Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The authors investigated the clinical impact of OSNA analysis for colorectal cancer. These findings will be of interest to surgeon as well as researchers in the field.

I have following concerns.

1. The authors state that surgical treatment using the OSNA method leads to organ sparing surgery. Please describe the authors' opinion on how to resect the lymph nodes, the number of lymph nodes to be resected, the identification of the lymph nodes to be resected, and whether pOSNA or cOSNA is appropriate for organ-sparing surgery.

We added in the article:

"Both ex vivo and in vivo Indocyanine green fluorescence-imaging (ICG-FI) are reported to be feasible for the detection of SLNs in CRC. The submucosal injection technique and subserosal was both used. The mesocolon was inspected with conventional imaging and additionally with a near-infrared (NIR) 30° laparoscope (Olympus, Tokyo, Japan). All fluorescent lymph nodes identified with the NIR camera were considered SLN(s), intraoperatively harvested and presented to the pathologist separately from the rest of the specimen.

More work needs to be done to define protocols, indications for its use, a standard number of lymph nodes that need to be removed and to test its efficacy in larger patient populations.".

The average number of SLN found was 0-4.

Given the limited number of lymph nodes found with the SLN technique, cOSNA is sufficient in organ sparing surgery

2. Please describe the frequency of upregulation of CK19 mRNA in lymph node metastases of colorectal cancer.

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 micro metastases. 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 breast cancer patients even when its expression is not observed in primary tumors. Targeted studies on the upregulation of CK19 mRNA in lymph node metastases of colorectal cancer are needed [Yamamoto 2013].

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Title: accurately reflects the topic and contents of the paper. Abstract: is appropriate, not structured, 201 words. Core tip: is appropriate, 62 words. Key words: 7 key words (phrases), precisely define the content of the paper. Introduction: 277 words, the reader is acquainted with OSNA known facts. The purpose of the rewiew is also clearly stated. Methods: the methodology is explained, MEDLINE, SCOPUS, ClinicalTrials.gov and Cochrane Database were used to conduct a comprehensive computerized literature search. Review: the content is reasonably divided into chapters, with an appropriate description of the content and research findings at the present time. The authors appropriately point out the advantages, disadvantages and limitations of this method, including cost analysis. The text of the review is supplemented by 4 tables. Conclusion: 149 words, authors conclude with a clear message: "OSNA may be considered as the route to tailor-made surgery". References: 204, references are appropriate. Conflict of interest: none declared. Opinion of the reviewer The manuscript is interesting, presents a modern diagnostic method in the treatment of patients with colorectal cancer, unfortunately it is only available in some institutions.

Many Thanks for your valuable comments.

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments to Authors: The manuscript entitled "State of the art on the role of one-step nucleic acid amplification (OSNA) in colorectal cancer lymph node metastases detection: A Minireview" reports a review on the role of OSNA in detecting metastasis of CRC. The authors have summarized a lot of works in the relevant fields. The writing can be improved with better formatting strategy, to present the review in a logic way.

Many Thanks for your valuable comments and suggestions that we have used to improve our article.

Reviewer #4:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Thank you for inviting me to evaluate the minireview titled"State of the art on the role of one-step nucleic acid amplification (OSNA) in colorectal cancer lymph node metastases detection: A Minireview". It is an interesting paper, their findings suggest that OSNA assay has a high diagnostic accuracy and negative predictive value in detecting metastatic LNs in colorectal malignancy. The short turnaround time renders OSNA an attractive intra-operative method. OSNA resulted in upstaging in about 25% of stage II colorectal cancer. Moreover, organ sparing surgery in early colorectal cancer and tailored lymphadenectomy in more advanced cases can be performed. The paper is well arranged and the logic is clear, and. The cited literature is comprehensive . The provided figure and tables are well composed and understandable. The quality of language of the manuscript is acceptable for me. So, I recommend to you that this manuscript may be accepted. There are some advices for author: 1)On page 14, "Several studies identified prognostic genes that may select high-risk patients for adjuvant treatment[105-110]. But none of these marker panels have made it into clinical practice so far.", the significance of MSI-H needs to be discussed.

We added in the article:

"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 tumour mutational burden and the number of tumour-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 as 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 tumours more immunogenic compared with microsatellite-stable (MSS) tumours. MSI-induced frameshift mutations produce a significant number of neoantigens. Accordingly, MSI-H/MMR-D tumours manifest a great number of TILs, many of which can be directed against tumour-related neoantigens".

2) On page 15, "The expression of CK-19 mRNA was observed in all pathologically positive lymph nodes; however, CEA and CK-20 mRNAs were not found in metastatic nodes" ? Could you tell us why ?

The study by Yamamoto et al. (2013) examined 98 candidate mRNA genetic markers which were from a genome-wide database by comparing an expression frequency in colon cancer. After four sequencing phases, CK-19, CEA and CK-20 mRNAs were evaluated using OSNA assay.

In the fourth screening phase, the ROC curve analyses showed that CK19 and CEA mRNAs had significantly better diagnostic performance than CK20 mRNA. Further analysis of the data indicated that the OSNA with CK19 mRNA would make it easier to detect a metastatic focus in a LN than using CEA mRNA because OSNA could detect CK19 mRNA (100 copy/μl) more reproducibly than CEA mRNA (1 copy/μl). Thus, CK19 mRNA has a great advantage over CEA and CK20 mRNAs for use in OSNA.

Reviewer #5:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This topic is interesting. The authors systematically describe the role of one-step nucleic acid amplification (OSNA) in colorectal cancer lymph node metastases detection. This minireview plays an important guiding role in clinical practice, especially in accurate assessment of the stage. However, the literatures are all from developed countries, and the price of this technology exceeds that of conventional technology, and the feasibility in developing countries needs to be discussed.

The average cost per patient (including capital, maintenance, and disposable costs) ranges from £568–£608, depending on the cost of disposables[199].

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 breast cancer (BC), with an estimated saving between 400 and 700 £ per patient[200,201].

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, the reduction in the treatment costs after surgery and the reduction in costs relating to the treatment of recurrences, this method could be also attractive for developing countries.

Reviewer #6:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Good work. Well done！ 1.The layout of the article could be further enhanced. 2.The tables in the article need further sprucing up. 3.It would be great to add an artwork to summarise the technique 4.The content should be reduced.

Many Thanks for your valuable comments and suggestions that we have used to improve our article. We provided also the standard three-line tables.