

Molecular Lymph Node Staging with One-Step Nucleic Acid Amplification in Colon Cancer and its Prognostic Value

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Abstract

Accurate identification of tumor deposits in lymph nodes is important for correct colon cancer staging. Molecular lymph node investigation with one-step nucleic acid amplification (OSNA) could allow more precise lymph node analysis. Promising results from breast cancer demonstrated better detection rates of tumor deposits when using OSNA compared to conventional lymph node staging. However, it was unknown whether this method translates into better survival rates of patients with colon cancer. The here presented study investigated a group of patients with colon cancer from three European centres on a long-term basis, staged with OSNA and Haematoxylin & Eosin (H&E). One-step nucleic acid amplification failed to outperform conventional H&E work-up regarding its prognostic value, contrasting findings in other cancer types. However, future research should focus on the informative value of the total tumor load of the resected lymph nodes and the respective mRNA thresholds in order to detect small but relevant metastatic deposits what may increase the prognostic value of this molecular detection method.

Short Communication

The introduction of molecular lymph node staging

One-step nucleic acid amplification (OSNA) was introduced as a method to detect tumor specific mRNA in sentinel lymph nodes (SLN) of patients undergoing surgery for breast cancer [1]. With OSNA, cytokeratin 19 (CK19) mRNA is amplified based on reverse transcription-loop-mediated isothermal amplification and a threshold of ≥ 250 mRNA copies / μL is considered as a positive lymph node [2]. In the evaluation of SLN from patients with breast cancer OSNA shows a sensitivity of 87.5-100% and a specificity of 90.5-100% [3,4]. Molecular lymph node work-up with OSNA is nowadays a validated diagnostic and prognostic adjunct in breast cancer surgery [5]. Therefore, its diagnostic and prognostic capabilities seemed to be promising for the staging of colon cancer.

The issue with colon cancer lymph node staging

In colon cancer, a positive lymph node status (stage III) is one of the most important prognostic factors determining the allocation of adjuvant chemotherapy [6]. To date, however, there is no defined gold standard for the identification of positive or negative lymph nodes in colon cancer staging [7]. The standard histopathological lymph node workup usually consists of an H&E staining of one to two slices of each retrieved lymph node. From this it becomes clear that the standard method of lymph node processing carries a high risk that lymph node metastases will be missed, which is referred to as understaging (i.e. a tissue allocation bias). In the staging of colon cancer most national cancer organizations therefore recommend to analyze at least 12 lymph nodes in each specimen [8,9]. In this context, however, it is important to note that the agreement to yield a minimum of 12 lymph nodes was proposed three decades ago and up to date clear evidence to justify this threshold is still missing [10,11]. From an oncological point of view, it actually seems logical to set the threshold higher, or to examine consequently all lymph nodes from the corresponding lymphatic drainage area in order to get an approximation of the real metastatic burden [12]. The question inevitably arises to how a certain number of examined lymph nodes can compensate for the possible tissue allocation bias. Understaging may therefore substantially contribute to

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the high recurrence rate (20-25%) of patients with a negative lymph node status [13]. In an attempt to fathom the problem of this unacceptably high recurrence rate, it was shown that multilevel lymph node sectioning combined with immunohistochemistry can improve the detection rate of small nodal tumor infiltrates (i.e. isolated tumor cells and micro metastases), providing a picture that comes closer to the “real” lymph node burden [14]. This however is a costly and protracted process and it is therefore mainly limited to the SLN, which are detected intraoperatively either by indocyanine green or methylene blue injection around the tumor and are marked or collected separately for the pathologist to undergo the in-depth analysis.

The introduction of OSNA in colon cancer

Considering the possible tissue allocation bias with H&E staining as well as the cost and time intensive multilevel sectioning with immunohistochemistry, OSNA seems to be appealing since it does not require microscopic examination and it is fast. Up to date only very few studies have evaluated the clinical performance of OSNA in colon cancer. These studies compared OSNA either with conventional pathological work-up or with immunohistochemistry techniques [15-19]. OSNA showed an upstaging rate of negative lymph nodes comparable to that of the SLN procedure with multilevel sectioning an immunohistochemistry, ranging from 15-25% [20]. Most studies did not consider the intensified histopathological observation after SLN extraction as a comparison method, but compared OSNA with the conventional H&E technique. And it is noticeable that in almost all studies the lymph nodes were selected at random from the mesentery and not according to the lymph node stations in relation to the primary tumor. This shows that H&E workup was considered by most authors as the gold standard against which the new technology was compared. However, as mentioned before, there exists no gold standard to reliably stage a lymph node in colon cancer. Interestingly, the prognostic value of OSNA has not been investigated until recently. This is, on the one hand due to the fact that no prospective observational studies were carried out and, on the other hand to the fact, that there is no sound standard with regard to lymph node staging. Assessing the prognostic value of a method for lymph node staging is challenging as one would need a reasonable standard for the definition of lymph negativity and positivity, against which the new technique is tested.

The issue with OSNA in colon cancer

In order to circumvent the above mentioned problem, the here presented study defined the event of disease recurrence or cancer related death as the gold standard to distinguish between true node negative and positive disease. This definition was made as it can be assumed, according to our current understanding of colon cancer behaviour, that “real” node negative colon cancer will be cured by surgery. Based on this understanding, adjuvant chemotherapy is generally not recommended to patients with node negative disease.

The first study to evaluate the prognostic value of OSNA analysis in patients with colon cancer revealed that OSNA does not offer prognostic advantage compared to conventional lymph node staging with H&E [21]. This finding was indeed very interesting as the previous studies identified OSNA as a promising diagnostic tool in colon cancer showing high concordance rates with conventional histopathological work-up with H&E [15-19]. The recently published study on the prognostic value of OSNA in colon cancer demonstrated that a positive lymph node status identified with H&E (UICC stage III) was a predictor of worse cancer specific survival (HR=10.77, 95% CI: 1.07-108.7, p=0.019), whereas identification of positive lymph nodes with OSNA did not predict worse outcome (HR=3.08, 95%CI: 0.26-36.07, p=0.197). Furthermore interesting in this study was the fact, that with such a distinct definition of true lymph node negativity and positivity also the diagnostic accuracy of H&E (sensitivity 46.7% and specificity 84.7%) as well as of OSNA (sensitivity 60.0% and specificity 75.0%) was markedly impaired compared to the previous reports [20]. This suggested that understaging is a persistent problem in colon cancer staging regardless of the technique used. In the study on the prognostic significance of OSNA, which is highlighted here, 50% of the SLN tissue was examined using OSNA and 50% using H&E, whereas the remaining lymph nodes (all non-SLN) were examined using H&E alone [21]. Here one could argue that a larger number of lymph nodes were examined with H&E and that this may have contributed to the results favoring H&E diagnostics. In this regard it should be noted that the SLN are the lymph nodes with the highest probability of containing metastases and these were examined with both methods.

It would theoretically be necessary to fully analyze all lymph nodes of each patient using H&E and OSNA to compare both techniques most equally. This is however technically not possible, as lymph nodes needed for H&E cannot be processed for OSNA and vice-versa. In a recently published study on breast cancer, the prognostic significance of OSNA in the investigation of lymph nodes was also examined [5]. In this study, however, all SLN were completely subjected to the OSNA analysis and the focus was on the total tumor load of the SLN and its relevant threshold instead of comparing two different staging techniques.

Despite the promising data in breast cancer, OSNA does not seem to offer the same opportunities in colon cancer. In addition to the demonstrated lack of prognostic significance, other critical points have to be considered. Analysis with OSNA relies on fresh tissue material always requiring the immediate availability of a pathologist for lymph node harvesting, compared to formalin-fixed resection specimens which can be processed at any time. Tissue processing also requires extreme care to avoid tissue contamination, a problem that usually can be resolved easily in conventional microscopy. Colorectal tumors can be large and friable; and, because CK19 is a panepithelial marker present in normal colonic epithelium and primary tumors, contamination of material for molecular analysis by dislodged tissue fragments during specimen processing has to be avoided at any circumstance as this could allocate a patient to unnecessary chemotherapy.

The here presented and recently published study is the first that evaluates the prognostic value of OSNA in patients with colon cancer and includes patients from three different centers in Europe. OSNA failed to outperform conventional H&E work-up regarding its prognostic value. Despite its relevance in other fields, the results of the here presented study do currently not support the adoption of OSNA in the routine staging of patients with colon cancer. However, it is conceivable that there will be a possible application for OSNA in the future as the prognostic role of isolated tumor cells (ITC) in colon cancer has still not been conclusively clarified and it has been shown that ITC can be associated with poorer survival in patients with node-negative colon cancer [22]. Because ITC is difficult to detect, even when using multilevel sectioning and

immunohistochemistry – OSNA could be of importance here. Future investigation should focus on the informative value of the total tumor load of the resected lymph nodes and respective mRNA thresholds. This could allow the detection of small but relevant metastatic deposits what in the end may increase the prognostic value of this molecular detection method.

Conclusion

Although OSNA is a well-established method in the staging of breast cancer, it has so far no significance in colon cancer staging. This is due to the relatively sparse evidence in colon cancer on the one hand and the difficulties involved in lymph node work up on the other. Even though the latest evidence could not prove any prognostic value of OSNA in colon cancer, the method remains attractive for scientific questions.

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