Original Article:

Quantitative data on red cell measures of iron status and their relation to the magnitude of the systemic inflammatory response and survival in patients with colorectal cancer

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Abbreviations

ASA American Society of Anesthesiologists

BMI body mass index

CI confidence interval

CRP C-reactive protein

CSS cancer specific survival

F female

FBC full blood count

Hb haemoglobin

HR hazard ratio

IL interleukin

LOD limit of detection

M male

MCH mean corpuscular haemoglobin

MCV mean corpuscular volume

mGPS modified Glasgow Prognostic Score

NLR neutrophil lymphocyte ratio

OS overall survival

RDW red cell distribution width

sTFR soluble transferrin receptor

TIBC total iron binding capacity

TSAT transferrin saturation,

Abstract

Background:

Inflammation is recognised to be associated with perturbation of serum measures of iron status. However, the impact of colorectal cancer associated host inflammation on red cell measures of iron status has not been previously quantified.

Methods:

Patients undergoing elective surgery with curative intent, for colorectal cancer, at a single centre between 2008 and 2017 were included (n=824). Blood samples taken for C-reactive protein (CRP), albumin, and full blood count (FBC) allowed patients to be grouped by modified Glasgow Prognostic Score (mGPS), and anaemia subtype (haemoglobin (Hb) M<130 mg/L and F<120 mg/L, with microcytic anaemia being mean corpuscular volume (MCV) <80 f/L, and normocytic anaemia with MCV 80-100 f/L). Relationships between these groupings and red cell measures iron status including Hb, MCV, mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW) were examined.

Results:

The combination of increasing T stage and increasing mGPS was associated with lower Hb, lower MCV, lower MCH, higher RDW, and higher prevalence of both microcytic and normocytic anaemia (all $p<0.001$). The combination of CRP >10mg/L and albumin <35g/L was associated with lower Hb, lower MCV, lower MCH, higher RDW, and higher prevalence of both microcytic and normocytic anaemia (all $p<0.010$). At multivariate Cox regression

only Hb remained significantly associated with cancer specific (HR 0.98, 95% CI 0.97-0.99, p<0.001), and overall survival (HR 0.98, 95% CI 0.97-0.99, p=0.001).

Conclusions:

The presence of a host systemic inflammatory response to colorectal cancer was associated with significant perturbation of red cell measure of iron status.

1.0 Introduction

Long term outcome following diagnosis with colorectal cancer is primarily related to disease stage at presentation. Although tumour factors remain key determinants of outcome, it is increasingly recognised that host factors also have a prognostic role [1]. In particular, the presence of an innate systemic inflammatory response prior to surgery is associated with poorer survival across a wide variety of solid tumours, including colorectal cancer [2]. The modified Glasgow Prognostic Score is a validated prognostic measure of the host systemic inflammatory response in colorectal cancer and other solid tumours [3].

At the time of diagnosis, around 30% of patients with colorectal cancer are found to be anaemic [4]. Whilst traditionally in colorectal cancer this has been thought to be mainly iron deficiency anaemia due to luminal blood loss, recent studies in fact suggest that normocytic anaemia, related to inflammation, is in fact more prevalent in this group of patients [5]. Furthermore, such normocytic anaemia has been reported to be a further host factor associated with poorer prognosis, more so than the microcytic anaemia of iron deficiency [4-5].

The presence of a systemic inflammatory response is recognised to significantly confound common measures of serum iron status including serum iron, transferrin saturation, and ferritin [6]. In addition, there are commonly measured parameters of the circulating erythrocyte population such as haemoglobin (Hb), mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW) [7]. These variables may reflect iron status but are also recognised to be influenced by systemic inflammation [8].

The aim of the present study was to quantify the impact of the host systemic inflammatory response to colorectal cancer on these measures of the circulating erythrocyte population,

subtypes of anaemia, and survival in patients undergoing potentially curative treatment for colorectal cancer.

2.0 Patients and Methods

2.1 Patients

This study was a retrospective observational analysis of a prospectively collected and maintained database of patients undergoing potentially curative surgery for colorectal cancer at a single centre. Patients undergoing elective surgery between January 2008 and June 2017 for histologically confirmed colorectal cancer were included. Patients undergoing emergency or palliative surgery, along with those with known existing inflammatory or haematological disorders were excluded.

All patients were discussed at a specialist colorectal cancer multidisciplinary team meeting before and after surgery. Tumours were staged according to the 5th edition of the TNM system [9].

This observational study was approved by the West of Scotland research ethics committee.

2.2 Methods

Haematological parameters were recorded from the electronic laboratory reporting systemic from Full Blood Count samples taken within the 2 weeks prior to surgery. Patients were classified as having anaemia based on WHO guidelines for males; haemoglobin (Hb) <130 mg/L and females; Hb <120 mg/L [10]. Furthermore, anaemic patients were classified as having microcytic anaemia with mean corpuscular volume (MCV) <80 f/L, normocytic anaemia with MCV 80-100 f/L, or macrocytic anaemia with MCV >100 f/L.

Both the modified Glasgow Prognostic Score (mGPS) and the neutrophil to lymphocyte ratio (NLR) were used as markers of the preoperative SIR and were obtained from preoperative

blood results. Serum concentrations of C-reactive protein (CRP) (mg/L) were measured using an autoanalyzer (Architect; Abbot Diagnostics, Maidenhead, UK) with a lower detectable limit of 0.2 mg/L as was serum albumin (normal range 35-50g/L). The preoperative modified Glasgow Prognostic Score (mGPS: CRP ≤10 mg/L = 0. CRP > 10 mg/L and albumin ≥35 g/L = 1, CRP >10 mg/L and albumin <35 g/L = 2), associated with cancer specific survival in multiple solid tumours, was calculated in patients for whom serum CRP and albumin concentrations were available [3].

2.3 Statistical Analysis

The associations between anaemia subtypes and clinicopathological characteristics, between T stage, mGPS and measures of the circulating erythrocyte population, and between CRP, albumin and measures of the circulating erythrocyte population were examined using the chi square test and chi square test of linear association where appropriate.

Deaths within 30 days of surgery or during the index admission were excluded from survival analysis. Cancer specific survival (CSS) was defined as time from date of surgery to death due to colorectal cancer while overall survival (OS) was defined as time from date of surgery to death of any cause. The association between measures of the circulating erythrocyte population, anaemia subtypes and survival were analysed using univariate Cox regression, and Kaplan Meier curves were used to examine the relationship between normocytic anaemia and survival in patients grouped by mGPS. Multivariate Cox regression using a backward conditional model was then applied to those variables found to be associated with CSS or OS at a univariate level of $p<0.1$.

All statistical analyses were performed using SPSS version 24 (IBM, IL, USA). P values <0.05 were considered statistically significant.

3.0 **Results**

3.1 Patients:

Eleven patients were found to have macrocytic anaemia and due to the small numbers were excluded from analysis. Therefore the study included 824 patients with colorectal cancer who underwent surgery with curative intent at a single centre during the study period (Table 1). Of these, the majority were male (55%), over 64 years old (65%), with colonic (63%) and node negative (63%) disease. Of the included patients, 321 (39%) were anaemic at diagnosis, with 69 (8%) having microcytic anaemia and 252 (31%) having normocytic anaemia.

3.2 Association between anaemia subtypes and clinicopathological variables:

When compared to no anaemia and microcytic anaemia (Table 1), normocytic anaemia was associated with the highest proportion of negative host characteristics including patients >75 years old ($p<0.001$), the highest proportion of patients with ASA grade 3 and 4 ($p<0.001$), the lowest proportion of patients with BMI >30 kg/m^2 ($p=0.001$), and the highest proportion of patients with mGPS 2 ($p<0.001$). However, microcytic anaemia was associated with the highest proportion of negative tumour characteristics including the highest proportion of T stage 4 ($p<0.001$) and poor differentiation ($p=0.004$). Those without anaemia had the highest proportion of rectal cancers ($p<0.001$).

3.3 Association between T stage, mGPS and measures of the circulating erythrocyte population:

There were statistically significant associations (Table 2) between median Hb, MCV, MCH, RDW, and both T stage and mGPS. In general, those patients with a higher T stage, and higher mGPS had lower Hb ($p<0.001$), lower MCV ($p<0.001$), lower MCH ($p<0.001$) and higher RDW ($p<0.001$). A significantly greater proportion of patients with higher T stage and higher mGPS were found to have either normocytic anaemia ($p<0.001$), or microcytic anaemia ($p<0.001$).

3.4 Association between CRP, albumin and measures of the circulating erythrocyte population:

There were statistically significant associations (Table 3) between median Hb, MCV, MCH, RDW and both CRP and albumin. In general, those patients with a higher CRP and lower albumin (i.e. greater inflammation), had lower Hb, ($p<0.001$), lower MCV ($p=0.001$), lower MCH ($p<0.001$), and higher RDW ($p<0.001$). A significantly greater proportion of patients with higher CRP and lower albumin were found to have either normocytic anaemia ($p<0.001$), or microcytic anaemia ($p=0.004$).

3.5 Association between measures of the circulating erythrocyte population and survival:

There were 12 deaths in the perioperative period, excluded from survival analysis. Long term survival data was available for 782 patients. There were 181 deaths (23%), of which 104 (13%) were due to colorectal cancer. The median length of follow up amongst those patients alive at the time of follow up was 52 months (range 12-122).

At univariate analysis (Table 4) preoperative Hb (HR 0.98, 95% CI 0.97-0.99, p<0.001) and normocytic anaemia (HR 1.98, 95% CI 1.34-2.89, p<0.001) were associated with cancer specific survival. When patients were grouped by inflammatory state (Figure 1) there was no significant association between normocytic anaemia and cancer specific survival in either patients with mGPS 0 (p=0.063) or mGPS 1-2 (p=0.086).

At univariate analysis (Table 4) preoperative Hb (HR 0.98, 95% CI 0.97-0.99, p<0.001), MCH (HR 1.01, 95% CI 1.00-1.02, p=0.006), RDW (HR 1.07, 95% CI 1.02-1.13, p=0.006), and normocytic anaemia (HR 1.94, 95% CI 1.45-2.60, p<0.001) were associated with overall survival. When patients were grouped by inflammatory state (Figure 2) normocytic anaemia was associated with significantly poorer overall survival in both patients with mGPS 0 (p=0.046) and mGPS 1-2 (p<0.001).

When those haematological factors which were significantly associated with cancer specific survival (Hb) and overall survival (Hb, MCH and normocytic anaemia) at univariate analysis were included in multivariate analysis along with established prognostic factors such as age, sex, ASA grade, tumour location, mGPS, TNM stage, differentiation (Table 5), none retained independent prognostic significance.

4.0 Discussion

The results of the present study report a significant association between T stage, the presence a host systemic inflammatory response, and red cell markers of iron status in patients with colorectal cancer. In addition, an increasing magnitude of innate systemic inflammation appears to be associated with progressive derangement of these measures. Furthermore, the presence of anaemia, and particularly normocytic anaemia, was associated with poorer cancer specific and overall survival independent of systemic inflammation in this cohort of patients.

These results are in keeping with recently published results from our own group [4], and others [5], which reported an association between systemic inflammation and anaemia in patients with colorectal cancer. It is well understood that the presence of systemic inflammation of any cause leads to significant derangement of common serum measures of iron status; including total iron, transferrin saturation, and ferritin [6]. This is thought to occur due to the action of hepcidin, upregulated by the proinflammatory cytokine IL 6, resulting in inhibition of enteric iron absorption and sequestration of iron and ferritin in the liver and reticuloendothelial system [11]. Indeed, this effect has in part lead to the search for novel measures of iron status which are not affected in such a way, and in other attempts to formulate correction factors and equations to account for inflammation when using these measures of iron status [12-13].

Inflammation is recognised to lead to anaemia through three broad mechanisms, the first being the inflammation related hypoferraemia, or functional iron deficiency, driven by hepcidin described above [11]. In addition, proinflammatory cytokines tend to have a suppressive effect on erythropoiesis through their inhibitory relationship with erythroid precursors [14]. Finally, activated macrophages reduce the life span of circulating red cells through enhanced phagocytosis [15]. In colorectal cancer, anaemia has traditionally been

attributed to frank or occult gastrointestinal blood loss. However, when the results of the present study are taken together with other recently published cohorts, it would appear that anaemia of inflammation is in fact far more prevalent in this group of patients than has been previously recognised [4-5].

The presence of host systemic innate inflammatory responses to colorectal cancer, and their negative prognostic impact, are well documented [16]. The presence of anaemia has also long been recognised to be associated with poorer outcomes [17]. Therefore, the increasing evidence of association between cancer related inflammation, and normocytic anaemia in particular, is of great significance [5]. Inflammation associated normocytic anaemia has been reported to be associated with poorer outcomes than classical microcytic anaemia of blood loss in several cohorts [4]. Unlike the previous studies [4-5, 8], multivariate survival analysis in the present cohort did not find normocytic anaemia to be independently prognostic. This is possibly due to the significant co-dependent relationship between Hb and normocytic anaemia, and the fact that a continuous variable was compared to a binary categorical variable. Alternatively, it may reflect the importance of the underlying host systemic inflammatory response as a mechanism underpinning anaemia, and disease progression. However, Figure 1 shows that a proportion of patients with mGPS 0 have normocytic anaemia, and that this still appears to impart a negative prognosis. Either other factors lead to normocytic anaemia in these patients, or much lower levels of inflammation than those thresholds used to determine mGPS are having an effect. The precise mechanisms linking these phenomena to poorer outcomes remain poorly understood.

This area is of further interest to those involved in surgery and perioperative medicine in this group of patients. This group of patients still require significant rates of perioperative blood transfusion, an intervention associated with significant cost, complications, and possibly a

negative oncologic impact [18]. There has been a drive to reduce perioperative blood transfusion rates in colorectal cancer patients using preoperative oral and parenteral iron replacement, with some initially encouraging results [19]. However, the large group of patients with normocytic anaemia of inflammation have never been considered in their own right [20]. Given the possibility that systemic inflammation will prevent the efficient utilisation of administered supplemental iron, this should form an important area of future research.

The present study has several limitations. The retrospective nature of the study means that not all patients had CRP and albumin recorded along with the red cell measures of iron status, leading to loss of data, especially in later analyses. Data were not immediately available regarding perioperative blood transfusion, a factor thought to relate to oncologic outcome. Furthermore, insufficient data regarding serum measures of iron status, such as ferritin or transferrin saturation, were collected to allow meaningful correlation between those and red cell measures of iron status. It should be noted that around 200 patients from the single centre within the present study are included in our previous published bowel screening cohort [4], however that cohort as a whole did not have measures of red cell iron status other than Hb and MCV available. Finally, the patients in the present paper were stages using the older TNM 5th edition. Using the more current 7th edition might result in an upstaging from node negative to node positive disease in less than 3% of cases so would hopefully have little impact on the results [21]. Another factor reducing this possible bias is that in the present study there was no association between N stage and preoperative anaemia.

5.0 Conclusions

In summary, the results of the present study report that the presence of a host systemic inflammatory response to colorectal cancer is associated with significant perturbation of red cell measures of iron status. Furthermore, the results from this cohort add to the existing evidence reporting the high prevalence of preoperative normocytic anaemia in this patient group, its association with systemic inflammation, and poorer cancer outcomes. Future investigation should aim to determine whether existing perioperative iron and blood management strategies are of use in this subgroup.

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Conflicts of interest

None

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Tables and footnotes

Table 1: Clinicopathological characteristics of patients undergoing elective surgery for colorectal cancer grouped by preoperative anaemia

Anaemia[£]	**None** n (%)	**Microcytic**[$] n (%)	**Normocytic*** n (%)	**p**
N	503 (61)	69 (8)	252 (31)	
Demographic				
Sex				
Male	263 (53)	42 (61)	150 (60)	0.118
Female	238 (47)	27 (39)	102 (40)	
Age				
<65	202 (40)	27 (39)	58 (23)	<0.001
64-74	212 (42)	22 (32)	90 (36)	
>75	89 (18)	20 (29)	104 (41)	
ASA				
1	128 (26)	11 (16)	30 (13)	<0.001
2	227 (46)	28 (42)	106 (44)	
3	127 (26)	24 (36)	91 (38)	
4	7 (1)	4 (6)	13 (5)	
BMI				
<20	19 (4)	8 (13)	24 (10)	0.001
20-25	154 (32)	20 (32)	83 (34)	
25-30	146 (30)	17 (27)	82 (34)	
>30	162 (34)	17 (27)	52 (22)	
Smoking status				
Never	240 (49)	32 (49)	117 (47)	0.099
Ex	177 (36)	27 (41)	108 (44)	
Current	74 (15)	7 (10)	22 (9)	
Neoadjuvant treatment				
Yes	83 (17)	4 (6)	38 (15)	0.069
No	415 (83)	64 (94)	210 (85)	
Pathological				
Tumour site				
Colon	269 (54)	62 (90)	187 (75)	<0.001
Rectum	234 (47)	7 (10)	64 (25)	
T stage				
0/1	101 (20)	3 (5)	16 (7)	<0.001
2	93 (19)	2 (3)	23 (9)	
3	236 (47)	39 (58)	157 (63)	
4	69 (14)	23 (34)	53 (21)	
N stage				
0	327 (66)	36 (54)	154 (62)	0.408
1	121 (24)	22 (33)	66 (27)	
2	59 (10)	9 (13)	28 (11)	
Differentiation				
Well / Mod	453 (93)	54 (82)	221 (90)	0.004
Poor	27 (7)	12 (18)	23 (10)	
Venous invasion				
Present	286 (58)	44 (67)	148 (59)	0.381
Absent	209 (42)	22 (33)	101 (41)	
Systemic inflammation				
NLR				
≥5	57 (12)	9 (14)	41 (17)	0.143
<5	438 (88)	57 (86)	204 (83)	
mGPS				
0	401 (85)	43 (72)	163 (72)	<0.001
1	48 (10)	5 (8)	15 (7)	
2	21 (5)	12 (20)	48 (21)	

ASA American Society of Anesthesiology, *BMI* body mass index, *mGPS* modified Glasgow Prognostic Score, *NLR* neutrophil-to-lymphocyte ratio

[£]Males = Hb <130g/L, females <120g/L

[$]Anaemia and MCV <80fL

*Anaemia and MCV 80-100fL

Table 2: Relationship between T stage, systemic inflammation and haematological parameters in

Number of patients, n				
		T stage		
	All	0-2	3-4	
mGPS				
All	748	225	523	
0	601	202	399	
1-2	147	23	124	
Haemoglobin, median, range (mg/L)				
		T stage		p
	All	0-2	3-4	
mGPS				
All	130 (63 – 194)	137 (94 – 172)	125 (63 – 194)	<0.001
0	132 (81-194)	138 (94 – 172)	129 (81 – 194)	<0.001
1-2	123 (74– 162)	130 (108 – 154)	120 (74 – 162)	<0.001
p	<0.001	0.070	<0.001	<0.001
MCV, median, range (fL)				
		T stage		p
	All	0-2	3-4	
mGPS				
All	89 (64 – 105)	91 (69 – 104)	89 (64 – 105)	<0.001
0	90 (68 – 105)	91 (69 – 104)	89 (68 – 105)	<0.001
1-2	87 (64 – 103)	90 (80 – 100)	87 (64 – 103)	<0.001
p	<0.001	0.483	0.001	<0.001
MCH, median, range (g/dL)				
		T stage		p
	All	0-2	3-4	
mGPS				
All	29.4 (18.0 – 46.7)	30.2 (20.6 – 36.4)	29.1 (18.0 – 46.7)	<0.001
0	29.7 (19.2 – 36.4)	30.2 (20.6 – 36.4)	29.4 (19.2 – 35.7)	<0.001
1-2	28.0 (18.0 – 35.4)	29.2 (24.8 – 35.2)	27.8 (18.0 – 35.4)	0.009
p	<0.001	0.173	<0.001	<0.001
RDW, median, range (%)				
		T stage		p
	All	0-2	3-4	
mGPS				
All	14.0 (11.7 – 27.1)	13.5 (11.8 – 24.2)	14.3 (11.7 – 27.1)	<0.001
0	13.8 (11.7 – 27.1)	13.5 (11.8 – 24.2)	14.1 (11.7 – 27.1)	<0.001
1-2	14.9 (12.1 – 23.8)	13.8 (12.1 – 15.9)	15.1 (12.3 – 23.8)	0.015
p	0.002	0.801	0.013	<0.001
Microcytic[$] anaemia[£], n (%)				
		T stage		p
	All	0-2	3-4	
mGPS				
All	59 (8)	5 (2)	54 (10)	<0.001
0	42 (7)	5 (3)	37 (9)	0.002
1-2	17 (11)	0 (0)	17 (13)	0.057
p	0.075	0.436	0.166	<0.001

Normocytic* anaemia[£], n (%)				
		T stage		p
	All	0-2	3-4	
mGPS				
All	223 (29)	36 (16)	187 (35)	<0.001
0	161 (27)	28 (14)	133 (33)	<0.001
1-2	62 (41)	8 (33)	54 (43)	0.401
p	<0.001	0.014	0.046	<0.001

mGPS modified Glasgow Prognostic Score, MCV mean corpuscular volume, MCH mean corpuscular haemoglobin, RDW red cell distribution width

[£]Males = Hb <130g/L, females <120g/L

[$]Anaemia and MCV <80fL

*Anaemia and MCV 80-100fL

Table 3: Relationship between systemic inflammation as measured by C-reactive protein (mg/L) and albumin (g/L), and anaemia in patients undergoing elective surgery for colorectal cancer (n=615)

Number of patients, n				
		CRP (mg/L)		
	All	<10	>10	
Albumin (g/L)				
All	615	470	145	
>35	432	369	101	
<35	183	101	82	
Haemoglobin, median, range (mg/L)				
		CRP (mg/L)		p
	All	<10	>10	
Albumin (g/L)				
All	130 (74 – 18.2)	132 (81 – 182)	122 (74 – 162)	<0.001
>35	135 (88 – 182)	135 (88 – 182)	132 (90 – 162)	0.190
<35	118 (74 – 162)	125 (81 – 162)	111 (74 – 151)	0.001
p	<0.001	<0.001	<0.001	<0.001
MCV, median, range (fL)				
		CRP (mg/L)		p
	All	<10	>10	
Albumin (g/L)				
All	89 (64 – 105)	90 (68 – 105)	87 (64 – 103)	<0.001
>35	90 (68 – 105)	90 (68 – 105)	87 (71 – 103)	0.019
<35	88 (64 – 103)	89 (70 – 102)	87 (64 – 103)	0.055
p	0.029	0.355	0.387	0.001
MCH, median, range (g/dL)				
		CRP (mg/L)		p
	All	<10	>10	
Albumin (g/L)				
All	29.4 (18.0 – 36.4)	29.7 (19.2 – 36.4)	28.0 (18.0 – 35.4)	<0.001
>35	29.7 (19.2 – 36.4)	29.8 (19.2 – 36.4)	28.9 (21.9 – 35.4)	0.013
<35	28.3 (18.0 – 35.7)	28.9 (19.3 – 35.7)	27.6 (18.0 – 35.2)	0.018
p	<0.001	0.003	0.015	<0.001
RDW, median, range (%)				
		CRP (mg/L)		p
	All	<10	>10	
Albumin (g/L)				
All	14.0 (11.8 – 27.1)	13.8 (11.8 – 27.1)	14.9 (12.1 – 23.8)	0.006
>35	13.8 (11.8 – 27.1)	13.8 (11.8 – 27.1)	14.0 (12.1 – 23.3)	0.666
<35	15.2 (12.6 – 24.8)	14.4 (12.8 – 24.8)	16.0 (12.6 – 23.8)	0.011
p	<0.001	0.048	0.001	<0.001
Microcytic[$] anaemia[£], n (%)				
		CRP (mg/L)		p
	All	<10	>10	
Albumin (g/L)				
All	46 (8)	30 (6)	16 (11)	0.063
>35	25 (6)	21 (6)	4 (6)	0.852
<35	21 (12)	9 (9)	12 (15)	0.234
p	0.011	0.215	0.102	0.004

Normocytic* anaemia[£], n (%)				
		CRP (mg/L)		p
	All	<10	>10	
Albumin (g/L)				
All	191 (31)	128 (27)	63 (43)	<0.001
>35	97 (22)	82 (22)	15 (23)	0.813
<35	94 (52)	46 (47)	48 (59)	0.087
p	<0.001	<0.001	<0.001	<0.001

MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, RDW - red blood cell distribution width, CRP - C-reactive protein

[£]Males = Hb <130g/L, females <120g/L,

[$]Anaemia and MCV <80fL,

*Anaemia and MCV 80-100fL

Table 4: Univariate and multivariate survival analysis of haematological parameters in patients with colorectal cancer treated with curative intent

Variable	Univariate survival		Multivariate survival	
	HR (95% CI)	p	HR (95% CI)	p
CSS				
Haemoglobin (mg/L)	0.98 (0.97-0.99)	<0.001	0.98 (0.97-0.99)	<0.001
MCV (fL)	1.00 (0.98-1.03)	0.859	-	-
MCH (g/dL)	0.98 (0.92-1.04)	0.402	-	-
RDW (%)	1.06 (0.99-1.14)	0.111	-	-
Microcytic[$] anaemia[£]	1.27 (0.68-2.37)	0.458	-	-
Normocytic* anaemia[£]	1.98 (1.34-2.89)	<0.001	-	0.152
OS				
Haemoglobin (mg/L)	0.98 (0.97-0.99)	<0.001	0.98 (0.97-0.99)	0.001
MCV (fL)	1.00 (0.98-1.02)	0.879	-	-
MCH (g/dL)	1.01 (1.00-1.02)	0.006	1.01 (1.01-1.02)	<0.001
RDW (%)	1.07 (1.02-1.13)	0.008	-	0.383
Microcytic[$] anaemia[£]	1.20 (0.74-1.95)	0.461	-	-
Normocytic* anaemia[£]	1.94 (1.45-2.60)	<0.001	1.43 (0.97-2.12)	0.069

MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, RDW - red blood cell distribution width, CRP - C-reactive protein
[£]Males = Hb <130g/L, females <120g/L,
[$]Anaemia and MCV <80fL,
*Anaemia and MCV 80-100fL

Table 5: Univariate and multivariate survival analysis comparing haematological parameters to established prognostic factors

Survival	Variable	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
CSS	Age	1.52 (1.19-1.94)	0.001	1.38 (1.06-1.81)	0.018
	Male Sex	1.24 (0.85-1.82)	0.264	-	-
	ASA	1.38 (1.08-1.75)	0.010	-	0.394
	mGPS	1.61 (1.25-2.08)	<0.001	1.37 (1.06-1.78)	0.017
	Rectal	0.92 (0.62-1.35)	0.649	-	-
	Differentiation	2.34 (1.40-3.93)	0.001	2.40 (1.36-4.22)	0.002
	Venous invasion	1.83 (1.22-2.75)	0.004	-	0.869
	TNM stage	2.46 (1.91-3.18)	<0.001	2.42 (1.83-3.20)	<0.001
	Haemoglobin (g/L)	0.98 (0.97-0.99)	<0.001	-	0.342
	Adjuvant treatment	1.03 (0.68-1.56)	0.899	-	-
OS	Age	1.77 (1.47-2.14)	<0.001	1.58 (1.27-1.96)	<0.001
	Male Sex	1.38 (1.03-1.85)	0.029	1.33 (0.97-1.83)	0.081
	ASA	1.63 (1.36-1.96)	<0.001	1.34 (1.09-1.65)	0.006
	mGPS	1.78 (1.48-2.14)	<0.001	1.54 (1.27-1.87)	<0.001
	Rectal	0.82 (0.61-1.10)	0.182	-	-
	Differentiation	1.95 (1.28-2.97)	0.002	1.79 (1.09-2.94)	0.022
	Venous invasion	1.41 (1.05-1.89)	0.021	-	1.000
	TNM stage	1.75 (1.46-2.09)	<0.001	1.72 (1.40-2.11)	<0.001
	Haemoglobin (g/L)	0.98 (0.97-0.99)	<0.001	-	0.294
	MCH (g/dL)	1.01 (1.00-1.02)	0.006	-	0.125
	Normocytic* anaemia[£]	1.94 (1.45-2.60)	0.001	-	0.266
	Adjuvant treatment	0.78 (0.56-1.08)	0.138	-	-

ASA American Society of Anesthesiology, HR Hazard Ratio, *CI* Confidence Interval , *CSS* disease specific survival, *OS* overall survival, *mGPS* modified Glasgow Prognostic Score, *POD* postoperative day *MCH* mean corpuscular haemoglobin

[£]Males = Hb <130g/L, females <120g/L,

[$]Anaemia and MCV <80fL,

*Anaemia and MCV 80-100fL

Figures and Legends

Figure 1: Kaplan Meier charts of (A) cancer specific survival amongst patients with modified Glasgow Prognostic Score 0 ($p=0.063$), (B) cancer specific survival amongst patients with modified Glasgow Prognostic Score 1-2 ($p=0.086$), (C) overall survival amongst patients with modified Glasgow Prognostic Score 0 ($p=0.046$), and (D) overall survival amongst patients with modified Glasgow Prognostic Score 1-2 ($p<0.001$), grouped by preoperative normocytic anaemia