Categorization of occult tumour cells in lymph nodes in patients with colon cancer not reliable enough

We examined the interobserver variability among Dutch pathologists in classifying occult tumour cells in lymph nodes in colon cancer.
Introduction

Lymph node status is the most important predictor for recurrence and survival in patients with colorectal cancer, and thus an essential part of the TNM-classification. After resection of colon carcinoma hematoxylin and eosin (H&E) staining of lymph nodes is used to detect lymphogenic metastases. However, 15-25% of patients with favourable early-stage I and II colon cancer (according to the American Joint Committee on Cancer (AJCC) absence of lymph node metastases) will have local recurrences or distant metastases within five years. This has prompted the use of sentinel lymph node (SN) mapping for colorectal cancer in an experimental setting. This procedure identifies lymph nodes most likely to harbour metastases and additional immunohistochemical analyses can be performed to detect occult tumour cells (OTC). Recently, OTC in lymph nodes were associated with a reduced 5-year rate of disease-free survival in women with breast cancer. Also, the use of the SN procedure in patients with breast cancer showed that 20% of the histologically negative patients were restaged. In the most recent TNM classification for breast cancer there is a distinction between macro metastases (≥2mm, pN1), micrometastases (0.2-2mm, pN1mi) and isolated tumour cells (ITC)(<0.2mm, pN0). The International Union Against Cancer (UICC) TNM-classification uses both quantitative and qualitative criteria. According to this definition OTC are ITC without clinical consequence if they are smaller than 0.2mm, or show no sign of activity (no proliferation, no desmoplastic stromal-reaction) and are localised in the lymphatic sinus. On the other hand if ITC are detected at the parenchyma of the lymph node, they are considered micrometastases (pN1mi+). However, in the 6th edition of the American Joint Committee on Cancer (AJCC) TNM-classification only quantitative criteria are used: clusters of tumour cells between 0.2mm and 2.0mm are micrometastases pN1(mi+) and all lesions smaller than 0.2mm are defined as ITC pN0(itc+). Furthermore, in immunohistochemical techniques sometimes a false positive staining of hematopoietic stem cells arise that has no meaning in staging.

If the occurrence of OTC in the SN demonstrated by immunohistochemical staining will be used for the administration of adjuvant chemotherapy in patients with colorectal cancer, it is crucial that these findings are consistent and reproducible. Therefore, we have examined the interobserver variability among Dutch pathologists in classifying OTC in lymph nodes in colon cancer. Characteristics that are associated with inconsistent diagnoses were identified in order to describe recommendations which could clarify the guidelines of the TNM classification.
Patients & methods

Patients

The study population consisted of a single centre consecutive series of patients operated on for colorectal cancer with curative intent between November 2006 and July 2007. After conventional H&E staining 82 patients were histologically staged as pN0. All lymph nodes of these patients were immunohistochemically analysed for the occurrence of OTC.

Immunohistochemical analysis

Serial sectioning was performed at 500μm intervals of all lymph nodes from formalin-fixed and paraffin-embedded archival tissue blocks. To decrease false positive staining three different monoclonal antibodies were used: The anti-epithelial cell antibody Ber-EP4 (DAKO, The Netherlands) was combined with two anti-cytokeratin antibodies: the anti-CK20 antibody, with its expression limited to gastrointestinal epithelial cells (Euro Diagnostica, Arnhem, The Netherlands), and the anti-cytokeratin marker Cam5.2 directed against cytokeratin 7 and 8 expressed in all epithelial cells (Becton and Dickinson, Alphen aan den Rijn, The Netherlands). Details of the immunohistochemical analysis are described elsewhere.13

Study design

Of 30 immunohistochemically detected lesions representable pictures were made by a digital camera (Olympus) fixed on a conventional light microscope. One to three images with different magnifications and a scale for each photo were placed on a secured website (http://gelre.wockey.nl). Together with these pictures seven pictures were added of small volume lesions detected by H&E staining. Forty randomly selected pathologists were asked to judge the pictures and to categorise the lesions into ‘micrometastases’, ‘isolated tumour cells’ or ‘different’ and whether they were certain of their diagnosis. At each photograph they had the opportunity to comment. A selection of pictures to be judged is shown on Figure 1.

Definitions

According to the latest TNM classification OTC found in lymph nodes are divided into clinically relevant micrometastases (pN1mi+) and ITC without clinical consequences (pN0itc+).8-11 The pathologists had the option to use the UICC TNM-classification based on both quantitative and qualitative criteria, or the AJCC TNM-classification which uses quantitative criteria only.7,11

Figure 1 Examples of pictures placed at the website. A. Picture 1 (classified as lymph-angio invasion, isolated tumour cell or micrometastasis), B. Picture 6 (unanimously classified as micrometastasis), C. Picture 11 (mostly classified as isolated tumour cell, sometimes as micrometastasis based on localization and stroma cell reaction), D. Picture 15 (classified as isolated tumour cell or false-positive staining), E. Picture 21 (unanimously classified as isolated tumour cell), F. Picture 25 (classified as isolated tumour cell or contamination based on localization), G. Picture 33 and H. Picture 34 (classified as micrometastasis or isolated tumour cell, depending on whether or not lesions can be added up).
Statistical analysis

The interobserver agreement between pathologists judging OTC was calculated by the Kendall-W coefficient. This coefficient calculates the reproducibility of responses and risk-corrected level of agreement in the case of multiple evaluators. The Kendall-W coefficient of agreement has a range of 0.0 to 1.0. A coefficient greater than 0.8 stands for (almost) excellent agreement between multiple observers (Table 1).14

All statistical analyses were performed using SPSS version 15.0 (SPSS INC., Chicago, IL, USA).

### Table 1
Kendall-W coefficient; risk-corrected level of agreement in the case of multiple evaluators.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Level of agreement</th>
</tr>
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<tbody>
<tr>
<td>0.00 - 0.20</td>
<td>Poor</td>
</tr>
<tr>
<td>0.21 - 0.40</td>
<td>Fair</td>
</tr>
<tr>
<td>0.41 - 0.60</td>
<td>Moderate</td>
</tr>
<tr>
<td>0.61 - 0.80</td>
<td>Good</td>
</tr>
<tr>
<td>0.81 - 1.0</td>
<td>Excellent</td>
</tr>
</tbody>
</table>

Results

Thirty-seven out of 40 pathologists took part in the survey (response rate of 93%). Two responses were excluded; one case because the query was incomplete, and the second case because the pathologist did not classify any lesion, arguing that in colon cancer the distinction between micrometastasis and ITC cannot be made. In total 35 responses could be used for analysis. All participants were certified pathologists, three pathologists were specialised in gastro-intestinal oncology. At the time of participation eight pathologists worked at a university hospital or oncological centre and 27 pathologists at a general hospital.

In five cases (14%) there was complete agreement in classification of the lesion. Three lesions were categorised unanimously as micrometastases and two small clusters of tumour cells localised in the lymphatic sinus as isolated tumour cells. In 17 out of the 37 pictures (46%) the degree of agreement was poor to moderate, nine pictures (24%) showed a moderate degree of agreement and in 11 pictures (30%) a good to perfect agreement was found (Kendall-W coefficient > 0.61). When the analysis was performed only on diagnoses in which the pathologists were confident about their judgment, the amount of pictures with a good to perfect agreement increased to 18 out of 37 pictures (49%) (Table 2).

### Table 2
Kendall-W degree of agreement of pathologists judging small volume lesions.

<table>
<thead>
<tr>
<th>Kendall-W</th>
<th>All pictures</th>
<th>Pictures marked as certain*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>0.00 - 0.20</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>0.21 - 0.40</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>0.41 - 0.60</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>0.61 - 0.80</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>0.81 - 1.0</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

*Pictures marked by the pathologists as convinced about their diagnosis.

Tumour cell characteristics correlated with discrepant diagnoses

Characteristics of the judged lesions of 26 pictures with a Kendall-W coefficient < 0.6 were analysed. In 16 lesions it was a relevant difference (ITC pN0 or artefact versus micrometastasis pN1). Predominantly this was associated with the occurrence of multifocal lesions in eight pictures, in which the pathologists indicated that it appeared unclear if these multifocal lesions should be added up. In three lesions the poor agreement was caused by the fact that some pathologists classified lesions less than 0.2 mm consciously as micrometastasis on the basis of tumour cell characteristics or localisation in lymphatic parenchyma. Two lesions with clinically relevant difference in diagnosis were approximately 0.2 mm and therefore discrepancy was probably based on differences of measurement. One lesion was scored by some pathologists as a micrometastasis while others classified these cells as contamination based on their position at the border of the lymph node. Two pictures with a poor agreement (Kendall-W 0.06) showed a tumour embolism at a blood vessel. This lesion was judged in three different manners; as a micrometastasis based on its localisation, as an isolated tumour cell because of its size and as a vascular invasion (no metastatic disease). The other ten lesions with a poor agreement were predominately different judgments in isolated tumour cells, artefacts or contamination.
Comments on the pictures

Although the pathologists confirmed that the survey consisted of clear pictures and a representative series of lesions, it appeared to be more difficult to judge these lesions on pictures compared to the use of a light microscope in daily practice. They addressed that this was mainly caused by the inability to see depth and they could not compare the lesion with the primary tumour. Off course this will influence the poor agreement shown in ten lesions for which they doubted between isolated tumour cells, contamination or artefact. However this will only partially explain the poor to moderate agreement in 16 pictures diagnosing micrometastasis or ITC. This is because for this distinction the main criteria are the localisation and the size of the lesion and whether or not multiple tumour clusters should be measured as one. These aspects can be well assessed on a picture with a reference line. Besides, the pathologists also stated in their comments that it is unclear which criteria should be used in daily practice.

Discussion

We show that OTC are difficult to classify reliably in lymph nodes of patients with colon cancer. In 70% of the 37 pictures the judgement of 35 pathologists showed a poor to moderate risk-corrected level of agreement (Kendall-W < 0.6). Two recent studies describing the reproducibility of the classification of OTC also showed disappointing results.17, 18 The pathologists who participated indicated that it was difficult to give a reliable diagnosis because they could not see depth at the pictures and a comparison with the original tumour was not possible either. This is partly an explanation for the moderate reliability. However, in contrast to daily practice, in our study investigators did not have to search for the lesions themselves which should enhance the reliability of immunohistochemically stained lesions in lymph nodes. Previously performed studies showed that the detection of small volume lesions is difficult and depends on concentration and experience of the pathologist.19,20 Besides, the pathologists had the opportunity to indicate at each picture whether they were confident about the diagnosis. If the impaired judgment of lesions at the pictures was the cause of the poor agreement, one would expect results to improve if only those lesions had been analysed in which the pathologists had indicated that they were convinced about the diagnosis. Although the percentage of pictures with a good agreement (Kendall-W coefficient 0.6-1.0) increased from 30% to 49%, the agreement in half of the judged pictures remained poor to moderate.

Two groups within the 26 pictures of moderate agreement were distinguished during analysis of the results: clinically relevant disagreement in diagnosis ITC (pN0ictc) versus micrometastasis (pN1mi) (n=16) and the discrimination of false-positive staining of ITC (n=10). In the first group the moderate agreement was related to the uncertainty in the TNM classification concerning adding up multifocal lesions. More specific guidelines could solve this problem. Another problem classifying small volume lesion is the difference in AJCC and UICC criteria. In the 6th edition of the AJCC TNM-classification only quantative criteria are used; all lesions smaller than 0.2 mm are defined as ITC pN0(ict+).7 Besides this criterion the UICC also uses malignant activity and localisation in the lymph node to describe OTC, lesions smaller than 0.2 mm can also be classified as clinically relevant micrometastasis.9 Some pathologists addressed that they deliberately used the latter classification because some lesion smaller than 0.2 mm were yet judged as clinically relevant. To come to a uniform judgement it is desirable to classify small lesions with the use of the AJCC criteria and to judge all lesions smaller than 0.2 mm as ITC. This is also consistent with the TNM classification; in doubt a lower category should be chosen.7

In patients with breast cancer a previous research showed that an uniform division between ITC and micrometastases can be made by establishing clear guidelines and training of pathologists.18 This does not apply to distinguishing false-positive staining cells from ITC. Although this difference is of little relevance, the poor agreement in ten pictures indicates that selective monoclonal antibodies are mandatory to prevent false positive staining.22

Conclusion

We concluded that categorisation of micrometastases and ITC in lymph nodes of patients with colon cancer is not unambiguous. In case occurrence of OTC will be used for the decision to give systemic chemotherapy in patients with colon cancer, better definitions and a more accurate classification for OTC are needed.
References


