNA increased as the number of histologically positive LNs increased. Indeed, the median value of CK19 mRNA was significantly smaller in patients with < 3 regional LNMVs than in those with ≥ 4 regional LNMVs. In the study of Aldecoa et al [133], the TTL was related to pT stage (P = 0.01) and tumor size (P < 0.01) in low-grade tumors. In this study, TTL correlates with classical high-risk factors in stage
I–II CC patients. These findings indicate that the sum of CK19 mRNA assessed by OSNA displays a trend compatible to the current pathological diagnosis system. These findings suggest the future possibility of novel molecular staging using OSNA, based on metastasis volume (amount of CK19 mRNA) rather than the number of LNM.

Archilla et al.[145] have suggested the correlation of TTL with TB and PDCs. TTL could be used as a new prognostic factor in CRC as it is related to the outcome and. The combination of the TTL as a new prognostic factor, TB and PDC, could help to better stratify and manage patients with early-stage CRC at risk of recurrence.

The application of OSNA in addition to the current standard pathology would likely provide an additional risk factor for disease recurrence regarding patients with stage II CRC. LNM is a reliable prognostic marker of CRC; it is used as the “gold standard” for post adjuvant chemotherapy after curative surgery. One may question whether OSNA-positive CRC patients should receive post-adjuvant chemotherapy after curative surgery. Further clinical trials are needed to determine if adjuvant therapy is beneficial in this upstaged group. On the other hand, the short turnaround time renders OSNA an attractive intra-operative method. Based upon the BC accumulated experience, the turnaround time is less than 40 min for one LN, whereas it ranges from 50 to 62 min for assessing four LNs.[7] The OSNA rapid turnover time may potentially be useful in circumventing the major issues associated with SLN biopsy. Moreover, OSNA is automated and the results are quantifiable; hence, easily reproducible, less operator-dependent with short learning curve[201-203]. Therefore, implementation of OSNA in routine clinical practice may ease the burden on pathologists.

Finally, it is laborious and rather expensive to perform molecular tests. Nonetheless, the OSNA use may reduce the re-interventions and could allow earlier commencement of adjuvant treatment. The financial implications of OSNA have been previously investigated in BC.[199,200] The results showed that pOSNA can simplify the process of cutting harvested LNs in half while reducing the equipment-related costs associated with OSNA assays used in clinical practice. Additionally, pOSNA demonstrated an upstaging rate for pNNCC equivalent to that reported in previous studies, suggesting its feasibility for molecular staging in clinical practice. With pOSNA, the possibility of fewer measurements per patient and of studying more cases simultaneously with the same panel further reduces costs.[33,125]. In an era of stringent economics, health systems should undergo cost-effectiveness analyses upon which a progressive integration of OSNA in their daily clinical practice could be based on.[109,200]

**Limitations**
The few OSNA limitations should be acknowledged. OSNA can be used to analyze LNs more than 50 mg; if the LN diameter is inferior to 3-4 mm, it cannot be divided and analyzed with OSNA and conventional histology (Table 3). The examination of the whole LN by OSNA inevitably precludes FFTS examination. One limitation of all OSNA studies performed in CRC, is that the analysis has been performed using only a part of the LNs, while using the rest of the LN tissue for conventional histological analysis and pN staging.[145].

The technique of nodal division must be taken into account when evaluating the results because it can vary widely between different studies. Discordant results between OSNA and FFTS were reported. The three main reasons for these discrepancies are: tissue allocation bias, low CK19 expression, and tissue contamination. Moreover, discordances may also arise from the presence of metastases from primary tumors that do not express CK19.[147]. The latter is considered an important limitation of OSNA. The accuracy of OSNA is seemingly higher amongst CK19 positive primary tumors by IHC compared to those with CK19-negative primary tumors.[183]. As such, the positive CK19 IHC in primary tumors has been proposed as a prerequisite for the OSNA use by some authors.[183]. An additional challenge is the fact that 36% and 49% of CK19-negative primary tumors have CK19-positive LNs[183]. Peigné et al.[203] also reported that CK19 mRNA can also be detected by OSNA even in cases with CK19-negative primary lesion.

The tendency toward a loss of CK19 expression in poorly differentiated cancers may represent a challenge for assays using CK19 IHC or PCR for detecting MMs. It is of note that upregulation of CK19 in tumors derived from cells that are CK19-negative can also be linked to unfavorable tumor features. CK19 is highly expressed in positive LNs from BC patients even when its expression is not observed in primary tumors. Targeted studies on the upregulation of CK19 mRNA in LNM of CRC are needed[15].

In light of the potential for false-negative results, the incorporation of additional markers would be a possible direction in improving the diagnostic performance of OSNA. The cut-off point of 250 copies/mL is established in BC and seemingly sensitive in CRC while the optimal diagnostic cut-off point is a matter of debate.[184]. The aforementioned pitfalls remain to date a field of contention necessitating a shift in the focus of future research into incorporation of novel biomarkers and evaluation of the optimal diagnostic cut-off point.

At this time there is no exact definition of true LN positives or negatives, cancer-related relapse and death were used as real positive LN indicators, disease-free survival as negative real LN.[147]. The false-negative rate of pOSNA is a point to be considered when applying OSNA in clinical practice (Table 2). However, Aldecoa et al.[133] observed that high-grade (G3) tumors or tumors with vascular invasion presented lower levels of TTL making it not a reliable prognostic tool for these specific pathologic features.
Finally, in this review, we found few reports dealing with CRC and SLN evaluation and there is obviously the need for future research in this field.

CONCLUSION

OSNA analysis is potentially more accurate than conventional pathologic methods for identifying metastasis because it solubilizes the entire LN and analyzes CK19 mRNA levels in the resulting sample. The advantages of OSNA include a short analysis time of approximately 30–40 min from start to completion, and the ability to automate the OSNA assay eliminates interlaboratory differences based on pathologist skill and experience. The short turnaround time renders OSNA an attractive intra-operative method. Patients with pN0 OSNA-positive CRC might also need chemotherapy after curative surgery. To achieve this goal, it needs several studies to compare the recurrence rate between the groups of no treatment or adjuvant chemotherapy after surgery both in OSNA-positive pStage II CRC patients. The result would clarify whether adjuvant chemotherapy is beneficial to patients with OSNA-positive pStage II CRC. Anyway, it can be suggested that OSNA may be considered as the route to tailormade surgery.

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FOOTNOTES

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REFERENCES

1. André T, de Gramont A, Vernerey D, Chibaudel B, Bonnetain F, Tijeras-Raballand A, Scriva A, Hickish T, Tabernero J, Van Laethem JL, Banzu M, Maartense E, Shmuely E, Carlsson GU, Scheithauer W, Papatheodorou Z, Mochler M, Landolfi S, Demetter P, Colote S, Tourignand C, Louvet C, Duval A, Fléjou JF. Adjuvant Fluorouracil and Oxaliplatin in Stage II to III Colon Cancer: Updated 10-Year Survival and Outcomes According to BRAF Mutation andMismatch Repair Status of the MOSAIC Study. J Clin Oncol. 2015; 33: 4176-4187 [PMID: 26527776 DOI: 10.1200/JCO.2015.63.4238]

2. Compton CC. Optimal pathologic staging: defining stage II disease. Clin Cancer Res 2007; 13: 6862s-6870s [PMID: 18006791 DOI: 10.1158/1078-0432.CCR-07-1398]

3. Choi HK, Law WL, Poon JT. The optimal number of lymph nodes examined in stage II colorectal cancer and its impact of on outcomes. BMC Cancer 2010; 10: 267 [PMID: 20529352 DOI: 10.1186/1471-2407-10-267]

4. Maguire A, Sheahan K. Controversies in the pathological assessment of colorectal cancer. World J Gastroenterol 2014; 20: 9850-9861 [PMID: 25110416 DOI: 10.3748/wjg.v20.i29.9850]

5. Meyer JE, Cohen SJ, Ruth KJ, Sigurdson ER, Hall MJ. Young Age Increases Risk of Lymph Node Positivity in Early-Stage Rectal Cancer. J Natl Cancer Inst 2016; 108 [PMID: 26719881 DOI: 10.1093/jnci/djv284]
6 Resch A, Langner C. Lymph node staging in colorectal cancer: old controversies and recent advances. *World J Gastroenterol* 2013; 19: 8515-8526 [PMID: 24379568 DOI: 10.3748/wjg.v19.i46.8515]

7 Tsujimoto M, Nakabayashi K, Yoshidome K, Kaneko T, Iwase T, Akiyama F, Kato Y, Tsuda H, Ueda S, Sato K, Tamaki Y, Noguchi S, Kataoka TR, Nakajima H, Komeike Y, Inaji H, Tsugawa K, Suzuki K, Nakamura S, Daioh M, Otomo Y, Matsuura N. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients. *Clin Cancer Res* 2007; 13: 4807-4816 [PMID: 17699859 DOI: 10.1186/1078-0432-CCR-06-2512]

8 Hunter-Smith AE, Rauter Z. One-step nucleic acid amplification: the possible value in assessing sentinel lymph node metastasis during mastectomy. *Breast Cancer (Dove Med Press)* 2018; 10: 13-21 [PMID: 29416374 DOI: 10.2147/BCTT.S113737]

9 Kumagai K, Yamamoto N, Miyaishi I, Tomita Y, Kaitai H, Kushima R, Tsuda H, Kitagawa Y, Takeuchi H, Mukai M, Mano M, Mochizuki H, Kato Y, Matsuura N, Sano T. Multicenter study evaluating the clinical performance of the OSNA assay for the molecular detection of lymph node metastases in gastric cancer patients. *Gastric Cancer* 2014; 17: 273-280 [PMID: 23743877 DOI: 10.1007/s10120-013-0271-9]

10 Hayama M, Chida M, Karube Y, Tamura M, Kobayashi S, Oyaizu T, Homma K. One-step nucleic acid amplification for detection of lymph node metastasis in lung cancer. *Ann Thorac Cardiovasc Surg* 2014; 20: 181-184 [PMID: 23603642 DOI: 10.5761/atcs.oa.12.02224]

11 Croner RS, Schellerer V, Demund H, Schildberg C, Papadopulos T, Naschberger E, Störl M, Matzel KE, Hohenberger W, Schlabrakowski A. One step nucleic acid amplification (OSNA): a new method for lymph node staging in colorectal carcinomas. *J Transl Med* 2010; 8: 3-36 [PMID: 20819209 DOI: 10.1186/1479-5876-8-36]

12 Güller U, Zettl A, Worni M, Langer I, Cabalzar-Wondberg D, Viehl CT, Demartines N, Zuber M. Molecular investigation of lymph nodes in colon cancer patients using one-step nucleic acid amplification (OSNA): a new road to better staging? *Cancer* 2012; 118: 6039-6045 [PMID: 22668496 DOI: 10.1002/cncr.27667]

13 Yamamoto N, Daito M, Hiyama K, Ding J, Nakabayashi K, Otomo Y, Tsujimoto M, Matsuura N, Kato Y. An optimal mRNA marker for OSNA (One-step nucleic acid amplification)-based lymph node metastasis detection in colorectal cancer patients. *World J Gastroenterol* 2013; 13: 43: 264-270 [PMID: 23937311 DOI: 10.3748/wjg.v19.i6.933]

14 Croner RS, Geppert CI, Bader FG, Nitsche U, Späth C, Rosenberg J, Zettl A, Galler J, Güller U, Störl M, Zuber M. Molecular staging of lymph node-negative colon carcinomas by one-step nucleic acid amplification (OSNA) results in upstaging of a quarter of patients in a prospective, European, multicentre study. *Br J Cancer* 2014; 110: 2544-2550 [PMID: 24722182 DOI: 10.1038/bjc.2014.170]

15 Vogelaa FJ, Reimers MS, van der Linden RL, van der Linden JC, Smit VT, Lips DJ, van de Velde CJ, Bosscha K. The diagnostic value of one-step nucleic acid amplification (OSNA) for sentinel lymph nodes in colon cancer patients. *Ann Surg Oncol* 2014; 21: 3924-3930 [PMID: 24219126 DOI: 10.1245/s10434-014-3820-5]

16 Yamamoto H, Sekimoto M, Oya M, Yamamoto N, Konishi F, Sasaki J, Yamada S, Taniyama K, Tominaga H, Tsujimoto M, Akamatsu H, Yanagisawa A, Sakakura C, Kato Y, Matsuura N. OSNA-based novel molecular testing for lymph node metastasis in colorectal cancer patients: results from a multicenter clinical performance study in Japan. *Ann Surg Oncol* 2011; 18: 1891-1898 [PMID: 21290195 DOI: 10.1245/s10434-010-1539-5]

17 Yamamoto H, Tomita N, Inomata M, Furuhata T, Miyake Y, Noura S, Kato T, Murata K, Hayashi S, Igarashi S, Babashi M, Kameoka S, Matsuura N. OSNA-Assisted Molecular Staging in Colorectal Cancer: A Prospective Multicenter Trial in Japan. *Ann Surg Oncol* 2016; 23: 391-398 [PMID: 26384840 DOI: 10.1245/s10434-015-4880-x]

18 Itabashi M, Yamamoto H, Tomita N, Inomata M, Murata K, Hayashi S, Miyake Y, Igarashi S, Kato T, Noura S, Furuhata T, Ozawa H, Takenaka I, Yasui M, Takeyama H, Okamura S, Ohno Y, Matsuura N. Lymph Node Positivity in One-Step Nucleic Acid Amplification: A Prognostic Factor for Postoperative Cancer Recurrence in Patients with Stage II Colorectal Cancer: A Prospective, Multicenter Study. *Ann Surg Oncol* 2020; 27: 1077-1083 [PMID: 31722872 DOI: 10.1245/s10434-019-0971-y]

19 Weiser MR. AJCC 8th Edition: Colorectal Cancer. *Ann Surg Oncol* 2015; 22: 1454-1455 [PMID: 29614622 DOI: 10.1245/s10434-018-6462-1]

20 Hari DM, Leung AM, Lee JH, Sim MS, Vuong B, Chiu CG, Bilchik AJ. AJCC Cancer Staging Manual 7th edition criteria for colon cancer: do the complex modifications improve prognostic assessment? *J Am Coll Surg* 2013; 217: 181-190 [PMID: 23768788 DOI: 10.1016/j.jamcollsurg.2013.04.018]

21 Hashiguchi Y, Hase K, Kotake K, Ueno H, Shintou E, Mochizuki H, Yamamoto J, Sugihara K. Evaluation of the seventh edition of the tumour, node, metastasis (TNM) classification for colon cancer in two nationwide registries of the United States and Japan. *Colorectal Dis* 2012; 14: 1065-1074 [PMID: 22176600 DOI: 10.1111/j.1463-1318.2011.02917.x]

22 Shida D, Kanemitsu Y, Hamaguchi T, Shimada Y. Introducing the eighth edition of the tumour-node-metastasis classification as relevant to colorectal cancer, anal cancer and appendiceal cancer: a comparison study with the seventh edition of the tumour-node-metastasis classification and the Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma. *Jpn J Clin Oncol* 2019; 49: 321-328 [PMID: 30680547 DOI: 10.1093/jjco/hyy193]

23 Nam J, Ng M, Roy-Chowdhury S, Morgan JW, Lum SS, Wong JH. Quantitating the impact of stage migration on staging accuracy in colorectal cancer. *J Am Coll Surg* 2008; 207: 882-887 [PMID: 19183353 DOI: 10.1016/j.jamcollsurg.2008.08.019]

24 Li J, Guo BC, Sun LR, Wang JW, Fu XH, Zhang SZ, Poston G, Ding KF. TNM staging of colorectal cancer should be reconsidered by T stage weighting. *World J Gastroenterol* 2014; 20: 5104-5112 [PMID: 24803826 DOI: 10.3748/wjg.v20.i17.5104]

25 Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Faraks L, Garrido-Laguna I, Grem JL, Gunn A, Hecht JR, Hoffie S, Hubbard J, Hunt S, Jhong KL, Klirkuc K, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Narkin S, Overman MJ, Parikh A, Patel H, Pedersen K, Saltz L, Schneider C, Shibata D, Skibber JM, Sofoceleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Gregory KM, Gurski LA. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2021; 19: 329-359 [PMID: 33724754 DOI: 10.1093/jjco/hjct001.0012]

26 Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Garrido-
Crafa F et al. OSNA in colorectal cancer

Laguna I, Grem JL, Gunn A, Hofie S, Hubbard J, Hunt S, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Nurkin S, Overman MJ, Parikh A, Patel H, Pedersen K, Sultz L, Schneider C, Shibata D, Skibber JM, Sofocleous CT, Stoffel FM, Stotsky-Himmelfarb E, Willett CG, Johnson-Chilla A, Gurski LA. NCCN Guidelines Insights: Rectal Cancer, Version 6.2020. J Natl Compr Canc Netw 2020; 18: 806-815 [PMID: 32634771 DOI: 10.6049/jncn.2020.00332];

Ueno H, Mochizuki H, Agaki Y, Kusumi T, Yamada K, Ikegami M, Kawachi H, Kameoka S, Okhara Y, Masaki T, Kushima R, Takahashi K, Ajijoka Y, Hase K, Ochihi A, Wada R, Iwaya K, Shimazaki H, Nakamura T, Sugihara K. Optimal colorectal cancer staging criteria in TNM classification. J Clin Oncol 2012; 30: 1519-1526 [PMID: 22430272 DOI: 10.1200/jco.2011.39.4692];

Japanese Society for Cancer of the Colon and Rectum. Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma: the 3rd English Edition [Secondary Publication]. J Anus Rectum Colon 2019; 3: 175-195 [PMID: 31768468 DOI: 10.23922/jarc.2019.018];

Hashiguchi Y, Muro K, Saito Y, Ito Y, Ajijoka Y, Hamaguchi T, Hasegawa K, Hotta K, Ishida H, Ishiguro M, Ishihara S, Kanemitsu Y, Kinugasa Y, Murofushi K, Nakajima TE, Oka S, Tanaka T, Taniguchi H, Tsuji A, Uehara K, Ueno H, Yamakata T, Yamazaki Y, Yoshida M, Yoshino T, Itabashi M, Sakamaki K, Sano K, Shimada Y, Tanaka S, Uetake H, Yamaguchi S, Yamaguchi N, Kobayashi H, Mutsuda K, Kotake S, Sugihara K. Japanese Society for Cancer of the Colon and Rectum. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer. Int J Clin Oncol 2020; 25: 1-42 [PMID: 31203527 DOI: 10.1007/s10147-019-01485-3];

Bilchik AJ, Hoon DS, Saha S, Turner RR, Wiese D, DiNonne M, Koyanagi K, McCarter M, Shen P, Iddings D, Chen SL, Gonzalez M, Elshoff D, Morton DL. Prognostic marker of micrometastases in colon cancer: interim results of a prospective multicenter trial. Ann Surg 2007; 246: 568-75; discussion 575 [PMID: 17893493 DOI: 10.1097/SLA.0b013e31815550c7];

Sloothaak DAM, van der Linden RLA, van de Velde CJH, Benelman WA, Lips DI, van der Linden JC, Doornenwaard H, Tanis PJ, Bosseka K, van der Zaag ES, Buskens CJ. Prognostic implications of occult nodal tumour cells in stage I and II colon cancer: The correlation between micrometastasis and disease recurrence. Eur J Surg Oncol 2017; 43: 1456-1462 [PMID: 28576463 DOI: 10.1016/j.ejso.2017.04.012];

Park SJ, Lee KY, Kim SY. Clinical significance of lymph node micrometastasis in stage I and II colon cancer. Cancer Res Treat 2008; 40: 75-80 [PMID: 19688052 DOI: 10.4143/ct.2008.40.2.75];

Tani K, Itabashi M, Okuwa K, Okita K, Takemasa I, Tomita N, Ogawa S, Nagashima Y, Yamamoto M. Feasibility of Pooled One-Step Nucleic Acid Amplification for Molecular Staging of Pathologically Node-Negative Colon Cancer: A Prospective Multicenter Study. Ann Surg Oncol 2021; 28: 8804-8812 [PMID: 34086123 DOI: 10.1245/s10434-021-10140-9];

Lino-Silva LS, Xinaxtile DL, Salcedo-Hernández RA. Tumor deposits in colorectal cancer: the need for a new "pN" category. Ann Transl Med 2020; 8: 733 [PMID: 32647658 DOI: 10.21037/amt.2020.03.175];

Nagtegaal ID, Knijn N, Hugen N, Marshall HC, Sugihara K, Tot T, Ueno H, Quirke P. Tumor Deposits in Colorectal Cancer: Improving the Value of Modern Staging-A Systematic Review and Meta-Analysis. J Clin Oncol 2017; 35: 1119-1127 [PMID: 28029327 DOI: 10.1200/JCO.2016.68.9091];

Mirkin KA, Kulaylat AS, Hollenbeak CS, Messaris E. Prognostic Significance of Tumor Deposits in Stage III Colon Cancer. Ann Surg Oncol 2018; 25: 3179-3184 [PMID: 30083822 DOI: 10.1245/s10434-018-6661-9];

Yamano T, Semba S, Noda M, Yoshimura M, Kobayashi M, Hamaoka M, Beppu N, Yano A, Tsukamoto K, Matsubara N, Tomita N. Prognostic significance of classified extramural tumor deposits and extracapsular lymph node invasion in T3-4 colorectal cancer: a retrospective single-center study. BMC Cancer 2015; 15: 859 [PMID: 26545360 DOI: 10.1186/s12885-015-1885-6];

Nagayoshi K, Ueki T, Nishioka Y, Manabe T, Mizuochi Y, Hirahashi M, Oda Y, Tanaka M. Tumor deposit is a poor prognostic indicator for patients who have stage II and III colorectal cancer with fewer than 4 lymph node metastases but not for those with 4 or more. Dis Colon Rectum 2014; 57: 467-474 [PMID: 24604303 DOI: 10.1097/DCR.0000000000000595];

Li J, Yang S, Hu J, Liu H, Du F, Yin J, Liu S, Li C, Xing S, Yuan J, Lv B, Fan J, Leng S, Zhang X, Wang B. Tumor deposits counted as positive lymph nodes in TNM staging for advanced colorectal cancer: a retrospective multicenter study. Oncotarget 2016; 7: 18269-18279 [PMID: 26934317 DOI: 10.18632/oncotarget.7756];

Basnet S, Lou QF, Liu N, Rana R, Shah A, Khadka M, Warrier H, Sigdel S, Dhakal S, Devkota A, Mishra R, Sapkota G, Zheng L, Ge HY. Tumor deposit is an independent prognostic indicator in patients who underwent radical resection for colorectal cancer. J Cancer 2018; 9: 3979-3985 [PMID: 30416062 DOI: 10.7150/jca.27475];

Athanasakis E, Xenaki S, Venianaki M, Chalkiadakis G, Chrysos E. Newly recognized extratumoral features of colorectal cancer challenge the current tumor-node-metastasis staging system. Ann Gastroenterol 2018; 31: 525-534 [PMID: 30174388 DOI: 10.20524/aog.2018.0284];

Gröne J, Loch FN, Taupitz M, Schmidt C, Kreis ME. Accuracy of Various Lymph Node Staging Criteria in Rectal Cancer with Magnetic Resonance Imaging. J Gastrointest Surg 2018; 22: 146-153 [PMID: 28900855 DOI: 10.1097/gjss.0000000000000659];

Kim MJ, Hur BY, Lee ES, Park B, Joo J, Kim MJ, Park SC, Baek JY, Chang HJ, Kim DY, Oh JH. Prediction of lateral pelvic lymph node metastasis in patients with locally advanced rectal cancer with preoperative chemoradiotherapy: Focus on MR imaging findings. PLoS One 2018; 13: e0195815 [PMID: 29649321 DOI: 10.1371/journal.pone.0195815];

Zhang H, Zhang C, Zheng Z, Ye F, Liu Y, Zou S, Zhou C. Chemical shift effect predicting lymph node status in rectal cancer using high-resolution MR imaging with node-for-node matched histopathological validation. Eur Radiol 2017; 27: 3848-3855 [PMID: 28168360 DOI: 10.1007/s00330-017-4738-7];

Choi J, Oh SN, Yeo DM, Kang WK, Jung CK, Kim SW, Park MY. Computed tomography and magnetic resonance imaging evaluation of lymph node metastasis in early colorectal cancer. World J Gastroenterol 2015; 21: 556-562 [PMID: 25593474 DOI: 10.3748/wjg.v21.i2.556];

Kaur H, Choi H, You YN, Rauch GM, Jensen CT, Hou P, Chang JG, Skibber JM, Ernst RD. MR imaging for
preoperative evaluation of primary rectal cancer: practical considerations. Radiographics 2012; 32: 389–409 [PMID: 22419139 DOI: 10.1148/radiographics.3211511222]

Koh DM, Brown G, Husbands JE. Nodal staging in rectal cancer. Abdom Imaging 2006; 31: 652–659 [PMID: 16897279 DOI: 10.1007/s00261-006-9021-3]

Wang C, Zhou Z, Wang Z, Zheng Y, Zhao G, Yu Y, Cheng Z, Chen D, Liu W. Patterns of neoplastic foci and lymph node micrometastases within the mesorectum. Langenbecks Arch Surg 2005; 390: 312–318 [PMID: 16049726 DOI: 10.1007/s00243-005-0562-7]

Hadfield MB, Nicholson AA, MacDonald AW, Farouk R, Lee PW, Duthie GS, Monson JR. Preoperative staging of rectal carcinoma by magnetic resonance imaging with a pelvic phased-array coil. Br J Surg 1997; 84: 529–531 [PMID: 9112099]

Kim NK, Kim MJ, Yun SH, Sohn SK, Min JS. Comparative study of transrectal ultrasonography, pelvic computerized tomography, and magnetic resonance imaging in preoperative staging of rectal cancer. Dis Colon Rectum 1999; 42: 770–775 [PMID: 10378601 DOI: 10.1007/BF02236933]

Roy C, Bierry G, Matau A, Bazille G, Pasquali R. Value of diffusion-weighted imaging to detect small malignant pelvic lymph nodes at 3 T. Eur Radiol 2010; 20: 1803–1811 [PMID: 20182732 DOI: 10.1007/s00330-010-1736-4]

Zhou J, Zhan S, Zhu Q, Gong H, Wang Y, Fan D, Gong Z, Huang Y. Prediction of nodal involvement in primary rectal carcinoma without invasion to pelvic structures: accuracy of preoperative CT, MR, and DWIBS assessments relative to histopathologic findings. PLoS One 2014; 9: e92779 [PMID: 24695111 DOI: 10.1371/journal.pone.0092779]

Beets-Tan RGH, Lambregs DM, Maas M, Bipat S, Barbaro B, Curvo-Semedo L, Fenlon HM, Gollub MJ, Gourtsoyianni S, Halligan S, Hoeftel C, Kim SH, Laghi A, Maier A, Rafaelsen SR, Stoker J, Taylor SA, Torkzad MR, Blomqvist L. Magnetic resonance imaging for clinical management of rectal cancer: Updated recommendations from the 2016 European Society of Gastrointestinal and Abdominal Radiology (ESGAR) consensus meeting. Eur Radiol 2018; 28: 1465–1475 [PMID: 29043428 DOI: 10.1007/s00330-017-5025-2]

Fritz S, Kilguss H, Schaad A, Lazarou L, Sommer CM, Richter GM, Köper-Steffen R, Feilhauer K, König J. Preoperative versus pathological staging of rectal cancer-challenging the indication of neoadjuvant chemoradiotherapy. Int J Colorectal Dis 2021; 36: 191-194 [PMID: 32955607 DOI: 10.1007/s00259-020-03751-3]

Catalano OA, Lee SL, Parente C, Cauley C, Ferrando FS, Striar R, Sossi V. Resolution modeling in PET imaging: theory, practice, benefits, and pitfalls. Med Phys 2013; 40: 043401 [PMID: 23718620 DOI: 10.1118/1.4808006]

Kijima S, Sasaki T, Nagata K, Utano K, Lefor AT, Sugimoto H. Preoperative evaluation of colorectal cancer using CT colonography, MRI, and PET/CT. World J Gastroenterol 2014; 20: 16964-16975 [PMID: 25493009 DOI: 10.3748/wjg.v20.i45.16964]

Lu YY, Chen JH, Ding HJ, Chien CR, Lin WY, Kao CH. A systematic review and meta-analysis of pretherapeutic lymph node staging of colorectal cancer by 18F-FDG PET or PET/CT. Nucl Med Commun 2012; 33: 1127-1133 [PMID: 22800029 DOI: 10.1097/NMN.0b013e328357e2b9]

Abdel-Nabi H, Doerr RJ, Lamonica DM, Cronin VR, Galantowicz PJ, Carbone GM, Spaulding MB. Staging of primary colorectal carcinomas with fluorine-18 fluorodeoxyglucose whole-body PET: correlation with histopathologic and CT findings. Radiology 1998; 206: 755–760 [PMID: 9494497 DOI: 10.1148/radiology.206.3.9494497]

Furukawa H, Ikuma H, Seki A, Yokoe K, Yuen S, Aramaki T, Yamagushi S. Positron emission tomography scanning is not superior to whole body multidetector helical computed tomography in the preoperative staging of colorectal cancer. Gut 2006; 55: 1007–1011 [PMID: 16361308 DOI: 10.1136/gut.2005.076273]

Mukai M, Sadahiro S, Yasuda S, Ishida H, Tokunaga N, Tajima T, Makuchi H. Preoperative evaluation by whole-body 18F-fluorodeoxyglucose positron emission tomography in patients with primary colorectal cancer. Oncol Rep 2000; 7: 85-87 [PMID: 10601597]

Shin SS, Jeong YY, Min JJ, Kim HR, Chung TW, Kang HK. Preoperative staging of colorectal cancer: CT vs. integrated FDG PET/CT. Abdom Imaging 2008; 33: 270–277 [PMID: 17610107 DOI: 10.1007/s00261-007-9262-9]

Bae SU, Won KS, Song BI, Jeong WK, Baek SK, Kim HW. Accuracy of F-18 FDG PET/CT with optimal cut-offs of maximum standardized uptake value according to size for diagnosis of regional lymph node metastasis in patients with rectal cancer. Cancer Imaging 2018; 18: 32 [PMID: 30217167 DOI: 10.1186/s40464-018-0165-5]

Rahmin A, Qi J, Sossi V. Resolution modeling in PET imaging: theory, practice, benefits, and pitfalls. Med Phys 2013; 40: 064301 [PMID: 23718620 DOI: 10.1118/1.4808006]

Catalano OA, Coutinho AM, Sahani DV, Vangel MG, Gee MS, Hahn PF, Witzel T, Sosoi C, Salvatore M, Li Y, Umifta L, Catalano OA, DM, Blaskowsky LS, Ryan DP, Clark JW, Hong TS, Blaszkowsky L, Berger DL, Ricciardi R, Clark JW, Ryan DP, Wo J. OSNA in colorectal cancer: a systematic review and meta-analysis. PLoS One 2014; 9: e92779 [PMID: 24695111 DOI: 10.1371/journal.pone.0092779]
Clinical impact of PET/MRI in oligometastatic colorectal cancer. *Br J Cancer* 2021; 125: 975-982 [PMID: 34282295 DOI: 10.1038/s41416-021-01494-8]

Catalano OA, Daye D, Signore A, Iannace C, Vangel M, Luongo A, Catalano M, Filomena M, Mansi L, Soriciella A, Salvatore M, Fuin N, Catana C, Mahmood U, Rosen BR. Staging performance of whole-body DWI, PET/CT and PET/MRI in invasive ductal carcinoma of the breast. *Int J Oncol* 2017; 51: 281-288 [PMID: 28335000 DOI: 10.3892/ijo.2017.4012]

Incoronato M, Grimaldi AM, Cavaliere C, Inglese M, Mirabelii P, Monti S, Ferbo U, Nicolai E, Soricielli A, Catalano OA, Aiello M, Salvatore M. Relationship between functional imaging and immunohistochemical markers and prediction of breast cancer subtype: a PET/MRI study. *Eur J Nucl Med Mol Imaging* 2018; 45: 1680-1693 [PMID: 29696443 DOI: 10.1007/s00259-018-4010-7]

Nessa F, Beiderwellen K, Heusch P, Wetter A. Clinical applications of PET/MRI: current status and future perspectives. *Diagn Interv Radiol* 2014; 20: 438-447 [PMID: 25010371 DOI: 10.5152/dir.2014.14008]

Lambregts DM, Maas M, Cappendijk VC, Prompers LM, Mottaghy FM, Beets GL, Beets-Tan RG. Whole-body diffusion-weighted magnetic resonance imaging: current evidence in oncology and potential role in colorectal cancer staging. *Eur J Cancer* 2011; 47: 2107-2116 [PMID: 21664810 DOI: 10.1016/j.ejca.2011.05.013]

Chen H, Zhao L, Ruan D, Pang Y, Hao B, Dai Y, Wu X, Guo W, Fan C, Wu J, Huang W, Lin Q, Sun L, Wu H. Usefulness of [68Ga]Ga-DOTA-FAPI-04 PET/CT in patients presenting with inconclusive [18F]FDG PET/CT findings. *Eur J Nucl Med Mol Imaging* 2021; 48: 73-86 [PMID: 32588089 DOI: 10.1007/s00259-020-04940-e]

Pang Y, Zhao L, Luo Z, Hao B, Wu H, Lin Q, Sun L, Chen H. Comparison of [68Ga]Ga-DOTA-FAPI and [18F]FDG-Uptake in Gastric, Duodenal, and Colorectal Cancers. *Radiology* 2021; 298: 393-402 [PMID: 33258746 DOI: 10.1148/radiol.2020203275]

Bedrikovetski S, Dudi-Venkata NN, Kroom HM, Seow V, Vatter R, Carneiro G, Moore JW, Sannour T. Artificial intelligence for pre-operative lymph node staging in colorectal cancer: a systematic review and meta-analysis. *BMC Cancer* 2021; 21: 1058 [PMID: 34565533 DOI: 10.1186/s12885-021-08773-w]

Wood TF, Nora DT, Morton DL, Turner RR, Rangel D, Hutchinson W, Bilichik A. One hundred consecutive cases of sentinel lymph node mapping in early colorectal cancer: detection of missed micrometastases. *J Gastrointest Surg* 2002; 6: 322-9, discussion 229 [PMID: 12022982 DOI: 10.1016/s1091-255x(02)00163-3]

Saha S, Monson KM, Bilichik A, Beutler T, Dan AG, Schochet E, Wiese D, Kaushal S, Desai D. Comparative analysis of nodal upstaging between colon and rectal cancers by sentinel lymph node mapping: a prospective trial. *Dis Colon Rectum* 2004; 47: 1767-1772 [PMID: 15622567 DOI: 10.1016/s1053-0004-0661-5]

Cahill RA, Bembenek A, Sirop S, Waterhouse DF, Schneider W, Leroy J, Wiese D, Beutler T, Bilichik A, Saha S, Schlag PM. Sentinel node biopsy for the individualization of surgical strategy for cure of early-stage colon cancer. *Ann Surg Oncol* 2009; 16: 2170-2178 [PMID: 19472012 DOI: 10.1245/s10434-009-0510-9]

Tiffet O, Kaczmarek D, Chambonnière ML, Guillain T, Baccot S, Prévot N, Bageacu S, Bourgeois E, Cassagnau E, Lehur A, Dubois F. Combining radioisotopic and blue-dye technique does not improve the false-negative rate in sentinel lymph node mapping for colorectal cancer. *Dis Colon Rectum* 2007; 50: 962-970 [PMID: 17468975 DOI: 10.1016/s10350-007-0236-3]

Bianchi PP, Petz W, Casali L. Laparoscopic lymphatic roadmapping with blue dye and radioisotope in colon cancer. *Colorectal Dis* 2011; 13 Suppl 7: 67-69 [PMID: 22090523 DOI: 10.1111/j.1463-1318.2011.02786.x]

Tan KY, Kawamura YJ, Mizokami K, Sasaki J, Tsujinaka S, Maeda T, Nobuki M, Komishi F. Distribution of the first metastatic lymph node in colon cancer and its clinical significance. *Colorectal Dis* 2010; 12: 44-47 [PMID: 19438890 DOI: 10.1111/j.1463-1318.2009.01924.x]

Bilichik AJ, Saha S, Wiese D, Stonecipher JA, Wood TF, Sostrin S, Turner RR, Wang HJ, Morton DL, Hoon DS. Molecular staging of early colon cancer on the basis of sentinel node analysis: a multicenter phase II trial. *J Clin Oncol* 2001; 19: 1128-1136 [PMID: 11181678 DOI: 10.1200/JCO.2001.19.4.1128]

Saha S, Johnston G, Korant A, Shaik M, Kanaan M, Johnston R, Gatana B, Kaushal S, Desai D, Mannam S. Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation. *Am J Surg* 2013; 205: 302-5, discussion 305 [PMID: 23414953 DOI: 10.1016/j.amjsurg.2012.10.029]

Iddings D, Bilichik A. The biologic significance of micrometastatic disease and sentinel lymph node technology on colorectal cancer. *J Surg Oncol* 2007; 96: 671-677 [PMID: 18081169 DOI: 10.1002/jso.20918]

Bianchi PP, Cerianzi C, Rottoli M, Torzilli G, Roncalli M, Spinelli A, Montorsi M. Laparoscopic lymphatic mapping and sentinel lymph node detection in colon cancer: technical aspects and preliminary results. *Surg Endosc* 2007; 21: 1567-1571 [PMID: 17285373 DOI: 10.1007/s00464-006-9152-1]

Wiese DA, Saha S, Badin J, Ng PS, Gauthier J, Ahsan A, Yu L. Pathologic evaluation of sentinel lymph nodes in colorectal carcinoma. *Arch Pathol Lab Med* 2000; 124: 1759-1763 [PMID: 11100053 DOI: 10.5855/2000-124-1759-PEOSLN]

Bilichik AJ, Trocha SD. Lymphatic mapping and Sentinel node analysis to optimize laparoscopic resection and staging of colorectal cancer: an update. *Cancer Control* 2003; 10: 219-223 [PMID: 12794620 DOI: 10.1397/07327480310063003]

Argiñés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, Laurent-Puig P, Quag P, Yosino T, Taieb J, Martínez E, Arnold D. ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Localised colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2020; 31: 1291-1305 [PMID: 32702383 DOI: 10.1016/j.annonc.2020.06.022]

Shinagawa T, Tanaka T, Nozawa H, Emoto S, Murono K, Kaneko M, Sasaki K, Otani K, Nishikawa T, Hata K, Kawai K, Watanabe T. Comparison of the guidelines for colorectal cancer in Japan, the USA and Europe. *Ann Gastroenterol Surg* 2018; 2: 6-12 [PMID: 29863118 DOI: 10.1002/ags3.12047]

Hohenberger W, Weber K, Matzel K, Papadopoulos T, Merkel S. Standardized surgery for colorectal cancer: complete mesocolic excision and central ligation--technical notes and outcome. *Colorectal Dis* 2009; 11: 354-64, discussion 364 [PMID: 19016817 DOI: 10.1111/j.1463-1318.2008.01735.x]

West NP, Kobayashi H, Takahashi K, Perrakis A, Weber K, Hohenberger W, Sugihara K, Quirke P. Understanding optimal colonic cancer surgery: comparison of Japanese D3 resection and European complete mesocolic excision with
central vascular ligation. *J Clin Oncol* 2012; 30: 1763-1769 [PMID: 22473170 DOI: 10.1200/JCO.2011.38.3992]

92 Heald RJ, Husband EM, Ryall RD. The mesorectum in rectal cancer surgery--the clue to pelvic recurrence? *Br J Surg* 1982; 69: 613-616 [PMID: 7651457 DOI: 10.1002/bjs.1800691019]

93 MacFarlane JK, Ryall RD, Heald RJ. Mesorectal excision for rectal cancer. *Lancet* 1993; 341: 457-460 [PMID: 8094488 DOI: 10.1016/0140-6736(93)90207-w]

94 Lowry AC, Simmang CL, Boulus P, Farmer KC, Finan PJ, Hyman N, Killingback M, Lubowski DZ, Moore R, Penfold C, Savoca P, Stitz R, Tjandra JJ. Consensus statement of definitions for anorectal physiology and rectal cancer: report of the Tripartite Consensus Conference on Definitions for Anorectal Physiology and Rectal Cancer, Washington, D.C., May 1, 1999. *Dis Colon Rectum* 2001; 44: 915-919 [PMID: 11496067 DOI: 10.1007/BF02554575]

95 Fujita S, Mizusawa J, Kanemitsu Y, Ito M, Kinugasa Y, Komori K, Ohsue M, Ota M, Akazai Y, Shinzawa M, Yamaguchi T, Bandou H, Katsumata K, Murata K, Akagi Y, Takiguchi N, Saida Y, Nakamura K, Fukuda H, Akasu T, Moriya Y; Colorectal Cancer Study Group of Japan Clinical Oncology Group. Mesorectal Excision With or Without Lateral Lymph Node Dissection for Clinical Node Stage II/III Lower Rectal Cancer (JCOCG0212): A Multicenter, Randomized Controlled, Noninferiority Trial. *Ann Surg* 2017; 266: 201-207 [PMID: 28288057 DOI: 10.1097/SLA.0000000000002212]

96 Sakai Y, Hida K. Real-World Situation of Lateral Lymph Node Dissection for Rectal Cancer in Japan. *Dis Colon Rectum* 2019; 62: e629 [PMID: 31094963 DOI: 10.1097/DCR.000000000001369]

97 Yokoyama S, Takifuki K, Hotta T, Matsuda K, Watanabe T, Mitani Y, Ieda J, Yamaue H. Survival benefit of lateral lymph node dissection according to the region of involvement and the number of lateral lymph nodes involved. *Surg Today* 2014; 44: 1079-1083 [PMID: 24370948 DOI: 10.1007/s00595-013-0815-y]

98 Cahill RA, Leroy J, Mareseaux J. Localized resection for colon cancer. *Surg Oncol* 2009; 18: 334-342 [PMID: 18835772 DOI: 10.1016/j.suronc.2008.08.004]

99 Creavin B, Ryan E, Martin ST, Hanly A, O’Connell PR, Sheahan K, Winter DC. Organ Preservation with local excision or active surveillance following chemoradiotherapy for rectal cancer. *Br J Cancer* 2017; 116: 169-174 [PMID: 27997526 DOI: 10.1038/bjc.2016.417]

100 Augestad KM, Merok MA, Ignatovic D. Tailored Treatment of Colorectal Cancer: Surgical, Molecular, and Genetic Considerations. *Clin Med Insights Oncol* 2017; 11: 1179559176907663 [DOI: 10.1177/1179559176907663]

101 Saha S, Sehgal R, Patel M, Doan K, Dan A, Bilchik A, Beutler T, Wiese D, Bassily N, Yee C. A multicenter trial of sentinel lymph node mapping in colorectal cancer: prognostic implications for nodal staging and recurrence. *Am J Surg* 2006; 191: 305-310 [PMID: 16490536 DOI: 10.1016/j.amjsurg.2005.10.028]

102 Wright FC, Law CH, Berry S, Smith AJ. Clinically important aspects of lymph node assessment in colon cancer. *Surg Oncol* 2009; 19: 248-255 [PMID: 19235179 DOI: 10.1016/j.suronc.2009.07.002]

103 van der Zaag ES, Bouma WH, Tanis PJ, Ubbink DT, Bemelman WA, Buskens CJ. Systematic review of sentinel lymph node mapping procedure in colorectal cancer. *Ann Surg Oncol* 2012; 19: 3449-3459 [PMID: 22645131 DOI: 10.1245/s10434-012-2417-0]

104 Quadros CA, Lopes A, Araujo I, Fregnani JH, Fahel F. Upstaging benefits and accuracy of sentinel lymph node mapping in colorectal adenocarcinoma nodal staging. *J Surg Oncol* 2008; 98: 324-330 [PMID: 18618578 DOI: 10.1002/jso.21112]

105 André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham C, Zanielli M, Clingen P, Bridgewater J, Tabah-Fisch I, de Gramont A; Multicenter International Study of Oxalaplatin5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) Investigators. Oxalaplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004; 350: 2343-2351 [PMID: 15175436 DOI: 10.1056/NEJMoa032709]

106 Kuebler JP, Wieand HS, O’Connell MJ, Smith RE, Colangelo LH, Yothers G, Petrelli NJ, Findlay MP, Seay TE, Atkins JN, Zapas JL, Goodwin JW, Fehrenbacher L, Ramanathan RK, Conley BA, Flynn PJ, Soori G, Colman LK, Levine EA, Lanier KS, Wolmark N. Oxalaplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: results from NSABP C-07. *J Clin Oncol* 2007; 25: 2198-2204 [PMID: 17470851 DOI: 10.1200/JCO.2006.06.2974]

107 Sahin IH, Akce M, Aleso O, Shaib W, Lesinski GB, El-Rayes B, Wu C. Immune checkpoint inhibitors for the treatment of MSI-H/MMR-D colorectal cancer and a perspective on resistance mechanisms. *Br J Cancer* 2019; 121: 809-818 [PMID: 31607751 DOI: 10.1038/s41416-019-0599-y]

108 Gray RG, Quirke P, Handley K, Lopatin M, Magill L, Baehner FL, Beaumont C, Clark-Langone KM, Yoshizawa CN, Lee M, Watson D, Shak S, Kerr DJ. Validation study of a quantitative multigene reverse transcriptase-polymerase chain reaction assay for assessment of recurrence risk in patients with stage II colon cancer. *J Clin Oncol* 2011; 29: 4611-4619 [PMID: 22067390 DOI: 10.1200/JCO.2010.32.8732]

109 Díaz-Mercedes S, Archilla I, Camps J, de Lacy A, Gorostiaga I, Momblan D, Ibarzabal A, Maurel J, Chic N, Bombí JA, Lopes A, Araujo I, Fregnani JH, Fahel F. Upstaging benefits and accuracy of sentinel lymph node mapping in colorectal cancer: a perspective on resistance mechanisms. *Br J Cancer* 2019; 121: 809-818 [PMID: 31607751 DOI: 10.1038/s41416-019-0599-y]

110 Salazar R, Roepman P, Capella G, Moreno V, Simon I, Dreezen C, Lopez-Doriga A, Santos C, Marijnen C, Westerja J, Bruin S, Kerr D, Kuppen P, van de Velde C, Morreau H, Van Velthuysen L, Glas AM, Van’t Veer LJ, Tollenaar R. Gene expression signature to improve prognosis prediction of stage II and III colorectal cancer. *J Clin Oncol* 2011; 29: 17-24 [PMID: 21093218 DOI: 10.1200/JCO.2010.30.1077]

111 Nielsen U, Rosenberg R, Balmert A, Schuster T, Slotta-Huspenina J, Herrmann P, Bader FG, Fries H, Schlag PM, Stein U, Janssen KP. Integrative marker analysis allows risk assessment for metastasis in stage II colon cancer. *Ann Surg* 2012; 256: 763-71; discussion 771 [PMID: 23095620 DOI: 10.1097/SLA.0b013e318272de87]

112 Mauk M, Simon I, Nitsche U, Roepman P, Snel M, Glas AM, Schuster T, Keller G, Zeestraaten E, Goossens I, Janssen KP, Fries H, Rosenberg R. Independent validation of a prognostic genomic signature (ColoPrint) for patients with stage II colon cancer. *Ann Surg* 2013; 257: 1053-1058 [PMID: 23295318 DOI: 10.1097/SLA.0b013e31827ec1180]

113 Rahbari NN, Bork U, Motschall E, Tholrand K, Büchler MW, Koch M, Weitz J. Molecular detection of tumor cells in regional lymph nodes is associated with disease recurrence and poor survival in node-negative colorectal cancer: a
systematic review and meta-analysis. J Clin Oncol. 2012; 30: 60-70 [PMID: 22124103 DOI: 10.1200/JCO.2011.36.9504]

114 Broderick-Vella G, Ko A, O’Connell TX, Guenther JM, Danial T, DiFrancesco LA. Does tumor burden limit the accuracy of systematic mapping and sentinel lymph node biopsy in colorectal cancer? Cancer J. 2002; 8: 445-450 [PMID: 12508853 DOI: 10.1097/00130404-200211000-00008]

115 Bembenek A, String A, Greschel S, Schlag PM. Technique and clinical consequences of sentinel lymph node biopsy in colorectal cancer. Surg Oncol. 2008; 17: 183-193 [PMID: 18579192 DOI: 10.1016/j.suronc.2008.05.003]

116 Bilichik AJ, DiNorome M, Saha S, Turner RK, Wiese D, McCarter M, Hoon DS, Morton DL. Prospective multicenter trial of staging adequacy in colon cancer: preliminary results. Arch Surg. 2006; 141: 527-33; discussion 533 [PMID: 16785352 DOI: 10.1001/archsurg.141.6.527]

117 Bilichik AJ, Nora DT, Sabin LH, Turner RB, Trocha S, Krasne D, Morton DL. Effect of lymphatic mapping on the new tumor-node-metastasis classification for colorectal cancer. J Clin Oncol 2003; 21: 668-672 [PMID: 12586804 DOI: 10.1200/JCO.2003.04.037]

118 Esser S, Reilly WT, Riley LB, Eyyavazadeh C, Arcona S. The role of sentinel lymph node mapping in staging of colon and rectal cancer. Dis Colon Rectum 2001; 44: 850-4; discussion 854 [PMID: 11391147 DOI: 10.1007/BF02234707]

119 Dionigi G, Castano P, Rovera F, Boni L, Annoni M, Villa F, Bianchi V, Carrafiole G, Bacuzzi A, Dionigi R. The application of sentinel lymph node metatasis for lymph nodes in colorectal cancer. Surg Oncol 2007; 16 Suppl 1: S129-S132 [PMID: 18023573 DOI: 10.1016/j.suronc.2007.10.024]

120 Notomi T, Okayama H, Masabuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000; 28: E63 [PMID: 1093928/12.6.e63]

121 Yao-moto H, Murata K, Fukunaga M, Ohnishi T, Noura S, Miyake Y, Kato T, Ohitsuka M, Nakamura Y, Takemasa I, Mizushima T, Ikeda M, Ohue M, Sekimoto M, Neya R, Matsurawa N, Monden M, Doki Y, Mori M. Micrometastasis Volume in Lymph Nodes Determines Disease Recurrence Rate of Stage II Colorectal Cancer: A Prospective Multicenter Trial. Clin Cancer Res 2016; 22: 3201-3208 [PMID: 26831719 DOI: 10.1186/1708-0432.CCR-15-2199]

122 Kostaczek J, Peksa M, Slama J, Slunécko R, Vlašák P, Bouda J, Novotný Z, Topolčan O, Kucéra R, Kulda V, Housková K, Berezovskiy D, Bartáková A, Presl J. One-step nucleic acid amplification vs ultrastaging in the detection of sentinel lymph node metastasis in endometrial cancer patients. J Surg Oncol 2019; 119: 361-369 [PMID: 30508294 DOI: 10.1002/jso.25322]

123 Aldecoa I, Montironi C, Planell N, Pellise M, Fernandez-Esparrach G, Gines A, Delgado S, Mombian D, Moreira L, Lopez-Ceron M, Rakislova N, Martinez-Palli G, Balus J, Bombi JA, de Lacy A, Castells A, Balague F, Cuatrecasas M. Endoscopic tattooing of early colon carcinoma enhances detection of lymph nodes most prone to harbor tumor burden. Surg Endosc. 2017; 31: 723-733 [PMID: 27324339 DOI: 10.1007/s00464-016-5026-3]

124 Rakislova N, Montironi C, Aldecoa I, Fernandez E, Bombi JA, Jimeno M, Balague F, Pellise M, Castells A, Cuatrecasas M. Lymph node pooling: a feasible and efficient method of lymph node molecular staging in colorectal carcinoma. J Transl Med. 2017; 15: 14 [PMID: 28088283 DOI: 10.1186/s12967-016-1114-3]

125 Brito MJ, Honavar M, Cipriano MA, Lopes J, Coelho H, Silva AR, Silva M, Guimarães S, Frutuoso A, Gomes A, Barbosa E, Carlos S. Molecular Staging of Patients with Colon Cancer. The C-Closer-II Study: A Multicentre Study in Portugal. Acta Med Port 2018; 31: 661-669 [PMID: 30521460 DOI: 10.20344/amp.9996]

126 Esposito F, Noviello A, Moles N, Coppola Bottazzi E, Baismonte M, Macione I, Verbo U, Lepore M, Miro A, Crafa F. Sentinel Lymph Node Analysis in Colorectal Cancer Patients Using One-Step Nucleic Acid Amplification in Combination With Fluorescence and Indocyanine Green. Ann Coloproctol 2019; 35: 174-180 [PMID: 31487764 DOI: 10.3393/ac.2018.07.21.1]

127 Tiernan JP, Vergheze ET, Nair A, Pathak S, Kim B, White J, Thygesen H, Horgan K, Hanby AM. Systematic review and meta-analysis of cytokeratin 19-based one-step nucleic acid amplification versus histopathology for sentinel lymph node assessment in breast cancer. Br J Surg. 2014; 101: 298-306 [PMID: 24536007 DOI: 10.1002/bjs.9386]

128 Colling R, Yeung T, Hompes R, Kraus R, Cahill R, Mortensen N, Wang LM. OSNA testing for lymph node staging in colorectal cancer. J Clin Pathol 2017; 70: 638-639 [PMID: 28348048 DOI: 10.1136/jclinpath-2016-204299]

129 Yeung TM, Wang LM, Colling R, Kraus R, Cahill R, Mortensen N. Intraoperative identification and analysis of lymph nodes at laparoscopic colorectal cancer surgery using fluorescence imaging combined with rapid OSNA pathological assessment. Surg Endosc. 2018; 32: 1073-1076 [PMID: 28643063 DOI: 10.1007/s00464-017-5644-4]

130 Hiyoshi Y, Akiyoshi T, Fukunaga Y. The advantage of one-step nucleic acid amplification for the diagnosis of lymph node metastasis in colorectal cancer patients. Ann Gastroenterol Surg. 2021; 5: 60-66 [PMID: 33532681 DOI: 10.1002/ags3.12392]

131 Viegas J, Ahmed A, Elashoff D, Bilchik A. The prognostic effect of micrometastases in previously staged lymph node negative (NO) colorectal carcinoma: a meta-analysis. Ann Surg Oncol 2006; 13: 1386-1392 [PMID: 17009147 DOI: 10.1245/s00034-006-9120-y]

132 Aldecoa I, Atares B, Tarragona J, Bernet L, Sardon JD, Pereda T, Villar C, Mendez MC, Gonzalez-Osado E, Elorriaga K, Alonso GL, Zamora J, Planell N, Palacios J, Castells A, Matesas-Guiu X, Cuatrecasas M. Molecularity determined total tumour load in lymph nodes of stage I-II colon cancer patients correlates with high-risk factors. A multicentre prospective study. Firehows Arch 2016; 385: 395-394 [PMID: 27447172 DOI: 10.1002/ags2.16-1990-1]

133 Lugli A, Kirsch R, Ajikiya Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, Flejou JF, Hansen TP, Hartmann A, Kakar S, Langner C, Nagtegaal I, Puppa G, Riddell R, Ristimäki A, Sheahan K, Smyrk T, Sugihara K, Terris B, Ueno H, Vieth M, Zlobec I, Quirke P. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol 2017; 30: 1299-1311 [PMID: 28548122 DOI: 10.1038/modpathol.2017.16]

134 Koelzer VH, Zlobec I, Lugli A. Tumor budding in colorectal cancer–ready for diagnostic practice? Hum Pathol 2016; 47: 4-19 [PMID: 26476568 DOI: 10.1016/j.humpath.2015.08.007]
Lee VWK, Chan KF. Tumor budding and poorly-differentiated cluster in prognostication in Stage II colorectal cancer. Pathol Res Pract 2018; 214: 402-407 [PMID: 29487008 DOI: 10.1016/j.prp.2017.12.019]

Ryan É, Khaw YL, Creavin B, Geraghty R, Ryan EJ, Gibbons D, Hanly A, Martin ST, O’Connell PR, Winter DC, Sheahan K. Tumor Budding and PDC Grade Are Stage Independent Predictors of Clinical Stage in Mismatch Repair Deficient Colorectal Cancer. Am J Surg Pathol 2018; 42: 60-68 [PMID: 29112018 DOI: 10.1097/PAS.0000000000000931]

Backes Y, Elias SG, Groen JN, Schwartz MP, Wolfhagen FHJ, Geesing MJ, Ter Borg F, van Bergeijk J, Spanier BW, de Vos Tot Nederveen Cappel WH, Kessels K, Seldenhuijzen CA, Raaiu MG, Drillenburg P, Milne AN, Kerkhof M, Seerden TJC, Siersema PD, Vleggaar FP, Offerhaus GJ, Lacle MM, Moons LMG; Dutch T1 CRC Working Group. Histologic Factors Associated With Need for Surgery in Patients With Pwnculated T1 Colorectal Carcinomas. Gastroenterology 2018; 154: 1647-1659 [PMID: 29366842 DOI: 10.1053/j.gastro.2018.01.023]

Mitrovic B, Schaeffer DF, Riddell RH, Kirsch R. Tumor budding in colorectal cancer: time to take notice. Mod Pathol 2012; 25: 1315-1325 [PMID: 22790014 DOI: 10.1038/modpathol.2012.94]

Grizzi F, Celesti G, Basso G, Laghi L. Tumor budding as a potential histopathological biomarker in colorectal cancer: hype or hope? World J Gastroenterol 2012; 18: 6532-6536 [PMID: 23236225 DOI: 10.3748/wjg.v18.i45.6532]

Barresi V, Reggiani Bonetti L, Branca G, Di Gregorio C, Ponz de Leon M, Tucciari G. Colorectal carcinoma grading by quantifying poorly differentiated cell clusters is more reproducible and provides more robust prognostic information than conventional grading. Virchows Arch 2012; 461: 621-628 [PMID: 23093109 DOI: 10.1007/s00428-012-1326-8]

Barresi V, Branca G, Ieni A, Reggiani Bonetti L, Baron L, Mondello S, Tucciari G. Poorly differentiated clusters (PDCs) as a novel histological predictor of nodal metastases in pT1 colorectal cancer. Virchows Arch 2014; 464: 655-662 [PMID: 24771119 DOI: 10.1007/s00428-014-1580-z]

Ueno H, Kijikawa Y, Shimazaki H, Shintou E, Hashiguchi Y, Nakashima K, Maekawa K, Katsurada Y, Nakamura T, Mochizuki H, Yamamoto J, Hase K. New criteria for histologic grading of colorectal cancer. Am J Surg Pathol 2012; 36: 193-201 [PMID: 22251938 DOI: 10.1097/PAS.0b013e31823e5ded]

Barresi V, Reggiani Bonetti L, Ieni A, Domati F, Tucciari G. Prognostic significance of grading based on the counting of poorly differentiated clusters in colorectal mucinous adenocarcinoma. Hum Pathol 2015; 46: 1722-1729 [PMID: 26344416 DOI: 10.1016/j.humpath.2015.07.013]

Archilla I, Diaz-Mercedes S, Aguirre JJ, Tarragona J, Machado I, Rodrigo MT, Lopez-Prades S, Gorostiaga I, Landolfi S, Alén BO, Balague F, Castells A, Camps J, Cuatrecessas M. Lymph Node Tumor Burden Correlates With Tumor Budding and Poorly Differen trying Clusters: A New Prognostic Factor in Colorectal Cancer? Clin Transl Gastroenterol 2021; 12: e00303 [PMID: 33939382 DOI: 10.14309/tcg.0000000000000303]

Wild JB, Iqbal N, Francombe J, Papettas T, Sanders DS, Ramcharan S. Is it time for one-step nucleic acid amplification (OSNA) in colorectal cancer? Tech Coloproctol 2017; 21: 693-699 [PMID: 28887714 DOI: 10.1007/s10150-017-1690-0]

Weixler B, Teixeira da Cunha S, Warschow R, Dameartes N, Guller U, Vehmeijeier A, van de Velde CJH, Viehl CT, Zuber M. Molecular Lymph Node Staging with One-Step Nucleic Acid Amplification and Its Prognostic Value for Patients with Colon Cancer: The First Follow-up Study. World J Surg 2021; 45: 1526-1536 [PMID: 33512566 DOI: 10.1007/s00268-020-05949-6]

Viehl CT, Guller U, Cecini R, Langer I, Oehsner A, Terracciano L, Rihele HM, Lafler U, Oetli D, Zuber M. Sentinel lymph node procedure leads to upstaging of patients with resectable colon cancer: results of the Swiss prospective, multicenter study sentinel lymph node procedure in colon cancer. Ann Surg Oncol 2012; 19: 1599-1605 [PMID: 22322921 DOI: 10.1245/s10434-012-2223-6]

Weixler B, Rickenbacber A, Raptis DA, Viehl CT, Guller U, Rueff J, Zettl A, Zuber M. Sentinel Lymph Node Mapping with Isosulfan Blue or Indocyanine Green in Colon Cancer Shows Comparable Results and Identifies Patients with Decreased Survival: A Prospective Single-Center Trial. World J Surg 2017; 41: 2378-2386 [PMID: 28508233 DOI: 10.1007/s00268-017-4051-2]

Tanaka S, Oka S, Chayama K. Colorectal endoscopic submucosal dissection: present status and future perspective, including its differentiation from endoscopic mucosal resection. J Gastroenterol 2008; 43: 641-651 [PMID: 18807125 DOI: 10.1007/s00353-008-2223-4]

Sakuragi M, Togashi K, Konishi F, Koinuma K, Kawamura Y, Okada M, Nagai H. Predictive factors for lymph node metastasis in T1 stage colorectal carcinomas. Dis Colon Rectum 2003; 46: 1626-1632 [PMID: 14668587 DOI: 10.1007/BF02660767]

Kudo S. Endoscopic mucosal resection of flat and depressed types of early colorectal cancer. Endoscopy 1993; 25: 455-461 [PMID: 8261988 DOI: 10.1055/s-2007-1010367]

Tanaka S, Oka S, Kaneko I, Hirata M, Mouri R, Kanao H, Yoshida S, Chayama K. Endoscopic submucosal dissection for colorectal neoplasia: possibility of standardization. Gastrointest Endosc 2007; 66: 100-107 [PMID: 17591481 DOI: 10.1016/j.gie.2007.02.032]

Koyama Y, Kudo SE, Miyachi H, Ichimasa K, Hisayuki T, Oikawa H, Matsudaira S, Kimura YJ, Misawa M, Mori Y, Kodama K, Kudo T, Hayashi T, Nakamura K, Katagiri A, Hidaka E, Ishida F, Hamanishi S. Practical problems of measuring depth of submucosal invasion in T1 colorectal carcinomas. Int J Colorectal Dis 2016; 31: 137-146 [PMID: 26428364 DOI: 10.1007/s00384-015-2403-7]

deBeche-Adams T, Hassan I, Haggerty S, Stefanidis D. Transanal Minimally Invasive Surgery (TAMIS): a clinical spotlight review. Surg Endosc 2017; 31: 3791-3800 [PMID: 28656337 DOI: 10.1007/s00464-017-5636-4]

Hiki N, Nunobe S. Laparoscopic endoscopic cooperative surgery (LECS) for the gastrointestinal tract: Updated indications. Ann Gastroenterol Surg 2019; 3: 239-246 [PMID: 31131352 DOI: 10.1002/ags3.12238]

Bues G, Kipfmüller K, Hack D, Grüssner R, Heintz A, Junginger T. Technique of transanal endoscopic microsurgery. Surg Endosc 1988; 2: 71-75 [PMID: 3413659 DOI: 10.1007/BF00704356]

Bues G, Mengtes B, Manncke K, Starlinger M, Becker HD. Technique and results of transanal endoscopic microsurgery in early rectal cancer. Am J Surg 1992; 163: 63-69; discussion 69 [PMID: 1733375 DOI: 10.1001/0002-9374(1992)163:1:63---69]

Saclarides TJ, Smith L, Ko ST, Orkin B, Bues G. Transanal endoscopic microsurgery. Dis Colon Rectum 1992; 35:
carcinomas by one-step nucleic acid amplification (OSNA) reduces time to adjuvant chemotherapy interval.

Predictive value of the sentinel lymph node procedure in the staging of non-metastatic colorectal cancer.

Management of early invasive colorectal cancer. Risk of recurrence and clinical guidelines. Dis Colon Rectum 1995; 38: 1286-1295 [PMID: 7497841 DOI: 10.1007/BF02049154]

Choi PW, Yu CS, Jang SJ, Jung SH, Kim HC, Kim JC. Risk factors for lymph node metastasis in submucosal invasive colorectal cancer. World J Surg 2008; 32: 2089-2094 [PMID: 18553050 DOI: 10.1007/s00268-008-9628-3]

Borschitz T, Heintz A, Junginger T. Transanal endoscopic microsurgical excision of T2 rectal cancer: results and possible indications. Dis Colon Rectum 2007; 50: 292-301 [PMID: 17222286 DOI: 10.1053/dcrs.2007.006-016-7]

Hahneloser D, Wolff BG, Larson DW, Ping J, Nivatvongs S. Immediate radical resection after local excision of rectal cancer: an oncologic compromise? Dis Colon Rectum 2005; 48: 429-437 [PMID: 15747069 DOI: 10.1053/dcrs.2005-006-9990-3]

Serra-Aracil X, Badia-Closa J, Pallisera-Lloveras A, Mora-Lopez L, Serra-Pla S, Garcia-Nalda A, Navarro-Soto S. Management of intra- and postoperative complications during TEM/TAMIS procedures: a systematic review. Minerva Surg 2021; 76: 343-349 [PMID: 33433070 DOI: 10.23736/S2724-5691.20.08405-9]

Dighie S, Purkayastha S, Swift I, Tekkis PP, Darzi A, A'Hern R, Brown G. Diagnostic precision of CT in local staging of colon cancers: a meta-analysis. Clin Radiol 2010; 65: 708-719 [PMID: 20096298 DOI: 10.1016/j.crad.2010.01.024]

Choi AH, Nelson RA, Schoellhammer HF, Cho W, Ko M, Arrington A, Oxner CR, Fakhri M, Wong J, Sentovich SM, Garcia-Aguilar J, Kim J. Accuracy of computed tomography in nodal staging of colon cancer patients. World J Gastrointest Surg 2015; 7: 116-122 [PMID: 26225194 DOI: 10.4240/wjgs.v7.i7.1116]

Slama J, Dunnd P, Dusek L, Cibula D. High false negative rate of frozen section examination of sentinel lymph nodes in patients with cervical cancer. Gynecol Oncol 2013; 129: 384-388 [PMID: 23595889 DOI: 10.1016/j.ygyno.2013.02.001]

Balasubramanian SP, Harrison BJ. Systematic review and meta-analysis of sentinel node biopsy in thyroid cancer. Br J Surg 2011; 98: 334-344 [PMID: 21246517 DOI: 10.1002/bjs.7425]

Hyslop T, Waldman SA. Molecular staging of node negative patients with colorectal cancer. J Cancer 2013; 4: 193-199 [PMID: 23459453 DOI: 10.7150/jca.5830]

Melfi FM, Lucchi M, Davini F, Viti A, Fontanini G, Boldrini L, Boni G, Mussi A. Intraoperative sentinel lymph node mapping in stage I non-small cell lung cancer: detection of micrometastases by polymerase chain reaction. Eur J Cardiothorac Surg 2008; 34: 181-186 [PMID: 18502662 DOI: 10.1016/j.ejcts.2008.03.059]

Miyashiro I, Hirasawa M, Sasaki M, Sano T, Mizusawa J, Nakamura K, Hashimoto A, Tsunuburya A, Fukushima N; Gastric Cancer Surgical Study Group (GCSSG). False negative biopsy of intraoperative histological examination as a serious problem for clinical application of sentinel node biopsy for gastric cancer: an oncologic compromise? Gastric Cancer 2015; 17: 334-344 [PMID: 23933782 DOI: 10.1016/j.ygcanc.2014.09.011]

Zhou M, Wang X, Jiang L, Chen X, Bao X. The diagnostic value of one step nucleic acid amplification (OSNA) in differentiating lymph node metastasis of tumors: A systematic review and meta-analysis. Int J Surg 2018; 56: 49-56 [PMID: 29753955 DOI: 10.1016/j.ijsu.2018.05.010]

Balagüe C, Pallarés JL. Preoperative and Intraoperative Lymphatic Mapping for Radioguided Sentinel Node Biopsy in Cancers of the Gastrointestinal Tract. In: Mariani G, Manca G, Orsini F, Vidal-Sicart S, Valdés Olmos RA. Atlas of Neoplasms. Springer, 2013 [DOI: 10.1007/978-4-431-255528-5_14]

Kusano M, Nozaki R, Fujiyoshi T, Uchida Y. Management of early invasive colorectal cancer. Dis Colon Rectum 2015; 38: 708-719 [PMID: 26225194 DOI: 10.1016/j.dcrs.2015.01.024]

Takano M, Takagi K, Fujimoto N, Nozaki R, Fujiyoshi T, Uchida Y. Management of early invasive colorectal cancer. In: Kusano M, Kokudo N, Toi M, Kaibori M. ICG Fluorescence Imaging and Navigation Surgery. Tokyo: Springer, 2013 [DOI: 10.1007/978-4-431-255528-5_14]

Daraï E, Balagué C, Pallarés JL. Preoperative and Intraoperative Lymphatic Mapping for Radioguided Sentinel Node Biopsy in Cancers of the Gastrointestinal Tract. In: Mariani G, Manca G, Orsini F, Vidal-Sicart S, Valdés Olmos RA. Atlas of Neoplasms. Springer, 2013 [DOI: 10.1007/978-4-431-255528-5_14]
detecting lymph node metastasis of head and neck squamous cell carcinoma.

Peigné L, Cserni G. 10.1245/s10434-011-1591-9

of sentinel node concept in gastric cancer.

Kumano I, Otomo Y, Mochizuki H, Yamamoto J, Hase K. 10.1016/j.ciresp.2011.04.013

OSNA for colon cancer staging-medtech innovation briefing (MIB77). 2016 Aug 24 [cited 26 February 2022]. In: 37

of Estimated Benefit from Lateral Lymph Node Dissection for Middle and Lower Rectal Cancer.

Takahashi T, Doki Y, Maeda I, Mori M, Yamamoto H. 10.1186/s12885-021-08480-6

Fujino S, Sugimura K, Wada H, Takahashi H, Omori T, Miyata H. 10.1186/s12885-017-3408-0

Sammour T, Chang GJ. 10.1016/j.oraloncology.2019.104553

Yaguchi Y, Cutress RI. 10.1093/annonc/mdx224

Glynne-Jones R, Yamaguchi T, Kinugasa Y, Shiomi A, Kagawa H, Yamakawa Y, Furutani A, Manabe S, Yamaoka Y. 10.1007/s00384-016-2641-3

Sammour T, Chang GJ. Lateral pelvic lymph node dissection and radiation treatment for rectal cancer: Mutually exclusive or mutually beneficial? 10.1016/j.oraloncology.2019.104553

Yanagita S, Uenosono Y, Arigami T, Kita Y, Mori S, Natsugoe S. Utility of the sentinel node concept for detection of lateral pelvic lymph node metastasis in lower rectal cancer. 10.1016/j.bmcancer.2017.07.015

Noura S, Ohue M, Miyoshi N. Sentinel Node Navigation Surgery for Rectal Cancer: Indications for Lateral Node Dissection. In: Kusano M, Kukudo N, Toi M, Kaibori M. ICG Fluorescence Imaging and Navigation Surgery. Tokyo: Springer, 2014 [DOI: 10.1007/978-4-431-55526-5_15]

Yasui M, Ohue M, Noura S, Miyoshi N, Takahashi Y, Matsuda C, Nishimura J, Haraguchi N, Ushigome H, Nakai N, Fujino S, Sugimura K, Wada H, Takahashi H, Omori T, Miyata H. Exploratory analysis of lateral pelvic sentinel lymph node status for optimal management of laparoscopic lateral lymph node dissection in advanced lower rectal cancer without suspected lateral lymph node metastasis. 10.1186/s12885-017-3408-0

Miyake Y, Mizushima T, Hata T, Takahashi H, Hanada H, Shoji H, Nomura M, Haraguchi N, Nishimura J, Matsuda C, Takemasa I, Doki Y, Maeda I, Mori M, Yamamoto H. Inspection of Perirectal Lymph Nodes by One-Step Nucleic Acid Amplification Predicts Lateral Lymph Node Metastasis in Advanced Rectal Cancer. 10.1186/s12885-016-0464-x

Glynn-Jones R, Wyrwicz L, Tietz E, Brown G, Rödel C, Cervantes A, Arnold D; ESMO Guidelines Committee. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. 10.1007/s10350-006-0714-z

Osana T, Akiyoshi T, Sugihara K. The important risk factor for lateral pelvic lymph node metastasis of lower rectal cancer is node-positive status on magnetic resonance imaging: study of the Lymph Node Committee of Japanese Society for Cancer of the Colon and Rectum. 10.1002/jso.26730

Ogawa S, Hida J, Ike H, Kinugasa T, Ota M, Shintö E, Itabashi M, Okamoto T, Sugihara K. The important risk factor for lateral pelvic lymph node metastasis of lower rectal cancer is node-positive status on magnetic resonance imaging: study of the Lymph Node Committee of Japanese Society for Cancer of the Colon and Rectum. Int J Colorectal Dis 2016; 31: 1719-1728 [PMID: 27576475] DOI: 10.1007/s00384-016-2641-3

Takahashi T, Ueno M, Azezuka K, Obta H. Lateral node dissection and total mesorectal excision for rectal cancer. Dis Colon Rectum 2000; 43: S59-S66 DOI: 10.1007/BF02237228

Numata M, Yamaguchi T, Kinugasa Y, Shiomi A, Kagawa H, Yamakawa Y, Furutani A, Manabe S, Yamaoka Y. Index of Estimated Benefit from Lateral Pelvic Lymph Node Dissection for Middle and Lower Rectal Cancer. Anticancer Res 2017; 37: 2549-2555 [PMID: 28476826] DOI: 10.21873/anticancerres.11598

OSNA for colon cancer staging-medtech innovation briefing (MIB77). 2016 Aug 24 [cited 26 February 2022]. In: National Institute for Health and Care Excellence. Available from: https://www.nice.org.uk/advice/mib77

Guillén-Paredes MP, Carrasco-González L, Chávez-Benito A, Campillo-Soto A, Carrillo A, Aguayo-Albaisini JL. [One-step nucleic acid amplification (OSNA) assay for sentinel lymph node metastases as an alternative to conventional postoperative histology in breast cancer: A cost-benefit analysis]. Cir Esp 2011; 89: 456-462 [PMID: 21664607] DOI: 10.1016/j.ciresp.2011.04.013

Cutress RI, McDowell A, Gabriel FG, Gill J, Jeffrey MJ, Agrawal A, Wise M, Raftery J, Cree IA, Yingou C. Observational and cost analysis of the implementation of breast cancer sentinel node intraoperative molecular diagnosis. J Clin Pathol 2010; 63: 522-529 [PMID: 20843932] DOI: 10.1136/jcp.2009.072942

Yaguchi Y, Sugawara H, Tsujimoto H, Takata H, Nakabayashi K, Ichikura T, Ono S, Hiraki S, Sakamoto N, Horio T, Kumano I, Otomo Y, Mochizuki H, Yamamoto J, Hase K. One-step nucleic acid amplification (OSNA) for the application of sentinel node concept in gastric cancer. Ann Surg Oncol 2011; 18: 2289-2296 [PMID: 21301968] DOI: 10.1245/s10434-011-1591-9

Cserni G. Intraoperative analysis of sentinel lymph nodes in breast cancer by one-step nucleic acid amplification. J Clin Pathol 2012; 65: 193-199 [PMID: 22090341] DOI: 10.1136/jcp-2011-200301

Peigné L, Godey F, Le Gallo M, Le Gall F, Fautrel A, Morcet J, Jégoux F. One-step nucleic acid amplification for detecting lymph node metastasis of head and neck squamous cell carcinoma. Oral Oncol 2020; 102: 104553 [PMID: 32004908] DOI: 10.1016/oraloncology.2019.104553
