The association between markers of tumour cell metabolism, the tumour microenvironment and outcomes in patients with colorectal cancer

Antonia K. Roseweir[1,2], Jennifer Clark[2], Stephen T McSorley[1], Hester C vanWyk[1], Jean Quinn[1,2], Paul G Horgan[1], Donald C McMillan[1], James H Park[1] Joanne Edwards[2].

1. Academic Unit of Surgery, School of Medicine, University of Glasgow, Glasgow, UK

2. Unit of Experimental Therapeutics, Institute of Cancer Science, University of Glasgow, Glasgow, UK

Corresponding author: Dr Antonia K. Roseweir, Lecturer, University of Glasgow, Wolfson Wohl Cancer Research Centre, Garscube Estate, Glasgow, G61 1QH. Email: antonia.roseweir@glasgow.a.c.uk Tel: 00 44 141 330 8607

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ABSTRACT

Introduction: Tumour cell anaerobic metabolism has been reported to be a prognostic factor in colorectal cancer. The present study investigated the association between monocarboxylate transporter (MCT) 1, MCT 2, lactate dehydrogenase (LDH) 1 and LDH 5, the tumour microenvironment, and outcome in patients with colorectal cancer.

Methods: A cohort of 150 patients with stage I-III CRC were utilised to assess tumour cell expression of MCT-1, MCT-2, LDH-1 and LDH5 by immunohistochemistry. Expression levels were dichotomised and associations with tumour factors, the tumour microenvironment and survival analysed.

Results: Nuclear LDH-5 associates with poor prognosis (HR 1.68 95% CI 0.99-2.84, p=0.050) and trends towards increased tumour stroma percentage (TSP, p=0.125). Cytoplasmic MCT-2 also trends towards increased TSP (p=0.081). When combined into a single score; nuclear LDH-5+TSP significantly associated with decreased survival independent of stage (HR 2.61 95% CI 1.27-5.35, p=0.009), increased tumour budding (p=0.002) and decreased stromal T-lymphocytes (p=0.014). Similarly, Cytoplasmic MCT-2+TSP significantly associated with decreased survival (HR 2.32 95% CI 1.31-4.11, p=0.003), decreased necrosis (p=0.039), and increased tumour budding (p=0.004).

Conclusions: The present study reports that the combination of TSP and nuclear LDH-5 was significantly associated with survival, increased tumour budding and decreased stromal T-lymphocytes. This supports the hypothesis that increased stromal invasion promotes tumour progression via modulation of tumour metabolism. Moreover, MCT and LDH may provide promising therapeutic targets for patients with stromal-rich CRC.

INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer death in developed countries[1]. Despite improvements in the diagnosis and treatment of CRC, outcomes remain poor with a 5-year survival rate of 60% with approximately half of patients dying of their disease despite curative resection[2]. TNM staging is currently the gold standard method to predict prognosis and aid treatment decisions for CRC. However, this staging method is suboptimal as seen by the variation in outcomes that exists amongst patients of the same stage. It is clear that other characteristics intrinsic to the tumour and patient may similarly affect oncological outcomes. Investigation of these characteristics as prognostic markers that could aid current staging, may allow for more accurate prediction of prognosis, better tailoring of treatment and development of novel therapies for CRC.

The tumour microenvironment, composed of blood vessels, stroma and immune cells that regulate paracrine and autocrine signalling to support tumour cells growth and spread is well recognised as an important factor in tumour development and outcomes in many solid tumours including CRC[3]. The mechanism by which tumour stroma facilitate tumour progression has not been fully elucidated however key theories include the stroma producing factors that can influence local and systemic inflammation, tumour pH, and tumour metabolism[4].

Of these, tumour metabolism has been a major focus of current research. Tumour cells favour glycolysis as a method of glucose metabolism, even in the presence of normal oxygen partial pressures[5]. Indeed, this phenomenon termed the Warburg effect may be facilitated by the tumour-supporting stroma. It has previously been reported that in patients with colorectal cancer, increased tumour cell expression of enzyme pathways associated with anaerobic

metabolism and lactate extrusion, including lactate dehydrogenase isoenzyme 5 (LDH 5) and monocarboxylate transporter 1 (MCT1), was associated with an increase in the ability of cancer-associated fibroblasts to uptake and oxidate lactate, suggesting a reciprocal role in supporting tumour cell metabolism[6]. It is of interest then that a high tumour stroma percentage has been reported to be associated with less tumour necrosis[7]. As both of these characteristics have previously been shown to be associated with increasing T stage, this supports the hypothesis that one of the mechanisms by which an expanded stroma facilitates disease progression is by the modulation of tumour cell metabolism, allowing continued tumour growth. It is clear however that further work is required.

The aim of the present study was to investigate the association between key pathways associated with tumour cell anaerobic metabolism, the tumour microenvironment, clinicopathological characteristics and outcome in patients with colorectal cancer.

MATERIALS AND METHODS

Patient Cohort

Patients were identified from a prospectively collected and maintained database of colorectal cancer resections at a single surgical unit within the Glasgow Royal Infirmary. Patients who were considered to have undergone potentially curative resection of Stage I–III CRC between 1997 and 2007 and whose tumour resection was included in a previously constructed CRC tissue microarray (TMA) were included. Patients who died within one month of surgery were excluded. The West of Scotland Research Ethics Committee approved the study.

Tumours were routinely staged using the fifth edition of the TNM classification[8]. Tumour differentiation was graded as well/moderate or poor in accordance with Royal College of Pathologists guidelines[9]. Venous invasion was measured using Elastica staining. Additional clinical data was taken from pathological reports following resection. Patients with stage III and high-risk stage II disease were considered for 5-fluorouracil-based adjuvant chemotherapy according to treatment guidelines at the time. Patients were routinely followed up for 5 years following surgery. Date and cause of death were crosschecked with the cancer registration system and the Registrar General (Scotland). Cancer-specific survival (CSS) was measured from date of surgery until the date of death from CRC.

Assessment of the systemic inflammatory response

Pre-operative C-reactive protein (CRP), serum albumin and differential white cell count measured within 30 days before surgery were recorded prospectively. The modified Glasgow Prognostic Score (mGPS) was calculated as previously described[10]; patients with a normal

CRP ($\leq 10\ mg\ l^{-1}$) were allocated a score of 0, an elevated CRP ($>10\ mg\ l^{-1}$) alone a score of 1 and an elevated CRP and low albumin ($<35\ g\ l^{-1}$) a score of 2.

Assessment of the tumour microenvironment

Using routine haematoxylin and eosin-stained sections of the deepest point of invasion, the generalised inflammatory cell infiltrate at the invasive margin was assessed using Klintrup–Mäkinen (KM) grade and the extent of tumour stroma was assessed using tumour stroma percentage (TSP), both as previously described[11,12]. Tumour-infiltrating T-lymphocyte density at the invasive margin and within the cancer cell nests was assessed using immunohistochemistry as previously described[3].

Western Blots

All antibodies were validated using western blotting (Figure S1). Standard lysates, HeLa whole cell lysate and NIH/3T3 whole cell lysate (SantaCruz Bio, CA, USA) and 293T whole cell lysate (Abcam, UK) were separated on a 10% (w/v) polyacrylamide gel and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, MA, USA). Membranes were blocked for 1hr and then incubated overnight at 4°C with primary antibodies for MCT-1 and MCT-2 at 1:500 (Santa Cruz Bio, CA, USA) or LDH1 and LDH5 at 1:5000 (Abcam, UK). The membranes were then incubated with the secondary antibody, Donkey anti-goat IgG-HRP (Santa Cruz Bio, CA, USA) for MCT1/2 and Anti-rabbit IgG-HRP (Cell Signaling Tech, USA) for LDH1/5. Bound antibodies were visualized by chemiluminescence (Thermoscientific, IL, USA).

Immunohistochemistry

Immunohistochemistry was used to examine four markers of cellular metabolism; LDH1, LDH5, MCT1 and MCT2, (Figure S2) in a pre-constructed CRC TMA. The TMA consisted of 0.6mm^2, 5µm thickness cores of CRC tissue in quadruplicate per patient. The TMA was dewaxed and rehydrated using histoclear and graded alcohols. Antigen retrieval was undertaken by heating under pressure for 5 mins with EDTA buffer (pH9) for MCT-1/2 or Citrate buffer (pH6) for LDH1/5. Endogenous peroxidases were quenched with 3% hydrogen peroxide for 20 mins and slides blocked with 5% normal horse serum (MCT-2) or 10% casein (MCT-1 and LDH1/5) for 30 mins. The slides were then incubated with primary antibody for MCT-1 (1:200) and MCT-2 (1:75) overnight at RT or for LDH-1 (1:600) and LDH-5 (1:300) for 75 mins at RT. Protein expression was amplified by incubation with ImmPress anti-goat IgG reagent (Vector laboratories, USA) for MCT1/2 or Envision (Dako) for LDH1/5. Protein expression was visualized using chromagen 3,3'-diaminobenzidine (Vector). The TMA was then counterstained with haemotoxylin, dehydrated and mounted in distrene, plasticizer, xylene (DPX).

Scoring Method

The stained TMAs were scanned using a Hamamatsu NanoZoomer (Welwyn Garden City, Hertfordshire, UK) at x20 magnification and visualized via Slidepath Digital Image Hub (Leica Biosystems, Milton Keynes, UK). Tumour cell expression was assessed using the weighted histoscore by examiners blinded to the clinical data (MCT 1 and 2 JC, LDH 1 and 5 SM). 15% of tumours were co-scored by the other examiner to ensure accuracy with a

minimum interclass correlation coefficient (ICCC) of 0.7[13]. Expression within the tumour cell membrane, cytoplasm and nucleus was scored separately. The weighted histoscore is calculated by multiplying the percentage density of cells stained by x0 if negative; x1 if weak; x2 if moderate; x3 if strong. The score gives a range from 0-300.

Statistical analysis

Cut off values to split each factor into low and high expression were determined using ROC analysis (Table S1). The relationship between clinicopathological characteristics, local and systemic inflammatory responses, and markers of tumour cell metabolism was examined using the Chi-squared test for linear trend. The relationship between markers of tumour metabolism and CSS was examined by Kaplan Meier curve analysis and the log rank test. Multivariate cox regression survival analysis was performed using a backward conditional model to assess prognostic independence. All analyses were performed using IBM SPSS version 22.0 (Chicago, IL, USA). Significance was set at $p<0.05$ and all data conforms to the REMARK criteria.

RESULTS

A total of 150 patients, who underwent potentially curative resection of stage I-III colorectal cancer and had a valid score for all markers, were included (Table S2). The majority of patients were male (55%) and were older than 65 at the time of surgery (61%). Pathological assessment confirmed stage I disease in 14 patients (9%), stage II disease in 72 patients (48%) and stage III disease in 64 patients (43%). 53 patients (35%) had right-sided colon cancer, 46 patients (31%) had left-sided colon cancer and 51 patients (34%) had rectal cancer. 39 patients (26%) received adjuvant therapy and mismatch repair deficiency was identified in 22 patients (15%). The median follow up of survivors was 11.0 years (range 6.2-16.1 years) with 62 cancer associated deaths and 27 non-cancer deaths.

Associations between metabolic markers and CSS are shown in Table 1. There was no significant association between CSS and MCT-1, MCT-2 and LDH-1 at any cellular location. However, nuclear LDH-5 significantly associated with decreased CSS (HR 1.68 95% CI 0.99-2.84, p=0.050, Figure 1A) and cytoplasmic LDH-5 showed a similar trend towards decreased CSS (HR 1.76 95% CI 0.973.20, p=0.058, Figure 1B). As it is hypothesised that metabolism may be a reason for increased stromal infiltrate, the relationship between the metabolic markers and tumour-stroma percentage (TSP) was investigated (Table S3). No significant associations were seen between any metabolic marker and TSP, however cytoplasmic MCT-2 trended towards associating with higher TSP (p=0.081) and membrane MCT-2 with lower TSP (p=0.112). Similarly, cytoplasmic LDH-5 (p=0.115) and nuclear LDH-5 (p=0.145) trended towards an association with lower TSP. Therefore, MCT-2 and TSP or LDH-5 and TSP were combined into a single prognostic score graded either as both low/one high or as both high. Both cytoplasmic MCT-2+TSP (HR 2.32 95% CI 1.31-4.11,

p=0.003, Figure 1C) and nuclear LDH-5+TSP (HR 3.70 95% CI 1.96-6.98, p<0.001, Figure 1D) significantly associated with poor CSS.

The relationship between nuclear LDH-5, cytoplasmic MCT-2+TSP, nuclear LDH-5+TSP and clinicopathological factors was investigated as shown in Table 2. Nuclear LDH-5 alone did not associate with any clinicopathological factors. However, cytoplasmic MCT-2+TSP showed significant associations with increased adjuvant therapy (p=0.040), decreased necrosis (p=0.039) and increased tumour budding (p=0.004). Trends were also noted towards increased stage (p=0.119) and decreased proliferation rate (p=0.134). Similarly, nuclear LDH-5+TSP showed significant associations with increased tumour budding (p=0.002) and trends towards decreased necrosis (p=0.063) and increased peritoneal involvement (p=0.083).

The relationship between nuclear LDH-5, cytoplasmic MCT-2+TSP, nuclear LDH-5+TSP and inflammatory response was then investigated as shown in Table 3. Nuclear LDH-5 alone showed significant associations with decreased regulatory T-cells at the invasive margin (p=0.039), within the stoma (p=0.004) and cancer cell nests (p=0.012). Conversely, cytoplasmic MCT-2+TSP did not associate with any markers of either the local or systemic inflammatory response. However, nuclear LDH-5+TSP showed a strong association with decreased stromal CD3+ T-cells (p=0.014) and trended towards associations with decreased regulatory T-cells at the invasive margin (p=0.107) and within the stroma (p=0.101) as well as decreased serum lymphocyte levels (p=0.080).

Next, nuclear LDH-5, cytoplasmic LDH-5, cytoplasmic MCT-2+TSP and nuclear LDH-5+TSP were entered into multivariate cox regression analysis along with common prognostic factors. Tumour budding (p<0.001), mGPS (p<0.001) and nuclear LDH-5+TSP (p=0.009) were independent prognostic factors for CSS in patients with stage I-III CRC

The only other thing I was thinking was how did necrosis fit in as the low oxygen as you discuss in the intro associates with necrosis which then also inversely associates with TSP.

DISCUSSION

The present study reports a significant association between tumour cell anaerobic metabolism, the tumour microenvironment, and survival following surgery for stage I-III CRC. In particular, the combination of TSP and tumour cell expression of cytoplasmic MCT-2 or nuclear LDH-5 was prognostic, with nuclear LDH-5+TSP being independent of stage. Furthermore, the combination of TSP and nuclear LDH-5 was significantly associated with tumour budding and CD3+ lymphocyte stromal density. Therefore, the results support the hypothesis that one mechanism by which increased stromal invasion promotes tumour progression is via modulation of tumour metabolism resulting in promotion of tumour budding and dampening of the local lymphocytic infiltrate.

The results of the present study are in keeping with the results of work reporting poorer prognosis in CRC patients with over expression of LDH[14]. Conversely, in the present study no association between MCT and prognosis was observed as has been reported in previous studies[15,16]. However, when cytoplasmic MCT-2 was combined with TSP it significantly associated with poorer prognosis, as did nuclear LDH-5+TSP. This suggests that metabolism is more active in stromal rich tumours. Previous work has reported that expression of cytoplasmic MCT-2 and nuclear LDH-5 was prognostic in CRC[17,18]. However, this is the first to directly assess the relationship between these metabolic markers and stromal invasion.

The presence of an expanded tumour stroma, of which the predominant cell type is fibroblasts, has widely been associated with poorer prognosis in CRC[7]. It has been suggested that a reciprocal relationship exists between tumour cells and stromal cells which facilitates survival and disease progression. Indeed, the metabolic markers in the present study may act to increase the ability of the tumour cell to carry out aerobic glycolysis, LDH by catalysing the conversion of pyruvate to lactate and back again, and MCT by lactate extrusion to protect

the tumour cell from its acidity[14]. It is thought that the lactate extruded into the stromal environment is then used as an energy source by the fibroblasts following its oxidation to pyruvate, which itself then returns to the tumour cell to be used as a glycolytic substrate[19]. Therefore, as the stroma expands, more pyruvate will be available for the tumour cells to utilise allowing them to thrive, suggesting that MCT-2 and LDH-5 may be potential therapeutic targets in CRC patients with high stromal infiltration. Preclinical studies of MCT inhibitors have shown that they induce apoptosis in CRC cell lines, potentiate the cytotoxicity of 5-fU chemotherapy, and slow tumour growth in murine xenograft models[20-22].

The presence of a strong lymphocytic inflammatory cell infiltrate is associated with improved survival in colorectal cancer[3]. Recently, TSP has been reported to further stratify survival in those patients with a weak inflammatory cell infiltrate measured by Klintrup-Makinen grade, leading to the creation of the Glasgow Microenvironment Score (GMS)[10,23]. In the present study, the combination of TSP and nuclear LDH-5 was associated with decreased CD3+ lymphocyte stromal density and trended towards associations with decreased regulatory T-lymphocytes. Furthermore, when nuclear LDH-5 was assessed alone it significantly associated with decrease regulatory T-lymphocytes within both the tumour and microenvironment. It may be that the highly metabolically active tumour cells remove metabolites needed by the regulatory T-cells from the microenvironment, causing them to move away from the tumour.

It was also of interest that the combination of TSP and nuclear LDH-5 or cytoplasmic MCT-2 was significantly associated with tumour budding. Tumour budding is associated with poor prognosis in colorectal cancer and is thought to be the histological representation endothelial-mesenchymal transition (EMT)[24,25]. It may be that highly metabolically active tumour cells in a rich stromal environment cause greater tumour budding and that this underpins the poor

prognosis imparted by an expanded tumour stroma and the expression of proteins associated with tumour cell metabolism.

The limitations of the present study include the size of the cohort, which leads to the possibility of type 2 error especially in the association between the combination of TSP and metabolic markers when assessing associations with characteristics of the tumour microenvironment. Furthermore, there were a relatively small number of CSS events, which may in part explain why MCT alone was not significantly associated with CSS in this study.

In conclusion, the present study reports that the combination of TSP and tumour cell expression of cytoplasmic MCT-2 or nuclear LDH-5 is associated with poor prognosis. Furthermore, the combination of TSP and nuclear LDH-5 was significantly associated with increased tumour budding and decreased stromal T-lymphocytes. Therefore, the results support the hypothesis that one mechanism by which increased stromal invasion promotes tumour progression is via modulation of tumour metabolism resulting in promotion of tumour budding and dampening of the local lymphocytic infiltrate. Moreover, MCT and LDH may provide promising therapeutic targets for patients with stromal-rich CRC.

Acknowledgements

The authors declare no conflicts of interest.

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Table 1. Metabolic markers and cancer-specific survival in patients undergoing potentially curative resection of colorectal cancer (n=150)

	Nuclear			Cytoplasmic			Membrane		
	N (%)	10yr CSS	***P***	***N (%)***	10yr CSS	***P***	***N (%)***	10yr CSS	***P***
MCT-1			0.281			0.527			0.191
Low expression	54	65 (7)		75	62 (6)		38	69 (8)	
High expression	97	54 (5)		75	54 (6)		112	55 (5)	
MCT-2			0.277			0.327			0.344
Low expression	107	60 (5)		38	67 (8)		146	59 (4)	
High expression	43	52 (8)		112	55 (5)		4	25 (22)	
LDH-1			0.596			0.605			
Low expression	52	60 (7)		28	61 (10)		-	-	-
High expression	98	57 (5)		122	57 (5)				
LDH-5			**0.050**			0.058			
Low expression	67	69 (6)		51	72 (6)		-	-	-
High expression	83	52 (6)		99	52 (5)				
MCT-2+TSP			0.684			**0.003**			
Both low or one high	124	62 (5)		105	68 (5)		-	-	-
Both high	11	55 (15)		30	39 (9)				
LDH-5+TSP			<**0.001**			0.069			
Both low or one high	119	68 (4)		116	65 (5)		-	-	-
Both high	16	19 (10)		19	40 (12)				

Table 2: Association of metabolic markers and stromal infiltrate in patients undergoing potentially curative resection of colorectal cancer (n=150)

	Tumour stroma %		
	Low (n=99)	**High** (n=36)	***P***
Membrane MCT-1			0.806
Low	24 (75)	8 (25)	
High	75 (73)	28 (27)	
Cytoplasmic MCT-1			0.815
Low	49 (74)	17 (26)	
High	50 (73)	19 (27)	
Nuclear MCT-1			0.589
Low	38 (73)	12 (27)	
High	61 (100)	24 (0)	
Membrane MCT-2			0.112
Low	95 (76)	36 (100)	
High	4 (72)	0 (0)	
Cytoplasmic MCT-2			0.081
Low	31 (84)	6 (16)	
High	68 (69)	30 (31)	
Nuclear MCT-2			0.464
Low	75 (75)	25 (25)	
High	24 (69)	11 (31)	
Cytoplasmic LDH-1			0.603
Low	18 (69)	8 (31)	
High	81 (74)	28 (26)	
Nuclear LDH-1			0.601
Low	35 (76)	11 (24)	
High	64 (72)	25 (28)	
Cytoplasmic LDH-5			0.115
Low	32 (65)	17 (35)	
High	67 (78)	19 (22)	
Nuclear LDH-5			0.125
Low	41 (67)	20 (33)	
High	58 (78)	16 (22)	

Table 3. Relationship between metabolic markers and clinicopathological characteristics in patients undergoing potentially curative resection of colorectal cancer (n=150).

	Nuclear LDH5			Cytoplasmic MCT-2 + TSP			Nuclear LDH-5 + TSP		
	Low (n=67)	High (n=83)	*P*	Both Low/One High (n=105)	Both High (n=30)	*P*	Both Low/One High (n=119)	Both High (n=16)	*P*
Age			0.682			0.152			0.705
<65	27 (40)	31 (37)		37 (35)	15 (50)		46 (39)	6 (37)	
>65	40 (60)	52 (63)		68 (65)	15 (50)		73 (61)	10 (63)	
Sex			0.231			0.359			0.162
Female	34 (51)	34 (41)		52 (49)	12 (40)		59 (50)	5 (31)	
Male	33 (49)	49 (59)		53 (51)	18 (60)		60 (50)	11 (69)	
Adjuvant			0.828			**0.040**			0.560
No	49 (73)	62 (75)		83 (79)	18 (60)		90 (76)	11 (69)	
Yes	18 (27)	21 (25)		22 (21)	12 (40)		29 (24)	5 (31)	
Tumour site			0.138			0.458			0.525
Colon (right-side)	22 (33)	31 (37)		36 (34)	11 (37)		43 (36)	4 (26)	
Colon (left-side)	26 (39)	20 (24)		30 (29)	11 (37)		35 (29)	6 (37)	
Rectum	19 (28)	32 (39)		39 (37)	8 (26)		41 (35)	6 (37)	
TNM-stage			0.292			0.119			0.640
I	9 (13)	5 (6)		12 (11)	2 (7)		13 (11)	1 (6)	
II	30 (45)	42 (51)		54 (52)	12 (40)		58 (49)	8 (50)	
III	28 (42)	36 (43)		39 (37)	16 (53)		48 (40)	7 (44)	
Differentiation			0.597			0.723			0.932
Mod/well	60 (90)	72 (87)		92 (88)	27 (90)		105 (88)	14 (88)	
Poor	7 (10)	11 (13)		13 (12)	3 (10)		14 (12)	2 (12)	
Venous invasion			0.701			0.367			0.233
Absent	44 (66)	52 (63)		69 (66)	17 (57)		78 (66)	8 (50)	
Present	23 (34)	31 (37)		36 (34)	13 (43)		41 (34)	8 (50)	
Margin involvement			0.726			1.000			0.362
No	62 (93)	78 (94)		98 (93)	28 (93)		112 (94)	14 (88)	
Yes	5 (7)	5 (6)		7 (7)	2 (7)		7 (6)	2 (12)	
Peritoneal involvement			0.095			0.833			0.083
No	54 (81)	57 (69)		79 (75)	22 (73)		92 (77)	9 (56)	
Yes	13 (19)	26 (31)		26 (25)	8 (27)		27 (23)	7 (44)	
Necrosis			0.374			**0.039**			0.063
Low	41 (62)	45 (55)		58 (56)	23 (77)		68 (58)	13 (81)	
High	25 (38)	37 (45)		45 (44)	7 (23)		49 (42)	3 (19)	
Tumour budding			0.401			**0.004**			**0.002**
Low	40 (71)	53 (65)		73 (72)	12 (43)		80 (71)	5 (31)	
High	16 (29)	29 (35)		28 (28)	16 (57)		33 (29)	11 (69)	
Mismatch repair status			0.236			0.400			0.289
Competent	53 (88)	64 (81)		81 (84)	26 (90)		93 (84)	15 (93)	
Deficient	7 (12)	15 (19)		16 (16)	3 (10)		18 (16)	1 (7)	
Proliferation Index			0.133			0.134			0.653
Low	24 (38)	40 (50)		74 (73)	24 (86)		86 (75)	12 (80)	
High	40 (62)	40 (50)		28 (27)	4 (14)		29 (25)	3 (20)	
Klintrup-Makinen grade			0.725			0.922			0.903
Strong	20 (30)	27 (33)		34 (32)	10 (33)		39 (33)	5 (31)	
Weak	47 (70)	56 (67)		71 (68)	20 (67)		80 (67)	11 (69)	
mGPS			0.161			0.946			0.818
0	42 (63)	41 (49)		61 (58)	17 (57)		70 (59)	8 (50)	
1	18 (27)	31 (37)		33 (31)	10 (33)		36 (30)	7 (44)	
2	7 (10)	11 (14)		11 (11)	3 (10)		13 (11)	1 (6)	

Table 4. Relationship between metabolic markers and inflammatory response in patients undergoing potentially curative resection of colorectal cancer (n=150).

	Nuclear LDH5			Cytoplasmic MCT-2 + TSP			Nuclear LDH-5 + TSP		
	Low (n=67)	High (n=83)	*P*	Both Low/One High (n=105)	Both High (n=30)	*P*	Both Low/One High (n=119)	Both High (n=16)	*P*
CD3+ lymphocytes - Margin			0.598			0.114			0.841
Low	35 (56)	45 (60)		59 (62)	13 (45)		63 (57)	9 (60)	
High	44 (28)	30 (40)		37 (38)	16 (55)		47 (43)	6 (40)	
CD3+ lymphocytes - Stroma			0.426			0.990			**0.014**
Low	29 (45)	41 (51)		47 (47)	14 (47)		49 (43)	12 (75)	
High	36 (55)	39 (49)		54 (53)	16 (53)		66 (57)	4 (25)	
CD3+ lymphocytes - Centre			0.854			0.529			0.555
Low	44 (68)	53 (66)		68 (67)	22 (73)		78 (68)	12 (75)	
High	21 (32)	27 (34)		33 (33)	8 (27)		37 (32)	4 (25)	
Cytotoxic T-cells - Margin			0.802			0.481			0.534
Low	39 (63)	45 (61)		59 (61)	15 (54)		63 (58)	10 (67)	
High	23 (37)	29 (39)		37 (39)	13 (46)		45 (42)	5 (33)	
Cytotoxic T-cells - Stroma			0.622			0.833			0.460
Low	45 (74)	56 (70)		71 (71)	20 (69)		81 (72)	10 (63)	
High	16 (26)	24 (30)		29 (29)	9 (31)		32 (28)	6 (37)	
Cytotoxic T-cells - Centre			0.213			0.801			0.460
Low	47 (76)	53 (66)		70 (70)	21 (72)		81 (72)	10 (63)	
High	15 (24)	27 (33)		30 (30)	8 (28)		32 (28)	6 (37)	
Memory T-cells - Margin			0.865			0.737			0.761
Low	35 (57)	44 (58)		56 (57)	15 (54)		62 (56)	9 (60)	
High	27 (43)	32 (42)		42 (43)	13 (46)		49 (44)	6 (40)	
Memory T-cells - Stroma			0.729			0.610			0.173
Low	29 (45)	38 (48)		46 (45)	11 (39)		48 (41)	9 (60)	
High	36 (55)	42 (52)		57 (55)	17 (61)		68 (59)	6 (40)	
Memory T-cells - Centre			0.694			0.532			0.947
Low	49 (75)	58 (73)		75 (73)	22 (78)		86 (74)	11 (73)	
High	16 (25)	22 (27)		28 (27)	6 (22)		30 (25)	4 (27)	
Tregs - Margin			**0.039**			0.377			0.107
Low	30 (49)	50 (67)		55 (57)	18 (67)		62 (56)	11 (79)	
High	31 (51)	25 (33)		41 (43)	9 (33)		47 (43)	3 (21)	
Tregs - Stroma			**0.004**			0.424			0.101
Low	31 (50)	58 (73)		59 (60)	19 (68)		66 (59)	12 (80)	
High	31 (50)	21 (27)		40 (40)	9 (32)		46 (41)	3 (20)	
Tregs - Centre			**0.012**			0.668			0.165
Low	25 (40)	48 (62)		48 (49)	15 (54)		53 (48)	10 (67)	
High	37 (60)	30 (38)		50 (51)	13 (46)		58 (52)	5 (33)	
Serum CRP			0.103			0.889			0.505
Normal	42 (63)	41 (49)		61 (58)	17 (57)		70 (59)	8 (50)	
High	25 (37)	42 (51)		44 (42)	13 (43)		49 (41)	8 (50)	
Serum Albumin			0.741			0.649			0.845
Normal	56 (84)	71 (86)		91 (87)	25 (83)		102 (86)	14 (88)	
Low	11 (16)	12 (14)		14 (13)	5 (17)		17 (14)	2 (12)	
Serum Neutrophils			0.422			0.441			0.666
Low	50 (86)	55 (81)		73 (83)	19 (76)		82 (82)	10 (77)	
High	8 (14)	13 (19)		15 (17)	6 (24)		18 (18)	3 (23)	
Serum Lymphocytes			0.734			0.193			0.080
Low	45 (78)	51 (75)		63 (72)	21 (84)		72 (72)	12 (92)	
High	13 (12)	17 (25)		25 (28)	4 (16)		28 (28)	1 (8)	
White Cell Count			0.423			0.789			0.631
Low	36 (62)	44 (60)		53 (58)	17 (68)		60 (58)	10 (77)	
Intermediate	16 (28)	16 (22)		26 (28)	3 (12)		29 (28)	0 (0)	
High	6 (10)	13 (17)		13 (14)	5 (20)		15 (14)	3 (23)	

Table 5. Clinicopathological characteristics of patients undergoing potentially curative resection of colorectal cancer and cancer-specific survival (n=150)

	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
Clinicopathological Characteristics				
Age (<65/>65)	1.14 (0.84-1.53)	0.403	-	-
Sex (Female/Male)	1.21 (0.72-2.01)	0.471	-	-
Adjuvant Therapy (No/Yes)	0.89 (0.50-1.60)	0.707	-	-
Tumour Site (Colon (right)/colon (left)/Rectum)	1.22 (0.90-1.65)	0.203	-	-
TNM-Stage (II/III)	2.03 (1.31-3.13)	**0.002**	1.36 (0.79-2.35)	0.268
Differentiation (Moderate or well/Poor)	1.41 (0.67-2.96)	0.366	-	-
Venous Invasion (Absent/Present)	1.93 (1.17-3.18)	**0.011**	1.74 (0.99-3.06)	0.053
Margin Involvement (No/Yes)	2.45 (1.05-5.72)	**0.038**	2.57 (0.97-6.81)	0.058
Peritoneal Involvement (No/Yes)	1.60 (0.94-2.72)	0.085	-	-
Necrosis (Low/High)	1.31 (0.79-2.17)	0.304	-	-
Mismatch Repair Status (Competent/Deficient)	1.06 (0.52-2.16)	0.879	-	-
Ki67 proliferation Index (Low/High)	0.52 (0.31-0.86)	**0.012**	0.91 (0.46-1.80)	0.791
Tumour budding (yes/no)	3.87 (2.27-6.60)	**<0.001**	3.19 (1.84-5.56)	**<0.001**
Inflammatory Characteristics				
Klintrup-Makinen Grade (Strong/Weak)	2.15 (1.14-4.04)	**0.018**	1.84 (0.95-3.59)	0.072
mGPS (0/1/2)	2.03 (1.45-2.82)	**<0.001**	2.21 (1.50-3.26)	**<0.001**
Metabolism markers				
Nuclear LDH-5	1.68 (0.99-2.84)	**0.050**	1.19 (0.62-2.29)	0.606
Cytoplasmic LDH-5	1.76 (0.97-3.20)	0.058	1.85 (0.97-3.53)	0.063
Cytoplasmic MCT-2+TSP	2.32 (1.31-4.11)	**0.003**	1.38 (0.71-2.71)	0.344
Nuclear LDH-5+TSP	3.70 (1.96-6.98)	**<0.001**	2.61 (1.27-5.35)	**0.009**

Figure 1. Metabolic markers are associated with poor prognosis in patients with colorectal cancer (n=150). Kaplan Meier curves showing associations between CSS and (A) Nuclear LDH-5, (B) cytoplasmic LDH-5, (C) cytoplasmic MCT-2+TSP and (D) nuclear LDH-5+TSP in 150 patients with stage I-III CRC.