Inflammatory cytokine network from blood plasma differs between clinical-pathological aspects of colorectal cancer patients

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ABSTRACT

Introduction: Colorectal cancer (CRC) is one of the most common solid neoplasms in the world. Inflammatory cytokines including interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNFα) have been controversially associated with the progression of the disease, but their clinical and prognostic

relevance remains poorly investigated. Objective: The aim of this work is to describe the blood plasma levels of IL-1β, IL-6, IL-8, IL-10 and TNFα, and their association with the clinical-pathological aspects of CRC patients at diagnosis. Methods: This is a descriptive and cross-sectional study, where peripheral blood samples were obtained from adult patients with CRC collected at diagnosis (T0). The blood plasma was processed for cytokine measurement using cytometric bead arrays (CBA) for flow cytometry. Sociodemographic and clinical-pathological characteristics were obtained from the medical records of patients. Results: There were significant differences in IL-1β levels in patients with well-differentiated and moderately differentiated tumors (p=0.0039). IL-6 levels differed when compared between patients with colon and rectal tumors (p=0.01) and between the 3 tumor locations: Colon, rectosigmoid junction, and rectum (p=0.03). There was a difference in IL-6 levels between groups in the initial (I/II) and more advanced (III/IV) stages of the disease (p=0.0005). IL-8 and TNFα levels were higher in patients with proximal tumors and moderately differentiated histology. IL-10, analyzed in parallel, showed higher levels in metastatic patients (p=0.0225). Conclusion: There are significant differences between the levels of inflammatory cytokines and the clinical-pathological characteristics of CRC at diagnosis, such as tumor location, histology, clinical stage, and metastasis. The antagonistic process of specific cytokines, such as TNFα and IL-10, may demonstrate relevant clinical value and longitudinal studies will be necessary to elucidate these events better.

Keywords: Colorectal câncer, Inflammatory cytokines, Clinical-pathological features.

1 INTRODUCTION

Colorectal cancer (CRC) is a malignant neoplasm characterized by the mutation and exacerbated cell proliferation in the colon and rectum, causing a marked imbalance in intestinal and systemic homeostasis. Currently, it is the third most diagnosed tumor in Brazil, with more than 20.500 cases annually and mortality regardless of gender. In Amazonas, incidence rates are set at 5.25 and 9.23/100,000 cases for men and women, respectively, according to the latest reports from the National Cancer Institute (INCA) (1). Many biological aspects of CRC have stimulated the investigation of the immune system and nonspecific inflammation has emerged as a factor that may underlie many features of this disease. In colorectal tumors, inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNFα have been controversially associated with these events, but their clinical and prognostic relevance in CRC has not yet been established (2). Therefore, the present study aims to describe the blood plasma levels of the cytokines IL-1β, IL-6, IL-8, IL-10 and TNFα in patients with CRC and their association with clinical-pathological aspects at diagnosis.

2 MATERIAL AND METHODS

This study was submitted to and approved by the Ethics Committee at Fundação Centro de Controle de Oncologia do Estado do Amazonas (FCECON), under registration number #5.180.654/2021. Before the inclusion of all patients in this study, they read and signed the informed consent form. The study was carried out in accordance with the principles of the Declaration of Helsinki and Resolution 466/2012 of the Brazilian National Health Council, which relates to research involving human participants. Between August 2022 and

Jule 2023, a total of 107 CRC patients were included in this study carried out at Fundação CECON, which is the reference center for cancer diagnosis and treatment in Manaus, Brazil. All patients were recently diagnosed with CRC, were of either sex (62 males/45 females) and had a median age of 63 years; interquartile range (IQR) = 53–71. The clinical-pathological characteristics of CRC patients include age, sex, previous cases of cancer, tumor location, histopathology, tumor stage, metastasis, and administered treatment. All the clinical and pathological characteristics are summarized in Table 1. Peripheral blood samples of CRC patients were obtained by venous puncture at T0 (Time 0, diagnosis). After collection, the samples were transferred to EDTA vacuum tubes (BD Vacutainer® EDTA K2) and submitted to centrifugation at 1260 rcf for 10 minutes. Subsequently, the plasma was collected and immediately stored at -80°C until processing for cytokine analysis. The cytokine concentrations (IL-1β, IL-6, IL-8, IL-10 and TNFα) were quantified using cytometric bead arrays (BD™ Human Chemokine and BD™ Human Cytokine Th1/Th2/Th17 kits, BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. Samples were acquired in a FACSCanto II (BD Biosciences, San Jose, CA, USA) and the FCAP-Array software v3 (BD Biosciences, San Jose, CA, USA) was used for data analysis, reported in picograms per milliliter (pg/mL) concentrations according to standard curves provided in the kits. GraphPad Prism, version 8.0.1 (GraphPad Software, San Diego, CA, USA), was used for the statistical analyses. To check the normality of the data, the Shapiro-Wilk test was used. Next, the parametric unpaired Student t-test and ANOVA test were applied, in addition to the non-parametric Kruskal-Wallis' test and the Mann-Whitney test for comparison between 2 and 3 groups, respectively. Statistical significance was considered in all cases where $p<0,05$.

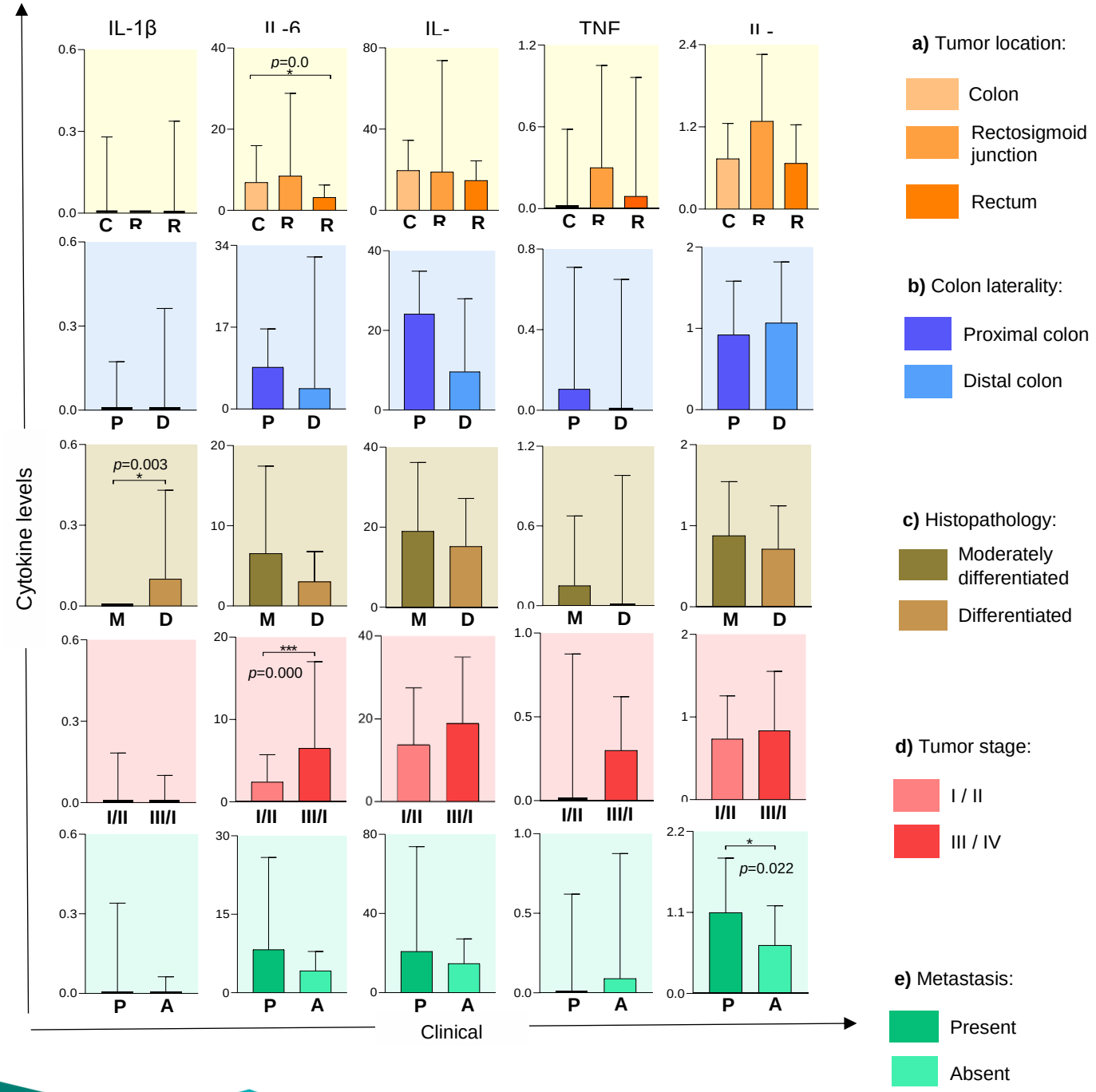
Table 1. Sociodemographic and clinical-pathological characteristics of the study population. Abbreviations: CRC (colorectal cancer); SURG (surgery); CT (chemotherapy); RT (radiotherapy); IQR (interquartile range).

Characteristics		**CRC (*n = 107*)**
Age (years), median (IQR)		63 (53-71)
Sex (male/female), relative value (%)		62M/45F (58% / 42%)
Previous history of cancer (yes/no), relative value (%)		48N/57S (45% / 55%)
Tumor location (n), relative value (%)	**Colon** **Proximal**	24 (23%)
	Distal	16 (15%)
	Rectosigmoid junction	17 (15%)
	Rectum	50 (47%)
Histopathology (n), relative value (%)	**Moderately Differentiated**	73 (68%)
	Differentiated	21 (20%)
	Others	13 (12%)
Tumor stage (n), relative value (%)	**I / II**	46 (43%)
	III / IV	61 (57%)
Metastasis (present/absent), relative value (%)		30P/77A (28% / 72%)
Treatment (n), relative value (%)	**Surgery**	30 (28%)
	Chemotherapy	11 (10%)
	SURG + CT	39 (36%)
	CT + RT	11 (10%)
	SURG + CT + RT	13 (12%)
	Others	3 (4%)

3 RESULTS AND DISCUSSION

The preliminary analysis of the inflammatory cytokine network in peripheral blood samples (n = 49) was performed on T0, in order to describe the profile of immunological molecules in newly diagnosed CRC and their behavior between clinical-pathological characteristics. The results demonstrated lower levels of the cytokines IL-1β, IL-6, and TNFα at the time of diagnosis, while the molecules IL-8 and IL-10 displayed a significant increase (Figure 1).

Figure 1. Cytokine levels in the peripheral blood plasma of CRC patients at diagnosis according clinical and pathological characteristics. Significant differences ($p<0.05$) are highlighted by connecting lines and asterisks (*).



When comparing groups, significant differences emerged in IL-1β levels in patients with differentiated and moderately differentiated tumors (p=0.0039). IL-6 levels showed significant differences when compared between patients with colon and rectal tumors (p=0.01) and between the 3 tumor locations: colon, rectosigmoid junction and rectum (p=0.03). The greatest difference found in IL-6 levels was between groups with initial (I and II) and more advanced (III and IV) stages of the disease (p=0.0005). IL-8 and TNFα levels were higher in patients with proximal tumors and moderately differentiated histology. IL-10, analyzed in parallel, showed higher rates in patients with metastasis (p=0.0225). High levels were also found when analyzing IL-6 and IL-8, while IL-1β and TNFα had lower circulating levels. It is not yet clear in the literature whether IL-1β levels differ depending on the tumor site, but low levels of this cytokine have been shown to counteract the increase in IL-6 and IL-8 in early stages. IL-6 and IL-8 are more related to tumor progression, linked to strong local expression of growth factors and inducing metastatic processes, a variable analyzed in our results (2). They are also investigated as indicators of resistance to treatment and clinical prognosis (3). Low circulating levels of TNFα are closely associated with the histological aspects of the tumor, in contrast to the presence of IL-10. The clinical impacts of this cytokine are still controversial, as high circulating levels are related to worse clinical status and high tumor levels to disease elimination (4). IL-10, as a TNFα antagonist, plays an immunoregulatory role at a local and systemic level, being used by cancer cells to suppress the Th1 inflammatory response (5).

4 CONCLUSION

The relationship between inflammatory cytokines and the clinical profile and prognosis of CRC is still a controversial topic. Our results suggest significant differences between these mediators and clinical-pathological characteristics of the disease, such as tumor location, histology, clinical stage, and metastasis. However, it is important that additional studies should be carried out regarding the mechanisms involved in cellular immunosuppression and the inflammatory cytokine network in the tumor microenvironment.

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