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Excellent prognosis of node negative patients after sentinel node procedure in colon carcinoma: A 5-year follow-up study

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Abstract

Aim: Investigate the prognostic impact and clinical relevance of the sentinel node (SN)-procedure in colon carcinoma.

Patients and methods: Between May 2002 and January 2004, the SN-procedure was performed in 55 patients that underwent elective resection for clinically non-advanced colon carcinoma. A control group of 110 patients was identified from a cohort between January 2000 and April 2002. All lymph nodes were analysed by conventional haematoxylin–eosin staining. All negative SNs underwent in-depth analysis using immunohistochemical-staining and automated microscopy with the Ariol-system. Patients with positive lymph nodes were offered adjuvant chemotherapy. All patients were routinely monitored at 6-month intervals and follow-up was more than 5 years.

Results: The SN was successfully identified in 98% of the patients, with 94% sensitivity. In-depth analysis with immunohistochemistry and automated microscopy (Ariol-system) upstaged 3 and 4 patients respectively. When only node-negative patients were analysed, overall 5-year-survival was significantly better in the SN group (91% vs. 76%, $p = 0.04$). Cancer-specific-mortality was even 0% (vs. 8%, $p = 0.08$). Disease-free-survival was significantly improved to 96% (vs. 77%, $p < 0.01$).

Conclusions: This study describes the prognostic impact of the SN-procedure in colon carcinoma after 5-year-follow-up. Only one patient had recurrent disease after a negative SN procedure (disease-free-survival 96%). These results indicate that the SN-procedure is of prognostic relevance and might be useful to select patients for adjuvant chemotherapy. Patients that are lymph node negative after an SN-procedure have an excellent prognosis and do not need adjuvant treatment.

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Keywords: Colorectal neoplasms; Colonic neoplasms; Sentinel lymph node biopsy; Lymphatic metastasis; Neoplasm staging; Follow-up studies

Introduction

The presence of lymph node (LN) metastases in colon carcinoma is one of the most important prognostic factors for disease recurrence and survival. About 25% of patients with stage II colon carcinoma develop recurrent disease.^{1,2} Even with current improved adjuvant treatment including

oxaliplatin, 16% of patients have a relapse.³ It is most likely that standard pathological examination misses clinically relevant occult (micro)metastases.

Accurate lymphatic staging is important since it has been shown that patients with evident LN metastases will benefit from adjuvant chemotherapy.^{3,4} However, adjuvant chemotherapy in patients without evidence of LN metastases remains debatable, and has been shown less beneficial with high numbers-needed-to-treat.^{2,3,5} This raises the question whether it would be possible to improve lymphatic staging to better

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identify which patients might benefit from adjuvant chemotherapy.

Various techniques have been investigated to improve LN-staging. Using fat clearance techniques it is possible to increase the number of identified LNs to 58 per specimen.⁶ Others tried to improve pathological examination itself by using techniques for in-depth analysis of the LNs by multi-sectioning, immunohistochemical (IHC)-staining or RT-PCR.^{7–12} Several groups showed improved prognosis of the node-negative-group, but these techniques are expensive and very labour intensive and therefore have not become widely accepted.

A technique for automated analysis (Ariol-system) of multiple sections was introduced in our institute, making in-depth analysis more applicable.¹³ Digitally acquired images from multiple sections are automatically analysed for IHC-stained tissue, whereupon only few sections need to be confirmed by conventional microscopy.

The sentinel node (SN)-procedure may facilitate pathologic examination, while only one or few nodes need in-depth analysis. Many studies have shown its technical feasibility in colon carcinoma with upstaging by the detection of (micro)metastases.^{14–19} However, there is a lack of follow-up studies to prove its clinical significance.^{10,19–23}

Currently, there are only two follow-up studies about the SN-procedure in colorectal carcinoma.^{11,24} Bilchik describes 25 months follow-up of 141 patients. Twelve patients had recurrent disease. Ten recurrences were in patients with micrometastasis in the SN, the other two were in patients without micrometastasis in the SN. These last two patients had rectal carcinoma in which the SN-technique is less accurate.^{25,26} None of the patients with colon carcinoma and a negative SN had recurrent disease. A recent study by Sirop briefly refers to a cohort of 109 patients; 5-year-cancer-specific-survival of the 62 node-negative patients was 96.2%. However, there were no numbers on recurrent disease. Interestingly, all patients with micrometastasis in the SN received adjuvant chemotherapy. None of these 14 patients had recurrent disease.

In 2002 we introduced the SN-technique in patients with colorectal carcinoma. Interim analysis showed the feasibility of the technique in colon carcinoma,¹⁷ but also showed it was not reliable in rectal carcinoma.²⁵ This is the first 5-year-follow-up study of the SN-procedure in colon carcinoma. By comparing the results with a control-group we show the potential clinical impact of the SN-procedure for patients with colon carcinoma.

Patients and methods

Patients and operation

Between May 2002 and January 2004, all patients who underwent elective resection for primary colon carcinoma were included in the study. Exclusion criteria were: distant metastases or gross invasion of other organs, clinically obvious LN

metastases at operation (multiple large and/or firm nodes), and multiple synchronous colon carcinomas.

After thorough examination of patient charts and operative reports from all consecutive patients operated for colon carcinoma between January 2000 and April 2002, we identified a control-group using the same in- and exclusion criteria as applied for the SN-group.

All patients underwent an oncological resection, including en-bloc resection of the mesocolon of the corresponding vascular trunk.

Sentinel node procedure

In the first 20 patients we used an 'ex-vivo'-technique; after resection the colon was opened at the antimesenteric border and 1–2 ml of Patent Blue V (Guerbet laboratories, Aulnay-Sous-Bois, France) was injected peritumourally in the submucosal layer. In the latter 35 patients an 'in-vivo'-technique was used, this to identify potential aberrant lymphatic drainage^{27–29}; Patent Blue V was injected peritumourally in the subserosal layer during operation. In both techniques lymphatics stained blue within few minutes and the first 1–4 blue LNs were categorized as SNs, and marked with a suture. The fresh specimen was sent for pathological examination.

Pathologic examination

In the SN-group, all LNs (non-SNs and SNs) were analysed by conventional haematoxylin–eosin (HE)-staining. LNs <0.5 cm were embedded whole, 0.5–1.0 cm were bisected, and >1.0 cm were cut at 0.3 cm intervals. One section of each level was analysed. If the SN was negative for metastases, serial sectioning was performed at 500 µm intervals. At each level one section was HE-stained and an adjacent section was IHC-stained against cytokeratin AE1/AE3 (Dako, Glostrup, Denmark) to reveal micrometastases. Metastases were described using the criteria applied in breast cancer³⁰; tumour cell deposits 0.2–2.0 mm were referred to as micrometastases and <0.2 mm as isolated tumour cells (ITC).

All SNs negative by HE-staining were analysed using automated microscopy on the Ariol-SL50 (Applied Imaging, a Genetix Company). This procedure has been described previously.¹³ In brief; all sections, previously analysed by IHC-staining against cytokeratin AE1/AE3, were automatically re-analysed with 50 slides at-the-time, using this Ariol-system. Based on our previous experiments with the Ariol-system, single IHC-stained cells were scored but counted as negative, whereas groups of two or more adjacent cells were regarded as positive for IHC.¹³

In the control-group, a standard pathological examination was performed with HE-staining at one level of all LNs.

Adjuvant chemotherapy

According to clinical guidelines at that time, all patients with LN metastases on conventional HE-staining, younger

Table 1
Patient demographics.

	Sentinel node group (n = 55)	Control group (n = 110)	p-value
Age (years)	Mean 67 (SD $\pm$ 12) Range 43–85	Mean 66 (SD $\pm$ 14) Range 22–100	0.56 ^a
Gender			
Female	26 (47%)	55 (50%)	0.74 ^b
Male	29 (53%)	55 (50%)	
Tumour localization			
Right colon	27 (49%)	59 (54%)	0.07 ^c
Transverse colon	1 (2%)	8 (7%)	
Left colon	5 (9%)	18 (16%)	
Sigmoid colon	22 (40%)	25 (23%)	
Tumour stage			
T1	1 (2%)	6 (5%)	0.64 ^c
T2	10 (18%)	15 (14%)	
T3	35 (64%)	73 (66%)	
T4	9 (16%)	16 (15%)	
Number of patients with positive lymph nodes on HE (AJCC stage III)	17 (31%)	37 (34%)	
Number of lymph nodes examined	Mean 9 (SD $\pm$ 5) Range 1–23	Mean 7 (SD $\pm$ 4) Range 0–23	0.03^a
Follow up (months)	Median 70 Mean 69 Range 32–82	Median 61 Mean 61 Range 37–83	

^a Student's *T*-test.

^b Pearson χ^2 test.

^c Fisher's exact test.

than 75 years of age and in clinically good condition, were offered adjuvant treatment with 5-fluorouracil 425 mg/m² and folonic acid (leucovorin) 20 mg/m² in six cycles of four weeks. These guidelines were the same and were followed for both the control- and SN-group.

Follow-up

All patients from the control- and SN-group were routinely monitored at 6-month-intervals. Besides routine physical examination, follow-up included measurement of carcinoembryonic antigen (CEA) level, and liver ultrasound or abdominal computed tomography. After three years all patients underwent total colonoscopy.

Statistical analysis

Statistical analysis was carried out using SPSS version 16.0. Categorical variables were analysed using Pearson χ^2 test and Fisher's exact test. Numerical variables were analysed using Student's *T*-test. Follow-up data (overall-survival, cancer-specific-survival and disease-free-survival) were analysed using Kaplan–Meier survival-curves, and differences between the curves were analysed with the Log–Rank test. *P*-values <0.05 (two-sided) were considered statistically significant. Cancer-specific-survival is defined as the percentage of patients that survive or died of causes other than cancer (=1-cancer-specific-mortality).

Results

Patients and patient demographics

Between May 2002 and January 2004, 81 consecutive patients underwent elective resection for primary colon carcinoma at the Isala klinieken, Zwolle, The Netherlands. Twenty-six patients were excluded for the following reasons: distant metastases or gross invasion of other organs (13), clinically obvious LN metastases at operation (4), two synchronous colon carcinomas (1), or none of the authors were available to perform the SN-procedure (8). Fifty-five patients were included in the study and an SN-procedure was performed.

Between January 2000 and April 2002, 140 consecutive patients underwent elective resection of a primary colon carcinoma at the Isala klinieken, Zwolle, The Netherlands. We identified 110 patients as a control-group using the same in- and exclusion criteria as applied for the SN-group. Thirty patients were excluded for the following reasons: distant metastasis or gross invasion of other organs (19), or clinically obvious LN metastases at operation (11).

Except for the mean number of identified LNs (9 vs. 7), there were no significant demographic differences between the SN-group and the control-group (Table 1).

The same clinical guidelines for adjuvant treatment were followed for both groups. Seventeen patients (31%) in the SN-group and 37 patients (34%) in the control-group had LN metastases on HE-staining. Of these, respectively, 6

Table 2
Sentinel node results.

Sentinel node group (n = 55)				
Number of patients with positive lymph nodes (on HE)	17		31%	
SN identified	54		98%	
	Only HE staining		With Ariol analysis	
Positive SN	14		18	
False-negative SN	2 ^a		1 ^a	
True-negative SN	38		35	
Accuracy	52/54	96%	53/54	98%
Sensitivity	14/16	88%	18/19	95%
False-negative rate	2/16	13%	1/19	5%
Negative predictive value	38/40	95%	35/36	97%
Number of patients upstaged by:				Recurrence ^c
SN (SN only positive LN)	8/55		0–15% ^b	4
IHC	3/55		5%	0
Ariol analysis	4/55		7%	1

^a Re-analysis of all negative SNs after immunostaining with anticytokeratin AE1/AE3 revealed in one of the two false negative SNs a micrometastasis just next to the node in an afferent lymphatic vessel, which could explain why this lymph node metastasis had not been identified before. This metastasis was also picked up by Ariol analysis.

^b In 8 patients the SN was the only lymph node positive for metastases, these could potentially have been missed on conventional pathologic examination and therefore upstaging by the SN procedure is somewhere between 0 and 15%. Follow-up data showed that 3 of these 8 patients actually had recurrent disease.

^c Six patients had recurrent disease of whom 4 had a positive SN on HE-staining. IHC-staining of the SN did not reveal the other two, however Ariol analysis did identify one.

and 12 patients were older than 75 and only 9 (53%) and 19 (51%) patients received adjuvant chemotherapy. One patient in the SN-group and 4 patients in the control-group were scheduled for but did not receive chemotherapy due to post-operative complications. One patient in the SN-group and 2 in the control-group received adjuvant chemotherapy based on tumour characteristics (T4 or lymphangio-invasive growth), although they did not have LN metastases.

Sentinel node-procedure and standard pathological examination

Results of the SN-procedure are shown in Table 2. In all but one patient at least one SN was found (mean 2.1, range 0–9), resulting in identification rate 98%. In the patient in whom no SN was identified, 15 LNs were found including one LN that contained metastases. In 14 patients the SN was positive for metastases on HE. In 2 cases the SN was false negative. This resulted in 17 patients (31%) with positive LN-status on HE-staining (AJCC-stage III). In 38 patients the SN was true negative. This resulted in accuracy 96%, sensitivity 88%, false-negative-rate 13% and negative-predictive-value 95%. Interestingly, in 8 patients the SN was the only LN positive for metastases. Because these could potentially have been missed on conventional pathological examination, upstaging by only the SN-procedure is somewhere between 0 and 15%.

In-depth pathological examination

IHC-staining identified ITCs (tumour cell deposits <0.2 mm) in one of the false-negative SNs. This increased

sensitivity of the SN-procedure to 95%. Furthermore, it revealed ITCs in 3 patients, node-negative on HE-staining, leading to upstaging 5%. No micrometastases (0.2–2.0 mm) were found. Automated analysis with the Ariol-system revealed positive SNs in 4 other patients and upstaged 7%. Interestingly, these 4 patients were not the same as the 4 patients identified by IHC-staining.

Follow-up and outcome

Median follow-up after surgery was 70 months in the SN-group (range 32–82) and 61 months in the control-group (range 37–83). Six patients in the SN-group had recurrent disease and all died. Four of these patients had a positive SN on HE-staining. The other two had a negative SN also on IHC-staining, but one was positive on Ariol analysis. Six patients died from other non-cancer-related diseases. Twenty-six patients in the control-group had recurrent disease of whom 15 died. Only 11 of these patients had positive LNs on HE-staining. Twenty-three patients died from other non-cancer-related diseases. One patient in the SN-group and 2 patients in the control-group had a second primary colorectal tumour; these cases were not counted as recurrent disease.

There was a significant difference in overall-5-year-survival in both groups, 83% in the SN-group vs. 69% in the control-group ($p = 0.03$) (Fig. 1). When non-cancer-related deaths were censored, both groups were more comparable. Cancer-specific-5-year-survival was 92% in the SN-group vs. 86% in the control-group ($p = 0.29$) (Fig. 1).

Because the hypothesis was that the SN-procedure would lead to upstaging and consequently improves

Figure 1. Kaplan–Meier curves of all patients included in the study (55 SN, 110 Control) (p -values; as compared to the control group with log rank test).

prognosis of the patients that remain node-negative, a separate analysis of all node-negative patients was performed. The SN-procedure with node-negative disease after HE-staining on multiple sections resulted in a significant higher overall-5-year survival of 92% vs. 76% in the control-group ($p = 0.04$) (Fig. 2). Remarkably the cancer-specific-5-year-survival was 100% in the node-negative patients in the SN-group vs. 92% in the control-group ($p = 0.16$) (Fig. 2). Disease-free-5-year survival was significantly higher in the node-negative patients in the SN-group, 94% vs. 77% ($p = 0.02$) (Fig. 2).

In-depth analysis of the SN using IHC-staining upstaged 3 patients, but did not identify the 2 patients that had recurrent disease and were missed on HE-staining. This resulted

in: overall-5-year-survival 91% of the node-negative patients in the SN-group ($p = 0.04$), cancer-specific-5-year-survival 100% ($p = 0.19$), and disease-free 5-year survival 93% ($p = 0.02$) (Fig. 2).

In depth analysis of the SN using the Ariol-system resulted in: overall-5-year-survival 91% of the node-negative patients ($p = 0.04$), cancer-specific-5-year-survival 100% ($p = 0.08$), and disease-free-5-year-survival 96% ($p < 0.01$) (Fig. 2).

Discussion

This study shows excellent prognosis of patients with colon carcinoma in whom no LN metastases are identified

Overall 5-year survival

SN with HE	92 %	$p=0.04$
SN with IHC	91 %	$p=0.04$
SN with Ariol	91 %	$p=0.04$
Control	76 %	

No. at risk				
SN with HE	38	35	32	31
SN with IHC	35	32	30	29
SN with Ariol	34	31	29	28
Control	73	61	56	30

Cancer-specific 5-year survival

SN with HE	100 %	$p=0.16$
SN with IHC	100 %	$p=0.19$
SN with Ariol	100 %	$p=0.08$
Control	92 %	

No. at risk				
SN with HE	38	35	32	31
SN with IHC	35	32	30	29
SN with Ariol	34	31	29	28
Control	73	61	56	30

Disease-free 5-year survival

SN with HE	94 %	$p=0.02$
SN with IHC	93 %	$p=0.02$
SN with Ariol	96 %	$p<0.01$
Control	77 %	

No. at risk				
SN with HE	38	34	31	29
SN with IHC	35	31	29	27
SN with Ariol	34	31	29	27
Control	73	58	48	26

Figure 2. Kaplan–Meier survival curves of node-negative patients (p -values; as compared to the control group with log rank test).

after an SN-procedure with standard pathologic examination as well as with in-depth analysis of the SN. This study confirms the studies by Bilchik and Sirop.^{11,24} After a relative short follow-up period of 25 month, Bilchik did not find recurrent disease nor cancer-related death in the SN-negative-group in patients with colon carcinoma.²⁴ Sirop refers to a 5-year-cancer-specific-survival of 96.2% in patients with colorectal carcinoma that had no evidence of disease in the SN, however this is not full description of the cohort.

This is the first study that describes a cohort with adequate long median follow-up of 70 months (range 32–82).^{31,32} To research the clinical impact of the SN-procedure, we compared the SN-group with a control-group, using the same in- and exclusion criteria.

Follow-up results show that patients with an SN negative for metastases after conventional HE, have an excellent prognosis. Compared with the control-group, node-negative patients have a significantly better overall 5-year-survival (92% vs. 76%, $p = 0.04$) and a significantly better disease-free-5-year-survival (94% vs. 77%, $p = 0.02$). Although IHC-staining was able to improve the SN-procedure itself (IHC-staining identified a false-negative SN and increased sensitivity of the SN-procedure to 95% (Table 2)), IHC-staining was not able to identify the two patients with recurrent disease and did not improve clinical outcome. Instead, the Ariol-system was able to identify one of the two patients with recurrent disease and further improved outcome of node-negative patients to a disease-free-5-year-survival of 96% ($p < 0.01$).

Interestingly, the single patient with recurrent disease, not identified after in-depth pathological analysis of the SN with the Ariol-system, was a patient with a carcinoma in the rectosigmoid that died after 72 months from lung and bone metastases. The tumour was initially described being located in the distal sigmoid colon, close to the rectum. The SN-procedure is less reliable in rectal carcinoma^{24–26} This could explain why we were not able to identify this patient with the SN-procedure.

In-depth pathological examination by various techniques as multi-sectioning, IHC-staining or RT-PCR have been shown to improve prognosis of the node-negative group,^{7–9,11,12} but because these techniques are expensive and labour intensive when applied to all harvested LNs, they have not become mainstream. Here we describe the use of the SN-procedure, which will make in-depth analysis more applicable. Interestingly, with the SN-procedure alone we were able to identify almost all patients at risk for recurrent disease and possibly, only for clinical use, in-depth analysis of the SN may be omitted. Three of the 8 patients in whom the SN was the only positive LN, had recurrent disease, making it reasonable to assume that the SN-technique is able to identify those clinically significant LN metastases.

For in-depth pathological examination of the SN, we used automated analysis of multiple sections with IHC-

staining against cytokeratin AE1/AE3. This Ariol-system is easy to apply and analyses 50 slides at once. In our series, another 4 patients were upstaged. One of these 4 patients had recurrent disease and the Ariol-system was able to further improve disease-free-survival of the node negative patients from 94% to 96%, which also was significantly better than the 77% in the control-group ($p < 0.01$) (Fig. 2).

Clearly, this study is not a randomized trial. We chose to use a control-group treated just prior to the SN-group, to make treatment variations negligible. Since 2000 pathological examination in our hospital was performed using a standardized protocol, which was the same in both groups. Patient demographics, except from number of LNs examined, were comparable in both groups. Also outcome, meaning cancer-specific-survival, was comparable in both groups (86% control, 92% SN, $p = 0.29$). Furthermore, outcome of the control-group (node-negative, stage I/II: cancer-specific-survival 92%, disease-free-survival 77%) was comparable and even better than described in the literature.³³ This was likely due to selection bias; patients were excluded in whom gross invasion of other organs or clinically obvious LN metastases were found. According protocol, such cases were excluded in the SN-group and thus also the control-group, because this could potentially interfere with the SN-procedure. But more importantly, recent literature suggests that these patients should receive adjuvant chemotherapy regardless their nodal status,^{2,3,31} making further lymph node analysis clinically insignificant.

Another potential limitation of this study is the relatively small number of patients included. Nevertheless, the SN-procedure showed significantly improved prognostic performance for overall-survival and disease-free-survival of the node-negative group as compared to a control-group, suggesting a high clinical relevance with only low numbers needed to treat. Still, these findings need to be reconfirmed in a larger cohort, for which the EnRoute + Study started.²³ Unfortunately, this study is stopped due to slow accrual, but its necessity remains.

Contrary to current practice where a minimum of 15 LNs is suggested for adequate nodal staging,³⁴ we had low numbers of LNs. In the control-group this was only 7, which seems rather low, but at the time this study was being performed a mean number of 8 or 9 LNs was usual practice.^{35–37} In the SN-group we found a mean number of 9 LNs. Likely the SN-procedure helps the pathologist to find more LNs. The excellent outcome of the SN-group clearly suggests that the SN-procedure (with or without in-depth analysis) is able to point out the right LN for pathological examination. Thus, although this was not our intention, the SN-procedure makes up for a low LN-yield.

In our opinion, introduction of the SN-procedure would be worthwhile in any clinic. Even without the use of in-depth pathological examination it could upstage those patients at risk for recurrent disease. The SN-technique is easy to learn and there is basically no downside in the

use of the procedure in colon carcinoma. The higher chance of a false-negative SN when introducing the procedure is clinically not relevant because the technique is only in addition to current practice, and there is certainly no increased risk of missing LN metastases.

Patients that will be upstaged by the SN-procedure (0–15%), and consequently are positive for metastases on standard pathological examination with HE-staining, should be offered adjuvant chemotherapy.^{3,4} Whether patients that will be upstaged by in-depth analysis of the SN should also be offered adjuvant chemotherapy, is suggested, but will need further investigation.^{11,12,23}

Conclusion

This study describes a 5-year-follow-up of the SN-procedure in colon carcinoma. By comparing the results with a control-group we show the potential clinical impact of the SN-procedure in the treatment of patients with colon carcinoma. Patients with colon carcinoma, who are node-negative after an SN-procedure with, but even without, in-depth pathological examination of the SN, have an excellent prognosis (100% cancer-specific-5-year-survival and 96% disease-free-5-year-survival in this study) and do not need adjuvant treatment.

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Conflicts of interest

The authors declare having no conflicts of interest.

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