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Prognostic indices of inflammatory markers, cognitive function and fatigue for survival in patients with localised colorectal cancer

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ABSTRACT

Background Inflammation promotes the development of malignancy, while a variety of systemic markers of inflammation predict for worse cancer outcomes including recurrence and survival. Here, we evaluate the prognostic impact of cytokine concentrations, full blood count (FBC) differential ratios, cognitive function and fatigue on survival in patients with localised colorectal cancer (CRC).

Patients and methods Data are from a prospective longitudinal study comparing cognitive function and fatigue in patients with CRC who did (n=173) and did not (n=116) receive adjuvant/neoadjuvant chemotherapy. Baseline blood results (prior to any chemotherapy) included cytokines and FBC from which neutrophil lymphocyte ratio, lymphocyte monocyte ratio, platelet lymphocyte ratio and platelet monocyte ratio were derived. Fatigue was measured with the Functional Assessment of Cancer Therapy-Fatigue subscale and cognitive function by a neuropsychological test battery. Kaplan-Meier methods were used to estimate disease-free survival (DFS) and overall survival (OS). Univariable and multivariable Cox regression analyses were performed to evaluate factors potentially prognostic of outcomes.

Results At a median follow-up of 91.2 months, 227 subjects (79%) are still alive, and 212 (73%) have no evidence of a recurrence. Five-year OS and DFS are 86% (95% CI 81% to 90%) and 77% (95% CI 71% to 82%), respectively. None of the cytokines (interleukin (IL-6), IL-1 and tumour necrosis factor) or differential ratios of blood components, fatigue or cognitive function was statistically related to DFS or OS. Patient educational status (P=0.018), stage of disease (P=0.032), alanine transaminase (P=0.003), lactate dehydrogenase (P=0.008) and carcinoembryonic antigen (P=0.002) were significant as prognostic covariates of OS in univariable analyses, with similar results for DFS.

Conclusion None of the a priori selected markers of inflammation, fatigue or cognitive function was associated with OS or DFS in this cohort of patients.

Trial registration number NCT00188331, Post-results.

Key questions

What is already known about this subject?

- Inflammation can lead to cancer development and may predict for recurrence.
- The Glasgow Prognostic Score and other markers of inflammation have been found to be prognostic of survival for a number of cancers.
- Neutrophil lymphocyte ratio is associated with survival in a number of solid tumours.

What are the new findings?

- In our colorectal cancer cohort, cognitive function and fatigue were not associated with survival.
- The baseline inflammatory cytokines were elevated in comparison to non-cancer controls but were not associated with survival in this cohort.
- Neutrophil lymphocyte ratio was not associated with survival.

How might this impact on clinical practice?

- The lack of association between baseline inflammatory markers and survival may have been due to timing of the blood collection.

BACKGROUND

Colorectal cancer (CRC) is the third leading cause of cancer-related death worldwide.[1] Localised disease has a good prognosis but depends on stage, with 5-year survival rates for subjects with stage I CRC of ~90%, stage II 63%–87% and stage III 53%–89%.[2]

There is increasing clinical and experimental evidence that inflammation can lead to cancer development,[3–6] and may be predictive for relapse and survival in a variety of cancers,[3–7] including CRC.[8 9] Elevation of systemic inflammatory markers is thought to be driven by release of cytokines and chemokines from the tumour microenvironment and/or the local immune environment, which then promote acute phase protein production from the liver. This results in elevation of C reactive protein (CRP) and a reduction in albumin levels.

Correlative biological markers of inflammatory pathways have been studied in an attempt to find prognostic biomarkers to

Table 1 Baseline characteristics for patients with localised stage colorectal cancer

Characteristic	Statistic	Results
Cohort: n (%)	Group A	173 (60)
	Group B	116 (40)
Age	Mean (SD)	58.7 (10)
Gender	N (%) male	183 (63)
Country	N (%) Australian	106 (37)
Education	Median, years (range)	14 (4–21)
Marital status: n (%)	Married/common law	188 (65)
	Separated/divorced	43 (15)
	Single	37 (13)
	Widowed	8 (3)
	Unknown	13 (5)
Alcohol: (glasses/day)	0–1	128 (44)
	2 – 4	94 (33)
	5+	29 (10)
	Unknown	38 (13)
Smoking status	Never	140 (48)
	Former	24 (8)
	Current	111 (38)
	Unknown	14 (5)
Time from surgery, weeks	Median (range)	7.4 (0.4–29)
Stage	I	50 (17)
	II	106 (37)
	III	128 (44)
	Unknown	5 (2)
Site of tumour	N (%) colon	193 (67)
	N (%) rectal	96 (33)
Chemotherapy: n (%)	Adjuvant	124 (43)
	Neoadjuvant	46 (16)
	None	119 (41)

Group A received adjuvant/neoadjuvant chemotherapy. Group B did not require chemotherapy.

assist in guiding therapeutic options and surveillance.[10] A prognostic inflammatory parameter evaluated in many studies is the modified Glasgow prognostic score (mGPS), which uses a three-point scale derived from categorical scoring of CRP and plasma albumin levels.[11] The mGPS has been shown to be prognostic of survival in patients with many types of cancer, including CRC.[11 12] However, its widespread use has been limited because CRP levels are not routinely available in patients with cancer.

Other inflammatory assessments that have been widely researched in patients with cancer using peripheral blood samples are cytokine concentrations and ratios derived from full blood count (FBC) parameters. Elevated plasma concentrations of cytokines (particularly interleukin (IL)-6) have been shown to be predictive for relapse and survival in several cancers.[3–7 13] Often there are changes in levels of both pro-inflammatory and anti-inflammatory cytokines in people with cancer. A limitation of using them routinely is that assays of multiple cytokines are expensive and difficult to interpret.

Differential blood counts are widely available and inexpensive. The neutrophil lymphocyte ratio (NLR) has been studied widely. A meta-analysis of 100 studies including 40 559 patients with solid tumours found an association of NLR with overall survival (OS) and disease-free survival (DFS).[14] Recent studies have found indices of inflammation such as platelet lymphocyte ratio (PLR) and lymphocyte monocyte ratio (LMR) to impact survival in CRC.[15–21] A meta-analysis of 33 studies (n=15 404 patients with CRC) evaluating the utility of pretreatment PLR and LMR as prognostic predictors in CRC found elevated PLR associated with poorer OS and DFS, while elevated LMR predicted a favourable outcome.[10]

Here, we investigate whether selected inflammatory markers, and measures of cognitive function and fatigue are predictors of survival in a CRC population we followed longitudinally to evaluate cognitive function and fatigue.[22–24] Our primary hypothesis was that baseline IL-6 (generally taken after surgery, and always prior to commencing adjuvant treatment) would be prognostic of OS and DFS in patients treated for localised CRC. Our exploratory hypotheses were that baseline cognitive function, fatigue, inflammatory cytokines, MLR, NLR, PLR and PMR levels would predict survival.

METHODS

Subjects were people with localised CRC who had participated in a prospective, longitudinal, multicentre study evaluating cognitive function and fatigue. Participants were recruited from November 2003 until September 2010. Patient characteristics and methods are described elsewhere.[22] The main study compared two groups of patients with localised CRC with each other: group A were those with stage III or high-risk stage II CRC, treated with surgery and adjuvant or neoadjuvant chemotherapy, while group B had surgery but did not receive chemotherapy for stage I or II CRC. Localised CRC subjects were also compared with healthy controls. Here, we evaluated survival of those with localised CRC, and compared their blood parameters at baseline with those of the healthy controls.

At baseline, participants were aged <75 years, had no prior invasive malignancy and were chemotherapy-naïve. Participants were recruited from hospitals in Toronto, Canada and Sydney, Australia. All subjects provided written informed consent. Control participants were recruited via patient participants and advertising within the hospital; eligibility criteria were the same as for the patients with CRC, with the exception that they could not have had invasive cancer or chemotherapy previously.

The CRC participants were assessed at baseline, 6, 12 and 24 months with cognitive testing, patient-reported

Table 2 Survival outcomes		
OS	N (%) deaths	62 (22)
	Median	Not reached
	2-year (95% CI) OS	94 (91– 97)
	5-year (95% CI) OS	86 (81– 90)
OS, group A	N (%) deaths	42 (24)
	2-year (95% CI) OS	93 (88– 96)
	5-year (95% CI) OS	82 (76– 88)
OS, group B	N (%) deaths	20 (17)
	2-year (95% CI) OS	96 (91– 99)
	5-year (95% CI) OS	91 (84– 95)
DFS	N (%) events	77 (27)
	2-year (95% CI) DFS	85 (80– 88)
	5- year (95% CI) DFS	77 (71– 82)
DFS, group A	N (%) events	50 (29)
	2-year (95% CI) DFS	82 (77– 87)
	5-year (95% CI) DFS	74 (67– 80)
DFS, group B	N (%) events	27 (23)
	2-year (95% CI) DFS	89 (81– 93)
	5-year (95% CI) DFS	81 (72– 87)

Group A received adjuvant/neoadjuvant chemotherapy. Group B did not require chemotherapy.
DFS, disease-free survival; OS, overall survival.

outcome (PRO) questionnaires and blood tests. In most patients, the baseline assessment occurred approximately 7 weeks after surgery and always prior to chemotherapy. In patients with rectal cancer who were to receive neoadjuvant chemoradiotherapy, the baseline assessment occurred prior to surgery, chemotherapy or radiotherapy. Cognitive function was assessed with a comprehensive battery of neuropsychological tests.[22] Impairment was defined as a Global Deficit Score of >0.5.[25] Fatigue was measured using the Functional Assessment of Cancer Therapy-Fatigue fatigue subscale.[26 27] Fatigue was defined as a standardised score of ≤68/100.[28]

Blood tests included FBC (with a differential white cell count), liver function tests (LFTs), carcinoembryonic antigen (CEA) and a panel of 10 cytokines. For patient convenience, the time of day of blood collection was not standardised. All cytokines were analysed in a central laboratory using Luminex technology with a human multiplex kit (Affymetrix, Santa Clara, California, USA). The median of the mean immunofluorescence levels is reported.

For the current analyses, we accessed participants' medical records between April and June 2017 to determine survival status of the CRC study patients, and where possible extracted information regarding survival status (OS and DFS), and dates of recurrence and/or death. Medical records were supplemented by a search of death notices and Google searches. The Ontario Cancer Registry was searched in Toronto, and in Sydney patients were contacted by mail and/or phone as part of an approved extension of the original study.

Statistical methods

The primary end point was OS with the secondary end point DFS.

The sample size of the original study was based on the primary end point of objective cognitive function, which targeted a sample size of 170 patients who received chemotherapy (group A) and 120 who did not (group B) to achieve 80% power (α=0.05, two-sided) to detect a difference in cognitive impairment of 8%. Here, all patients with localised CRC with baseline data are included.

Summary statistics were used to describe characteristics of participants and their responses to questionnaires. The Kaplan-Meier method was used to estimate DFS and OS. OS was defined as date of baseline assessment to date of death from any cause; living patients were censored at date of last follow-up. DFS was defined as date of baseline assessment to date of known recurrence of CRC. Univariable and multivariable Cox regression analyses were performed to evaluate factors potentially prognostic of outcomes. Analyses were stratified by patient cohort (group A vs group B). Multivariable model building was performed using forward selection and including all available patient, laboratory and disease factors as potential covariates. Given the large number of missing laboratory data, a second model was constructed using only patient and disease factors as potential covariates.

Logarithmic transformations were performed on selected laboratory values for statistical normalisation purposes. FBC ratios were analysed using both continuous and dichotomised data, with the later dividing participants into 'high' and 'low' groups based on cut-points from a previous study by one of the authors (SJC).[18] The cut-point of 3.19 was used for NLR, 2.38 for LMR and 258 for platelets. All tests were two-sided and statistical significance was defined as a P value of 0.05 or less. No correction was applied for multiplicity. Analyses were performed in SAS V.9.0 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Baseline data for 289 subjects with localised CRC were available: 173 received neoadjuvant/adjuvant chemotherapy (group A) and 116 received no chemotherapy (group B). Table 1 provides demographic and baseline characteristics. Mean (SD) age of subjects was 58.7 (10.4) years, and the majority were men (63%). The baseline assessment (including blood collection) occurred a median of 7.4 weeks after surgery, but prior to any treatment in those who received neoadjuvant chemotherapy.

As of 15 June 2017, at a median follow-up of 91 months (maximum of 152 months), 227 (78.5%) were alive and 212 (73.4%) had no evidence of a recurrence of their CRC. Median survival has not been reached, but 5-year OS is 86% (95% CI 81% to 90%); (group A=82% (95% CI 76% to 88%); group B=91% (95% CI 84% to 95%). The

Table 3 Median (range) laboratory values, by cohort

Characteristic	CRC group A	CRC group B	Healthy controls	P value
Number (N)	173	116	72	
IL-6	43 (2–11 500)	20 (2–8500)	9 (4–24)	<0.001
TNF-α	20 (3–11 700)	7 (2–900)	10 (3–30)	0.002
IL-1b	30 (3–8500)	13 (2–8000)	10 (4–41)	<0.001
WCC	7.1 (3.5–16.3)	6.7 (3.7–14.3)	6.6 (3.4–10.7)	0.21
Neutrophils	4.3 (1.8–13.0)	3.9 (1.6–11.1)	3.8 (1.6–7.0)	0.026
Lymphocytes	1.8 (0.8–5.2)	1.8 (0.8–7.1)	2.1 (0.7 4.5)	0.090
Platelets	279 (50–757)	282 (147–719)	268 (151–387)	0.39
Monocytes	0.5 (0.2–1.4)	0.5 (0.2–1.31)	0.5 (0.3–1.0)	0.26
Neutrophils-lymphocyte ratio	2.31 (0.75–7.22)	2.13 (0.51–7.07)	1.93 (0.64–5.71)	0.007
N (%), NLR>3.19	31/140 (22)	18/95 (19)	7/72 (10)	0.084
Platelet-lymphocyte ratio	151 (21–355)	155 (37–507)	132 (70–317)	0.007
N (%), PLR>258	12/140 (9)	10/95 (11)	2/72 (3)	0.16
Neutrophils-monocyte ratio	7.83 (2.5–36.5)	7.78 (3.2–17.25)	7.17 (4.0–14.75)	0.16
Lymphocyte-monocyte ratio	3.46 (1.29–9.67)	3.40 (1.3–11.83)	3.85 (1.3–9.75)	0.18
N (%), LMR>2.38	112/132 (85)	86/94 (92)	66/72 (92)	0.19
Albumin	43 (33–79)	44 (34–49)	46 (41–53)	<0.001
Haemoglobin	133 (103–169)	130 (96, 160)	142 (97, 187)	<0.001
Bilirubin	7 (2–21)	8 (3–33)	8 (3–31)	0.25
AST	20 (8–58)	21 (11–68)	21 (11–42)	0.24
ALT	20 (6–116)	22 (9–112)	19.5 (10–60)	0.53
ALP	78 (25–269)	75 (45–680)	67 (30–139)	<0.001
LDH	173 (116–769)	179 (112–710)	184 (137–271)	0.092
CEA	1.7 (0.3–31.4)	1.6 (0.4–7.5)	1.6 (0.3–12.3)	0.61

Group A received adjuvant/neoadjuvant chemotherapy.
Group B did not require chemotherapy.
ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; CEA, carcinoembryonic antigen; CRC, colorectal cancer; LDH, lactate dehydrogenase; IL, interleukin; LMR, lymphocyte monocyte ratio; NLR, neutrophils lymphocyte ratio; NMR, neutrophils monocyte ratio; PLR, platelet lymphocyte ratio; TNF, tumour necrosis factor; WCC, white cell count.

5-year DFS was 77% (95% CI 71% to 82%); (group A=74% (95% CI 67% to 80%); group B=81% (95% CI 72% to 87%)) (table 2).

Cognitive function and fatigue

Cognitive impairment and fatigue were significantly greater in both CRC groups at baseline than in controls, but with no significant difference between group A and group B. In total, 125/282 (44%) patients with CRC had cognitive impairment (group A 77/167 (46%) vs group B 48/115 (42%)) compared with 11/72 (15%) in the controls, with 148/283 (52%) having fatigue (group A 87/172 (51%) vs group B 61/113 (54%)) and 19/72 (26%) healthy controls (P<0.001).

Blood results

Table 3 outlines baseline blood results by cohort. Median levels of the cytokines were significantly higher in patients with CRC than healthy controls, and higher in patients with more advanced stage of disease and those whose baseline assessment was presurgical (ie, they were to receive neoadjuvant treatment). NLR and PLR were higher in patients with CRC than controls, but the percentage of participants above our designated cut-offs was not significantly different between groups.

Associations

Results of univariable Cox regression analyses for OS and DFS are shown in tables 4 and 5, respectively. None of the cytokines or laboratory values selected a priori to be of interest, nor cognitive function and fatigue, were observed to be statistically significant predictors of OS or DFS. Fewer years of patient education (P=0.018), more advanced stage of disease (P=0.032) and higher values of ALT (P=0.003), LDH (P=0.008) and CEA (P=0.002) were all associated with poorer OS in univariable analyses. Results were similar for DFS.

Including all factors as candidates for entry, education (HR 0.90, 95% CI 0.82 to 0.99, P=0.032), CEA on the

Table 4 Prognostic factors for overall survival

Characteristic	Comparison	N	HR (95% CI)	P value
Age	/year	289	1.00 (0.98 to 1.03)	0.93
Education	/year	289	0.92 (0.85 to 0.99)	0.018
Gender	M vs F	289	1.12 (0.66 to 1.91)	0.67
Drinks per day	2+ vs 0–1	251	0.83 (0.47 to 1.47)	0.52
Smoking status	Non-smoker Ex-smoker Smoker	275	1.23 (0.71 to 2.14) 1.26 (0.47 to 3.33) Reference	0.74
Marital status	Yes vs no	276	1.12 (0.64 to 1.98)	0.69
Site of disease	Colon vs rectum	289	0.69 (0.41 to 1.15)	0.16
Stage	I II III	284	0.18 (0.05 to 0.65) 0.57 (0.26 to 1.23) Reference	0.032
IL-6	Log-transformed	259	0.99 (0.82 to 1.19)	0.88
TNF-α	Log-transformed	259	0.96 (0.81 to 1.15)	0.68
IL-1b	Log-transformed	259	0.92 (0.75 to 1.13)	0.43
WCC	Log-transformed	238	1.79 (0.68 to 4.72)	0.24
Neutrophils	Log-transformed	238	1.36 (0.63 to 2.97)	0.43
Lymphocytes	Log-transformed	235	1.60 (0.70 to 3.65)	0.27
Monocytes	/unit	232	2.27 (0.62 to 8.38)	0.22
Neutrophils-lymphocyte ratio	Log-transformed High vs low	235	0.93 (0.48 to 1.80) 1.09 (0.56 to 2.15)	0.82 0.80
Platelet-lymphocyte ratio	Log-transformed High vs low	235	1.16 (0.56 to 2.39) 0.76 (0.27 to 2.13)	0.70 0.60
Neutrophils-monocyte ratio	Log-transformed	226	0.87 (0.38 to 2.00)	0.74
Platelet-monocyte ratio	Log-transformed	226	1.02 (0.50 to 2.07)	0.95
Lymphocyte-monocyte ratio	Log-transformed High vs low	226	0.78 (0.36 to 1.66) 0.87 (0.37 to 2.06)	0.51 0.75
Albumin	/unit	205	0.98 (0.90 to 1.07)	0.66
Haemoglobin	/unit	266	0.99 (0.98 to 1.01)	0.51
Bilirubin	Log-transformed	257	0.67 (0.36 to 1.24)	0.20
AST	Log-transformed	257	0.47 (0.18 to 1.20)	0.11
ALT	Log-transformed	252	0.38 (0.20 to 0.72)	0.003
ALP	Log-transformed	259	0.91 (0.39 to 2.09)	0.82
LDH	Log-transformed	219	3.21 (1.35 to 7.64)	0.008
CEA	Log-transformed	229	1.69 (1.22 to 2.34)	0.002
Homocysteine	Log-transformed	242	1.32 (0.66 to 2.66)	0.43
FACT-F	/unit ≤43 vs >43	285	0.99 (0.96 to 1.01) 0.96 (0.58 to 1.60)	0.27 0.88
Cognitive impairment on global deficit score	Yes vs no	282	1.52 (0.92 to 2.52)	0.10
Multivariable model (including laboratory data)				
Education	/year	189	0.90 (0.82 to 0.99)	0.032
CEA	Log-transformed		1.79 (1.23 to 2.62)	0.003
LDH	Log-transformed		10.25 (2.84 to 37.0)	<0.001
ALT	Log-transformed		0.35 (0.16 to 0.79)	0.011
Multivariable model (excluding laboratory data)				
Education	/year	289	0.92 (0.85 to 0.99)	0.018

ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; CEA, carcinoembryonic antigen; FACT-F, Functional Assessment Cancer Therapy-Fatigue subscale; IL, interleukin; LDH, lactate dehydrogenase; LMR, lymphocyte monocyte ratio; NLR, neutrophils lymphocyte ratio; NMR, neutrophils monocyte ratio; PLR, platelet lymphocyte ratio; TNF, tumour necrosis factor; WCC, white cell count.

logarithmic scale (HR 1.8, 95% CI 1.2 to 2.6, P=0.003), LDH on the logarithmic scale (HR 10.25, 95% CI 2.84 to 37.00, P<0.001) and ALT on the logarithmic scale (HR 0.35, 95% CI 0.16 to 0.79, P=0.011) were prognostic for OS in the regression model. However, only 189 patients had complete data for these four covariates. After excluding laboratory values, only education remained as a significant prognostic variable. CEA, stage of disease,

Table 5 Prognostic factors for disease-free survival

Characteristic	Comparison	N	HR (95% CI)	P value
Age	/year	289	1.00 (0.98 to 1.02)	0.95
Education	/year	289	0.91 (0.85 to 0.97)	0.002
Gender	M vs F	289	1.31 (0.81 to 2.12)	0.27
Drinks per day	2+ vs 0–1	251	0.86 (0.52 to 1.43)	0.56
Smoking status	Non-smoker Ex-smoker Smoker	275	1.11 (0.68 to 1.81) 1.26 (0.55 to 2.88) Reference	0.84
Marital status	Yes vs no	276	1.44 (0.86 to 2.43)	0.17
Site of disease	Colon vs rectum	289	0.70 (0.44 to 1.12)	0.13
Stage	I II III	284	0.14 (0.05 to 0.46) 0.44 (0.20 to 0.95) Reference	0.005
IL-6	Log-transformed	259	0.88 (0.74 to 1.05)	0.16
TNF-α	Log-transformed	259	0.89 (0.76 to 1.05)	0.18
IL-1b	Log-transformed	259	0.86 (0.71 to 1.04)	0.11
WCC	Log-transformed	238	1.76 (0.76 to 4.12)	0.19
Neutrophils	Log-transformed	238	1.35 (0.68 to 2.66)	0.39
Lymphocytes	Log-transformed	235	1.53 (0.75 to 3.15)	0.24
Monocytes	/unit	232	1.89 (0.61 to 5.86)	0.27
Neutrophils-lymphocyte ratio	Log-transformed High vs low	235	0.94 (0.53 to 1.68) 1.09 (0.60 to 1.98)	0.84 0.77
Platelet-lymphocyte ratio	Log-transformed High vs low	235	1.06 (0.57 to 1.98) 0.77 (0.31 to 1.93)	0.86 0.58
Neutrophils-monocyte ratio	Log-transformed	226	0.79 (0.39 to 1.63)	0.53
Platelet-monocyte ratio	Log-transformed	226	0.97 (0.53 to 1.79)	0.93
Lymphocyte-monocyte ratio	Log-transformed High vs low	226	0.84 (0.44 to 1.62) 0.97 (0.44 to 2.15)	0.60 0.94
Albumin	/unit	205	1.01 (0.95 to 1.07)	0.71
Haemoglobin	/unit	266	1.00 (0.99 to 1.02)	0.96
Bilirubin	Log-transformed	257	0.80 (0.46 to 1.38)	0.42
AST	Log-transformed	257	0.50 (0.22 to 1.14)	0.099
ALT	Log-transformed	252	0.54 (0.31 to 0.95)	0.032
ALP	Log-transformed	259	0.79 (0.37 to 1.69)	0.54
LDH	Log-transformed	219	2.62 (1.22 to 5.63)	0.014
CEA	Log-transformed	229	1.66 (1.24 to 2.24)	<0.001
FACT-F	/unit ≤43 vs >43	285	0.99 (0.97 to 1.02) 0.95 (0.61 to 1.50)	0.55 0.83
Cognitive impairment on global deficit score	Yes vs no	282	1.26 (0.80 to 1.98)	0.32
Multivariable model (including laboratory data)				
CEA	Log-transformed	194	1.85 (1.32 to 2.58)	<0.001
Stage	I II III		0.08 (0.02 to 0.38) 0.22 (0.07 to 0.63) Reference	0.004
Education	/year		0.88 (0.80 to 0.96)	0.003
LDH	Log-transformed		3.12 (1.19 to 8.13)	0.020
Multivariable model (excluding laboratory data)				
Education	/year	284	0.92 (0.87 to 0.98)	0.011
Stage	I II III		0.14 (0.04 to 0.45) 0.39 (0.18 to 0.84) Reference	0.004
Site of disease	Colon vs rectum		0.58 (0.36 to 0.94)	0.025

ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; CEA, carcinoembryonic antigen; FACT-F, Functional Assessment Cancer Therapy-Fatigue subscale; IL, interleukin; LDH, lactate dehydrogenase; LMR, lymphocyte monocyte ratio; NLR, neutrophils lymphocyte ratio; NMR, neutrophils monocyte ratio; PLR, platelet lymphocyte ratio; TNF, tumour necrosis factor; WCC, white cell count.

education and LDH were prognostic for DFS in the regression model.

Given the large number of potential prognostic factors evaluated, and the lack of correction for multiplicity, the above associations should be regarded as hypothesis generating rather than definitive.

DISCUSSION

In our cohort of patients with CRC, the cytokines IL-6, IL-1 and TNF, and most of the FBC differential ratios evaluated were elevated in comparison to a non-cancer control group but they were not associated with OS or DFS. Similarly, cognitive impairment and fatigue at baseline were significantly greater in patients with localised CRC than controls but were not associated with OS or DFS. The only baseline variables that were associated were stage of disease, education, CEA, ALT and LDH.

This is contrary to the findings of a number of studies evaluating similar inflammatory markers in patients with localised CRC collected prior to surgery, or in studies of patients with CRC with metastatic disease. The most likely reason for the difference is that our baseline assessment in most patients occurred ~7 weeks after surgery, so the tumour had already been removed. In the patients scheduled for neoadjuvant therapy, who had baseline assessment prior to any treatment the results were also negative, although the sample size was small.

An Australian study of 1623 consecutive patients with CRC who had potentially curative surgery found elevated LMR to be associated with improved OS (HR 0.57, 95% CI 0.48 to 0.68, P<0.001) in multivariate analysis, independent of age, T-stage, N-stage and grade.[18] NLR, PLR were not independently significant. Furthermore, they found low LMR to be associated with more advanced T-stage and tumour grade, but not nodal status, leading them to suggest that the low LMR may be associated with tumour proliferation rather than metastasis. A subgroup analysis of patients with mGPS (n=389) found LMR to be the only biomarker that was an independent and significant predictor of survival.[18] The mechanism by which the above blood indices are associated with survival is poorly understood, but they are probably indicative of a chronic inflammatory response.

In contrast to our findings, a recent Canadian study of 692 subjects with stage II and III CRC found that only 29% reported high levels of fatigue as measured by the brief psychosocial screen for cancer. However, they reported that increased fatigue was associated with worse OS (HR 1.99; P=0.00007) and DFS (HR 1.63; P=0.03).[29] Poorer survival was also associated with increased age. No other study has evaluated the relationship between objectively measured cognitive function in patients with localised cancer with survival.

An important limitation of our analysis is that the baseline assessment occurred after surgery in most patients, but prior to surgery in patients with rectal cancer receiving neoadjuvant chemoradiotherapy. We recognise that the effect of cytokines might be confounded by whether or not the tumour is in situ or by postsurgical effects.

Although all participants have been followed for a minimum of 7 years (and up to 14 years), median OS has not yet been reached and there may be too few events to accurately determine predictors. Missing data were an issue. Data regarding date of death for OS were relatively complete but information on date of recurrence is less robust, due primarily to patients not continuing follow-up in tertiary cancer centres longer term, or moving out of area. Cytokine collection times were not standardised to account for circadian rhythm and blood analysis was by a multiplex platform. The sample size of the study was determined based on the primary end point of cognitive differences between the groups and a larger sample size would have given greater statistical power to determine predictors of survival status. We have explored a number of potential predictors in our exploratory hypotheses and acknowledge that some results may be due to chance.

Strengths of the study include that data, with the exception of survival, were collected prospectively, with patients followed up regularly for 2 years as part of the main study. We also have an aged-matched, non-cancer control group for comparison of baseline blood results.

A predictive marker such as an FBC differential ratio that is easy to obtain and inexpensive and that could help to guide treatment and surveillance for localised CRC would be useful clinically. Our results, however, suggest that these markers when taken at the time patients are presenting to medical oncologists for consideration of treatment are not helpful in determining prognosis. These potential biomarkers have mainly been studied either retrospectively or as a secondary analysis in patients on clinical trials, who are not necessarily representative of the general population of people with CRC. They need to be evaluated further in a large prospective study with blood collection taken prior to surgery.

In conclusion, although inflammatory cytokines, NLR, PLR were raised at baseline and there was more cognitive impairment and fatigue, in comparison to a non-cancer control group, none of the variables was predictive of OS or DFS in this CRC cohort of patients with localised disease. For the blood parameters, this may be due to the timing of the sample collection.

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