Rácio neutrófilos-linfócitos pré-operatório como preditor de recidiva no cancro do cólon estádio II

Preoperative neutrophil-to-lymphocyte ratio as a predictor of recurrence for stage II colon cancer

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RESUMO

Introdução: O cancro colo-rectal é a terceira causa de morte mais comum por cancro em homens e a segunda em mulheres. Tem sido proposto que a reacção do hospedeiro à doença, como a resposta inflamatória sistémica elevada, pode estar associada à redução da sobrevivência em várias neoplasias.

O objetivo deste estudo foi realizar uma análise combinada de factores clinico-patológicos e do rácio neutrófilos-linfócitos (RNL), como preditores de sobrevivência e recorrência específicas da neoplasia após ressecção curativa em doentes com cancro do cólon estádio II.

Métodos: Estudo retrospectivo unicêntrico de doentes com neoplasia do cólon estádio II, submetidos a ressecção curativa entre janeiro de 2010 e setembro de 2014. O RNL medido 30 dias antes da cirurgia foi registado. Os resultados avaliados foram a sobrevivência específica de cancro, sobrevivência global e sobrevivência livre de doença (SLD).

Resultados: Num serviço de Cirurgia foram estudados 103 doentes submetidos a ressecção potencialmente curativa de cancro do cólon estádio II.

A mediana do seguimento foi 51 meses. Registaram-se, aos 3 anos, 7 mortes relacionadas com cancro e 26 mortes não relacionadas com cancro.

Na análise multivariável, os tumores T4 (p = 0,003) associaram-se a menor SLD, mas um RNL > 3 (p = 0,043) também revelou um pior prognóstico. A curva ROC revelou que um RNL > 3 consegue predizer pior SLD para os tumores do estádio II, com uma precisão razoável.

Conclusão: A avaliação sistemática dos parâmetros inflamatórios, como o RNL, pode estratificar os resultados em doentes submetidos a ressecção potencialmente curativa de cancro do cólon estádio II. A combinação da resposta inflamatória sistémica do hospedeiro e os sistemas tradicionais de estadiamento de tumores pode aumentar a precisão da estratificação.

ABSTRACT

Background: *Colorectal cancer is the third commonest cause of cancer death in men and the second in women. Some authors proposed that host reaction to disease, such as an elevated systemic inflammatory response, might be associated with reduced survival in several cancers.*

The aim of this study was to perform a combined analysis of several clinicopathological factors and neutrophil-to-lymphocyte ratio (NLR) as predictors of cancer-specific survival and recurrence after curative resection of stage II colon cancer.

Methods: *Retrospective study of patients with stage II colon cancer submitted to curative resection in a single surgical unit from january 2010 to september 2014. NLR measured 30 days before surgery was recorded. The outcomes measured were cancer-specific survival, overall survival and disease free survival (DFS).*

Results: *We studied 103 patients undergoing potentially curative resection for stage II colon cancer in a single surgical unit.*

Median follow-up was 51 months. Seven cancer-related deaths and 26 non cancer-related deaths were reported at 3-years.

On multivariable analysis, T4 (p = 0.003) was associated with reduced DFS, but NLR > 3 (p = 0.043) also indicated a worse prognosis. ROC curve revealed that NLR > 3 predicted a worse DFS for stage II tumors with a reasonable accuracy.

Conclusion: *Routine evaluation of inflammatory parameters, such as NLR, may stratify outcomes in patients undergoing potentially curative resection for stage II colon cancer. The combination of host systemic inflammatory response with traditional tumor staging systems might increase the stratification accuracy.*

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BACKGROUND

Colorectal cancer (CRC) is the third commonest cause of cancer death in men (745,000 cases) and the second in women (614,000 cases) worldwide and the first cause of

death by cancer in Portugal.[1] Overall 5-year survival rates under standardized treatments for localized, regional, and distant diseases are 91%, 70% and 11%, respectively.[2]

Distant tumor recurrence is the *major* cause of death after curative treatment in CRC patients. Several staging systems have been proposed to define prognosis and to identify patients that benefit from adjuvant treatments after curative surgery. Regional lymph node involvement has been accepted as one of the main prognostic features after curative resection and the main indication for adjuvant chemotherapy. However even in the absence of lymph node involvement, adjuvant chemotherapy appears to benefit patients with some clinical or pathological features, but traditional staging systems fail to correctly identify that subgroup of patients.[3]

Numerous authors have been studying the prognostic value of molecular markers in this setting.[3] However due to high costs, difficulties in availability or lack of standardization, few have been incorporated into clinical practice; therefore, a need to investigate clinically relevant prognostic factors still remains.

Some authors proposed that the host reaction to disease, such as an elevated systemic inflammatory response (evidenced by rising of circulating acute phase proteins or myeloid cells) may be associated with reduced survival in several cancers.[4,5] Elevated serum C-reactive protein (CRP), decreased serum albumin[5] and high NLR[4] have been reported to have a stage-independent impact on prognosis.[6] Inflammatory proteins induce local cellular injury through oxidative damage which may result in genetic mutations or disruption of DNA repair mechanisms, and thus be considered as carcinogenics.[7]

The aim of this study was to perform a combined analysis of several clinicopathological factors and NLR as predictors of cancer-specific survival and recurrence after curative resection of stage II colon cancer.

METHODS

We conducted a retrospective study of patients with stage II colon cancer submitted to curative resection in a single surgical unit from january 2010 to september 2014. The study was approved by the Research Ethics Committee.

All patients with stage II (TNM classification) submitted to curative resection were selected. Exclusion criteria were defined as: patients undergoing non-elective surgery, patients submitted to adjuvant chemotherapy, those who died within 30 days after surgery, lacking preoperative inflammation parameters or lost to follow-up.

TABLE 1 Clinicopathological characteristics an 3-year overall survival

Parameter	n	3-year OS	*p value*
Gender			
Male	61	79.0%	0.336
Female	42	70.7%	
Age			
≤ 70 years	39	84.6%	0.101
> 70 years	64	70.3%	
ASA			
1-2	58	78.7%	0.519
3-4	44	73.2%	
Tumor location			
Right	63	74.6%	0.572
Left	39	79.5%	
Laparoscopy			
Yes	38	86.5%	0.057
No	65	69.7%	
Anastomotic leak			
No	97	76.3%	0.594
Yes	5	60.0%	
T stage			
< 4	90	77.8%	0.296
≥ 4	13	61.5%	
Lymphatic invasion			
No	69	79.1%	0.276
Yes	34	69.4%	
Venous invasion			
No	56	80.0%	0.279
Yes	47	70.8%	
Differentiation			
Well/moderate	95	75.8%	1.000
Poor	6	83.3%	
NLR			
≤ 3	48	89.4%	**0.003**
> 3	55	64.3%	

Tumors were staged using the seventh edition of TNM classification.[8] Pathological reports issued after surgical resection were reviewed to retrieve tumor data.

The following clinicopathological factors were analyzed: age, gender, American Society of Anesthesiologists (ASA) classification, tumor location, TNM stage, histologic

TABLE 2 **Relationship between clinicopathological characteristics and survival (CSS and DFS) of patients undergoing potentially curative resection of stage II colorectal cancer (Cox Regression)**

	Cancer-specific survival				Disease-free surviva			
Parameters	**Univariable (HR 95%CI)**	***p***	**Multivariable (HR 95%CI)**	***p***	**Univariable (HR 95%CI)**	***p***	**Multivariable (HR 95%CI)**	***p***
Gender: female	1.215 (0.272-5.431)	0.799			0.948 (0.464-1.935)	0.883		
Age > 70 years	4.219 (0.508-35.054)	0.183			**2.251 (1.004-5.047)**	**0.049**	-	-
ASA ≥ 3	1.862 (0.416-8.329)	0.416			1.812 (0.878-3.737)	0.108		
Tumor location: right	1.195 (0.267-5.341)	0.815	-	-	0.989 (0.469-2.084)	0.976	-	-
Open approach	1.550 (0.301-7.993)	0.600	-	-	**2.433 (1.044-5.667)**	**0.039**	-	-
Anastomotic Leak	3.529 (0.424-29.335)	0.243	-	-	1.297 (0.308-5.467)	0.723	-	-
T stage: 4	**9.808 (2.193-43.868)**	**0.003**	**10.596 (2.368-47.413)**	**0.002**	**3.705 (1.636-8.389)**	**0.002**	**3.575 (1.548-8.257)**	**0.003**
Lymphatic invasion	2.978 (0.666-13.309)	0.153	-	-	**2.078 (1.005-4.294)**	**0.048**	-	-
Venous invasion	1.176 (0.384-7.670)	0.479	-	-	1.390 (0.686-2.817)	0.360	-	-
Poor differentiation	2.621 (0.351-21.778)	0.372	-	-	1.492 (0.354-6.285)	0.585	-	-
NLR > 3	6.469 (0.778-53.782)	0.084	-	-	**2.402 (1.104-5.225)**	**0.027**	**2.348 (1.028-5.363)**	**0.043**

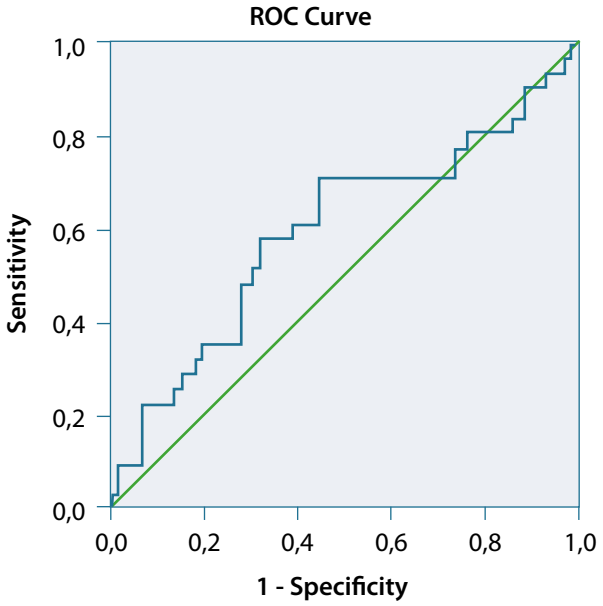
grading, preoperative laboratory data, operative report, complications and follow-up status. "Right side colon" was defined as both ascending and transverse colon and "left side colon" was defined as both descending and sigmoid colon.

Neutrophil-to-lymphocyte ratio was defined as the absolute neutrophil count divided by the absolute lymphocyte count. NLR ratio was calculated based in the values recorded within 30 days before surgery. For the analysis, cut-off value was selected by using receiver operating characteristic (ROC) curve analysis.

Patients were routinely followed-up after surgery according to a previously defined protocol: after curative resection, all patients had outpatient visits every 3 to 6 months for physical examination and CEA test. They also received chest x-ray, abdominal ultrasound or computed tomography annually and colonoscopy on the first year, then every 3 years and if normal every 5 years. Records were complete until september 30, 2017, which acted as the censor date.

Overall survival (OS) was measured until the date of last follow-up or date of death from any cause, whichever came first. Cancer-specific survival (CSS) was measured from the date of surgery until death from recurrent or metastatic colon cancer. Disease free survival was measured until the diagnosis of local recurrence and/or distant metastasis confirmed by histology, reoperation or radiologic studies.

All analyses were performed using SPSS version 20.0 (IBM SPSS, IL). The relationship between clinicopathological characteristics, inflammatory markers and survival was evaluated using Qui-square test and univariable Cox proportional hazards regression to calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs). Va-



Stage II	
AUC (CI) p	0.596 (0.469-0.724) 0.122
Cut-off value Sensitivity Specificity	3 71.0% 55.6%

FIGURE 1 ROC curve of NLR prognostic impact in recurrence for stage II colon cancer.

riables observed to be statistically or clinically significant on univariable analysis were subsequently entered into a multivariable model using a backwards-conditional method. A p value ≤ 0.05 was considered statistically significant. The NLR adequate cut-off value (best sensitivity and specificity) for predicting disease-free survival on stage II colon cancer was examined through ROC curves.

RESULTS

We studied 103 consecutive patients undergoing potentially curative resection for stage II colon cancer in a single surgical unit, after applying the aforementioned exclusion criteria; baseline characteristics are described on Table 1.

Male to female ratio was 1.5:1 and median age of the patients was 75 years (range 28 to 95). According to T staging, patients were divided into T2 (2 patients), T3 (88 patients) and T4 (13 patients) and most tumors were well or moderately differentiated (94%). The cut-off NLR value of 3, obtained by ROC curve analysis, was similar to the cut-offs described in the literature.

Median follow-up was 51 months (range 5 to 85 months). Seven (6.8%) cancer-related deaths and 26 (25.2%) non cancer-related deaths were reported at 3-years.

Univariable and multivariable analysis of the relationship between clinicopathological characteristics and survival were performed (Table 2). On multivariable analysis, only T4 (p = 0.002) predicted reduced CSS. Regarding DFS, T4 (p = 0.003) surfaced as a parameter of poorer prognosis, but NLR > 3 (p=0.043) was also associated to reduced DFS.

Furthermore we obtained a ROC curve for NLR as a recurrence predictor. For the cut-off value of 3, NLR revealed a reasonable accuracy to predict DFS for stage II tumors, with 71.0% sensitivity and 55.6% specificity (Figure 1).

DISCUSSION

It is well known that inflammation suppresses the immune response to cancer cells and also modifies the tissue microenvironment that warrants those cells to become more aggressive.[9] The use of routinely available inflammatory biomarkers has two possible advantages: stratification of prognosis, which may contribute to select patients for adjuvant chemotherapy, and identification of patients that may benefit from future perioperative therapies targeting the systemic inflammatory response.

Adjuvant chemotherapy has a clear survival advantage for patients with stage III colon cancer but only a subgroup of patients with stage II will obtain the same benefit. A controversial and highly debated topic, therefore, persists: how to correctly identify stage II patients

for adjuvant chemotherapy[10], with only few reports having investigated this issue.[11,12] High-risk pathological features, such as tumor differentiation and presence of lymphovascular invasion, have shown to stratify outcome within TNM and have been used to predict the need for adjuvant therapy.[13] However, the importance of these pathological prognostic factors reminds us that other factors, such as host characteristics (inflammation status), might also be useful in further stratification of outcomes. Simple analytical parameters have further advantages such as objective measurement, wide availability and low cost, whereas assessment of pathological features is often subjective and underreported.[14]

In our study NLR > 3 predicted disease-free survival for stage II tumors. This parameter should be assessed in clinical practice, in order to further stratify the outcome and investigate patterns of response to chemotherapy, in patients with stage II colon cancer. Lymphopenia is the marker of a depressed cell-mediated immunity while neutrophilia occurs during systemic inflammation in response to various stressful events.[15] The prognostic value of these markers in malignancy might be explained by higher tumor angiogenesis[16], lymphocyte anergy associated with disease severity[17], and immune escape of tumor cells from tumor-infiltrating lymphocytes.[18]

Other possible advantage of this biomarker is to select patients that may benefit from modulation of inflammatory response. It is now supported that systemic inflammation is associated with cancer cachexia[19] and may be attenuated by the use of non-steroidal anti-inflammatory drugs[20], although the effective dose of the drugs is not currently known.[21]

A study demonstrated that the majority (80%) of patients with high inflammatory response before surgery do not revert to normal post-operatively, and these findings seem to have impact on prognosis.[22] Therefore, changes to the perioperative management of patients that may reduce inflammatory response, such as enhanced recovery protocols, could be studied as a mean to modulate the inflammatory state and improve survival.

These results confirm that routine evaluation of inflammatory parameters (such as NLR) may stratify outcomes in patients undergoing potentially curative resection for stage II colon cancer. The importance of these parameters in predicting recurrence has already been shown in other retrospective studies.[12,23] Furthermore, the combination of host systemic inflammatory response with traditional tumor staging systems might increase the stratification accuracy.

Limitations to this study include its single-center, retrospective nature and small sample. Since we have excluded patients submitted to adjuvant chemotherapy for the purpose of this study, this could lead to a potential selection bias. The analysis of other possible markers (such as platelet-to-lymphocyte ratio[24], fibrinogen[25], CRP and albumin) was not performed due to the low statistical power of our sample and also because fibrinogen, CRP and albumin are not routinely evaluated before surgery in our institution.

To clarify these assumptions, randomized controlled trials incorporating both routine assessment of systemic inflammatory response markers and use of anti-inflammatory agents are required. Similarly, molecular characterization of tumors will be informative in future examinations of colon cancer survival.

CONCLUSION

Routine evaluation of inflammatory parameters, such as NLR, may stratify outcomes in patients undergoing potentially curative resection for stage II colon cancer. The combination of host systemic inflammatory response with traditional tumor staging systems might increase the stratification accuracy.

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