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Derived neutrophil to lymphocyte ratio as a prognostic factor in patients with advanced colorectal cancer according to RAS and BRAF status: a post-hoc analysis of the MRC COIN study

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Abstract

Background—The phase III COntinuous or INtermittent (COIN) trial failed to demonstrate a benefit in overall survival of cetuximab in combination with chemotherapy for patients with metastatic colorectal cancer (mCRC). High derived neutrophil to lymphocyte ratio (dNLR) has been shown to be prognostic in patients with mCRC. The aim of this analysis is to evaluate dNLR as a predictive biomarker of the survival according to RAS and BRAF mutations status within the COIN trial.

Methods—A post-hoc exploratory analysis of the COIN trial arms A and B was performed. All patients with available white blood cell (WBC) and neutrophil data were analysed. The dNLR was calculated using a formula which has previously demonstrated predictive power in cancer patients: dNLR=ANC/(WBC-ANC). A high dNLR was defined as a value of ≥2.22. dNLR was correlated with clinical outcomes using Kaplan-Meier and cox regression analysis.

Results—A total of 1603 patients were assigned to the oxaliplatin based chemotherapy (arm A, N=815) or oxaliplatin based chemotherapy plus cetuximab (arm B, N=815) arms. There was a strong association between dNLR level and overall survival using Kaplan-Meier analysis. In all mutation groups, dNLR<2.2 was associated with better overall survival (OS) compared to

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Availability of data and materials
Data and materials are available from the corresponding authors upon request.

Competing interests
The authors declare that they have no competing interests.

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dNLR≥2.2. Median OS in patients with wild type disease (dNLR<2.2 vs dNLR≥2.2) was 22.8 vs 13.1 months (HR 1.33); 16.9 vs 11.8 months (HR 1.36) in patients with RAS mutant tumours; and 12.6 vs 6.8 (HR 1.67) in patients with BRAF mutant tumours.

In patients with dNLR<2.2, the median OS was 19.2 months in arm A compared to 18.0 months in arm B (HR 1.11). Among patients with dNLR≥2.2, the median OS was 13.0 months in arm A compared to 13.1 months in arm B (HR of 0.96).

Conclusion—dNLR is strongly prognostic for survival in all mutations groups. dNLR does not predict for benefit from the addition of cetuximab.

Keywords

Colorectal cancer; neutrophil lymphocyte ratio; Cetuximab; RAS; BRAF

Background

The Continuous or Intermittent (COIN) phase III randomised study demonstrated a prognostic effect of BRAF, KRAS, and NRAS mutations on the outcome of patients with advanced colorectal cancer. However, benefit of additional cetuximab treatment to oxaliplatin based chemotherapy in first line treatment of these patients was not proved. [1] Comparable studies have demonstrated mixed response outcome data for patients with RAS wild-type tumours in the context of chemotherapy combinations with epidermal growth factor receptor (EGFR) inhibitors. [2–5] To further clarify sub-group sensitivity to EGFR inhibition prospective testing is needed. [6–7]

The tumour microenvironment and the inflammatory response have been shown to play a vital role in cancer development. Measurable serum parameters of C-reactive protein, neutrophil/lymphocyte ratio (NLR) and platelet-lymphocyte ratio have been associated with poor outcomes in patients with colorectal cancer. [8–10] NLR is a marker of host inflammation and may reflect cytokine activation and therefore be a surrogate marker of more aggressive disease. A recently reported meta-analysis of 100 studies comprising 40559 patients with various solid tumours, found that NLR >4 was associated with poorer OS (HR 1.81; 95% CI = 1.67 to 1.97; $p < 0.001$). This effect was observed in all of the disease sites, subgroups and stages. [11] Within this meta-analysis, 6 prospective studies, contained a total of 1817 patients with mCRC.

The COIN trial did not collect lymphocyte count data, however the derived NLR (dNLR) has been shown to possess similar prognostic value. [12] In a previous analysis of the COIN trial we have determined that dNLR is predictive of survival when administering intermittent versus continuous treatment. [13] In this study, we examined dNLR as a prognostic factor and assessed its' predictive power regarding the potential benefit of EGFR inhibition, particularly in the RAS and BRAF populations.

Methods

The phase III COIN trial was undertaken by the Medical Research Council Clinical Trials Unit and was overseen by an independent trial steering committee. The trial was approved

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by national research ethics committees in the UK and Ireland and both the Medicines and Healthcare Regulatory Agency and Irish Medicines Board. The trial design and eligibility criteria have been reported previously. [1]

COIN trial's primary objective was to assess the effect of the addition of EGFR-targeted monocloncal antibody (cetuximab) to continuous oxaliplatin and fluoropyrimidine combination chemotherapy on survival. Shortly after COIN completed recruitment, external evidence showed that anti-EGFR antibodies were unlikely to benefit mCRC patients whose tumours carry KRAS mutations. [14]

Treatment allocation was non-blinded and randomly assigned (1:1) to the control arm of continuous oxaliplatin based (oxaliplatin plus capecitabine or oxaliplatin plus fluorouracil and folinic acid) chemotherapy (arm A) or continuous chemotherapy plus cetuximab (arm B). The treatment was continued until progression of disease, development of cumulative toxicities or patient choice. [1]

We have performed a post-hoc exploratory analysis of the prognostic and predictive power of dNLR in the COIN trial arms A and B. All patients with available white blood cell (WBC) and neutrophil data were analysed. Unfortunately, lymphocyte data was not collected at patient entry to the COIN trial.

Derived Neutrophil/lymphocyte ratio (dNLR) calculation

WBC and absolute neutrophil count (ANC) were collected on all patients at enrolment to the COIN trial. dNLR was calculated using this formula - dNLR=ANC/(WBC-ANC). [8,12]

Statistical methods

All statistical analyses were performed by the Cancer Research UK and University College London Cancer Trials Centre. Stats version 12.1 was used to analyse data.

A high dNLR was defined as ≥2.2. dNLR was correlated with clinical outcomes including overall survival (OS), progression-free survival (PFS) and objective response rate (ORR). Kaplan-Meier survival curves were generated based on dNLR. Comparison between groups was performed using cox-regression analysis adjusted for treatment, age, sex, tumour status (resected, unresected, or local recurrence), primary site (colon, rectum, rectosigmoid junction, multiple growths), liver-only metastases (yes vs no), number of metastatic sites (0, 1, 2, ≥3), platelets (<400,000 vs ≥400,000 μL), alkaline phosphatase (<300 vs ≥ 300 U/L). Prognostic value was assessed with ROC analysis, using one year survival as the outcome, and reporting the estimate of AUC. [13]

Results

1,630 of 2,445 patients in the COIN trial were randomised to arm A (chemotherapy) and arm B (chemotherapy plus cetuximab). Our total cohort was 1,603 patients (accounting for 98.3% of the total study population), excluding 9 patients with no WBC and ANC data and

18 patients with other missing data. The median value of dNLR was 2.2; baseline characteristics within each dNLR group are shown in table 1.

dNLR as a prognostic marker

There was a strong association between dNLR level and outcome. We found that patients with dNLR ≥ 2.2 had a hazard ratio (HR) of 1.35 (95% CI 1.20-1.52; p<0.001) for OS (figure 1) and 1.25 (95% CI 1.13-1.40; p<0.001) for PFS.

In patients with dNLR<2.2, the median overall survival was 19.2 months in arm A and 18.0 months in arm B - HR 1.11 (figure 2a). Among patients with dNLR≥2.2, the median overall survival was 13.0 months in arm A compared to 13.1 months in arm B- HR 0.96 (figure2b). A differential treatment effect between the two dNLR groups was not seen (p = 0.21).

The AUC for dNLR was 63.9% (95% CI 61.1-66.7). Dichotomising the data at the median value of dNLR (2.2) resulted in a true detection rate of 57.7% and a false positive rate of 38.5% for one year survival.

RAS/RAF mutations

RAS and BRAF mutation status was available for 1,263 (78.8%) patients. Of these, 575 (45.1%) were RAS/RAF wild type; 587 (46.5%) were RAS mutated and 101 (8.0%) were BRAF mutated. There was clear evidence of an association between low dNLR and improved overall survival in each of these four groups (table 2). No evidence for a beneficial effect of additional cetuximab was demonstrated in any group of patients.

Discussion

Inflammation is well reported to contribute to tumour formation and is now a recognised hallmark of cancer. It is known that the tumour microenvironment can attract, educate and control invading leukocytes to promote angiogenesis, viability, motility and invasion. [16] The stroma around solid cancers has been compared with a poorly healing wound and its' associated chronic inflammation. [17] The association of high NLR with worse survival is more pronounced in metastatic than localised disease and therefore may reflect greater tumour burden or a more prolonged chronic inflammatory process. [11] It is uncertain why NLR is more strongly associated with outcome than neutrophil or lymphocyte counts alone. This biological mechanism requires further investigation. Neutrophils may act as tumour-promoting leukocytes through TGF-β, IL-10 and regulatory T-cells induced signal pathways and circulating neutrophils can also secrete the vascular endothelial growth factor (VEGF), resulting in higher levels of VEGF in the tumours. [18] High NLR may also represent a relatively depleted lymphocyte count, potentially impairing the host immune response to malignancy and therefore negatively impact outcomes.

There is also evidence that RAS mutations influence the host immune response. KRAS and NRAS are critical components of intracellular signalling. Functional specificity of mutated RAS isoforms has been demonstrated and the role of mutant proteins in onset and progression of disease continues to be investigated. [19,20] NRAS activation has been shown to suppress stress-induced apoptosis in human colorectal cancer cells lines and

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therefore contribute to colorectal cancer development. Mouse models also indicated that NRAS mutations enhance colon cancer development in the context of inflammation. [20]

Recently, retrospective analysis investigated the relationship of NLR with molecular alterations (KRAS/NRAS/BRAF/PIK3CA/CIMP) and circulating cytokines. [21] High NLR was associated with a poor prognosis in metastatic colorectal cancer, independent of the common molecular alterations. Similarly, in our study, the correlation between dNLR and survival was seen in all mutation groups. These results were consequently not predictive of benefit from the addition of cetuximab in any particular mutation group. Although modest, our results have shown a numerically poorer survival for patients with dNLR≥2.2 treated with additional cetuximab compared to those treated with chemotherapy alone. The BRAF mutated cohort was relatively small in number and underpowered. This data should therefore be interpreted with caution as this limits the ability to differentiate between prognostic and predictive value of dNLR in the context of chemotherapy with cetuximab. A meta-analysis with similar BRAF mutated cohorts may be of value.

CRC patients with elevated NLR have been characterised by aggressive biology and distinctive expression profile of cytokines involved in angiogenesis, inflammation and regulation of the epidermal growth factor axis. In the retrospective analysis, elevated NLR was >5. [21] There is ongoing statistical uncertainty with respect to the cut-off of elevated NLR and the subsequent interpretation of NLR as a prognostic and predictive biomarker. Our analysis confirms the prognostic value of dNLR in advanced colorectal cancer. We have demonstrated a strong association between dNLR with OS and PFS. dNLR is therefore moderately prognostic for one-year survival in the COIN trial. This was independent of the treatment allocation arm.

Conclusion

Our study gives further support for the use of dNLR as a readily available, inexpensive biomarker for prediction of survival in MCRC. We have demonstrated that in the randomised phase III COIN trial, dNLR was a reliable prognostic marker in patients with mCRC that received first line oxaliplatin based chemotherapy with or without additional cetuximab. dNLR was strongly prognostic for survival in all mutations groups especially in patients with BRAF mutant tumours. dNLR was not predictive of benefit from cetuximab.

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List of abbreviations

ANC	absolute neutrophil count
AUC	area under curve

CI	confidence interval
CRC	colorectal cancer
dNLR	derived neutrophil to lymphocyte ratio
EGFR	epidermal growth factor receptor
HR	hazard ratio
IL2	interleukin 2
mCRC	metastatic colorectal cancer
MRC	Medical Research Council
NLR	Neutophil to lymphocyte ratio
OS	overall survival
PFS	progression free survival
ROC	receiver operating characteristic
TGF-β	transforming growth factor beta
VEGF	vascular endothelial growth factor
WBC	white blood cells count

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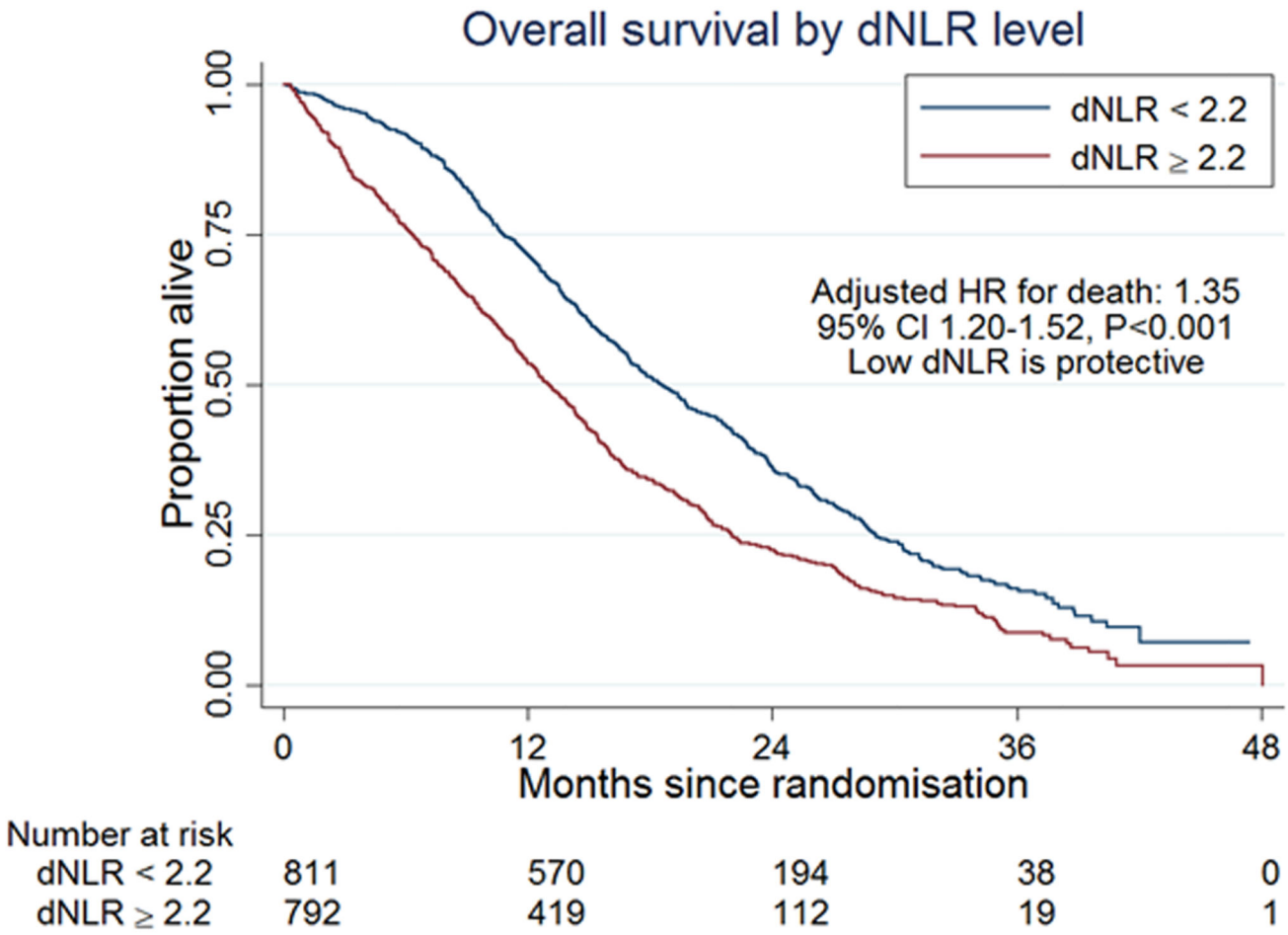

Figure 1.
Kaplan–Meier curves for overall survival according to dNLR.

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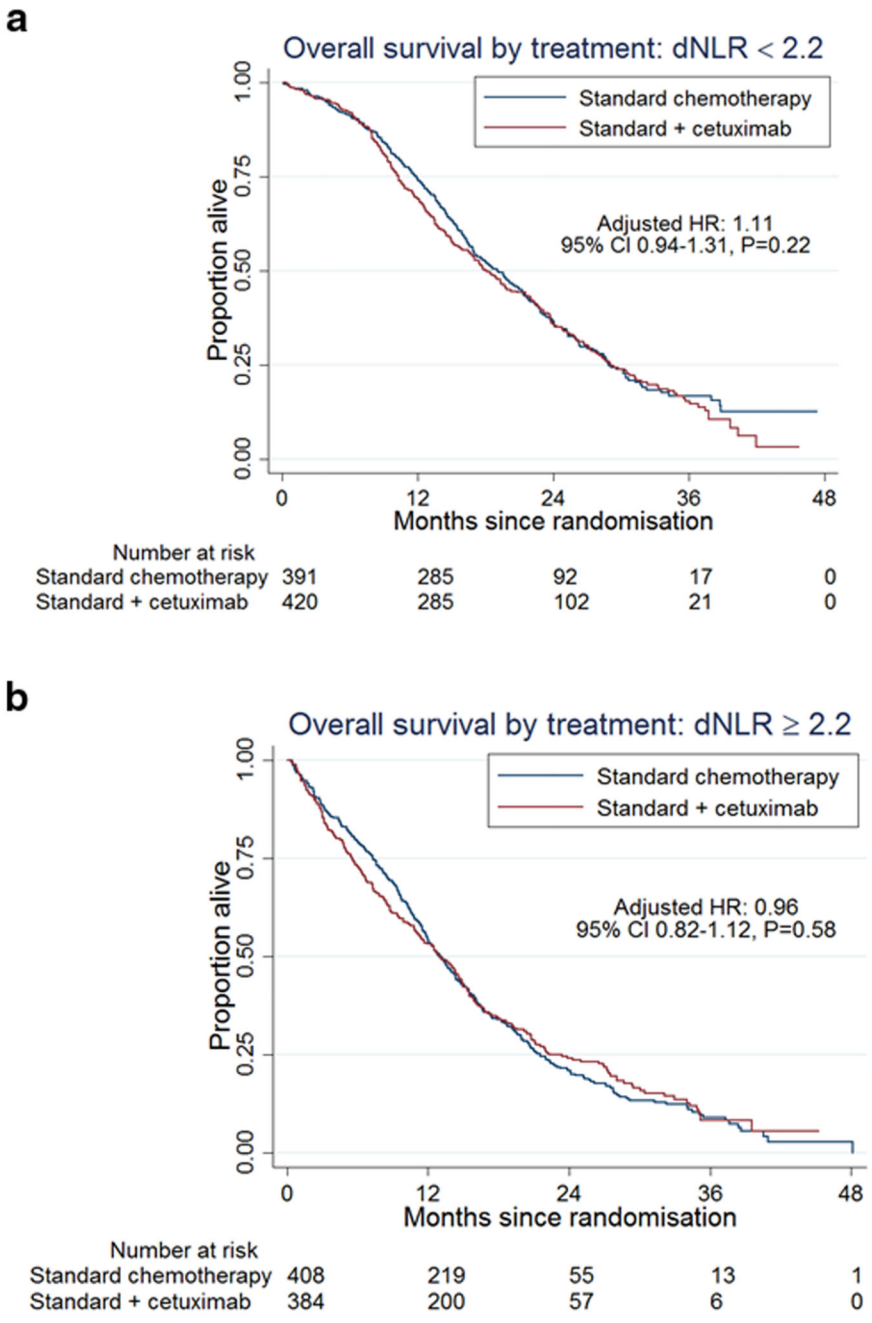

Figure 2.

Kaplan–Meier curves for overall survival according to treatment, in low (figure2a) and high (figure2b) dNLR.

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Table 1

Demographic characteristics of patients

	dNLR < 2.2 (N=811)	dNLR >= 2.2 (N=792)
Treatment		
A - Standard chemotherapy	391 (48.2)	408 (51.5)
B – Cetuximab	420 (51.8)	384 (48.5)
Age; median (range)	64.3 (22-82)	63.6 (25-87)
Sex		
Male	543 (67.0)	507 (64.0)
Female	268 (33.0)	285 (36.0)
WHO Performance Status		
0	422 (52.0)	312 (39.4)
1	350 (43.2)	400 (50.5)
2	39 (4.8)	80 (10.1)
Status of primary tumour		
Resected	502 (61.9)	350 (44.2)
Local recurrence	43 (5.3)	43 (5.4)
Unresected/unresectable	266 (32.8)	399 (50.4)
Number of metastatic sites		
0	4 (0.5)	9 (1.1)
1	304 (37.5)	274 (34.6)
2	310 (38.2)	314 (39.6)
3+	193 (23.8)	195 (24.6)
Liver-only metastases		
No	628 (77.4)	614 (77.5)
Yes	183 (22.6)	178 (22.5)
Platelet count > 400,000 μL		
Normal	633 (78.1)	470 (59.3)
Elevated	178 (21.9)	322 (40.7)
CEA cutoff at 100 U/L		
Low	408 (66.1)	319 (51.0)
High	209 (33.9)	307 (49.0)
Alkaline cutoff at 300 U/L		
Normal	726 (89.5)	624 (78.8)
High	85 (10.5)	168 (21.2)
All wild-type mutations		
Any mutation	360 (54.7)	328 (54.2)
All wild-type	298 (45.3)	277 (45.8)

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Table 2

Median survival by dNLR

	Median survival time (months)		
	dNLR < 2.2	**dNLR ≥ 2.2**	**HR (95% CI)**
All patients	18.6	13.1	1.35 (1.20-1.52)
All wild type	22.8	16.6	1.33 (1.07-1.64)
KRAS wild type	21.3	14.7	1.32 (1.10-1.59)
BRAF mutant	12.6	6.8	1.67 (1.02-2.75)
RAS mutant	16.9	11.8	1.36 (1.12-1.65)