TUMOUR INFLAMMATORY INFILTRATE PREDICTS SURVIVAL FOLLOWING CURATIVE RESECTION FOR NODE-NEGATIVE COLORECTAL CANCER

Campbell SD Roxburgh[1], Jonathan M Salmond[2], Paul G Horgan[1], Karin A Oien[2], Donald C McMillan[1]

[1]University Department of Surgery and [2]University Department of Pathology: both Division of Cancer Sciences and Molecular Pathology, Faculty of Medicine, University of Glasgow, Glasgow Royal Infirmary, Glasgow, United Kingdom

Correspondence to:

Mr Campbell Roxburgh,

University Department of Surgery,

Glasgow Royal Infirmary,

Glasgow G31 2ER, United Kingdom.

Tel No. 0141 211 5435

Fax No. 0141 552 3229

E-mail: campbellroxburgh@doctors.net.uk

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Abstract

Background: A pronounced tumour inflammatory infiltrate is known to confer a good outcome in colorectal cancer. Klintrup and colleagues reported a structured assessment of the inflammatory reaction at the invasive margin scoring low or high grade. The aim was to examine the prognostic value of tumour inflammatory infiltrate in node-negative colorectal cancer.

Methods: 200 patients had surgery for node-negative colorectal cancer between 1997-2004. Specimens were scored with Jass and Klintrup criteria for peritumoural infiltrate. Pathological data was taken from reports at the time.

Results: Low-grade inflammatory infiltrate assessed using Klintrup's criteria was an independent prognostic factor in node negative disease. In patients with a low-risk Petersen Index (n=179), low-grade infiltrate carried a 3-fold increased risk of cancer death. Low-grade infiltrate was related to increasing T stage and an infiltrating margin.

Conclusion: Assessment of inflammatory infiltrate using Klintrup's criteria provides independent prognostic information in node-negative colorectal cancer. A high-grade local inflammatory response may represent effective host immune responses impeding tumour growth.

Introduction

Colorectal cancer is the second most common cause of cancer death in Western Europe and North America. Each year in the UK, there are approximately 35,000 new cases and 16,000 deaths attributable to this disease [1]. Overall survival is poor; even in those who undergo resection with curative intent, only half survive five years [2]. Prognosis and thereby optimal treatment can be partly predicted by clinico-pathological criteria. Tumour stage, in terms of the extent of local spread and lymph node status, has long been recognised to predict outcome. This is the basis of the Dukes' and TNM staging systems which have four main categories [3 4].

However some stages, e.g. Stage II (T3/4 N0, Dukes B) include patients with a wide range of clinical outcomes. Additional clinically applicable prognostic features have therefore been sought. Pathologically determined criteria which provide independent prognostic information following resection of node-negative colorectal cancer have been identified by Shepherd's group [4]. These are: peritoneal involvement, venous spread (both submucosal and extramural), spread to involve a surgical margin, and perforation through the tumour. The so-called Petersen prognostic index (PI) combines these four factors in a simple cumulative scoring system. Patients have a worse outcome when more of these features are present. Thus in lymph node negative disease, a group which makes up 40-50% of surgical resections for colorectal cancer [6 7], 5 year survival of 75-90% is reported; however, where high risk pathological features are present survival is similar to node positive disease [8 9]. The Petersen Index can select patients at high risk of tumour recurrence who may benefit from adjuvant treatment and is validated in Dukes B and C (single positive node) colorectal cancer [5 10].

Since the first study by Jass and colleagues [11], the presence of a pronounced tumour inflammatory infiltrate has also been recognised as an important determinant of good

outcome following potentially curative resection for colorectal cancer [12 13 14]. More recently, Galon and co-workers [15] concluded that the type and density of immune cells in and around the tumour were a primary determinant of tumour progression. However, assessment of the tumour inflammatory infiltrate is not routinely performed: for example, presence of tumour infiltrating lymphocytes is listed as non-core data within the RCPath guidelines [4]. This is largely because its assessment has been perceived to be more subject to inter-observer variation than the standard staging and Petersen parameters [16 17]. Recently, however, Klintrup and colleagues described a simplified method for structured scoring of the inflammatory reaction at the tumour invasive edge [18]. This includes all white cell types and results in a binary score of low-grade or high-grade.

The aim of the present study was to examine the prognostic value of a simple assessment of the tumour inflammatory infiltrate in addition to routinely reported tumour pathological criteria including the Petersen Index in patients undergoing curative resection for node negative colorectal cancer. Furthermore the prognostic value of inflammatory infiltrate and tumour margin characteristics in node negative disease will be examined.

Materials and methods

Consecutive patients with histologically proven colorectal cancer who, on the basis of laparotomy findings and pre-operative abdominal computed tomography, were considered to have undergone potentially curative resection for node-negative colorectal cancer (Stage I and II) between January 1997 and June 2004 in a single surgical unit at Glasgow Royal Infirmary, were included in the study. Patients were identified from a prospectively maintained database. Clinical conditions known to acute or chronically evoke a systemic inflammatory response were excluded from the present study. These include (i) emergency presentation (ii) clinical evidence of infection such as pyrexia and elevated white cell count (iii) presence of a chronic inflammatory condition. Patients receiving pre-operative radiotherapy were excluded from the study since radiotherapy has been reported to evoke an inflammatory response [19 20]. Patients who died within 30 days of surgery were excluded from the analysis. The tumours were staged using the conventional Tumour, Node and Metastases classification (from the 5th edition and according to the Royal College of Pathologists Dataset 2007) [4]. All other pathological data were taken from the pathology reports issued at the time of resection.

The routine haematoxylin and eosin slides were retrieved from the pathology archives. A minimum of three slides per specimen were selected from the deepest area of tumour invasion and scored according to both Jass [11] and Klintrup [18] criteria. The Klintrup method is based on the deepest point of invasion identified from the 3 slides and this provides the overall score for the specimen.

Jass scoring of slides was carried out as described previously [11 17]. Briefly, the term "peritumoural lymphocytic infiltrate" was applied to the stromal response at the tumours' invasive edge. A specific and important feature of this response is the presence of a loose connective tissue lamina or cap (resembling lamina propria) at the deepest point of tumour

penetration. The tumour glands are often heavily infiltrated by neutrophils but lymphocytes are not necessarily present in large numbers: it is the connective tissue stroma which is the most important feature. The tumours were scored on a 2-point scale as peritumoural infiltrate either present or absent.

Klintrup scoring of slides was carried out as described previously [18]. Briefly, tumours were scored according to a 4 point score. Scores were based on appearances at the deepest area of tumour invasion. A score of 0 indicated there was no increase in inflammatory cells at the deepest point of the tumour's invasive margin; score 1 denoted a mild and patchy increase in inflammatory cells; score 2 denoted a prominent inflammatory reaction forming a band at the invasive margin with some evidence of destruction of cancer cell islands and score 3 denoted a florid cup-like inflammatory infiltrate at the invasive edge with frequent destruction of cancer cell islands. These scores were then subsequently classified as low-grade (scores 0 and 1) or high-grade (scores 2 and 3) (Figures 1 and 2).

Assessment of tumour margin characteristics was also undertaken in accordance with Jass criteria [11 17]. Briefly, specific features of a diffusely infiltrating tumour margin include 'streaming dissection' of tumour cells through the muscularis propria, or dissection of mesenteric adipose tissue by small glands or irregular clusters of cords of cells on microscopic examination.

A total of 100 tumour specimens were scored independently by 2 observers (CSDR and JMS), who were blinded to patient outcome, to confirm consistency of scoring. Training was provided by a consultant pathologist (KO). The inter-observer intraclass correlation coefficients (ICCC) provides a measure of interobserver agreement. The ICCCs for assessment of peritumoural inflammatory cell infiltrate were: 0.71 for Jass and 0.81 for Klintrup. For assessment of tumour margin characteristics (expanding or infiltrating), the ICCC was 0.83 (ICCC values of ≥ 0.6 are considered acceptable and >0.7

is considered good). CSDR then scored all slides (n=200) and these data were used in the analysis.

The Petersen Index was generated from scores allocated to the four selected pathological variables present in a tumour specimen. Intra or extramural vascular invasion, peritoneal involvement and surgical margin involvement were allocated a score of 1. Tumour perforation was allocated a score of 2. The total score is calculated and the PI considered low risk where the score is 0 or 1 and high risk from 2 to 5 [5 10].

In the Royal Infirmary, patients undergo regular follow-up (3 months, 6 months and then yearly to five years) with yearly CT scanning and regular colonoscopic surveillance until 5 years post surgery. Information on date and cause of death was checked with that received by the cancer registration system and the Registrar General (Scotland). The study was approved by the Research Ethics Committee, Royal Infirmary, Glasgow.

Statistics

Grouping of the variables was carried out using standard thresholds. Univariate survival analysis and multivariate survival analysis with calculation of hazard ratios (HR) were performed using Cox's proportional-hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.1. Deaths up to August 2008 were included in the analysis. Inter-relationships between variables were assessed using contingency table analysis with the chi-squared test for trend as appropriate. Because of the number of statistical correlations, a P value of <0.01 was considered to be significant. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Baseline clinico-pathological characteristics and relationship with cancer specific survival of the patients with node negative disease (n= 200) who underwent curative surgery for colorectal cancer are shown in Table 1. The majority of patients were 65 or older (68%), were male (55%), and had colon cancer (66%). Eighteen (9%) patients received adjuvant chemotherapy. Median number of lymph nodes sampled was 14 (range 1-41) for colonic tumours and 12 (range 1-41) for rectal tumours. 62% of patients had twelve or more lymph nodes harvested (Table 1). Overall the mean number of lymph nodes sampled was 14, the frequency of extramural venous invasion was 24% and serosal involvement was 22%, in line with current audit standards set by the Royal College of Pathologists [4].

On routine pathological analysis, the minority of patients had evidence of the 'high-risk' pathological features including poor tumour differentiation (9%), extramural vascular invasion (24%), peritoneal involvement (22%), margin involvement (8%), tumour perforation (3%) or perineural invasion (8%). The minority of patients were thus classed as low risk by the Petersen Index (11%). On assessment by Jass' criteria, 61% (n=121) had tumour margins classed as expanding and the majority (74%, n=148) had no peritumoural inflammatory infiltrate. On assessment using Klintrup's criteria, 59% (n=118) patients were given scores of 0 or 1 (low grade inflammation) and 41% (n=82) were given scores of 2 or 3 (high grade inflammation).

The minimum follow-up was 43 months; the median follow-up of the survivors was 73 months. No patients were lost to follow up. During this period 76 patients died, 36 of their cancer. On univariate survival analysis of individual variables, age ($P<0.01$), fewer than 12 nodes sampled ($P<0.1$), vascular invasion ($P<0.01$), peritoneal involvement ($P<0.1$), tumour perforation ($P=0.05$), presence of an infiltrating invasive tumour margin

(P<0.05) and absent or low grade peritumoural inflammatory infiltrate score assessed by Klintrup's criteria (P<0.05) were associated significantly with cancer-specific survival (Table 1).

On multivariate survival analysis of significant variables for cancer-specific survival, age (HR 2.26, 95% CI 1.42-3.61, P=0.001), high risk Petersen Index (HR 2.45, 95% CI 1.08-5.45, P=0.032), perineural invasion (HR 3.62, 95% CI 1.48-8.89, P=0.005) and a low-grade peritumoural inflammatory infiltrate assessed by Klintrup's criteria (HR 2.61, 95% CI 1.19-5.77, P=0.017) were all independently associated with cancer specific survival (Table 1).

179 patients with node-negative colorectal cancer were classed as low risk by the Petersen Index. In this group, on univariate survival analysis of individual variables, age (P<0.001), poor tumour differentiation, (P<0.1), perineural invasion (P<0.05), presence of an infiltrating invasive margin (P<0.1) and absent or low grade peritumoural inflammatory infiltrate score assessed by Klintrup's criteria (P<0.05) were associated with cancer-specific survival (Table 2).

On multivariate survival analysis for cancer-specific survival in the low risk Petersen Index group, age (HR 2.31, 95% CI 1.37-3.89, P=0.002), perineural invasion (HR 3.55, 95% CI 1.20-10.55, P=0.022) and a low-grade peritumoural inflammatory infiltrate assessed by Klintrup's criteria (HR 3.04, 95% CI 1.22-7.58, P=0.017) were all independently associated with cancer specific survival (Table 2).

When those patients who received adjuvant chemotherapy were removed the from analysis, age (P<0.1), less than 12 nodes sampled (P<0.1), poor tumour differentiation (P<0.05), vascular invasion (P<0.05), a high risk Petersen Index (P<0.1), perineural invasion (P<0.005), an infiltrating tumour margin (P<0.1), and low-grade peritumoural

inflammatory infiltrate assessed by Klintrup's criteria ($P<0.05$) were associated with cancer specific survival on univariate analysis. On multivariate analysis of these significant variables in patients who did not receive adjuvant chemotherapy, only age (HR 1.81, 95% CI 1.14-2.88, P=0.011) and low-grade peritumoural inflammatory infiltrate assessed by Klintrup's criteria (HR 2.46, 95% CI 1.10-5.48, P=0.028) were independently associated with cancer specific survival.

The inter-relationships between the individual pathological criteria in the low risk Petersen Index group (n=179) are shown in Table 3. Increasing T stage was directly related to the presence of peritoneal involvement ($P<0.001$), an infiltrating tumour growth pattern and a low-grade peritumoural infiltrate assessed by both Jass ($P<0.01$) and Klintrup criteria ($P<0.01$). A lymph node sample less than 12 was directly related to the presence of peritoneal involvement ($P<0.01$). The presence of vascular invasion was directly associated with presence of perineural invasion ($P<0.001$). Peritoneal involvement was directly associated with an infiltrating tumour growth pattern ($P<0.01$). The presence of an infiltrating tumour growth pattern was associated with a low-grade inflammatory cell infiltrate assessed with Jass (P=0.001) and Klintrup criteria ($P<0.001$). Both Jass and Klintrup criteria were directly associated ($P<0.001$)

Discussion

Following potentially curative resection for colorectal cancer there are a number of pathological features which guide treatment. Principal among these is nodal disease which indicates a high likelihood of disease recurrence and the need for adjuvant therapy [3 21]. In patients with no evidence of nodal disease, other pathological criteria have been proposed by Shepherd's group which identify patients at high risk of recurrence and which are now routinely reported [5]. These include vascular invasion, peritoneal involvement, margin involvement and tumour perforation. These criteria have been combined in a score, the Petersen Index (PI), which reliably predicts poorer cancer-specific outcome in Dukes' B colon cancer [5]. These findings have recently been validated in colorectal cancer by Quirke's group [10].

In the present study of patients with node-negative colorectal cancer we have confirmed the finding that a high risk PI is associated with poorer cancer specific survival. However, the majority of patients (89%) were identified by the PI as low risk. Therefore it was of interest that the presence of a low-grade tumour inflammatory cell infiltrate (Klintrup criteria) was associated with an approximately 3-fold poorer cancer specific survival in these low-risk patients. In the present study, tumour inflammatory cell infiltrate assessed using Jass criteria was also examined. However, in terms of predicting cancer specific survival, the Klintrup criteria were superior to that of Jass. Taken together these results would suggest that tumour inflammatory cell infiltrate, in particular using Klintrup criteria, should be considered for inclusion in routine pathological reporting of colorectal cancer.

In the present study it was interest that both Jass and Klintrup criteria were inversely associated with T stage and an infiltrating tumour margin. The presence of infiltrating growth pattern appears to be similar to the phenomenon known as de-

differentiation, where there is a dissociation of tumour cells at the invasive front allowing these cells to migrate away from the main tumour, which may represent a morphological assessment of 'tumour aggressiveness' [22]. This feature has also recently been described as 'tumour budding' where there is a transition from glandular structures to single cells or clusters of up to four cells the invasive margin of colorectal tumours [23]. A high-grade local immune response may represent effective host cellular immune responses preventing the invasive margin from developing an infiltrating or budding appearance. These results are consistent with the detailed analysis of the tumour immune response in patients with colorectal cancer by Nielsen and co-workers [13] and Galon and co-workers [15]. Furthermore, the results of the present study are consistent with the concept that the type, density, and location of a variety of immune cells, and not an individual immune cell type, is an important independent determinant of cancer specific survival in patients with colorectal cancer.

The Klintrup method used in the present study differs from previous methods of assessing immune cells within the tumour as it is a structured assessment of all white cell types at the invasive margin. The technique can be applied to routine H&E specimens with no special staining required. Previous assessments of peritumoural inflammation have been criticised for the subjective nature of the scoring methodology [16 17]. In the present study the simple scoring method described by Klintrup was applied with relative ease and, as the low inter-observer variation demonstrates, it is reproducible. In the present study, Klintrup's scoring was undertaken using the original 4-point method, subsequently modified to a 2-point score (high and low grade) (Figures 1 and 2). This methodology was similar to the original Klintrup study and we would recommend a similar method be applied when classifying tumours in future studies.

With the introduction of whole population screening for colorectal cancer, earlier diagnosis should result in a higher proportion of early stage or node negative disease being treated by clinicians. Therefore, the identification of high-risk pathological features in this cohort will be important in guiding provision of adjuvant therapy. The results of the present study have demonstrated that in addition to existing routinely reported pathological features, other criteria such as low-grade peritumoural inflammation and the presence of an infiltrating tumour margin (which are currently listed in the Royal College of Pathologists guidelines but as non-core data items) are significantly related to poor survival. These criteria may therefore provide additional prognostic information, which could be used to inform multidisciplinary teams on treatment decisions. Currently, it is not known whether patients with pathological features such as low-grade inflammatory cell infiltrate would benefit adjuvant systemic treatment; however the same is true of other criteria such as vascular invasion and poor tumour differentiation [9 24]. Which patients benefit from adjuvant therapy may become clearer as large adjuvant chemotherapy trials publish further results.

In summary, assessment of peritumoural inflammatory cell infiltrate provides independent prognostic information in node negative disease and should therefore be considered for future inclusion in routine pathological reporting of colorectal cancer.

Conflict of Interest Statement

All authors agree there are no conflicts of interest to declare.

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Figure 1: Low grade inflammatory cell infiltrate at the tumours invasive edge.

Figure 2: High grade inflammatory cell infiltrate at the tumours invasive edge.

Table 1: The relationship between clinico-pathological variables and cancer specific survival in patients undergoing curative surgery for node-negative colorectal cancer.

		Patients n=200 (%)	**Univariate Analysis Hazard Ratio (95% CI)**	**P-value**	**Multivariate Analysis Hazard Ratio (95% CI)**	**P-value**
Age	<65 years	64 (32)				
	65-74years	70 (35)				
	>75years	66 (33)	1.92 (1.24-2.98)	0.004	2.26 (1.42-3.61)	0.001
Sex	female	90 (45)				
	Male	110 (55)	0.86 (0.45-1.66)	0.659		
Site	Colon	132 (66)				
	Rectum	68 (34)	0.75 (0.36-1.55)	0.437		
Number of nodes harvested						
	≥12	123 (62)				
	<12	77 (38)	1.88 (0.97-3.62)	0.060		0.134
T Stage	T1	7 (4)				
	T2	22 (11)				
	T3	126 (63)				
	T4	45 (22)	1.48 (0.87-2.52)	0.152		
Adjuvant therapy	no	182 (91)				
	yes	18 (9)	1.42 (0.50-4.03)	0.507		
Differentiation	Mod-well	183 (91)				
	Poor	17 (9)	2.17 (0.84-5.61)	0.110		
Vascular invasion	no	152 (76)				
	yes	48 (24)	2.63 (1.34-5.17)	0.005		
Peritoneal involvement	no	157 (78)				
	yes	43 (22)	1.81 (0.90-3.61)	0.095		
Margin involvement	no	186 (92)				
	yes	14 (8)	0.84 (0.20-3.50)	0.811		
Tumour Perforation	no	195 (97)				
	yes	5 (3)	4.23 (1.00-17.89)	0.050		
Petersen Index	Low Risk	179 (89)				
	High Risk	21 (11)	3.36 (1.52-7.46)	0.003	2.45 (1.08-5.45)	0.032
Perineural Invasion	no	185 (92)				
	yes	15 (8)	3.63 (1.59-8.29)	0.002	3.62 (1.48-8.89)	0.005
Invasive Margin						
Expanding		121 (61)				
Infiltrating		79 (39)	1.95 (1.01-3.76)	0.046		0.407
Peritumoural Infiltrate (Jass criteria)						
Cap-like	yes	52 (26)				
Cap-like	no	148 (74)	2.00 (0.83-4.80)	0.122		
Peritumoural Infiltrate (Klintrup criteria)						
High grade inflammation		82 (41)				
Low grade inflammation		118 (59)	2.65 (1.21-5.82)	0.015	2.61(1.19-5.77)	0.017

Table 2: The relationship between clinico-pathological variables and cancer specific survival in patients with low risk Petersen Index following curative surgery for node-negative colorectal cancer.

	Patients n=179 (%)	**Univariate Analysis Hazard Ratio (95% CI)**	**P-value**	**Multivariate Analysis Hazard Ratio (95% CI)**	**P-value**
Age <65 years	57 (32)				
65-74years	63 (35)				
>75years	59 (33)	2.01 (1.21-3.33)	0.005	2.31 (1.37-3.89)	0.002
Sex female	77 (43)				
Male	102 (57)	0.96 (0.45-2.02)	0.907		
Number of nodes harvested					
≥12	109 (61)				
<12	70 (39)	1.80 (0.86-3.79)	0.121		
T Stage T1	7 (4)				
T2	22 (12)				
T3	125 (70)				
T4	25 (14)	1.04 (0.57-1.88)	0.908		
Adjuvant therapy no	166 (93)				
Yes	13 (7)	0.46 (0.06-3.40)	0.487		
Differentiation Mod-well	165 (92)				
Poor	14 (8)	2.51 (0.86-7.32)	0.091		0.198
Vascular invasion no	146 (82)				
yes	33 (18)	1.46 (0.59-3.62)	0.409		
Peritoneal involvement no	154 (74)				
yes	25 (26)	1.15 (0.44-3.03)	0.776		
Margin involvement no	176 (93)				
yes	3 (7)	1.87 (0.25-13.94)	0.540		
Tumour Perforation no	179 (97)				
yes	0 (3)				
Perineural Invasion no	169 (94)				
yes	10 (6)	3.39 (1.18-9.79)	0.024	3.55 (1.20-10.55)	0.022
Invasive Margin					
Expanding	111 (62)				
Infiltrating	68 (38)	2.07 (0.98-4.36)	0.055		0.246
Peritumoural Infiltrate (Jass criteria)					
Cap-like yes	48 (27)				
Cap-like no	131 (73)	2.40 (0.83-6.93)	0.105		
Peritumoural Infiltrate (Klintrup criteria)					
High grade inflammation	76 (43)				
Low grade inflammation	103 (57)	2.83 (1.15-6.98)	0.024	3.04 (1.22-7.58)	0.017

Table 3: Inter-relationships between pathological variables in patients with a low risk Petersen Index following potentially curative resection for node-negative colon cancer (n=179).

	Node sample (<12/ >12)	Diff (mod-well/ poor)	VI (no/ yes)	Perit Inv (no / yes)	Margin Inv (no / yes)	Perineur Inv (no / yes)	Tumour Margin (exp/inf)	Jass criteria (Yes/ No)	Klintrup criteria (Yes/ No)
T stage (1/ 2/ 3/ 4)	0.943	0.218	0.993	<0.001	0.868	0.846	0.002	0.001	0.004
Node sample (>12/<12)		0.386	0.124	0.006	0.837	0.545	0.044	0.339	0.017
Differentiation (poor/ mod/well)			0.310	0.403	0.612	0.141	0.697	0.637	0.975
Vascular invasion (yes/ no)				0.011	0.408	<0.001	0.170	0.095	0.434
Perit. involvement (yes/ no)					0.483	0.572	0.004	0.022	0.015
Margin Involved (yes/ no)						0.672	0.867	0.292	0.135
Perineural inv. (yes/ no)							0.422	0.218	0.140
Tumour margin (exp/ infiltrating)								0.001	<0.001
Inflammatory cell infiltrate present (Jass criteria) (yes/ no)									<0.001