Systemic neutrophil-to-lymphocyte ratio in colorectal cancer: the relationship to patient survival, tumour biology and local lymphocytic response to tumour

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Background: Colorectal cancer (CRC) is a major cause of mortality and morbidity. The impact of inflammatory biomarkers (C-reactive protein etc.) on CRC is increasingly studied including systemic neutrophil-to-lymphocyte ratio (NLR) as they seem to predict outcome.

Methods: All patients who underwent curative resection for CRC from 2000 to 2004 at Leeds Teaching Hospitals NHS Trust had pre-operative NLR calculated. Demographic, histopathological and survival data were collected. Tissue microarrays were created and stained to determine the mismatch repair (MMR) protein status of each tumour. Local lymphocytic response to the tumour was assessed and graded.

Results: About 358 patients were eligible. Of these 88 had an NLR $\geqslant 5$, which predicted lower overall survival and greater disease recurrence. A high NLR is associated with higher pT- and pN-stage and a greater incidence of extramural venous invasion. MMR protein status was not associated with NLR. A pronounced lymphocytic reaction at the invasive margin (IM) indicated a better prognosis and was associated with a lower NLR.

Conclusion: Neutrophil-to-lymphocyte ratio predicts disease-free and overall survival and is associated with a more aggressive tumour phenotype. The lymphocytic response to tumour at the IM is associated with NLR however dMMR is not. Neutrophil-to-lymphocyte ratio is a cheap, easy-to-access test that predicts outcome in CRC.

Colorectal cancer (CRC) is the second most common cause of cancer death in Western Europe and North America. In the UK in 2010, there were 40 695 newly diagnosed CRCs and 15 708 CRC-related deaths (CRUK, 2010). Advances in surgical technique, screening and adjuvant oncological therapies have improved outcomes but it remains a significant cause of mortality (Martling et al, 2000; Gill et al, 2004). Adjuvant therapies, while improving survival in certain groups of patients, cause significant morbidity and it is therefore vital to identify prognostic factors that indicate which patients would benefit from this type of treatment. Traditionally, prognosis in CRC is predicted by histopathological parameters, including staging criteria such as Dukes' or TNM staging (Wood, 1971; Deans et al, 1994). Other factors such as circumferential resection margin involvement, depth of extramural

invasion and extramural venous invasion (EMVI) are also important (Courtney *et al*, 2009). More recently, increasing attention has been paid to genetic and epigenetic factors and their influence on outcome. The presence of microsatellite instability has been shown to be associated with better prognosis (Gryfe *et al*, 2000; Hutchins *et al*, 2011). The identification of specific mutations can determine prognosis (Hutchins *et al*, 2011) and response to treatment, such as RAS mutations predicting a poor response to cetuximab therapy (Lievre *et al*, 2006).

The role of the immune system in cancer pathogenesis has been given an increasingly prominent role. There is a link between inflammation and outcome in a number of solid tumours (Nozoe *et al*, 2001; McKeown *et al*, 2004; Hilmy *et al*, 2005; Jamieson *et al*, 2005; Gerhardt *et al*, 2006; Lamb *et al*, 2008). The relationship between the various inflammatory factors in the tumour micro-environment and tumour growth, invasion and metastasis has been explored (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; de Visser *et al*, 2006; Hanahan and Weinberg, 2011). Cancer cells generate an immune response in the host. The strength and type of this response may have an impact on disease progression.

A number of studies have examined the systemic inflammatory response to tumour and its impact on prognosis. Elevated C-reactive protein (CRP), produced as part of the acute-phase response, has been associated with poor outcome in multiple solid tumours including CRC (McMillan *et al*, 2003; Wong *et al*, 2007). During an acute inflammatory response the ratio of different leucocyte subsets is altered. There is a neutrophilia often accompanied by a relative lymphocytopenia (Zahorec, 2001). Therefore a simple measure of systemic inflammation can be generated by calculating the neutrophil-to-lymphocyte ratio (NLR). In 2005 an association was demonstrated between NLR and outcome in CRC but this was not significant on multivariate analysis (Walsh *et al*, 2005). This marker has already been demonstrated to be an independent prognostic factor following resection of CRC liver metastases (Halazun *et al*, 2008). Since then multiple studies have indicated that NLR predicts outcome in a variety of stages in CRC and following chemotherapy (Ding *et al*, 2010; Chua *et al*, 2011; Hung *et al*, 2011; Chiang *et al*, 2012; Kaneko *et al*, 2012; Mallappa *et al*, 2013; Absenger *et al*, 2013; Malietzis *et al*, 2014). However, none have attempted to examine the underlying mechanisms.

A morphological sub-type of CRC associated with mismatch repair (MMR) protein deficiency, as a consequence of microsatellite instability, has been noted to provoke a strong host response to tumour in the form of a pronounced lymphocytic infiltrate (Dolcetti *et al*, 1999; Guidoboni *et al*, 2001; Drescher *et al*, 2009). These cancers also seem to have a better prognosis compared with other types of CRC (Popat *et al*, 2005). The presence of tumour-infiltrating lymphocytes in CRC has also been shown to confer a better prognosis (Pages *et al*, 2005; Galon *et al*, 2006).

The aim of this study was to examine the relationship between pre-operative NLR and clinico-pathological features in a cohort of CRC patients undergoing curative resection and outcome. We also determined the MMR status and local lymphocytic response to tumour and its association with NLR.

MATERIALS AND METHODS

Patients who underwent potentially curative resection for CRC at Leeds Teaching Hospitals NHS Trust between September 2000 and January 2004 were identified. All patients included in the study had a full blood count (including a white cell differential count) performed a maximum of 7 days prior to their surgery. Exclusion criteria included any evidence of colonic perforation or documented sepsis. Sepsis was defined as a temperature $>38\,^{\circ}\text{C}$ in combination with a positive microbiological culture sample within 48 h.

Ethical approval for this project was granted by Leeds (East) Ethics Committee (reference number: 08/H1306/86).

Data collection. All participants had histopathologically proven colorectal adenocarcinoma. A variety of data was collected from the histopathology reports including TNM/Dukes' staging, evidence of EMVI and of resection margin status.

The white cell count and differential was obtained via the Siemens Advia 2120 (Siemens, Erlangen, Germany) flow cytometer. Overall neutrophil count was measured in $10^9\,\text{l}^{-1}$ (normal range 2.0–$7.5 \times 10^9\,\text{l}^{-1}$). Overall lymphocyte count was measured in $10^9\,\text{l}^{-1}$ (normal range 1.0–$4.5 \times 10^9\,\text{l}^{-1}$). Neutrophil-to-lymphocyte ratio was calculated and a predefined value of $\geqslant 5$ was taken to represent an elevated NLR (Walsh *et al*, 2005).

Survival data. Data regarding patient survival were collected from the Northern and Yorkshire Cancer Registry and Information Service (NYCRIS). This was used to calculate the overall patient survival. Overall survival was defined as the time from date of first surgery to date of death from any cause. Censor points were last attendance at hospital or date of death. Data regarding disease recurrence were collected from radiological, histopathological and endoscopic records, and disease-free survival was calculated. Disease-free survival was defined as date of first surgery to date on which disease recurrence, either local or distant, was first detected. Follow-up protocol was standardized within the colorectal department at Leeds Teaching Hospitals NHS Trust.

Determination of MMR status. Tissue microarrays (TMAs) were constructed using three individual 0.6 mm cores of formalin-fixed paraffin-embedded tumour tissue for each patient. Sections were taken from the TMAs and stained for the MMR proteins, hMLH-1 and hMSH-2, using pre-determined immunohistochemical protocols. The glass slides were scanned using an Aperio XT slide scanner (Aperio Technologies Inc, San Diego, CA, USA) and visualised using Aperio ImageScope (Aperio Technologies Inc). Each individual tumour core was scored as positive or negative, with negative defined as the absence of any staining within tumour cells. Staining within immune cells was used as an internal positive control. Cases scored as negative for either marker were taken to be MMR-deficient cancers. In cores with no tumour tissue, assessment of MMR status was not possible and these were classed as unknown.

Assessment of peritumoral lymphocytes. Slides were cut from each of the whole tissue blocks and stained with haematoxylin and eosin. These were scanned and viewed digitally so that the lymphocytic response to tumour could be assessed.

The scoring system was based on the study by Klintrup *et al* (2005). This assessed the tumour centre and invasive margin (IM). Invasive margin was defined as the interface between the normal host tissue and the invading edge of the tumour. At the margin the deepest point of invasion was chosen. Each area was scored as 1 to 3 where 1 was none or low density (no reaction or a mild patchy lymphocytic response), 2 moderate density (band-like infiltrate with some cancer cell destruction) and 3 high density (prominent inflammatory reaction with a cup-like zone and destruction of cancer cells). All slides were scored by the author based on the described parameters. To confirm the consistency of scoring, a total of 120 tumour specimens from the CRC group were scored by two independent histopathologists, who were blinded to outcome (NPW and GGH).

Statistical analysis. Data are presented as mean or median (range). Comparisons between the means of normally distributed groups were performed using Student's *t*-test. Non-parametric data was analysed using the χ^2-test.

Univariate survival trends were compared using Kaplan–Meier curves and significant differences determined via the log rank test. To determine whether variables were independently linked to prognosis, the Cox proportional hazard model was used. Significant factors on univariate analysis were entered into a multivariate model. A P-value <0.05 was considered significant. All statistical analysis was performed using SPSS version 16 (SPPS Inc., Chicago, IL, USA).

RESULTS

From the cohort, 400 patients with pre-operative bloods taken within 1 week prior to surgery were identified. About 42 patients were excluded due to the presence of bowel perforation or sepsis preoperatively, leaving 358 patients in the study group. All patients have follow-up of >4 years therefore patients were censored at 4 years for the survival analysis.

Demographics. The study population comprised of 202 (56.4%) males and 156 (43.6%) females. The median age was 74 years (range 32–95). About 261 cases were cancers of the colon and 97 were rectal. Of the colonic cancers, 141 were right sided and 120 were left sided.

Clinico-pathological data. Clinico-pathological data is presented in Supplementary Table 1. About 38 (10.6%) patients were staged as Dukes' A, 163 (45.5%) as Dukes' B, 119 (33.4%) were Dukes' C and 37 (10.5%) were stage D. About 138 (38.5%) demonstrated evidence of EMVI. About 310 (86.6%) patients had complete R0 resections, while in 37 (10.3%) the resection margin was involved microscopically (R1). R1 was defined as a margin $\leqslant 1$ mm clear of tumour.

Neutrophil-to-lymphocyte ratio. Neutrophil-to-lymphocyte ratio was calculated for each patient producing two groups: high NLR (NLR $\geqslant 5$) and low NLR (NLR <5). This cut-off was used as previously cited in the relevant literature (Walsh *et al*, 2005). The median age for both groups was 74 years. The distribution of gender in each group is similar (Table 1). The site of tumour in the low NLR group was 81.2% colon and 18.8% rectum compared with 89.4% colon and 10.6% rectum in the high NLR group ($P = 0.186$).

Table 1 contains data comparing clinico-pathological factors within the high and low NLR groups. Median maximum tumour diameter in the NLR <5 group was 4.0 cm compared with 3.5 cm in the NLR $\geqslant 5$ group ($P = 0.153$). The grade of differentiation in each group was similar. It is clear from Table 1 that NLR is associated with a more advanced, aggressive tumour type. The NLR $\geqslant 5$ group tumours are more likely to have a higher pT-stage ($P < 0.001$) and pN-stage ($P = 0.014$). They are more likely to be associated with more positive lymph nodes and evidence of EMVI. The percentage of the NLR $\geqslant 5$ group that was stage D/IV was 20.5% compared with 8.6% in the low NLR group.

Patient survival. Overall survival for the entire cohort is demonstrated (separated by Dukes' stage) in Supplementary Figure 1. The difference between the four groups is significant ($P < 0.001$). When the overall survival for the high NLR group is compared with the low NLR group the outcomes for those with an NLR $\geqslant 5$ is significantly poorer (Figure 1A). Thirty-day mortality (3.7 *vs* 19.3%, $P < 0.001$), 90-day mortality (7.4 *vs* 24.0%, $P < 0.001$) and 4-year survival (59.6 *vs* 27.3%, $P < 0.001$) are significantly worse in the high NLR group. Disease-free survival also demonstrated poorer survival in the NLR $\geqslant 5$ group (Figure 1B). Overall survival in the NLR <5 and $\geqslant 5$ groups by Dukes' stage is shown in Supplementary Figure 2. This indicates that the prognostic effect of NLR may be independent of Dukes' stage.

Univariate/multivariate analysis. Univariate analysis for overall survival was performed on age, gender, NLR and clinico-pathological factors. The results can be seen in Table 1 with significant factors including age, NLR, CRP level, number of positive lymph nodes, EMVI, pT- and pN-stage, and Dukes' stage. The hazard ratio (HR) for NLR was 2.62 (95% CI 1.93–3.55, $P < 0.001$) making it one of the stronger predictors on univariate analysis. Significant factors ($P < 0.05$) were put through a multivariate model and the results are shown in Table 1. The factors that remained significant were age, NLR and Dukes' stage C and stage D.

A similar analysis was performed for disease-free survival. On univariate analysis, NLR, number of positive lymph nodes, EMVI, complete resection and Dukes' stage were significant. On multivariate analysis, NLR, EMVI and Dukes stage C remained significant (Supplementary Table 2).

MMR status and NLR

Demographics. The incidence of MMR deficiency compared with NLR of CRC patients is shown in Table 2. Demographic data shown in Supplementary Table 3 demonstrates some of the features associated with MMR-deficient tumours, namely female gender and right-sided tumours.

Association between MMR status and NLR. The ratios of NLR <5 and $\geqslant 5$ against the presence, or otherwise, of the MMR proteins are shown in Table 2. There appears to be no association between the two groups.

Tumour-associated lymphocytes in CRC. The intraclass correlation co-efficient (ICCC) between the author and the observers was 0.70 (GGH) and 0.87 (NPW) at the IM and 0.88 (GGH) and 0.85 (NPW) at the tumour centre. Inter-observer ICCCs >0.7 are considered acceptable (Streiner and Norman, 1995).

Based on the original grading system (Klintrup *et al*, 2005), Kaplan–Meier curves for overall survival were constructed using the results obtained from the IM (Figure 2A). These demonstrated a significant difference in patient outcomes for each group with a more pronounced lymphocytic reaction being associated with a better prognosis ($P = 0.004$). The same curves were produced for the lymphocytic reaction seen at the tumour centre but no significant difference was observed between the groups (Figure 3).

Characteristics of the mild *vs* pronounced lymphocytic reaction (at the IM) groups. These characteristics are shown in Table 3. There was no difference in age or gender between the two groups. There is a trend towards colonic tumours being more likely to produce a moderate-to-severe lymphocytic reaction than rectal tumours but this did not reach statistical significance. A moderate-to-severe lymphocytic reaction at the IM is associated with MMR deficiency ($P = 0.040$), decreased incidence of EMVI ($P = 0.010$) and lower Dukes' stage ($P = 0.001$). There is a significant association between raised NLR and a milder degree of lymphocytic infiltration at the IM with 15.3% of the moderate and severe lymphocytic response groups having an NLR $\geqslant 5$ compared with 29.2% of the mild lymphocytic response group ($P = 0.005$).

On univariate analysis, grade of lymphocytic reaction at the IM was a significant prognostic factor (HR 1.75, 95% CI 1.24–2.47, $P = 0.001$). However, when it is included in the multivariate model for overall survival in this group it is not significant (Table 4).

Disease-free survival by grade of lymphocytic reaction at the IM. Disease-free survival for the two lymphocytic response groups (none/mild *vs* moderate/severe) was calculated. The survival curves (Figure 2B) show a clear difference between groups ($P = 0.010$). On univariate analysis, grade of lymphocytic reaction at the IM was a

Table 1. Univariate and multivariate analysis for overall survival (covariates included in multivariate model highlighted in bold)

Covariate	N	Univariate			Multivariate		
		P-value	HR	95% CI	P-value	HR	95% CI
NLR							
<5	270	—	1.00	—	—	1.00	—
⩾5	88	<0.001	2.616	1.925–3.553	<0.001	1.819	1.310–2.526
Age (years)							
⩽70	129	—	1.00	—	—	1.00	—
>70	229	<0.001	2.549	1.799–3.612	<0.001	2.964	2.045–4.297
Gender							
M	202	—	1.00	—	—	—	—
F	156	0.920	1.015	0.758–1.360	—	—	—
CRP (U)							
⩽10	74	—	1.00	—	—	—	—
>10	90	0.025	1.640	1.063–2.528	—	—	—
Total lymph nodes found							
0–15	178	—	1.00	—	—	—	—
>15	178	0.020	1.419	1.057–1.904	—	—	—
EMVI							
Y	138	—	1.00	—	—	1.00	—
N	213	<0.001	2.210	1.641–2.976	0.139	1.294	0.920–1.820
pT-stage							
1	11	—	1.00	—	—	—	—
2	32	0.596	0.731	0.229–2.330	—	—	—
3	212	0.757	1.171	0.430–3.188	—	—	—
4	98	0.036	2.931	1.070–8.024	—	—	—
pN-stage							
0	203	—	1.00	—	—	—	—
1	88	0.048	1.454	1.002–2.108	—	—	—
2	65	<0.001	3.712	2.615–5.272	—	—	—
Complete resection							
Y	310	—	1.00	—	—	—	—
N	37	0.130	1.435	0.917–2.246	—	—	—
Site of tumour							
Colon	261	—	1.00	—	—	—	—
Rectum	97	0.161	0.783	0.556–1.102	—	—	—
Dukes' stage							
A	38	—	1.00	—	—	1.00	—
B	163	0.510	1.223	0.672–2.228	0.999	0.999	5.33–1.875
C	119	0.02	2.701	1.430–5.100	0.025	2.097	1.098–4.007
D	37	<0.001	6.776	3.408–13.474	<0.001	6.665	3.241–13.707

Abbreviations: CI = confidence interval; CRP = c-reactive protein; EMVI = extramural vascular invasion; F = female; HR = hazard ratio; M = male; N = no; NLR = neutrophil-to-lymphocyte ratio; Y = yes.

significant prognostic factor (HR 1.87, 95% CI 1.07–3.30, $P = 0.029$). On multivariate analysis this was not significant (Table 5).

DISCUSSION

This study assessed the impact of NLR on a retrospective series of CRC patients. The cohort consisted of a variety of patients presenting at Dukes' stage A to C (with a small proportion of patients with stage IV disease and resectable liver metastases). Histopathological parameters such as the number of positive lymph nodes and evidence of EMVI were shown to be prognostic. As expected various staging methods (TNM, Dukes') also predict outcome. These data demonstrated that pre-operative NLR predicted survival. By splitting the cohort into two groups (NLR <5 and NLR ⩾5) the difference in outcome was illustrated by survival curves for overall and disease-free survival (Figure 1A and B). On univariate analysis of overall survival, NLR was shown to be prognostic (HR 2.62, 95% CI 1.93–3.55, $P = <0.001$). Neutrophil-to-lymphocyte ratio, along with age and Dukes' stage, remains significant in a multivariate model. When the high and low NLR groups are analysed it becomes apparent that NLR is associated with more advanced, aggressive tumour biology with more lymph node metastases and EMVI.

To further assess the effect of tumour biology on NLR, we investigated the role of MMR status. Our MMR-deficient cancers had typical characteristics of this tumour type: female gender and right-sided lesions (Lanza et al, 2006). Unlike many series MMR status did not demonstrate any differences in survival. Mismatch repair-deficient cancers were associated with a more prominent peritumoral lymphocytosis. It is thought that the anti-tumoural effect of T-lymphocytes within the infiltrative population may account for the better outcomes observed in this group. As a component of NLR is circulating lymphocytes, we postulated that the lymphocytic reaction seen in MMR cancers may be associated with NLR. However, on analysis no correlation between MMR status and NLR was observed. This may be a type II error and larger series/meta-analyses would be required to confirm or refute this hypothesis.
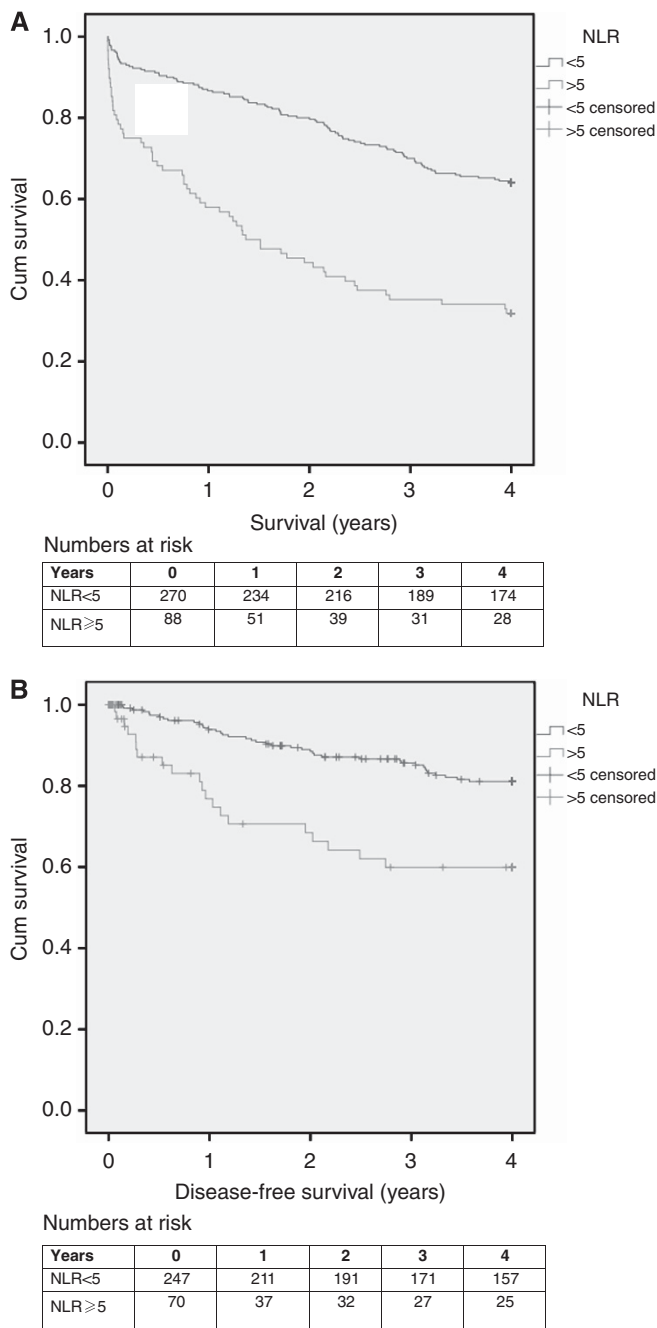
Figure 1. Kaplan–Meier curves comparing survival for NLR<5 vs NLR⩾5. (**A**) Overall survival ($n = 358$, $P < 0.001$, HR 2.616, 95% CI 1.925–3.553). (**B**) Disease-free survival ($n = 317$, $P < 0.001$, HR 2.547, 95% CI 1.521–4.265). NLR = neutrophil-to-lymphocyte ratio.

Table 2. MMR protein deficiency/proficiency in the NLR groups

		NLR		
	Total	<5	⩾5	P-value[a]
hMLH-1				
Negative	45 (12.6%)	37 (13.7%)	8 (9.1%)	0.247
Positive	291 (81.3%)	216 (80.0%)	75 (85.2%)	—
Unknown	22 (6.1%)	17 (6.3%)	5 (5.7%)	—
hMSH-2				
Negative	12 (3.4%)	8 (3.0%)	4 (4.5%)	0.489
Positive	326 (91.1%)	246 (91.1%)	80 (90.9%)	—
Unknown	20 (5.6%)	16 (5.9%)	4 (4.5%)	—
Final MMR status				
Deficient	55 (15.4%)	44 (16.3%)	11 (12.5%)	0.356
Proficient	286 (79.9%)	212 (78.5%)	74 (84.1%)	—
Unknown	17 (4.7%)	14 (5.2%)	3 (3.4%)	—

Abbreviations: NLR = neutrophil-to-lymphocyte ratio; MMR = mismatch repair.
[a] χ^2-test.

tumour biology) makes comparisons between groups difficult and increases the risk of individual factors skewing the data. We were unable to separate elective and emergency resections. We attempt to correct for this by identifying and excluding patients with evidence of sepsis or bowel perforation. However, emergency presentations have worse outcomes, particularly in the early post-operative period (Ueberrueck *et al*, 2013). This may bias our results and may contribute to the early mortality seen in the NLR high group.

The relationship between NLR and cancer has been explored elsewhere in the literature. Cho *et al* (2009) demonstrated that not only was NLR more likely to be elevated in patients with epithelial ovarian cancer, compared with patients with benign conditions and normal controls, but that the pre-treatment value predicted patient survival (Cho *et al*, 2009). Similar findings have been seen in non-small cell lung cancer (Sarraf *et al*, 2009). In CRC Walsh *et al* (2005) examined a cohort of 230 CRC patients over a 2-year period. They found that pre-operative NLR >5 correlated with overall and cancer-specific survival on univariate analysis. However, on multivariate analysis it was not independent of Dukes' stage (Walsh *et al*, 2005). NLR also predicts outcome in stage II (Ding *et al*, 2010; Hung *et al*, 2011) and stages I–III CRC (Chiang *et al*, 2012; Mallappa *et al*, 2013; Malietzis *et al*, 2014). It is prognostic in metastatic CRC patients and those with metastatic disease undergoing palliative chemotherapy (Chua *et al*, 2011; Kaneko *et al*, 2012). This suggests that NLR is an indicator of prognosis in CRC and, when used with more established predictors, can identify patients who may benefit from adjuvant treatment or more frequent surveillance.

There are a number of mechanisms by which the prognostic effect of NLR may be produced. It is thought to be a simple measure of the systemic inflammatory response and an elevated value may represent tumour-generated inflammation (O'Mahony *et al*, 1984). If pro-inflammatory cytokines, such as TNF-α and IL's 1, 6 and 8, are upregulated as part of an inflammatory response this can have important effects on tumour growth and progression. These inflammatory mediators activate intracellular pathways, such as those mediated by NF-κB, modulate cellular proliferation and angiogenesis and may have a profound effect on tumour growth and invasion.

Lymphocytopenia is an important component of a high NLR and its value may be indicative of the type and degree of lymphocytic response to tumour. A low level of blood-borne lymphocytes may reflect a muted or absent lymphocytic response to tumour. Tumours with an increased population of intrae-pithelial T-lymphocytes, particularly the CD8+ cytotoxic phenotype, have better outcomes (Dolcetti *et al*, 1999). The NLR may reflect the type of T-cell response. Evidence suggests
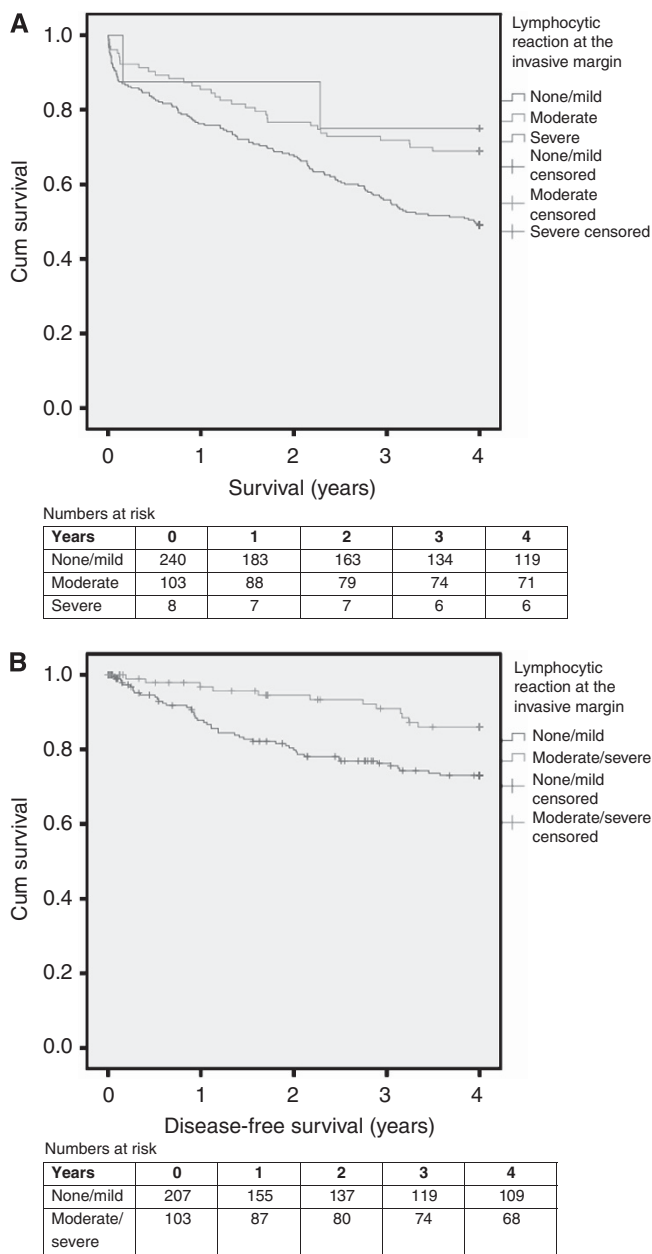
We examined the local lymphocytic reaction to tumour. We found that when absent/mild, moderate and severe lymphocytosis at the IM was compared the patient outcomes differed markedly with the moderate and severe groups having a significant survival advantage. It was demonstrated that a moderate-to-severe lymphocytic infiltrate at the IM was associated with a less advanced Dukes' stage, less EMVI and MMR-deficient cancers. The lymphocytic reaction also correlated with NLR in that a moderate-to-severe reaction was significantly less likely to exhibit a raised NLR ($P = 0.005$).

Our study comprises a retrospective cohort of consecutive patients treated in Leeds between 2000 and 2004. There are limitations to the study. The small size of the cohort makes it difficult to draw comprehensive conclusions. The heterogeneous nature of the data (variation in patient physiology, treatment and

Figure 2. Kaplan–Meier curves showing survival by grade of lymphocytic reaction at the invasive margin. (A) Overall survival (n = 344, P = 0.004, HR 1.751, 95% CI 1.241–2.469). (B) Disease-free survival (n = 310, P = 0.010, HR 1.873, 95% CI 1.065–3.295).

Figure 3. Kaplan–Meier curves showing overall survival by grade of lymphocytic reaction at the tumour centre (n = 344, P = 0.380).

Table 3. Data by grade of lymphocytic reaction at the invasive margin				
	Total	None/low density (Group 1)	Moderate/ severe density (Groups 2 and 3)	P-value
N	351	240 (68.4%)	111 (31.6%)	—
Median age (years)	73	73	73	0.946[a]
Gender				
Male	200 (57.0%)	142 (59.2%)	58 (52.3%)	0.224[b]
Female	151 (43.0%)	98 (40.8%)	53 (47.7%)	—
Site of tumour				
Colon	256 (72.9%)	168 (70.0%)	88 (79.3%)	0.069[b]
Rectum	95 (27.1%)	72 (30.0%)	23 (20.7%)	—
Dukes' stage				
A	37 (10.5%)	17 (7.1%)	20 (18.0%)	0.001[b]
B	157 (44.7%)	100 (41.7%)	57 (51.4%)	—
C	119 (33.9%)	91 (37.9%)	28 (25.2%)	—
D	37 (10.5%)	32 (13.3%)	5 (4.5%)	—
EMVI				
Yes	137 (39.0%)	105 (43.8%)	32 (28.8%)	0.01[b]
No	208 (61.0%)	132 (56.2%)	76 (71.2%)	—
MMR status				
Deficient	55 (15.7%)	31 (12.9%)	24 (21.6%)	0.04[b]
Proficient	281 (84.3%)	198 (87.1%)	83 (78.6%)	—
NLR				
<5	264 (%)	170 (70.8%)	94 (84.7%)	0.005[b]
≥5	87 (%)	70 (29.2%)	17 (15.3%)	

Abbreviations: EMVI = extramural vascular invasion; MMR = mismatch repair; NLR = neutrophil-to-lymphocyte ratio.
[a]Mann–Whitney U-test.
[b]χ^2-test.

that even when lymphocytic aggregates are present in a tumour their immune responsiveness is often blunted by cytokines within the microenvironment (Zippelius et al, 2004). Cells are often polarised away from the anti-tumour Th-1 phenotype towards a more immunosuppressive Th-2 type. This blunts the anti-tumour response and creates a milieu in which tumour cell populations can develop unimpeded (Balkwill and Mantovani, 2001). Cytokines associated with Th-1 polarisation, such as IFN-γ or IL-12, and associated pro-inflammatory cytokines, such as IL-2, may also have a role.

As well as being implicated in the inflammatory response and its associated cancer promoting pathways, NLR may also offer an indication of the physiological condition of the patient. In more advanced CRC causing large bowel obstruction, a neutrophilia would be generated. Such patients may have a greater mortality
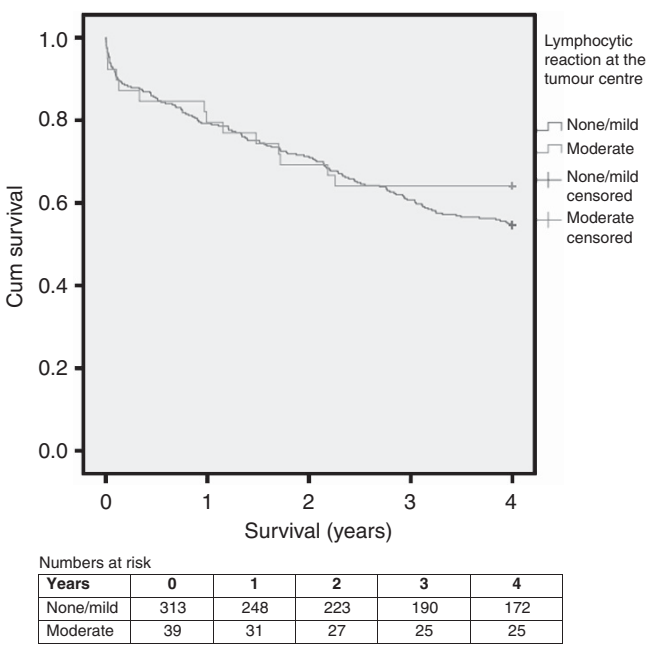
rate than patients without such problems. This may contribute to the impact of NLR on overall survival; however, an elevated NLR is also seen in patients with early stage cancer.

Like others we have demonstrated that the pre-operative systemic NLR predicts both overall survival and disease recurrence following surgery for CRC. However, we have gone onto

Table 4. Multivariate analysis for overall survival including lymphocytic reaction at invasive margin

Covariate	N	P-value	HR	95% CI
NLR				
<5	260	—	1.00	—
⩾5	84	0.002	1.712	1.227–2.389
Age (years)				
⩽70	123	—	1.00	—
>70	221	<0.001	3.008	2.067–4.376
EMVI				
Yes	138	—	1.00	—
No	212	0.144	1.294	0.916–1.827
Dukes' stage				
A	36	—	1.00	—
B	152	0.635	0.857	0.454–1.619
C	119	0.089	1.763	0.916–3.392
D	37	<0.001	5.649	2.731–11.683
Lymphocytic reaction (grade)				
None/mild	237	—	1.00	—
Moderate/severe	107	0.135	0.757	0.526–1.090

Abbreviations: EMVI = extramural vascular invasion; HR = hazard ratio; NLR = neutrophil-to-lymphocyte ratio.

Table 5. Multivariate analysis for disease-free survival including lymphocytic reaction at invasive margin

Covariate	N	P-value	HR	95% CI
NLR				
<5	234	—	1.00	—
⩾5	65	0.007	2.185	1.233–3.871
Complete resection				
Yes	106	—	1.00	—
No	193	0.355	0.727	0.370–1.429
EMVI				
Yes	270	—	1.00	—
No	29	0.007	2.187	1.234–3.874
Dukes' stage				
A	36	—	1.00	—
B	150	0.797	0.864	0.284–2.631
C	113	0.084	2.660	0.877–8.064
Lymphocytic reaction (grade)				
None/mild	201	—	1.00	—
Moderate/severe	98	0.253	1.420	0.778–2.589

Abbreviations: EMVI = extramural vascular invasion; HR = hazard ratio; NLR = neutrophil-to-lymphocyte ratio.

demonstrate a strong association of NLR with histopathology features of the local immune response rather than MMR status and the frequency of nodal involvement and EMVI. While the immunological mechanism for this phenomenon remains unclear, this represents a cheap, easily accessible test that can provide valuable prognostic information prior to surgical intervention. Further work is needed to investigate the immunological basis for these observations, as well as whether they affect the developing immunotherapeutic approaches in such patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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