Surgery for colorectal cancer: improving staging by the sentinel lymph node procedure
van der Zaag, E.S.

Citation for published version (APA):
van der Zaag, E. S. (2011). Surgery for colorectal cancer: improving staging by the sentinel lymph node procedure
Summary

In this thesis the role of the sentinel lymph node (SN) procedure in colorectal surgery is described. The aim of our research was to assess the accuracy of SN mapping in staging patients with colorectal cancer (CRC).

In Chapter 1 we determined the accuracy of this procedure from published data and identified factors that contribute to the conflicting reports in the literature. A systematic search of the Medline, Embase and Cochrane databases from 1999 to July 2011 revealed 98 potentially eligible studies, of which 57 were analysed including 3934 patients. The pooled SN identification rate was 91% with a higher identification rate in studies including more than 100 patients or studies using the ex vivo SN technique. The pooled sensitivity of the SN procedure was 70% with an overall accuracy of 88%. Subgroups with higher sensitivity were identified: 4 SNs versus <4 SNs (85% versus 66%, p=0.003), colon versus rectal cancer (78% versus 66%, p=0.04), and T1/T2 versus advanced T3/T4 carcinomas (93% versus 59%, p=0.01). Serial sectioning and immunohistochemical analysis resulted in a mean upstaging of 19%. The rate of upstaging, defined as a micrometastasis (pN1mi+) rather than isolated tumour cells (ITC) (pN0itc+) was 8%. If one included the immunohistochemical findings, the pooled sensitivity of the SN procedure increased to 80%. Our meta-analysis demonstrates an overall disappointing sensitivity of SN mapping in colorectal patients. However, in early staged colon cancer the SN procedure has acceptable accuracy rates and refines staging.

To compare the predictive value of SN mapping between patients with colon and rectal cancer a prospective comparison was performed, described in Chapter 2. An ex vivo SN procedure was performed in 100 patients with colon and 32 patients with rectal cancer. If the SN was negative, immunohistochemical analyses using two different antibodies against cytokeratins (Cam5.2, and CK 20) and one antibody against epithelial cells (BerEp-4) were performed to detect occult tumour cells (OTC). According to the America Joint Committee on Cancer (AJCC) ITC (< 0.2mm) were discriminated from micrometastases (0.2-2mm). A SN was identified in 117 patients and it accurately predicted nodal status in 106 patients (accuracy 91%). Both sensitivity and negative predictive value were higher in colon carcinomas than in rectal carcinomas (83% versus 57%, p=0.06 and 93% versus 65%, p=0.002 respectively). In patients with extensive lymph node metastases the SN procedure was unsuccessful. Eleven of the 13 unsuccessful SN procedures occurred in patients with rectal cancer who had had pre-operative radiotherapy. After immunohistochemical analysis, 21 of the 73 N0 patients had OTC in their SN; eight patients had micrometastases and 13 patients had ITC. The SN mapping accurately predicted nodal status in patients with colon cancer. Immunohistochemical analysis demonstrated micrometastatic disease in eight out of 73 N0 patients, with a true upstaging rate of 11%. However, SN mapping was less reliable in patients with rectal cancer after pre-operative radiotherapy.
Most studies on the SN procedure in patients with CRC include immunohistochemical analysis of the SN only. To evaluate the real diagnostic accuracy of the SN procedure with immunohistochemical analysis, the presence of OTC in all histologically negative lymph nodes was compared to the presence of these cells in SNs. Also the reproducibility of diagnosing OTC and the sensitivity of three different antibodies was assessed and described in **Chapter 3**. Between November 2006 and July 2007, an SN procedure was performed in 58 histologically N0 patients with CRC. All lymph nodes (n=908, mean 16) were step-sectioned and immunohistochemistry was performed. Tumour cells were identified in 19 of 58 patients, with micrometastases (0.2-2 mm) in seven and ITC (<0.2 mm) in 12 patients. The overall agreement in diagnosing OTC between two independent pathologists was 86%. A SN was identified in 53 of 58 patients. All micrometastases were found in the SNs. In two patients with negative SNs, ITC were demonstrated in non-SNs (sensitivity 88%, overall accuracy 96%). Additional immunohistochemical analysis of histologically negative lymph nodes demonstrated OTC in 33% of the patients resulting in an upstaging rate of 12%. If occult tumour cells are present they can predominantly be found in the SN of patients with colorectal cancer.

According to the latest version of the TNM-classification OTC are divided in micrometastases (pN1mi+) and ITC (pN0itc+). The question is whether this division can be made reliably. In **Chapter 4** we assessed the interobserver agreement between pathologists in judging photographs of OTC in lymph nodes of patients with colon cancer. All lymph nodes of 82 pN0 patients with colon cancer were analysed immunohistochemically with three different monoclonal antibodies against epithelial cells. Digital pictures of the 37 detected lesions were placed on a secured website. Forty randomly selected pathologists were asked to categorize the lesions into 'micrometastases', 'isolated tumour cells' or 'different' and they had the opportunity to comment. The degree of agreement was calculated by the Kendall W coefficient. Thirty-five pathologists (88%) categorised the 37 lesions. Five lesions (14%) were categorised unanimously as micrometastases or ITC. In 26 pictures (70%) the agreement was poor to moderate. When the analysis was performed only on those diagnoses of which the pathologists were confident about their judgment, the percentage of lesions with good agreement rose to 49%. Differences in agreement were principally associated with multifocal lesions, clusters of tumour cells <0.2 mm with proliferation characteristics in the parenchyma of the lymph node and lymphangio invasion. The differentiation between micrometastases and ITC in lymph nodes of patients with colon cancer is not uniform.

Laparoscopic surgery has the potential of less tumour cell spread because of the no touch isolation technique. In **Chapter 5** we assessed the effect of the surgical approach (open versus no touch laparoscopic) on the presence of tumour cells in SNs of patients with stage I and II CRC. A single centre consecutive prospective series of patients operated on for CRC was analysed. After conventional H&E staining 107 patients without lymphatic metastases were included, 59 patients had open surgery, 48 patients underwent laparoscopic resection. Patients in the laparoscopic group underwent a no touch medial to lateral approach whereas the conventional lateral to medial approach was applied in open surgery. A SN procedure was performed in all patients. The SNs were analysed for the presence of OTC. These tumour cells were divided into micrometastases (0.2-2 mm) or ITC (<0.2 mm). In ten patients micrometastases were found equally distributed over the groups. However, ITC were more often found after open surgery (18 versus 5 patients, p=0.03). The presence of OTC was related to the depth of tumour invasion and tumour diameter (>3.5 cm). Logistic regression analysis identified lymphangio invasion as a predictor for micrometastases (OR 18.4) whereas open resection was predictive for the presence of ITC (OR 3.3). The no touch medial to lateral laparoscopic surgery resulted in less isolated tumour cells in lymph nodes compared to open surgery in patients with stage I and II colorectal cancer. Every attempt should be made to prevent worsening of the prognosis of patients during surgery. In that respect laparoscopic resection with the no touch technique may have benefits over open surgery in patients with stage I and II colorectal cancer.

SN mapping for staging in patients with CRC remains controversial because survival analyses are lacking. In **Chapter 6** we prospectively assessed the effect of SN mapping on nodal staging and its implication on survival in these patients. Between November 2005 and July 2009, 331 patients underwent a resection. In 189 patients (group A) an SN procedure was performed with immunohistochemical analysis of the SN. Tumour cell deposits between 0.2 mm and 2.0 mm were referred to as micrometastases (pN1mi+). The remaining patients (n=142, group B) had standard nodal staging. Multivariate cox regression analysis was performed to identify prognostic factors for disease recurrence. The average number of harvested lymph nodes was higher in group A than in group B (16 versus 12, p<0.0001). After conventional staging, 81 patients (43%) had nodal metastasis in group A. This increased to 89 patients (47%) when immunohistochemically detected micrometastases were included. In group B 50 patients (35%) had nodal metastasis. During follow-up, a lower recurrence rate was seen in true pN0 patients after SN mapping revealed no tumour cells when compared to the conventional staging group (4% versus 15%, p=0.04). The SN procedure (hazard ratio: 4.1) was an independent strong predictor of disease recurrence. The SN procedure results in a more accurate staging of patients with CRC. This is reflected by a better prognosis of pN0 patients after SN mapping.

In patients with CRC colonoscopic tattooing is performed to mark the tumour site before surgery. Possibly this pre-operative tattooing can also serve as a SN procedure. The aim of our study described in **Chapter 7** was to determine if colonoscopic tattooing can contribute to staging accuracy by increasing the lymph node (LN) yield.
Conclusions and future perspectives

The SN procedure accurately predicts nodal status in patients with early staged colon cancer. However, SN mapping is less reliable in patients with rectal cancer after pre-operative radiotherapy. If occult tumour cells are present they are predominantly found in the SN. Additional immunohistochemical analysis of the SN of patients with histologically negative lymph nodes demonstrates OTC in 33% of these patients, resulting in an upstaging rate of 12%. Therefore, the SN procedure results in a more accurate staging of patients and is an independent predictor of disease-free survival. This is reflected by a better prognosis of pN0 patients after SN mapping. Since the ex-vivo SN mapping is an easy and safe procedure we recommend that the SN procedure should always be considered in addition to conventional resection in patients with colon cancer.

However, the differentiation between micrometastases and ITC in lymph nodes of patients with colon cancer is not uniform. If patients with OTC in their SN are treated with systemic chemotherapy, better definitions are needed. Especially, multifocal lesions and clusters of tumour cells < 0.2 mm with malignant proliferation characteristics in the parenchyma of the lymph node should be discussed and uniform criteria for these items should be made. It is very important that the prognostic clinical relevance of these OTC will be assessed. Further survival analyses of patients with CRC are needed but with a clear division of micrometastases and ITC. Research should be performed into the relation of the nature and behaviour of these cells compared to cancer stem cells. It is also, very interesting to assess the relation between OTC detected by immunohistochemical analysis, tumour cell characteristics and genetic tumour cell profile on survival of these patients. If repeated survival analyses show a decreased disease free survival in patients with micrometastases randomised trials should be performed to assess if survival of these patients improves after administration of systemic chemotherapy. In the Netherlands such a randomised trial is currently recruiting patients (EnROUTE; NCT01097265).