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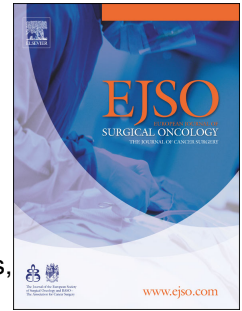
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**Improving lymph node detection in colon cancer in community hospitals and their pathology department in southern Netherlands**

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**ABSTRACT**

**Aim:** The aim was to investigate whether a set of measures directed at increasing lymph node (LN) detection among colon cancer patients led to clinically relevant changes in LN detection rate.

**Methods:** Data of all patients with curative colon cancer (pT<sub>any</sub> N<sub>any</sub> M0) diagnosed in 1999-2007 whose resection specimens were evaluated by the Institute for Pathology and Medical Microbiology in Eindhoven, (n=1,501) were included. Feedback to specialists, increased fixation time, and ex-vivo injection of the specimen with Patent blue V dye were used to increase LN detection rate. Trends in the proportion of patients with insufficient LNs examined were investigated; moreover, the Patent blue stained patients (n=86) were compared with a group of unstained patients (n=84). Based on the decrease in the proportion of high-risk node-negative patients, a calculation of chemotherapy-related costs saved was made.

**Results:** The proportion of patients with <12 LNs examined decreased from 87% in 1999 to 48% in 2007 ( $p_{\text{trend}} < 0.0001$ ). In the stained group this was 37%, versus 56% for the unstained group ( $p = 0.010$ ). In 1999, 79% of stage II patients were high-risk compared to 55% in 2007, which translates to a saving of almost 1,000,000 euro based on 92 stage II patients diagnosed in 2007.

**Conclusion:** A diverse set of measures increased the number of examined lymph nodes among patients with colon cancer. Large savings can be made due to the reduced proportion of high-risk node-negative patients who would otherwise have received adjuvant chemotherapy.

**Keywords:** colon cancer, cost-effectiveness, lymph node detection, Patent blue V, trend

## INTRODUCTION

Colon cancer is one of the most frequent cancers in the Netherlands with over 6,000 new cases annually.[1] In 2007 over 3,800 patients died of colon cancer [2]. Resection of the tumour with adequate margins and the associated mesentery, including draining lymph nodes, is the primary modality of treatment of colon cancer. Generally, only patients with positive lymph nodes benefit from adjuvant chemotherapy.[3] Therefore, lymph node analysis is one of the critical factors for therapeutic decision-making. A minimum number of 12 identified lymph nodes is defined as adequate assessment by the International Union Against Cancer (UICC). Recently, in the Netherlands the minimum number of lymph nodes that should be examined was reduced to 10.[4]

The 5-year survival for patients with pT<sub>3-4</sub> N0 M0 (stage II) is approximately 59%, versus only 42% for pT<sub>any</sub> N+ M0 (stage III) patients.[5] For obvious reasons, high-risk node-negative patients often receive adjuvant chemotherapy. The Dutch guidelines define high risk as stage II patients with pT4 or poorly differentiated tumours, tumours with angio-invasion, or patients with fewer than 10 lymph nodes evaluated.[4] Recently, the QUASAR trial showed a small survival benefit for these patients compared to high-risk node-negative patients who were not treated with adjuvant chemotherapy.[6] However, a proportion of patients with an insufficient number of lymph nodes examined truly has negative lymph nodes and has received adjuvant chemotherapy unnecessarily. Besides avoiding the potential burden of this treatment for the individual patient, large savings could be made by reducing the proportion of patients eligible for chemotherapy.

The number of lymph nodes examined in colon cancer patients in southern Netherlands is often inadequate, since 70% of the patients had fewer than 12 lymph nodes examined.[7] This was communicated to these departments by means of individual feedback, discussions in multidisciplinary working groups and educational presentations, which increased awareness and resulted in an increased fixation time which has shown to improve lymph node detection.[8] Besides, a subset of colon specimens were injected ex-vivo with Patent blue V dye in the mesocolon next to the tumour. The purpose of this study was to investigate whether this set of measures directed at increasing lymph node detection among colon cancer patients since 1999 led to clinically relevant changes in lymph node detection rate, and to evaluate the costs saved by these interventions.

## MATERIALS AND METHODS

### *Data collection and patients*

Population-based data from the Eindhoven Cancer Registry (ECR), which is maintained by the Comprehensive Cancer Centre South, was used to investigate the trend in lymph nodes examined and to select a control group for the comparison with the Patent blue stained group. The ECR collects data on all patients newly diagnosed with cancer in the southern part of the Netherlands. Information about patient characteristics, such as gender and date of birth, and tumour characteristics, such as date of diagnosis, subsite (International Classification of Diseases for Oncology (ICD-O-3)), grade, pathological stage, and number of lymph nodes examined were recorded. Tumour subsite was categorized into two sites; proximal colon, consisting of tumours in the coecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure (C18.0-C18.5); and distal colon consisting of tumours in the descending colon and sigmoid colon (C18.6-C18.7). This information is obtained routinely from the medical records. The quality of the data is high, due to thorough training of the registration team and computerized consistency checks at regional and national levels. Completeness has been estimated to be at least 95%. [9]

All cases of primary colon cancer stage I to III ( $pT_{any} N_{any} M0$ ) registered between 1999 and 2007 treated in one of the hospitals served by the regional department of pathology in Eindhoven were included in the lymph node detection trend analysis ( $n=1,501$ ). For the Patent blue-staining study 86 consecutive patients who underwent elective or nonelective curative surgical resection for colon cancer between May 2007 and February 2008 were included as cases. A subset of 84 patients from the ECR evaluated at the Department of Pathology in Eindhoven and diagnosed in 2007, whose specimen were not stained, was used as the control group for the Patent blue staining study. These were patients who underwent surgery in 2007 before the start of the study and patients who were missed due to logistic reasons. All patients included in the Patent blue staining study, both cases and controls, underwent a standard surgical resection and lymphadenectomy according to the location of the tumour. Patients with rectal cancer were not included, since lymph node detection in rectal cancer is hindered by neoadjuvant treatment.

### *Lymph node detection technique*

Immediately after resection, the surgeon injected 0.25-1.0 ml of Patent blue dye V in the mesocolon neighbourhood of the tumour. The injection site was gently massaged for 30 seconds. After 42-48 hours in 4% buffered formalin, the specimen was examined by a pathologist in the routine setting. Afterwards, the colon is cut transversally into slices 0.5 to 1.0 cm thick. All slices of the specimen were examined by the pathologist for lymph nodes, which were routinely processed for histological examination using conventional methods.

#### *Statistical analysis*

The median number of lymph nodes examined from 1999 to 2007 was assessed in order to determine the percentage of cases with insufficient lymph nodes examined. A Cochrane-Armitage trend test was used to investigate the trend in the proportion of patients with insufficient lymph nodes examined. Differences between the group who underwent the new lymph node technique and a control group with standard pathology examination were described, focusing mainly on the number of lymph nodes examined and tumour characteristics. Chi-square tests were conducted to test the differences in percentages of patients with insufficient lymph nodes evaluated. A Mann-Whitney test was conducted to test the difference in the number of lymph nodes examined between the Patent blue dye and the control group. SAS/STAT® statistical software (SAS system 9.1, SAS Institute, Cary, NC) was used for the analyses.

## RESULTS

### *Trend in lymph node examination*

The proportion of colon cancer patients with an insufficient ( $<12$ ) number of lymph nodes examined decreased ( $p_{\text{trend}} < 0.0001$ ) (Fig 1). The proportion of patients with less than 10 lymph nodes examined decreased from 79% in 1999 to 35% in 2007. Considering patients with N0 stage only, resulted in a similar proportion of patients with insufficient lymph nodes examined.

### *Patent blue-staining*

The median age of the Patent blue-stained group was 73 years and 72 years for the control group. The control group contained slightly more patients with stage I disease and less stage II. In the Patent blue-stained group less tumours were located in the proximal colon compared to the control group. However, these differences were not found significant (Table 1).

The median number of lymph nodes examined in the Patent blue-stained group was significantly higher compared to the control group (Fig 2). In the Patent blue-stained group 37% of the patients had fewer than 12 lymph nodes examined, while this was significantly higher in the control group (Table 2). After excluding patients with a stage I ( $T_{1-2}$  N0 M0) tumour, the percentage of patients with fewer than 12 lymph nodes examined in the Patent blue-stained group was 37% and in the control group 47% ( $p=0.40$ ). With 10 lymph nodes as a cut-off point,[4] 21% of the patients in the Patent blue-stained group and 45% of the control patients had an insufficient number of lymph nodes examined (Table 2). No significant difference was found between the proportion of N+ patients in the Patent Blue and the control group. Considering only patients with a pT3 colon tumour, 40% of patients in the Patent blue-stained group versus 34% of patients in the control group had fewer than 12 lymph nodes examined ( $p=0.66$ ). A median number of 15 (range 2-45) lymph nodes were examined for patients with a tumour in the proximal colon in the Patent blue-stained group and 13 for patients with a tumour in the distal colon (results not shown). In the Patent blue-stained group as well as the unstained group, the majority of patients with insufficient lymph nodes examined had stage N0, especially in the categories of 6-8 and 9-11 lymph nodes examined (Fig 3).

Almost 70% of the lymph nodes examined coloured blue, whereas only 9% of the blue lymph nodes being positive in the total study population. In 69% of the N+ patient group non-stained positive lymph nodes were found; however the large majority (78%) of these patients also had blue-stained positive lymph nodes.

#### *Cost-effectiveness*

In 1999, 79% of the patients with stage II disease were N0 with fewer than 10 lymph nodes examined compared to 55% in 2007. According to the Dutch guidelines these patients are considered high-risk and should receive adjuvant chemotherapy at the cost of approximately €35,000 per person [10, 11]. Extrapolating these results to all patients diagnosed with colon cancer stage II in the department of pathology in Eindhoven in 2007 (n=92), 22 patients would have been rendered ineligible for adjuvant chemotherapy, because of a sufficient lymph node detection. This would have saved about  $22 * €35,000 = €770,000$  per 92 patients. One third of this saving can be attributed to the Patent blue staining. However, some patients might be upgraded from node negative to node positive and should receive chemotherapy anyway.

## DISCUSSION

We demonstrated a significant increase in the number of lymph nodes examined in colon cancer patients by a regional pathology department between 1999 and 2007. Staining the resection specimen ex-vivo with Patent blue V dye clearly increased lymph node detection in colon cancer patients, in addition to the gradual increase observed in the number of lymph nodes examined over time, especially after 2006 when fixation time was increased. In the Patent blue-stained group the median number of lymph nodes examined was higher compared to the control group. The proportion of patients with fewer than 12 lymph nodes decreased from 56% in the unstained control group to 37% in the Patent blue-stained group. Enormous chemotherapy-related savings could be attained by better lymph node detection, and therefore reducing the proportion of high-risk node-negative colon cancer patients. The Patent blue intervention was aimed at increasing the number of lymph nodes examined. The detection of a possible sentinel node was therefore not the aim of this study.

### *Improvement of lymph node detection*

Lymph node detection in a population-based study of colon cancer patients diagnosed in 1999-2002 in southern Netherlands was poor with a median of 6 lymph nodes examined in the ECR region, with the median for the department of pathology in Eindhoven being 8.[7] This result was communicated to the departments of pathology in October 2005 in the region by means of individual feedback and discussions in multidisciplinary working groups. Educational presentations create awareness among pathologists and surgeons, which result in an improvement in lymph node staging practice. To further increase the number of lymph nodes examined, several steps were taken by various regional pathology departments involved. In 2006 they increased fixation time to 42-48 hours, which has been reported by several studies to lead to an increase in lymph node detection.[8, 13] The closer collaboration between surgeons and pathologists was expressed in 2007 with the start of the Patent blue staining method in the department of pathology in Eindhoven described in this study. Other pathology departments in the ECR region also studied comparable methods to increase lymph node yield.[14] Population-based studies reported a median number of examined lymph nodes of 6 to 12 in the period between 1990 and 2005,[7, 15, 16, 17, 18, 19, 20] while single-hospital studies reported median numbers up to 18.[21, 22, 23]

The experiences of the pathologists with the new staining technique varied and there was some resistance initially. However, this decreased when the technique was well implemented and the surgeons got more experience with the injection of Patent Blue V dye. Some pathologists emphasized the simplified detection of lymph nodes, due to the increased solidness and colouring of the lymph nodes. Whereas other pathologists preferred the traditional method with a more extensive search for lymph nodes to increase the detection rate. To reduce the workload for pathologists and to ensure adequate lymph node detection in the future, technicians will be trained to search for lymph nodes in the specimen.

There are several ways to increase the lymph node detection rate, including fat clearance methods [24] which are rather labour-intensive and hazardous chemicals are needed.[25] Re-fixation of a specimen in a lymph node-revealing solution resulted in a higher detection rate, [26] although it is an extra step for the pathologist. The median number of lymph nodes increased from 5 to 13 [27] and from 10 to 17 [28] with different modified fixatives. Another research group used a blue marking liquid injected into the rectal artery in 24 patients with rectal cancer with 27 lymph nodes examined in the stained group versus 14 in the controls.[29]

The Patent blue staining method did not change the stage distribution significantly. However, a larger proportion of patients had sufficient lymph nodes examined. Three-year overall survival for stage II patients with sufficient compared to stage II patients with insufficient lymph nodes examined in 1999-2007 in the ECR region was 83% vs. 69%. At hospital level, number of lymph nodes examined was not associated with stage distribution or use of adjuvant chemotherapy.[22, 30] However, at the patient level, higher lymph node count was associated with improved survival, relative to fewer than 12 nodes.[31]

The lymph node detection rate is not only affected by the thoroughness of the surgeon during the lymphadenectomy to remove all potential lymph node metastases, but also to the extent and diligence of the pathologists' examination which is provoked by medical oncologists. The inter-individual differences in biological behaviour of the tumour and/or host also affect the lymph node detection rate.[17, 32] Furthermore, more lymph nodes were examined during right than left colectomies,[17, 33] which was in line with our results.

#### *Cost-effectiveness*

Patients with insufficient lymph nodes examined are considered high risk patients who should receive chemotherapy.[4] Therefore, a large reduction in chemotherapy costs can be achieved by increasing the proportion of patients with a sufficient number of lymph nodes examined. We calculated a saving of almost €1,000,000 per year for one department of pathology comparing the number of node-negative patients with less than 10 lymph nodes examined in 1999 with 2007. One third of this saving can be attributed to the Patent blue staining. However, some patients might be upgraded from node negative to node positive, and should receive chemotherapy anyway, reducing the saving. No previous studies are known in which chemotherapy-related savings are shown like in our study. Thus, the set of measures as described in this study can be considered highly cost-effective.

In conclusion, a diverse set of measures including increased awareness of pathologists and surgeons, improved communication between pathologists and surgeons, increased fixation time, and Patent blue V dye staining increased the number of examined lymph nodes among patients with colon cancer. This would reduce the proportion of node-negative patients who would otherwise have received unnecessary adjuvant chemotherapy. Besides avoiding the potential burden of this treatment for the individual patient, large savings can be made due to the reduced proportion of high-risk node-negative patients.

**CONFLICT OF INTEREST**

The authors have no conflict of interest.

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**Conflict of interest**

The authors state that they have no conflict of interest.

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**FIGURE LEGENDS**

Figure 1: Colon cancer patients with an insufficient number of lymph nodes examined in the region of the Department of Pathology in Eindhoven since 1999 (n=1,501)

Figure 2: Number of lymph nodes examined in the Patent blue V stained group (n=86) and the control group (n=84)

Figure 3: Number of lymph nodes examined in the Patent blue V stained group per N stage (n=86)

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Table 1: General characteristics for patients in the Patent blue stained study group and the control group

	Patent blue V stained group (n=86 (%))	Control group (n=84 (%))	p-value
Mean age (years)	71 (SD: 10)	70 (SD: 11)	0.6
Gender			
- male	44 (51)	40 (48)	0.7
- female	42 (49)	44 (52)	
Depth of penetration			0.1
- pT1	4 (5)	9 (11)	
- pT2	13 (15)	8 (9)	
- pT3	58 (67)	46 (55)	
- pT4	11 (13)	20 (24)	
Stage			0.7
- I (pT1/2 N0 M0)	13 (15)	16 (19)	
- II (pT3/4 N0 M0)	38 (44)	33 (39)	
- III (pT <sub>any</sub> N1/2 M0)	35 (41)	35 (42)	
Tumour site			0.3
- Proximal colon	44 (53) <sup>1</sup>	51 (61)	
- Distal colon	39 (47)	33 (39)	

<sup>1</sup> For 3 patients, tumour site was missing

Table 2: Lymph node involvement in newly diagnosed colon cancer patients in the Patent blue stained study group (n=86) and the control group (n=84)

	Patent blue V stained group (n=86)	Control group (n=84)	p-value
Median number of examined lymph nodes (range)	14 (2-45)	11 (0-39)*	<0.0001
N0	51 (59%)	45 <sup>1</sup> (56%)	0.7
N+	35 (41%)	35 (44%)	
<10 lymph nodes examined	18 (21%)	38 (45%)*	<0.0001
<12 lymph nodes examined	32 (37%)	47 (56%)**	0.014

<sup>1</sup> In the control group 4 patients had Nx stage

