COMPARISON OF THE PROGNOSTIC VALUE OF INFLAMMATION BASED PATHOLOGICAL AND BIOCHEMICAL CRITERIA IN PATIENTS UNDERGOING POTENTIALLY CURATIVE RESECTION FOR COLORECTAL CANCER

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Objective: To examine inter-relationships between the local inflammatory response (Klintrup and Jass scores) and the systemic inflammatory response (Glasgow Prognostic Score (mGPS)), and compare their prognostic value in patients undergoing curative resection for colorectal cancer.

Background: Both localised peritumoral inflammatory cell infiltrate and the host systemic inflammatory response are known to have prognostic value in colorectal cancer. However, the inter-relationships of biochemical and cellular components of the systemic inflammatory response and the local inflammatory response are poorly understood.

Methods: Retrospective study of 287 patients who underwent surgery between 1997 and 2004. Data was collected from routine pre-operative blood tests. Routine pathology specimens were scored according to Jass and Klintrup criteria for peritumoural infiltrate.

Results: Increased Dukes stage was associated with less peritumoural infiltrate (Jass criteria $P<0.001$, Klintrup criteria $P<0.01$). Increased mGPS was associated with increased circulating white cell ($P<0.01$) and neutrophil ($P<0.01$) counts and low lymphocyte counts ($P<0.01$). Increased circulating white cell count was associated with increased neutrophil count ($P<0.001$) and low grade peritumoural infiltrate ($P<0.05$, Klintrup criteria). Jass and Klintrup criteria for peritumoural infiltrate were directly associated ($P<0.001$). On univariate survival analysis of patients with node negative disease (Dukes A and B), age ($P<0.01$), mGPS ($P<0.01$), neutrophil count ($p<0.05$) and Klintrup criteria ($P<0.05$) and were associated with cancer-specific survival. On multivariate survival analysis in node negative disease, the mGPS (HR 2.60, 95% CI 1.27-5.33, $P<0.01$) and Klintrup criteria (HR 6.35, 95% CI 1.41-28.53, $P<0.05$) and were

independently associated with cancer specific survival.

Conclusion: The present study's results suggest low peritumoural infiltrate (Klintrup criteria) and increased systemic inflammation (mGPS criteria) are linked through the cell mediated immune system. Furthermore, both pathological (Klintrup) and biochemical (mGPS) measures of the inflammatory response predict survival following colorectal cancer surgery.

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].

It has long been recognised that disease progression in cancer patients is not solely determined by the local characteristics of the tumour but also by the systemic host response. Indeed, there is increasing evidence that both local and systemic inflammatory responses play an important role in the progression of a variety of common solid tumours [3 4].

In terms of the localised tumour, there is good evidence that in patients with colorectal cancer, the presence of a pronounced lymphocytic infiltrate around the infiltrating tumour, on simple H&E staining of sections, is associated with improved survival [5 6 7]. Futhermore, Galon and co-workers have provided further persuasive evidence that the type, density and location of immune cells in colorectal tumours may provide prognostic information superior to that of tumour staging [8].

Klintrup and co-workers have simplified the subjective measurement of the tumour inflammatory infiltrate by including all white blood cell types and classifying the inflammatory infiltrate as either low or high grade [9]. They showed that a high grade inflammatory infiltrate was associated with improved survival in patients undergoing potentially curative resection of node negative colorectal cancer.

In terms of systemic inflammation, there is now good evidence that the presence of a systemic inflammatory response, as evidenced by a simple objective score (Glasgow Prognostic Score, GPS), based on elevated circulating concentrations of C-reactive protein and

hypoalbuminaemia, is independently associated with poor outcome in patients with colorectal cancer [10 11 12].

The aim of the present study was to examine the inter-relationships between these local subjective pathological (Jass and Klintrup criteria) and systemic objective biochemical (GPS criteria) inflammatory scores and to compare their prognostic value in patients undergoing potentially curative resection for colorectal cancer.

Materials and methods

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 between January 1997 and June 2004 in a single surgical unit at Glasgow Royal Infirmary, were included in the study. Exclusion criteria were (i) emergency presentation, (ii) clinical evidence of infection, (iii) presence of a chronic inflammatory condition, (iv) pre-operative radiotherapy or (v) death within 30 days of surgery. The tumours were staged using conventional Dukes classification [13].

The routine haematoxylin and eosin slides were retrieved from the pathology archives. A minimum of three slides from the deepest area of tumour invasion were selected and scored according to both Jass [5] and Klintrup [9] criteria. Jass scoring of slides were carried out as described previously [5 14]. Briefly, the term "peritumoural lymphocytic infiltrate" was applied to the stromal response at the tumours' invasive edge. Specific features of this response are: the presence of a loose connective tissue lamina or cap at the deepest point of tumour penetration; a heavy infiltration of neutrophils, macrophages, eosinophils lymphocytes and plasma cells between glands; and most importantly the presence of loose connective tissue stroma. The tumours were scored on a 2-point scale as either peritumoural infiltrate present or absent.

Klintrup scoring of slides were carried out as described previously [9]. Briefly, tumours were scored according to a 4 point score. Scores were allocated based on appearances at the deepest area of tumour invasion. A score of 0 was given where there was no increase in inflammatory cells at the deepest point of the tumours' invasive margin; 1 denoting a mild and patchy increase in inflammatory cells; 2 denoting a prominent inflammatory reaction forming a band at the invasive margin with some evidence of destruction of cancer cell islands; 3 denoting 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) and high grade (scores 2 and 3) (Figures 1 and 2).

A total of 162 tumour specimens were scored independently by 2 observers (CSDR and JMS), who were blinded to patient outcome, to confirm consistency of scoring. The inter-observers intraclass correlation coefficients (ICCC) were as follow; Jass =0.71 and Klintrup =0.81 (ICCC values $\geq$ 0.6 were considered acceptable). CSDR scored all slides (n=287) and these data were used in the analysis.

Blood samples were taken for routine laboratory measurements of albumin, C-reactive protein and differential white cell count measurement prior to surgery. The coefficient of variation for these methods, over the range of measurement, was less than 5% as established by routine quality control procedures.

The GPS was constructed as previously described [15]. Briefly, patients with both an elevated C-reactive protein (>10mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of 2. Patients in whom only one of these biochemical abnormalities was present were allocated a score of 1. Patients in whom neither of these abnormalities was present were allocated a score of 0. The GPS has recently been modified based on evidence that hypoalbuminaemia, in patients with colorectal cancer without an elevated C-reactive protein concentration, had no significant association with cancer specific survival. Therefore, patients with an elevated C-reactive protein were assigned a modified GPS score (mGPS) of 1 or 2 depending on the presence of hypoalbuminaemia [10].

The study was approved by the Research Ethics Committee, Royal Infirmary, Glasgow.

Grouping of the variables was carried out using standard thresholds. Inter-relationships between variables were assessed using contingency table analysis with the chi-squared test for trend as appropriate. Univariate and multivariate survival analysis and 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 0.05. Deaths from colorectal cancer up to September 2007 were included in the analysis. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Two hundred and eighty seven patients undergoing potentially curative resection for colorectal cancer were studied. The majority of patients were 65 or older (68%), were male (54%), had colonic tumours (61%) and Dukes A/B disease (59%). Median number of lymph nodes sampled was 14 (range 1-41) for Dukes B tumours and 13 (range 3-34) for Dukes C tumours. One hundred and sixteen (40%) patients had an elevated C-reactive protein concentration (>10 mg/l), and 50 (17%) patients had hypoalbuminaemia (<35g/l). Of the 50 patients with hypoalbuminaemia, 34 (68%) had an elevated C-reactive protein. The majority of tumours had no evidence of peritumoural inflammatory infiltrate using either Jass (76%) or Klintrup (65%) criteria. Sixty-seven (23%) patients received adjuvant therapy.

The inter-relationships between inflammation-based pathological and biochemical criteria are shown in Table 1. Old age was associated with a greater proportion of females ($P<0.01$), colonic tumours ($P<0.01$) and elevated mGPS ($P<0.01$). Increased Dukes stage was associated with less peritumoural infiltrate (Jass criteria $P<0.001$, Klintrup criteria $P<0.01$). Increased mGPS was associated with increased circulating white cell ($P<0.01$) and neutrophil ($P<0.01$) counts and low lymphocyte counts ($P<0.01$). Increased circulating white cell count was associated with increased neutrophil count ($P<.001$) and low grade peritumoural infiltrate ($P<0.05$, Klintrup criteria). Jass and Klintrup criteria for peritumoural infiltrate were directly associated ($P<0.001$).

The minimum follow-up was 34 months; the median follow-up of the survivors was 71 months. No patients were lost to follow up. During this period, 67 patients died of their cancer and a further 49 patients died of intercurrent disease. The relationship between clinical, pathological and biochemical characteristics and cancer specific survival in patients undergoing potentially curative resection for colorectal cancer is shown in Table 2.

On univariate survival analysis, age (P<0.001), Dukes stage (P<0.001), mGPS (P<0.001), total white cell count (P<0.01), neutrophil count (P<0.01) and a low grade or absent peritumoural inflammatory cell infiltrate assessed by either Jass criteria (P<0.01) or Klintrup criteria (P<0.001) were associated significantly with cancer-specific survival. On multivariate survival analysis the significant individual variables of age (HR 1.60, 95% CI 1.06-2.41, P<0.05), Dukes stage (HR 2.20, 95% CI 1.17-4.13, P<0.05), the mGPS (HR 2.65, 95% CI 1.66-4.25, p<0.001) and Klintrup criteria (HR 3.70, 95% CI 0.18-3.05, P<0.05) were independently associated with cancer specific survival (Table 2).

On univariate survival analysis of patients with node negative disease (Dukes A and B), age (P<0.01), mGPS (P<0.01), neutrophil count (p<0.05) and Klintrup criteria (P<0.05) and were associated with cancer-specific survival. On multivariate survival analysis, the mGPS (HR 2.60, 95% CI 1.27-5.33, P<0.01) and Klintrup criteria (HR 6.35, 95% CI 1.41-28.53, P<0.05) and were independently associated with cancer specific survival.

Discussion

The present study, to our knowledge, shows for the first time the inter-relationships between the preoperative systemic inflammatory response (GPS criteria), peritumoural inflammatory infiltrate (Jass and Klintrup criteria) and cancer specific survival in patients undergoing potentially curative resection for colorectal cancer. In the present study, a low grade peritumoural infiltrate, measured by the Klintrup criteria, was associated with increased Dukes stage and increased circulating total white cell count and neutrophil count. Furthermore, peritumoural infiltrate (Klintrup criteria) was independently associated with cancer specific survival.

The results of the present study are consistent with those reported by Klintrup and co-workers [9]. In a study of 374 patients (229 with node-negative disease) who underwent surgery between 1986 and 1996, a significant relationship was observed between low grade inflammatory infiltrate at the invasive margin and poor survival. In the same study, in addition to a grading all white cell counts at the invasive margin, increased neutrophils, lymphocytes and macrophages were all correlated with an improved 5 year survival.

In contrast, the systemic inflammatory response, measured by mGPS criteria, was not associated with Dukes stage nor peritumoural infiltrate (Jass or Klintrup criteria), but was associated with increased circulating total white cell count, increased neutrophil count and decreased circulating lymphocyte count. The mGPS was also independently associated with cancer specific survival in patients undergoing potentially curative resection for colorectal cancer. Taken together the results of the present study suggest that both low peritumoural infiltrate (Klintrup criteria) and increased systemic inflammation (mGPS criteria) are linked through the cell mediated immune system, both acquired (lymphocytes) and innate immune (neutrophils)

cells. Furthermore, both pathological (Klintrup) and biochemical (mGPS) measures of the inflammatory response predict cancer specific survival following potentially curative resection for colorectal cancer.

Although colon and rectal tumours may be considered as separate entities there was, in the present study no significant association in colorectal cancer survival. In contrast, peritumoural infiltrate as measured by Jass and Klintrup criteria were associated with significant differences in colorectal cancer survival. Therefore, in the present study, we have not considered colon and rectum tumours separately. Also, there are a number of factors which might result in the presence of a pre-operative systemic inflammatory response as evidenced by the mGPS (i.e. C-reactive protein and albumin). These include emergency presentation [16], and clinical evidence of infection and other chronic inflammatory conditions. In the present study, patients with such factors were excluded from the analysis to obviate confounding results.

The basis of the relationship between the systemic inflammatory response (mGPS) and poorer cancer specific survival is not clear and likely to be complex. However, it is clear that systemic inflammatory markers, such as C-reactive protein, play a pivotal role in the tumour–host relationship, its elevation reflecting compromised cell-mediated immunity as it is associated with lymphocytopenia and impaired T lymphocytic response within the tumour [17 18]. In addition, elevated C-reactive protein and hypoalbuminaemia have also been shown to be associated with upregulation of components of innate immune system, including complement and macrophage function [17 19]. As part of the systemic inflammatory response, there is a release of pro-inflammatory cytokines and growth factors which may promote and maintain tumour growth [20 21]. Taken together, the apparent inverse relationship between markers of the systemic inflammatory

response and the local inflammatory response are likely to reflect imbalances in the innate and adaptive immune systems compromising effective host-tumour immune responses.

The results of the present study are consistent with previous studies in that where a high grade local inflammatory response exists, a variety of innate and adaptive immune cells are associated with long term survival [5 6 7 8 9]. Also, the results of the present study suggest that the systemic inflammatory response is associated with changes in the type, density and location of immune cells in colorectal tumours type. Further detailed investigation of this relationship may result in a better understanding of the loss immune control in patients with primary operable colorectal cancer.

In the present study although both pathological (Klintrup criteria) and biochemical (mGPS) measures of the inflammatory response had independent prognostic value it is more likely that the mGPS will be adopted for the routine clinical assessment of the inflammatory response alongside tumour staging because the mGPS is simple, objective, internationally well standardised and can be measured pre-operatively.

The systemic inflammatory response is also recognised to be a precursor to progressive involuntary loss of weight and lean tissue, both of which are key factors in determining the survival of the cancer patient [22]. Moreover, the mGPS, can be used, in addition to traditional risk factors, to stratify colorectal cancer patients into specific follow-up [11 12] and perhaps treatment regimes [23].

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References

[1] Cancerstats, 2004; www.cancerresearchuk.org.

[2] McArdle CS, Hole DJ. Outcome following surgery for colorectal cancer: analysis by hospital after adjustment for case-mix and deprivation. Br J Cancer.2002;86:331-5.

[3] Vakilla J, Lotze MT Inflammation and necrosis promote tumour growth. Nature Cancer Reviews 2004;4:641-8

[4] Mantovani A, Romero P, Palucka AK, Marincola FM. Tumour immunity: effector response to tumour and role of the microenvironment. Lancet 2008 371(9614):771-83.

[5] Jass Jr, Love SB, Northover JM. A new prognostic classification of rectal cancer. Lancet. 1987;1(8545):1303-6.

[6] Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E, Kosma VM. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol. 1997;182:318-24.

[7] Nielsen HJ, Hansen U, Christensen IJ, Reimert SM, Brunner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. J Pathol. 1999;189:487-95

[8] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313(5795):1960-4

[9] Klintrup K, Makinen JM, Kauppila S, Vare PO, Melkko J, Tuominen H, Tuppurainen K, Makela J, Karttunen TJ, Makinen MJ. European Journal of Cancer. Inflammation and prognosis in colorectal cancer. 2005;41:2645-54.

[10] McMillan DC, Crozier JE, Canna K, Angerson WJ, McArdle CS. Evaluation of an inflammation-based prognostic score (GPS) in patients undergoing resection for colon and rectal cancer. Int J Colorectal Dis. 2007;22:81-6.

[11] Leitch EF, Chakrabarti M, Crozier JEM, McKee RF, Anderson JHA, Horgan PG, McMillan DC. Comparison of the prognostic value of selected markers of the systemic inflammatory response in patients with colorectal cancer. Br J Cancer. 2007;97:1266-70.

[12] Ishizuka M, Nagata H, Takagi K, Horie T, Kubota K. Inflammation-based prognostic score is a novel predictor of postoperative outcome in patients with colorectal cancer. Ann Surg. 2007;246:1047-51.

[13] Dukes CE, Bussey HJR. The spread of rectal cancer and its effect on prognosis. Br J Cancer 1958;12:309–320

[14] Jass JR, Ajikoka Y, Allen JP, Chan YF, Cohen RJ, Nixon JM, Radojkovic M, Restall AP, Stables SR, Zwi LJ. Assessment of invasive growth pattern and lymphocytic infiltration in colorectal cancer. Histopathology 1996;28:543-548

[15] Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ. Evaluation of cumulative prognostic scores based on the systemic inflammatory response in patients with inoperable non-small-cell lung cancer. Br J Cancer. 2003;89:1028-30.

[16] Crozier JEM, Leitch EF, McKee RF, Anderson JH, Horgan PG, McMillan DC. Relationship between emergency presentation, systemic inflammatory response, and cancer specific survival in patients undergoing potentially curative resection for colon cancer. Am J Surg 2008 Jul 8 (Epub ahead of print)

[17] Du Klos TW, Mold C. C-reactive protein: an activator of innate immunity and a modulator of adaptive immunity. Immunol Res. 2004;30:261-77

[18] Nozoe T, Matsumata T, Sugimachi K. Preoperative elevation of serum C-reactive protein is related to impaired immunity in patients with colorectal cancer. Am J Clin Oncol. 2000;23:263-6.

[19] Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420(6917):860–867

[20] Abramovitch R, Marikovsky M, Meir G, Neeman M. Stimulation of tumour growth by wound-derived growth factors. Br J Cancer 1999;79:1392–1398.

[21] Canna K, Hilmy M, McMillan DC, Smith GW, McKee RF, McArdle CS, McNicol AM. The relationship between tumour proliferative activity, the systemic inflammatory response and survival in patients undergoing curative resection for colorectal cancer. Colorectal Dis. 2007 Nov 12; [Epub ahead of print]

[22] McMillan DC. An inflammation-based prognostic score and its role in the nutrition-based management of patients with cancer. Proc Nutr Soc. 2008;67:257-62.

[23] Crozier JEM, Mckee RF, Mcardle CS, Angerson WJ, Anderson JH, Horgan PG, McMillan DC. The presence of a systemic inflammatory response predicts poorer survival in patients receiving adjuvant 5-FU chemotherapy following potentially curative resection for colorectal cancer. Br J Cancer. 2006;94:1833-6.

Figure 1: Low grade or absent inflammatory cell infiltrate at the tumour's invasive margin

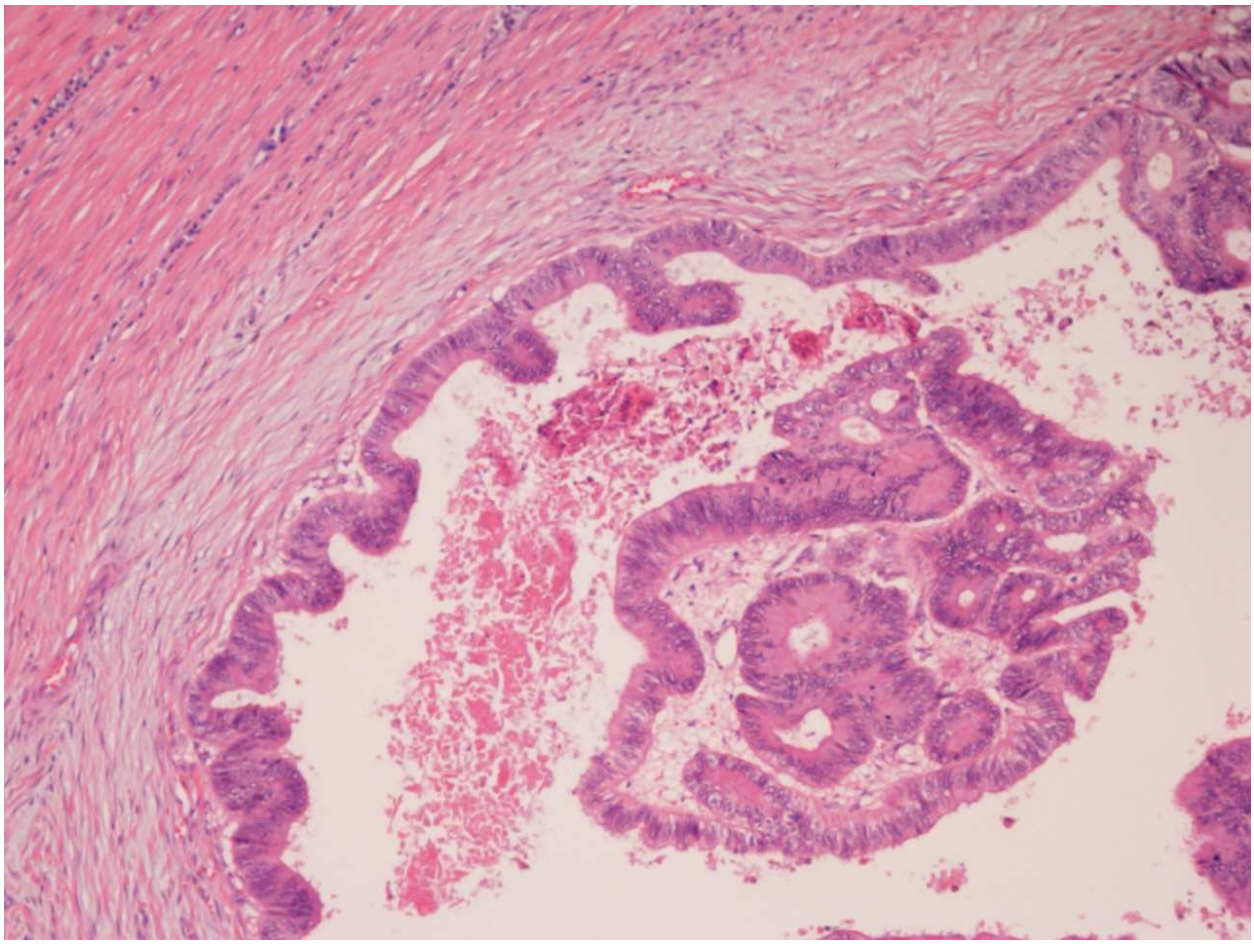

Figure 2: High Grade inflammatory cell infiltrate at the tumour's invasive margin.

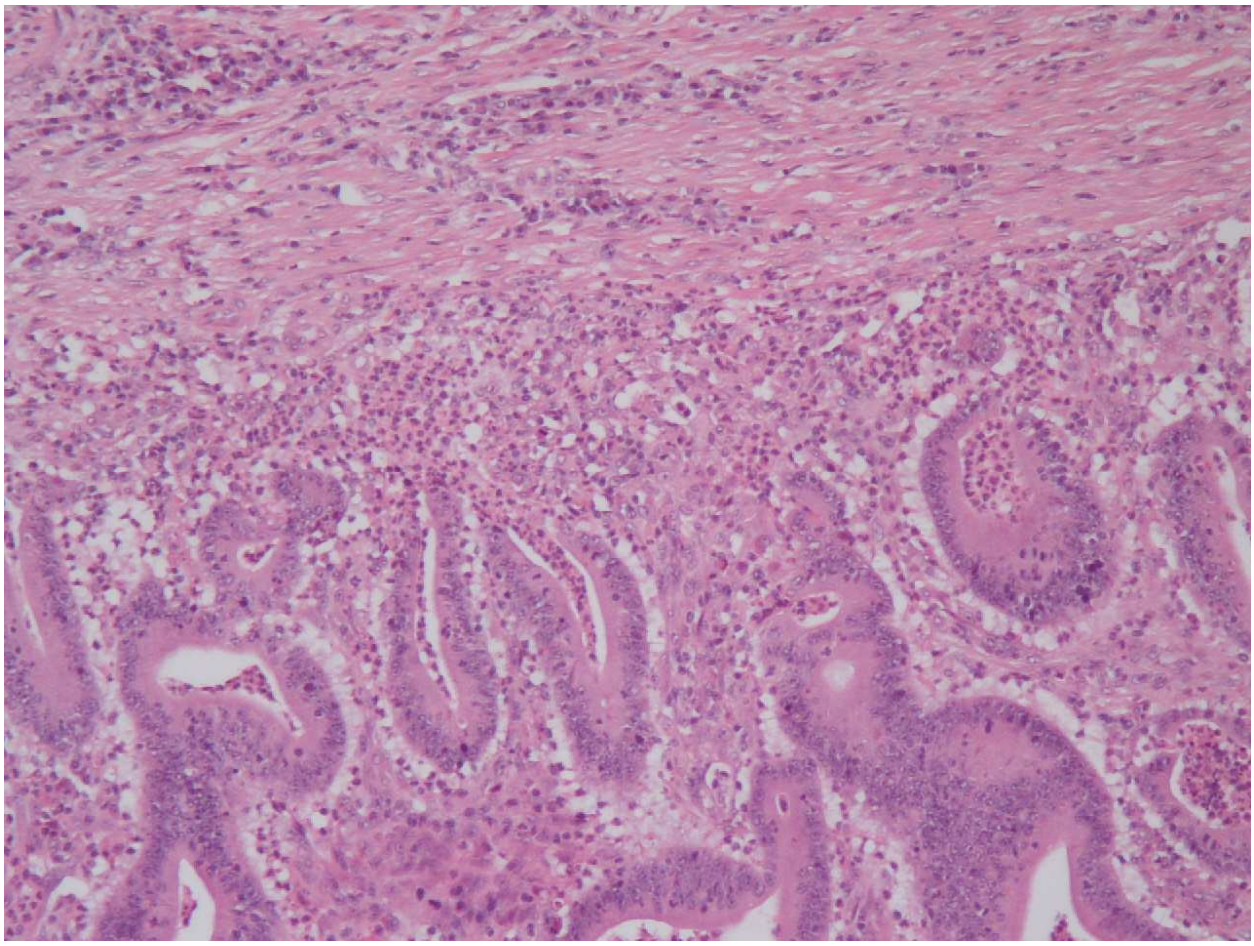

Table 1: Inter-relationships between the inflammation based pathological and biochemical criteria in patients undergoing potentially curative resection for colorectal cancer (n=287).

	Sex (F/ M)	Site (C/ R)	Dukes (A/ B/ C)	mGPS (0-2)	White cell count (<8.5/8.5-11/>11)	Neutrophils (<7.5/ ≥7.5)	Lymphocytes (<1/1-3/>3)	Jass criteria (Yes/ No)	Klintrup criteria (Yes/ No)
Age (<65/ 65-74/ >75yrs)	0.008	0.004	0.724	0.004	0.484	0.966	0.298	0.329	0.983
Sex (Female/ Male)		0.124	0.628	0.205	0.401	0.513	0.149	0.834	0.641
Tumour site (Colon/ Rectum)			0.813	0.082	0.303	0.438	0.070	0.448	0.443
Dukes stage (A/ B/ C)				0.871	0.197	0.271	0.960	<0.001	0.001
mGPS (0-2)					0.003	0.001	0.005	0.128	0.626
White Cell Count (<8.5/ 8.5-11/ >11x10^9/L)						<0.001	0.135	0.164	0.012
Neutrophils (<7.5/ ≥7.5x10^9/L)							0.684	0.241	0.067
Lymphocytes (<1/ 1-3/ >3 x10^9/L)								0.173	0.412
Peritumoural Infiltrate (Jass criteria)									<0.001
Cap-like (Yes/ No)									

Table 2. The relationship between clinical, pathological and biochemical characteristics and cancer specific survival in patients undergoing potentially curative resection for colorectal cancer: Univariate and multivariate analysis.

	Patients	Univariate Analysis		Multivariate Analysis	
	n=287 (%)	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Age <65 years	91 (32)				
65-74years	93 (32)				
>75years	103 (36)	1.81 (1.32-2.48)	<0.001	1.60 (1.06-2.41)	0.027
Sex Female	133 (46)				
Male	154 (54)	0.75 (0.47-1.22)	0.250		
Tumour site Colon	174 (61)				
Rectum	113 (39)	0.87 (0.53-1.43)	0.580		
Lymph nodes sampled ≥12	172 (60)				
<12	115 (40)	1.36 (0.84-2.20)	0.212		
Dukes Stage A	22 (8)				
B	146 (51)				
C	119 (41)	2.44 (1.56-3.83)	<0.001	2.20 (1.17-4.13)	0.014
mGlasgow Prognostic Score					
0	171 (60)				
1	82 (28)				
2	34 (12)	2.17 (1.61-2.92)	<0.001	2.65 (1.66-4.25)	<0.001
Adjuvant therapy no	220 (77)				
yes	67 (23)	1.24 (0.72-2.12)	0.443		
White Cell Count					
<8.5 $x10^9$/L	117 (62)				
8.5-11 $x10^9$/L	50 (26)				
>11 $x10^9$/L	23 (12)	1.76 (1.15-2.69)	0.009	1.04 (0.52-2.08)	0.905
Neutrophils <7.5 $x10^9$/L	158 (83)				
≥7.5 $x10^9$/L	32 (17)	2.80 (1.38-5.69)	0.004	1.40 (0.43-4.59)	0.582
Lymphocytes <1 $x10^9$/L	21 (11)				
1-3 $x10^9$/L	161 (85)				
>3 $x10^9$/L	8 (4)	1.32 (0.55-3.19)	0.553		
Peritumoural Infiltrate					
Jass criteria					
Cap-like yes	63 (24)				
Cap-like no	219 (76)	2.83 (1.29-6.20)	0.009	0.73 (0.18-3.05)	0.670
Klintrup criteria					
High grade inflammation	99 (35)				
Low grade inflammation	188 (65)	4.43 (2.12-9.27)	<0.001	3.701 (1.11-12.33)	0.033

Table 3. The relationship between clinical, pathological and biochemical characteristics and cancer specific survival in patients undergoing potentially curative resection for node-negative (Dukes A and B) colorectal cancer: Univariate and multivariate analysis.

	Patients	Univariate Analysis		Multivariate Analysis	
	n=169(%)	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Age <65 years	49 (29)				
65-74years	58 (34)				
>75years	62 (37)	2.29 (1.32-3.99)	0.003	1.99 (0.99-4.01)	0.055
Sex Female	75 (44)				
Male	94 (56)	0.47 (0.35-1.62)	0.467		
Tumour site Colon	107 (63)				
Rectum	62 (37)	0.91 (0.40-2.03)	0.808		
Lymph nodes sampled ≥12	101 (60)				
<12	66 (40)	1.98 (0.92-4.29)	0.082		
Dukes Stage A	22 (13)				
B	147 (87)	1.48 (0.35-6.32)	0.597		
mGlasgow Prognostic Score					
0	98 (58)				
1	48 (28)				
2	23 (14)	2.09 (1.31-3.34)	0.002	2.60 (1.27-5.33)	0.009
Adjuvant therapy no	155 (92)				
yes	14 (8)	1.06 (0.25-4.50)	0.935		
White Cell Count					
<8.5 x10^9/L	69 (63)				
8.5-11 x10^9/L	27 (25)				
>11 x10^9/L	13 (12)	1.83 (0.95-3.52)	0.070	0.87 (0.24-3.07)	0.822
Neutrophils <7.5 x10^9/L	91 (83)				
≥7.5 x10^9/L	18 (17)	3.32 (1.13-9.76)	0.029	3.55 (0.36-35.48)	0.281
Lymphocytes <1 x10^9/L	13 (12)				
1-3 x10^9/L	93 (85)				
>3 x10^9/L	3 (3)	1.61 (0.44-5.88)	0.470		
Peritumoural Infiltrate					
Jass criteria					
Cap-like yes	46 (27)				
Cap-like no	123 (73)	2.58 (0.59-11.34)	0.208		
Klintrup criteria					
High grade inflammation	68 (40)				
Low grade inflammation	101 (60)	3.99 (1.38-11.59)	0.011	6.35 (1.41-28.53)	0.016