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Analysis of neutrophil-lymphocyte ratio as a prognostic element in the response to neoadjuvant therapy in rectal cancer



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ABSTRACT

Introduction: The previous radio-chemotherapy approach is highly relevant in the management of rectal cancer, collaborating on organ functional preservation, being performed prior to surgery. The inflammatory response plays an important role in this treatment.

Objective: It consists in correlating the number of peripheral lymphocytes and the neutrophil/lymphocyte ratio in the peripheral blood with tumor response to neoadjuvant therapy.

Methods: Review of medical records of patients with rectal cancer in HMSJ and HSJ Oncology Services since 2009 – cases submitted to neoadjuvant treatment with radio-chemotherapy. **Results:** Of those 96 patients with this disease who underwent neoadjuvant therapy with radio-chemotherapy, 35 patients were eligible; complete tumor response was observed in 11 cases (31%), and 9 were submitted to surgical treatment. Comparing the leukocyte parameters between patients with complete response (CR) and incomplete response (IR) the following values were observed: total number of leukocytes (mean) CR 7390.9 × IR 7220.4 ($p=0.8$); total lymphocytes CR 2103 × IR 1960.9 ($p=0.4$); neutrophil/lymphocyte ratio CR 3.55 × IR 3.79 ($p=0.5$). The mean radiotherapy dose was 49.1 Gy, with CR 47.3 × IR 50.0 ($p=0.06$).

Conclusion: It was not possible to demonstrate in this study a significant relationship between complete tumor response to neoadjuvant therapy with respect to blood leukocyte parameters analyzed.

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Análise da relação neutrófilo-linfócito como elemento prognóstico na resposta à terapia neo-adjuvante no câncer do reto

R E S U M O

Palavras-chave:

Neoplasia
Reto
Radioterapia
Linfócitos
Quimioterapia

Introdução: A abordagem radio-quimioterápica previa apresenta grande relevância no manejo do câncer de reto, colaborando na preservação funcional do órgão, sendo realizada previamente à cirurgia. A resposta inflamatória tem papel importante neste tratamento.

Objetivo: Consiste em correlacionar o número de linfócitos periféricos e a relação neutrófilos/linfócitos no sangue periférico com a resposta tumoral à terapia neo-adjuvante.

Métodos: Revisão de prontuários dos pacientes portadores de câncer retal dos serviços de Oncologia do HMSJ e HSJ, desde 2009, casos submetidos ao tratamento neo-adjuvante com radio-quimioterapia.

Resultados: Do total de 96 pacientes portadores desta enfermidade, submetidos à terapia neo-adjuvante com radio-quimioterapia foram elegíveis 35 pacientes, tendo sido observada resposta completa tumoral em 11 casos (31%), e nove foram submetidos ao tratamento cirúrgico. Na comparação dos parâmetros leucocitários entre os pacientes com resposta completa (RC) e resposta incompleta (RI) foram observados os seguintes valores: número total de leucócitos (média) RC 7390,9 × RI 7220,4 ($p = 0,8$); linfócitos totais RC 2103 × RI 1960,9 ($p = 0,4$); relação neutrófilo/linfócito RC 3,55 × RI 3,79 ($p = 0,5$). A dose radioterápica média foi de 49.1 Gy, sendo RC 47,3 × RI 50,0 ($p = 0,06$).

Conclusão: Não foi possível demonstrar no presente estudo relação significativa entre resposta completa tumoral à terapia neo-adjuvante nos parâmetros analisados do perfil leucocitário.

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Introduction

The good results obtained with the association of chemotherapy with radiotherapy led to the adoption of neoadjuvant therapy in the treatment of rectal cancer, with the aim of promoting a reduction in tumor size, allowing better conditions of resectability and sphincter preservation; this has led some authors to suggest a non-surgical treatment in cases of complete response to neoadjuvant therapy. It was not thus far possible to identify prognostic factors that could contribute to the prognosis, with high levels of accuracy, to the occurrence of tumor regression in response to neoadjuvant therapy.

On the other hand, several studies have suggested that the host immune response plays a very important role in this response. Various aspects of immunology have been the subject of studies, taking into account factors related to the tumor, the host and their interaction. Among these factors, the most consistent results have been obtained in the evaluation of local and systemic lymphocytic response, characterized by lymphocyte infiltration into the tumor tissue and by a greater presence of lymphocytes in peripheral blood. The prognostic value of this greater systemic lymphocytic expression has been shown by studies suggesting a better tumor response to neoadjuvant therapy in patients who have an increased number of lymphocytes, in proportion to the total number of neutrophils, and this relationship is summarized by the relationship between neutrophils and lymphocytes.¹

Therefore, the aim of this study is to analyze a possible prognostic value of blood leukocytes in peripheral blood with

tumor response to neoadjuvant therapy, observed in a group of patients with rectal cancer.

Methods

Patients

Medical records of 96 patients with rectal cancer, treated in the Radiotherapy Services of the Hospital Municipal São José (HMSJ) in Joinville and of the Hospital São José (HSJ) in Jaraguá do Sul, were reviewed; these patients underwent neoadjuvant treatment with radiotherapy and chemotherapy.

Among these participants, those with incomplete data in their medical records (making it impossible to assess their outcomes) were excluded, as well as patients with no complete blood count (CBC) available for evaluation, patients who did not conclude the radiochemotherapy, or who were lost to oncological follow-up. Thus, 35 patients (14 males) with a mean age of 55 years were eligible.

Neoadjuvant therapy was indicated for patients with rectal adenocarcinomas located in the middle and lower third of the rectum, whose staging suggested parietal invasion (T3 or T4 stage), or by the presence of a possible perirectal lymphadenopathy. All patients underwent proctologic examination, endoscopic assessment and biopsy through colonoscopy or rectosigmoidoscopy, and an imaging examination by computed tomography or magnetic resonance was obtained, in addition to a chest X-ray, to rule out metastatic lesions.

Neoadjuvant therapy technique

All patients underwent two-dimensional radiotherapy with simulations using contrast-enhanced radiography of the rectum and bladder, or three-dimensional radiotherapy with simulations using computed tomography and intravenous contrast. The treatment volumes were: primary tumor and its local-regional lymphatic (perirectal, iliac and obturator) drainages, with dosages from 45 to 50.40 Gy administered over 25–28 days, from Monday to Friday. A four-field treatment was performed; the patient was placed in a prone position, and the belly-board technique was used for bowel loop removal and toxicity reduction. The patients treated by three-dimensional technique were evaluated according to a dose/volume histogram, and the two-dimensional treatment cases were evaluated with the use of treatment curves.

The chemotherapy procedure consisted of an infusional chemotherapy, including 5-fluorouracil (5-FU) and leucovorin (Lv) in the first and last five days of radiotherapy; or, alternatively, by continuous oral ingestion of capecitabine throughout radiotherapy.

With respect to whether or not performing the surgical treatment, the therapeutic conduct was defined by those surgeons responsible for each case.

Patients undergoing surgical treatment had their tumor response defined by a pathological analysis of the surgical specimen, with the response being classified as complete or incomplete.

Blood leukocyte profile analysis

The number of total leukocytes and the expression of lymphocytes were identified by reviewing a CBC performed before starting the treatment with radiotherapy and chemotherapy. The relationship between leukocytes and lymphocytes was obtained by the following calculation: “total leukocyte count divided by the total number of lymphocytes” (neutrophil/lymphocyte ratio; N/L).

Data procedure and statistical analysis

The data were stored in an Excel database (Microsoft Office 2003 package) and analyzed with the Statistical Package for the Social Sciences (SPSS) program, version 17.0.

Ethical aspects

The study was approved by the Ethics Committee of the Universidade da Região de Joinville (UNIVILLE). The study began after the Ethics Committee approval.

Results

Of the 35 patients analyzed, complete (clinical or pathological) responses were observed in 11 cases (31.4%). In two patients, we opted for a scheme of observation with frequent revisions (clinical response), while in nine cases surgical treatment (pathological response) was performed.

Table 1 – Total number of leukocytes.

Complete response	N	Mean	SD	p
Absent	24	7220	1999.2	0.838
Present	11	7390	2788.5	

Patients with complete response who did not undergo surgery

These two patients were not submitted to surgical treatment, as a complete tumor response, evaluated by digital rectal examination and sigmoidoscopy, was observed. These patients were followed for a median of 24 months.

In this group, the mean age was 82.5 years; one of these patients was a man.

In this observational period, there was no evidence of tumor recurrence in both patients.

Operated patients

Of the 32 patients who underwent surgical resection after neoadjuvant therapy, a complete pathological response was observed in nine (28%). This group had a mean age of 51.9 years; among those who achieved a complete response, 48.8 years; and for those cases of tumor persistence, 55 years.

Non-operated patients

One patient had the disease and could not be operated due to the clinical condition. This patient was considered as not respondent, and data were included in the analysis.

Blood leukocyte profile analysis

Considering the total number of patients with complete or incomplete response to neoadjuvant therapy, we observed the respective blood leukocyte profiles for each group, as follows.

Total number of leukocytes

As shown in [Table 1](#), the CBC obtained previously to neoadjuvant therapy showed that there was no statistical difference in total number of leukocytes between patients with and without complete response.

Table 2 – Lymphocyte expression.

Complete response	N	Mean	SD	p
Total lymphocytes				
Absent	24	1960	608.3	0.474
Present	11	2103	338.9	
% Lymphocytes				
Absent	24	27	6.0	0.193
Present	11	31	9.8	

Table 3 – Neutrophil/lymphocyte ratio.

Complete response	N	Mean	SD	p
N/L ratio				
Absent	24	3.7	0.88	0.502
Present	11	3.5	1.21	

Table 4 – Radiotherapy dosage.

Complete response	N	Mean	SD	p
Absent	24	50	4.4	0.065
Present	11	47	2.6	

Lymphocyte expression

As shown in Table 2, the CBC obtained previously to neoadjuvant therapy showed that there was no statistical difference regarding the absolute or relative number of lymphocytes between patients with or without complete response.

Neutrophil/lymphocyte (N/L) ratio

As shown in Table 3, the CBC obtained previously to neoadjuvant therapy showed that there was no statistical difference regarding the neutrophil/lymphocyte ratio between patients with or without complete response.

Treatment dose

Although the radiotherapy dose was not among the variables initially established for analysis in this study, the retrospectively observed difference between the means of complete and incomplete response groups (50 Gy × 47 Gy) led us to assess its possible prognostic value. As shown in Table 4, a borderline difference for significance ($p = 0.06$) was observed.

Excluded patients

After the selection of 96 patients (35 from the center of Joinville and 61 from the center of Jaragua do Sul), 61 cases that did not meet the criteria for inclusion in the study were excluded.

Discussion

Tumor antiinflammatory response plays an important role in tumor evolution response, including cases of colorectal cancer. Several studies have reported that tumor infiltration by CD8+ T cells, when activated, shows prognostic value and good correlation with disease staging, suggesting a possible action that induces tumor cell apoptosis. This lymphocyte activation can be demonstrated by the local expression of elements such as granzymes B, CD69+ or CD107+. According to Koch et al., an increase in the number of CD4+ T cells was observed in the mucous membrane of tumor cells, compared to normal tissue mucosa, suggesting an increased immune response in colorectal tumors. This prognostic value was also observed by Naito et al.; these authors studied 131 cases of colorectal cancer and correlated the location of CD8+ cells, which, when present in the vicinity of the cancer cell nidus, showed better survival.²⁻⁴

In addition of tumor response, there is compelling evidence of a relationship between systemic response and prognosis. Several authors have shown that the number of circulating lymphocytes, in particular through a neutrophil/lymphocyte (N/L) ratio analysis, has prognostic value in cases of colorectal cancer. Liu et al. noted a significant relationship between N/L value and survival of patients undergoing treatment of rectal cancer. Hung et al. analyzed 1040 patients with colon cancer (stage II), of whom 785 (75.5%) exhibited normal N/L ratios and 255 (24.5%) had high N/L ratios. Patients with high N/L had lower rates of overall survival and of disease-free interval, when compared to patients with normal N/L.⁵

Chiang et al. evaluated 3857 patients with colorectal cancer in stages I-III, noting that N/L ratios >3 were associated with higher sensitivity and specificity and lower survival after 5 years, both in colon cancer (66.3% × 78.9%, $p < 0.001$) and in colorectal cancer (60.5% × 66.2%) compared with patients with N/L ratios below this value. Furthermore, patients with N/L above this level also exhibited larger tumors (5 cm) and a more advanced local disease.⁶

Chua et al. analyzed 349 patients in two independent cohorts treated with first-line palliative chemotherapy and noted that N/L ratio is a positive prognostic factor.⁷

Relationship between systemic inflammatory response and response to neoadjuvant therapy in rectal cancer

Once detecting the prognostic value of the systemic inflammatory response, some authors have sought to demonstrate its possible influence on radiochemotherapy results performed in patients with rectal tumor. In a study that analyzed 73 patients operated for treatment of rectal cancer after neoadjuvant radiochemotherapy, 10 (14%) achieved complete tumor response. The authors observed a significantly higher peripheral lymphocyte count ($p = 0.02$) and a neutrophil/lymphocyte ratio tending to lower values ($p = 0.099$). Based on these findings, the authors suggested that the complete eradication of tumor cells is dependent on an immune reaction mediated by lymphocytes, and that the maintenance of the number of circulating lymphocytes is an important factor in this response to neoadjuvant therapy. The same authors, in a subsequent study, analyzed blood samples taken before and after radiotherapy and confirmed that patients who achieved a complete response, with disappearance of tumor cells (15/179), had elevated peripheral lymphocyte counts and low neutrophil/lymphocyte ratios.⁸

In a study that analyzed the tumor response to radiochemotherapy with barium enema, the authors also observed an association between this response and the proportion of lymphocytes in relation to leukocytes. Other authors related the results obtained in the treatment of rectal cancer in 115 patients undergoing neoadjuvant therapy with the use of radiochemotherapy with the neutrophil/lymphocyte ratio (N/L) obtained from peripheral blood, noting that N/L ratios >5 were associated with lower overall survival, shorter time to local recurrence and lower disease-free interval. Moreover, these patients showed a median survival of 18.8 months compared with 54.4 months for those patients with N/L <5 ($p < 0.001$).^{1,9}

In an interesting development of the findings mentioned above, a study was conducted in which the authors sought to correlate tumor response to neoadjuvant therapy in patients with rectal cancer with the apoptotic index obtained with *in vitro* irradiation of lymphocytes obtained from peripheral blood of these patients. As a result, these authors achieved a significant correlation between lymphocyte apoptosis and histological regression, concluding that tumor radiosensitivity can be estimated from the apoptotic index of lymphocytes *in vitro*.¹⁰

Relationship between lymphocytic infiltration and response to neoadjuvant therapy in rectal cancer

With the use of immunohistochemistry, Yasuda et al. analyzed the level of tumor infiltration (LTI) by CD4+ and CD8+ T cells in biopsies of 48 cases of advanced colorectal cancer before completion of neoadjuvant radiochemotherapy, and related their findings to the tumor reduction evaluated by barium enema and by biopsy after neoadjuvant therapy. The numbers of both CD4+ and CD8+ lymphocytes in biopsy samples previously to radiochemotherapy showed strong correlation with tumor reduction, as assessed by barium enema. Moreover, CD4+ and CD8+ LTI densities were significantly associated with histological grade after neoadjuvant therapy. CD8+ LTI density was also an independent prognostic factor in achieving a complete response.¹

In the present study, the occurrence of complete tumor response after neoadjuvant therapy for the treatment of rectal cancer was observed in 31.4% of cases, a result consistent with those observed in the literature. However, it was not possible to detect a relationship between this response and the analyzed elements in the leukocyte profile, including the total numbers of leukocytes and lymphocytes and the neutrophil/lymphocyte ratio. We believe that the small number of cases included in this study might have contributed to this finding, due to factors inherent to a retrospective study.

On the other hand, the strong trend for a significant correlation between the response and the dose of radiotherapy administered called our attention to the need for taking a possibly greater control, in order to reduce the variability between treatments, with the establishment of more rigid protocols for optimizing the neoadjuvant therapy results.

Conclusions

In this study, we could not demonstrate a significant relationship between complete tumor response and neoadjuvant therapy in the analyzed leukocyte profile parameters, including a previous total number of leukocytes, absolute and relative numbers of lymphocytes, and neutrophil/lymphocyte ratio.

Conflicts of interest

The authors declare no conflicts of interest.

Annex. Table of pathological response grading.

Dworak et al. (14)	Rodei et al. (17)
0. No regression	0. No regression
1. Predominantly tumor with significant fibrosis and/or vasculopathy	1. Regression <25% of tumor mass
2. Predominantly fibrosis with scattered tumor cells (slightly recognizable histologically)	2. Regression of 25–50% of tumor mass
3. Only scattered tumor cells in the area of fibrosis with/without acellular mucin	3. Regression >50% of tumor mass
4. No vital tumor cells detectable	4. Complete regression

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