In managing colon cancer, lymph node status is the most important prognostic factor and staging parameter. According to global and national treatment guidelines, adjuvant chemotherapy is not recommended for patients where metastases have not been found in one or more lymph nodes, except patients at high risk (T4 in combination with other tumour characteristics). Therefore, correct staging of the patient is of utmost importance in order to make optimal treatment decisions.

The current standard for nodal staging in colon cancer is postoperative histopathological examination of at least 12 lymph nodes with one section H&E. However, up to 30% of patients with stage II disease develop local recurrence or distant metastases within five years of surgery, which results in significantly poorer survival rates [1]. It can be estimated that this is caused by the limited analysis of lymph node tissue and so overlooking a relevant proportion of metastases, leading to a significant false negative rate between 11% and 24% [2].

The sentinel lymph node concept, which could facilitate nodal staging considerably by enabling intensive analysis of only a few nodes, is not currently established in colon cancer. Yet examining all resected lymph nodes in depth by serial sectioning and IHC staining is time-consuming and cost intensive and therefore not part of routine practice.
Lymph node analysis with OSNA®

OSNA® (One Step Nucleic-Acid Amplification), a molecular assay which is already used routinely for detecting lymph node metastases in breast cancer, is an optimal solution for the intensive analysis of lymph nodes in colon cancer and leads to improved staging. OSNA® is an automated procedure that uses rapid nucleic acid amplification technology (RT-LAMP®) to detect the expression levels of Cytokeratin 19 (CK19) mRNA. CK19 is an epithelial cell marker that normally is not present in healthy lymph node tissue. Lymph node tissue is simply homogenised and, after a short preparation step, samples are inserted into the RD-100i real-time detection system. Up to four samples can be analysed in parallel and results are available after about 30 minutes (Figure 1).

Results are displayed in three categories (++, +, –) with a direct relation to the measured copy numbers of CK19 mRNA. This not only allows differentiation between positive and negative samples but also provides an indication of the extent of the tumour burden. As this method enables the whole lymph node to be analysed, it delivers a maximum level of sensitivity and a reliable basis for treatment decisions.

- higher sensitivity than conventional method
- automated and standardised procedure
- improved staging
- faster availability of results, even intraoperative if desired
- less pathology workload

*RT-LAMP = reverse transcriptase loop-mediated isothermal amplification; licensed under the agreement of Eiken Chemical Co., Ltd
Clinical validation studies and results

The suitability of CK19 as a marker has been demonstrated in a pre-evaluation phase where CK19 was tested on histological positive and negative lymph nodes and in comparison to other markers (Figure 2). Results identified CK19 as the most sensitive and specific marker for lymph node metastasis in colon cancer.

In evaluation studies [3–5], the OSNA® method was compared to extensive histopathological examination where lymph nodes were cut into 4 slices. Alternate slices were analysed either by the OSNA® assay or used for multilevel histology of permanent sections. Sections were taken from five levels with skip ribbons of 200 μm. In summary, some 1000 lymph nodes have been analysed, generating the following performance data:

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<table>
<thead>
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<tbody>
<tr>
<td>Concordance rate</td>
<td>96.7 %</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95.0 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.1 %</td>
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</tbody>
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As part of the Japanese study, the method’s specificity was confirmed by the analysis of 136 lymph nodes from pNO patients.

The CK19 mRNA copy number of all negative lymph nodes tested in this setting was far below the cut-off value, resulting in a specificity of 100%. This strongly indicates that the risk of false positive results can be almost excluded (Figure 3). This performance evaluation provided an initial indication for the method’s upstaging potential. In another clinical trial, which has just been finished and where data will be published soon, this has been confirmed. Compared to conventional histology an upstaging rate of about 26% was demonstrated by analysing lymph nodes with OSNA®.
OSNA® in routine use

The easy sample preparation procedure combined with the high speed reaction offers a high degree of flexibility in terms of routine application of the system. It can be employed in intraoperative analysis of sentinel lymph nodes identified by in-vivo mapping, postoperative examination of sentinels (ex-vivo mapping) as well as ultrastaging of multiple lymph nodes.

The OSNA® system is a superb solution for different clinical needs but with a single aim of improved staging and decision making for colon cancer patients.

- fast and accurate results
- improved staging and patient management
- reliable basis for treatment decisions
- earlier start of therapy

<table>
<thead>
<tr>
<th>Intraoperative analysis</th>
<th>in-vivo SLNM</th>
<th>up to 4 LNs within 40 minutes</th>
</tr>
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<tbody>
<tr>
<td>Postoperative analysis</td>
<td>ex-vivo SLNM</td>
<td>up to 4 LNs within 40 minutes</td>
</tr>
<tr>
<td>Postoperative analysis</td>
<td>fresh or frozen LNs</td>
<td>12 LNs or more within 2 hours</td>
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</tbody>
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References


