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Title: Sentinel node procedure in colorectal carcinoma
Issue Date: 2013-12-05
Chapter 8

Summary
The aim of this thesis is to investigate whether lymph node staging in patients with colorectal carcinoma can be improved by using the sentinel node technique in combination with in-depth analysis of this sentinel node.

In patients without distant metastasis, the presence of lymph node metastasis is the most important prognostic factor for disease recurrence and survival. Even if after a complete, oncologic resection of the primary tumour, with adjacent colon/rectum and mesocolon/rectum, the pathologic examination shows a radical resection without lymph node metastasis, still 25% of patients develop recurrent disease\(^1\textsuperscript{2}\). It is presumed that standard pathological examination misses clinically relevant (micro)metastases (occult metastasis).

Patients with lymph node metastases benefit from adjuvant chemotherapy\(^3\textsuperscript{4}\). In patients without evidence of lymph node metastases, chemotherapy has been shown less beneficial with high numbers needed to treat, and therefore remains debatable\(^2\textsuperscript{4}\textsuperscript{3}\textsuperscript{5}\). This raises the question whether it would be possible to improve lymphatic staging and identify those occult micrometastasis. Patients with these lymph node micrometastasis could then be offered adjuvant chemotherapy to improve their prognosis\(^6\).

Chapter 1 is a general introduction.

Chapter 2 provides an overview of the literature about lymphatic staging in colorectal carcinoma. Accuracy of lymphatic staging depends on the number of lymph nodes assessed and the pathological procedure of this assessment, the in-depth analysis of the lymph nodes. Fat-clearance techniques have shown to increase the lymph node yield to a mean of 58\(^7\). For in-depth analysis of the lymph nodes, two different techniques are discussed; immunohistochemical (IHC) staining and reverse transcriptase polymerase chain reaction (RT-PCR). The studies, and exact techniques used, vary quite a bit; with or without multi-sectioning, used antibody or antigen mRNA and threshold for positivity. This reflects in the various results. Four studies showed clinical relevant upstaging using IHC\(^8\textsuperscript{11}\), as did all described studies using RT-PCR\(^12\textsuperscript{14}\).

A review of the literature (in 2005) about the sentinel node procedure in colorectal carcinoma is presented. Most studies show a successful procedure with high accuracy rates and acceptable sensitivity\(^15\textsuperscript{18}\). Upstaging, with the use of in-depth pathologic examination, varies from 4% to 25%.

Chapter 3 describes the successful use of Patent Blue V for the sentinel node procedure in colon carcinoma. Previous studies describing a successful sentinel lymph node detection in colorectal carcinoma (sensitivity > 90%), all used Lymphazurin (isosulfan blue) as blue dye\(^15\textsuperscript{19}\textsuperscript{20}\). Studies that used Patent Blue V had lower sensitivity\(^21\textsuperscript{25}\). This is the first study using Patent Blue V that has equally good results as with Lymphazurin; sentinel node identification 94% (33/35), accuracy 97% (32/33) and sensitivity 91% (10/11).

Chapter 4 shows that the sentinel node procedure after preoperative short-course radiotherapy in rectal carcinoma is not reliable. In most centres in The Netherlands,
patients with rectal carcinoma undergo a total mesorectal excision (TME) after preoperative short-course radiotherapy, because this has been shown to lower local recurrence and improve survival\textsuperscript{26,27}.

Contrary to studies that show a successful sentinel node procedure for rectal carcinoma\textsuperscript{15,28}, we here show that it is not reliable for rectal carcinoma when performing a total mesorectal excision (TME-procedure) after preoperative short-course radiotherapy. Identification of the sentinel node was successful in 76% (26/34) of patients. In 4 patients the sentinel node contained metastases, and in 6 patients the sentinel node was false negative. This resulted in a sensitivity of 40% (4/10), negative predictive value of 73% (16/22), and accuracy of 77% (20/26). Possible reasons for these poor results are discussed in this chapter and include the anatomy of the rectum with a mesorectum surrounding the rectum (which has to remain intact in the TME-procedure for adequate pathological examination), and the preoperative radiotherapy that may cause lymph nodes to disappear and obliterate lymphatic vessels causing altered lymphatic flow\textsuperscript{29,30}.

\textbf{Chapter 5} describes the successful introduction of the sentinel node procedure in colon carcinoma in six hospitals in The Netherlands. Identification of the sentinel node was successful in 97% (67/69) of patients, with a high sensitivity of 89% (24/27), negative predictive value of 93% (40/43), and accuracy of 96% (64/67). Sentinel nodes that were negative for metastasis on standard haematoxylin and eosin (HE) staining were further analysed using IHC staining. This resulted in an upstaging of 13% (9/69).

\textbf{Chapter 6} shows the possibilities of in-depth analysis of the sentinel node using a RT-PCR technique with CEA. In 12 patients with colon carcinoma that had sentinel nodes negative for metastasis on conventional HE staining and additional IHC staining, RT-PCR with CEA was performed on these sentinel nodes. Two patients had positive CEA expression levels, one patient had a doubtful CEA expression level (0.004). The other 9 patients had negative CEA expression levels and all other lymph nodes were also examined using RT-PCR with CEA. All non-sentinel nodes had negative CEA expression levels, this indicates that the sentinel node correctly reflects the lymph node status in colon carcinoma when using RT-PCR with CEA for in-depth analysis. Upstaging was 17–25% (2 or 3 /12), depending on the cut-off used for expression level.

Furthermore, this study shows the possibility of performing RT-PCR on mRNA extracted from paraffin-embedded tissue using the Specht-method\textsuperscript{31}. This is important for clinical applicability of the procedure because in general no frozen lymph node tissue is available, but usually paraffin blocks/sections are stored from all histological examinations.

\textbf{Chapter 7} describes the 5-year follow-up results of 55 patients who underwent elective resection for primary colon carcinoma, including a sentinel node procedure. Conventional pathologic examination, including the sentinel nodes, identified 17 patients (31%) with lymph node metastasis and 38 patients (69%) without lymph node metastasis.
After a median follow-up of 70 months, 6 patients died of non-cancer related disease, 6 patients had recurrent disease and all died. Four of these 6 patients with recurrent disease had a positive sentinel node on standard HE-staining, resulting in a disease-free 5-year survival of the patients without lymph node metastasis (stage I and II) of 94%. In depth pathological examination of the sentinel node with IHC identified 3 more patients with lymph node metastasis (upstaging 5% (3/55)), but was unable to identify the one with recurrent disease. The Ariol system identified 4 (other) patients with a positive sentinel node (upstaging 7% (4/55)), including one with recurrent disease, resulting in a disease-free 5-year survival of patients without lymph node metastasis (stage I and II) of 96%. These results were compared with a historical control group to show the potential clinical impact of the sentinel node procedure. Patients with colon carcinoma, who are node-negative after a sentinel node procedure with, but even without, in-depth pathological examination of the sentinel node, have an excellent prognosis and do not need adjuvant treatment.

**GENERAL DISCUSSION**

Prognosis and treatment of colorectal carcinoma depends on disease-stage. Patients with distant metastasis have the poorest prognosis (5-year survival 6%)\(^1\). Treatment depends highly on location and number of distant metastasis. Surgical approach and/or radiologic intervention have become more aggressive, resulting in improved overall 5-year survival up to 41% after liver resection\(^{33}\), and 54% after pulmonary resection\(^{34}\). When local treatment is not possible, because of location or number of distant metastasis, patients can, depending on physical state, be offered palliative chemotherapy with a median overall survival of about 16 months\(^{35}\).

In patients without distant metastasis, lymphatic staging is critical because this is the best predictor of prognosis, and indicates whether a patient should be offered adjuvant treatment. It has been shown that patients with lymph node metastasis on conventional pathological examination -single section with HE staining- (stage III) should be offered adjuvant treatment\(^{33,34}\). Currently, the most used chemotherapeutical regimen is a combination of 5-fluorouracil, folinic acid (leucovorin) and oxaliplatin or a combination of capecitabine and oxaliplatin\(^{36}\). Patients without lymph node metastasis on conventional pathological examination (stage I / II) have the best prognosis, but still about 25% develop recurrent disease\(^3\). Even if treated with chemotherapy, 16% of patients with stage II colon carcinoma have recurrent disease\(^4\). Therefore, chemotherapy in the absence of lymph node metastasis remains debatable with high numbers needed to treat\(^{45}\). Identifying those patients (with stage I / II disease) at risk for developing recurrent disease remains
key. It is most likely that standard pathological examination misses clinically relevant occult (micro)metastasis.

In-depth pathological examination with either IHC staining or RT-PCR has been shown to be able to identify such occult (micro)metastasis. A recent review and meta-analysis shows that molecular tumour-cell detection (micrometastases and ITCs) in regional lymph nodes indicate poor prognosis in node-negative patients with colorectal carcinoma. It seems logical to select these patients at risk for recurrent disease for adjuvant treatment, however no prospective randomized trials have been performed to proof such approach to be beneficial.

The downside of such in-depth pathological examination of all resected lymph nodes is that it is an expensive and very labour intensive procedure. Therefore, these techniques have not become mainstream.

The sentinel node procedure is based on the concept that tumour metastasis occur in an orderly and sequential pattern. The sentinel node is the first lymph node that receives lymphatic drainage directly from the tumour and therefore has the highest chance of harbouring metastases. Combining the sentinel node procedure with in-depth pathologic analysis makes such in-depth pathological examination less labour intensive and subsequently less expensive. Identification of micrometastasis, ITCs or molecular detected metastasis, that all have a proven worse prognosis, may become generally applicable. To date, however, no randomised trial has confirmed the hypothesis that adjuvant treatment of high risk micrometastatic disease leads to an improved prognosis for colon cancer patients.

This thesis describes the successful prognostication of the sentinel node procedure in colon carcinoma. However, it is not reliable in rectal carcinoma when these patients are treated with total mesorectal excision and preoperative short-course radiotherapy. The sentinel node procedure can be used in combination with in-depth pathological examination with IHC-staining, RT-PCR or the Ariol-system, resulting in an accuracy of 98% and sensitivity of 95%. The procedure can (and should) be easily introduced in every clinic, because it upstages patients with micrometastasis and consequently causes stage migration. Patients that remain node-negative have an excellent prognosis (100% cancer-specific 5-year survival and 96% disease-free 5-year survival) and do not need further treatment. Whether patients that have lymph node micrometastasis, and are upstaged from stage II to stage III, should be offered adjuvant chemotherapy has been suggested, but will need further research to be proven.

In The Netherlands, the En-Route+ Study was designed to elucidate this problem. This open label, multicenter, randomized controlled clinical trial started in November 2010 and randomizes patients with micrometastasis in the sentinel node between adjuvant chemotherapy or no adjuvant therapy. Unfortunately, this study is stopped because of slow accrual and fewer than expected eligible patients were being observed. Over the years many promising prognostic markers have failed to be implemented in routine clini-
cal practice. In fact, our tremendous improved knowledge of the biology of colorectal cancer has left us with disappointing little markers for risk stratification and subsequent adjuvant treatment. One of the main reasons for this may be the fact that large, multicenter randomized trials are necessary to evaluate molecular markers for treatment allocation. These trials are often hampered by slow accrual or are underpowered. This phenomenon, known as “Lasagna’s Law” – where researchers and clinicians invariably overestimate the number of patients available for study participation – has been observed by a number of commentators. It was exactly this, that led to the premature closure of the En-Route+ Study, notwithstanding the sound biological rationale of the study as described in this thesis and by others.

For the studies described in this thesis we used Patent Blue V (Guerbet laboratories, Aulnay-Sous-Bois, France) as blue dye for the sentinel node procedure. A disadvantage of using visible blue dyes is that these dyes cannot be easily seen through fatty tissue, which is one of the reasons that the identification of the sentinel node in rectal carcinoma is unreliable. An improved technique of the sentinel node biopsy may overcome this problem. Optical imaging using near-infrared (NIR) fluorescence has been introduced for real-time visualization of sentinel nodes during surgery. Advantages of NIR fluorescent (700-900 nm) light include high tissue penetration (up to 10 mm), thereby enabling the identification of nodes located deeper into the mesenteric fat. In a recent paper, combining blue dye staining with NIR imaging of colon cancer tissue specimens, NIR fluorescence was of added value to blue dye staining, as NIR fluorescence allowed detection of additional SNs (not seen with blue dye only) and allowed easy detection of SNs located deeper into the mesenteric fat.

Another new development is the use of ‘one-step nucleic acid amplification’ (OSNA) for pathological lymph node analysis. This is a semiautomatic system that detects cytokeratin 19 (CK 19) mRNA by amplification. CK 19 is an epithelial marker, which presence is highly suggestive of lymph node metastasis. Compared to histopathology, OSNA has high sensitivity (95%) and specificity (98%), and upstages 15% of patients. Although this is a very promising diagnostic procedure, there is yet no clinical data on the relevance of these patients upstaged by OSNA.

The key question remains whether detectable micrometastatic disease in colorectal cancer indicates the need for systemic treatment. With technical advancements, such as OSNA, the opportunities for routine clinical use become better. Nevertheless, clinical relevance of the upstaging effect of such techniques must come from long-term clinical follow-up. For breast cancer the same issues of relevance for micrometastasis have been discussed for years. In a large retrospective Dutch cohort it was found that patient with micrometastatic disease treated with chemotherapy had a better prognosis than the same patients without chemotherapy. Although this is no formal level 1 evidence (randomized controlled trial), in the current Dutch breast cancer guidelines, micrometastasis in...
lymph nodes can be weighed as a factor in calculating recurrence risk and subsequent need for adjuvant treatment.

Another emerging technique for prognostication is gene expression profiling\(^5\). However these techniques are expensive and not easily implemented in routine clinical practice. One can envision however that in a step-wise approach sentinel node procedure in colon cancer may serve as a first step in which true node negative patients have an excellent prognosis. These patients do not need further treatment. Patients with lymph node metastasis on routine HE staining are in need of adjuvant chemotherapy\(^3\).\(^4\). This leaves only a small proportion of patients with lymph node micrometastasis or ITC’s for whom it is still not clear whether adjuvant treatment is beneficial or where there is a high number of patients needed to treat (NNT)\(^4\).\(^5\). For this group, new imaging or molecular techniques may elucidate that clinical question.

Meanwhile, the sentinel node procedure can be generally introduced for patients with colon carcinoma as described in this thesis. There is basically no downside of the procedure and it will improve prognostication of those patients. Node-negative patients will not need any further treatment. Hopefully new trials, incorporating new and improved detection techniques, such as fluorescence imaging, one-step nucleic acid amplification (OSNA) or gene expression profiling, will enable us to show if patients with micrometastatic positive sentinel nodes will have a better prognosis when treated with adjuvant systemic therapy.

**CONCLUSION**

This thesis shows that the sentinel node procedure can be successfully performed in colon carcinoma, using Patent Blue V as blue dye (Chapter 3). However, it is not reliable in rectal carcinoma treated with total mesorectal excision after preoperative short-course radiotherapy (Chapter 4). The sentinel node procedure can be easily introduced in clinical practise in every clinic (Chapter 5). RT-PCR with CEA, on mRNA extracted from paraffin-embedded sentinel nodes, upstages 17 – 25% of patients and accurately predicts lymph node status (Chapter 6). A 5-year follow-up of the sentinel node procedure in colon carcinoma -with, but even without, in-depth pathological examination- shows excellent results of the patients in de node-negative group with 100% cancer-specific 5-year survival and 96% disease-free 5-year survival and these node-negative patients do not need further treatment (Chapter 7).
REFERENCES


Summary


