

## Serum and tumor microenvironment IL-8 values in different stages of colorectal cancer

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### Abstract

**Introduction:** Colorectal cancer represents a major cause of mortality and morbidity and occupies the third place as cancer incidence and the fourth place as cancer mortality. Colorectal cancer causes 608 000 deaths per year, despite correct applied treatment (surgery and chemotherapy). Interleukin 8 (IL-8) is an important proinflammatory cytokine with an important role in leukocyte chemoattraction. IL-8 also represents an important tumorigenic and proangiogenic factor. **Materials and Methods:** We have studied 68 patients aged between 55 and 70 years, hospitalized in the IInd Surgery Clinic of the Emergency County Hospital of Craiova, Romania. According to TNM grading, the patients were in stages II, III and IV. From these patients, we have prelevated two types of samples: serum and tumor fragment. **Results:** IL-8 values were significantly increased, both in serum and tumor supernatant. The highest IL-8 values were found in tumor supernatant and in advanced stages; IL-8 increased values were correlated with tumor growth and TNM stage. **Conclusions:** IL-8 has an important role in colorectal cancer growth and metastasis and represents an important therapeutic target in this type of cancer. IL-8 represents also a predictor for colorectal cancer prognosis.

**Keywords:** interleukin 8, colorectal cancer, tumor microenvironment.

### Introduction

Colorectal cancer represents a worldwide major mortality and morbidity cause [1]. Colorectal is the third most frequent cancer and occupies the fourth place as mortality rate, with 608 000 deaths per year [2]. Colorectal cancer represents 10% of all human malignant tumors worldwide [2, 3]. The countries with the highest incidence are Australia, New Zealand, Canada, United States of America and some areas within Europe. The lowest incidence of colorectal cancer can be found in China, India, some parts of Africa and South America [3–6]. In Romania, the incidence of colorectal cancer is increasing, fact caused by lifestyle changes, especially dietary modifications. These changes lead to obesity and physical inactivity, which represent two of the most important risk factors involved in colorectal cancer etiopathogenesis [7].

Many other risk factors are involved in colorectal cancer etiology and pathology, such as: age over 40, benign tumors of the colon and rectum (such as polyps), chronic inflammatory diseases of the colon and rectum, alcohol and hereditary factors [8–10].

Despite the correct treatment applied for this disease (surgery and chemotherapy), 30 to 50% of these tumors present recurrences. Unfortunately, many of them are discovered in advanced stages [11].

The immune system plays an important role in cancer evolution and cancer prognosis. The tumor's presence and the antitumoral therapy determine immune response changes because of the immunosuppression induced by both the tumor and the antitumoral therapy. Immune response changes include immune mediators' changes (cytokines, chemokines) that are correlated with tumor progression most importantly [12].

Interleukin 8 (IL-8) is known as a proinflammatory chemokine with tumorigenic and proangiogenic effects. IL-8 hyperexpression can be detected in many types of malignant tumors, including colorectal cancer. The IL-8 hyperexpression is frequently associated with unfavorable prognosis [13].

### Materials and Methods

Our study was performed on 68 patients, 40 males and 28 females, aged between 55 and 70 years, hospitalized in the II<sup>nd</sup> Surgery Clinic of the Emergency County Hospital of Craiova, Romania. The study took place between 2012 and 2013. The patients had a clinical, imaging and histopathological diagnosis.

The histopathological diagnosis has shown colorectal adenocarcinomas, well and moderately differentiated. According to TNM grading, 18 patients were in stage II,

26 patients were in stage III, and 24 patients were in stage IV.

The sampling was performed before surgical treatment and chemotherapy. Two samples were simultaneously taken from each patient: first sample was represented by a blood sample, which was centrifuged at 3000 rpm for 10 minutes and the serum was kept at  $-20^{\circ}\text{C}$  until the determination; second sample was represented by a 5–6 g tumor sample, which was homogenized with 1 mL phosphate buffered saline (pH 7.4) and then centrifuged at 1800 rpm for 10 minutes, in order to obtain the tumor supernatant. All tumor supernatants were kept at  $-20^{\circ}\text{C}$  until the determination.

The protein content of the supernatant was checked using Bradford technique (Bio-Rad protein assay kit, Bio-Rad Laboratory, CA, USA). The results were expressed in  $\mu\text{g/mL}$  [14].

IL-8 was determined using ELISA Sandwich technique, with the kit Human IL-8 ELISA (Krishgen BioSystem, Spain), in the presence of the concentration standards. Double sample testing was performed according to protocol. The results were expressed in  $\text{pg/mL}$ .

Statistical analysis was performed by the Department of Biostatistics, University of Medicine and Pharmacy of Craiova, Romania, using Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA) for processing the data.

Because the study involved numerical comparisons

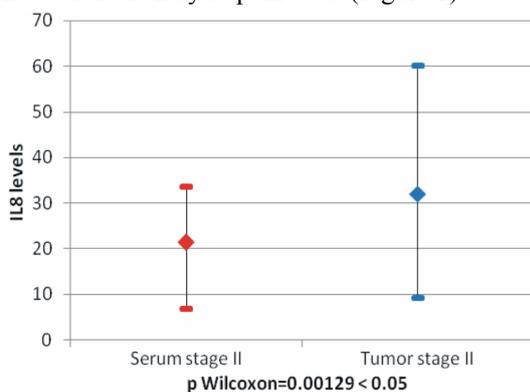
**Table 1 – IL-8 values for our studied group**

IL-8	Minimum value [pg/mL]	Median value [pg/mL]	Maximum value [pg/mL]	No. of cases	Mean value [pg/mL]	Standard deviation	<i>p</i> Wilcoxon	<i>p</i> Student
Serum stage II	6.900	21.35	33.600	18	21.011	8.714	0.00129	0.00078
Tumor stage II	9.200	32.05	60.100	18	32.739	16.028	S	
Serum stage III	7.200	28.4	80.100	26	32.185	20.155	$3.62 \times 10^{-7}$	$2.97 \times 10^{-9}$
Tumor stage III	5.300	77.25	150.000	26	68.819	34.219	HS	
Serum stage IV	18.300	79.8	210.000	24	88.496	48.892	0.00252	0.00576
Tumor stage IV	15.900	99.95	320.000	24	134.204	101.492	S	

S – Significant; HS – Highly significant.

### IL-8 values for patients with stage II TNM colorectal cancer

The average value of IL-8 for patients with stage II TNM colorectal cancer was 21.35  $\text{pg/mL}$  in serum and 32.05  $\text{pg/mL}$  in tumor supernatant, a significant difference, as the statistical analysis points out (Figure 1).



**Figure 1 – IL-8 levels for patients with stage II TNM colorectal cancer.**

between data sets of which not all had a normal (Gaussian) distribution, the non-parametric Wilcoxon and Kruskal–Wallis tests were primarily used, instead of Student's *t*-test or ANOVA, to detect significant differences between the values in the compared data series. These tests mainly assess if there is a difference between the median values of the compared data series (or between the ranks or locations of the data in the compared series), and not between the mean/average values, as Student's *t*-test and ANOVA, respectively, do.

## Results

The studied group counted 68 patients, aged between 55 and 70 years. According to TNM classification, 18 patients were in stage II, 26 patients were in stage III and 24 patients were in stage IV. Although the investigation methods (imaging methods, colonoscopy) would have allowed us to diagnose tumors in stage I TNM, none of the patients from our studied group were in stage I TNM. This fact can be explained by the prevention addressability, which is still low in our country.

In order to assess the differences between the IL-8 levels found in paired serum and tumor samples for same stage patients, we used Wilcoxon rank sum test. We found significant differences for second stage ( $p=0.00129$ ) and fourth stage ( $p=0.00252$ ) samples and a highly significant difference for the third stage ( $p=3.62 \times 10^{-7} < 0.001$ ), in all cases values for serum levels being lower than values for tumor levels (Table 1).

### IL-8 values for patients with stage III TNM colorectal cancer

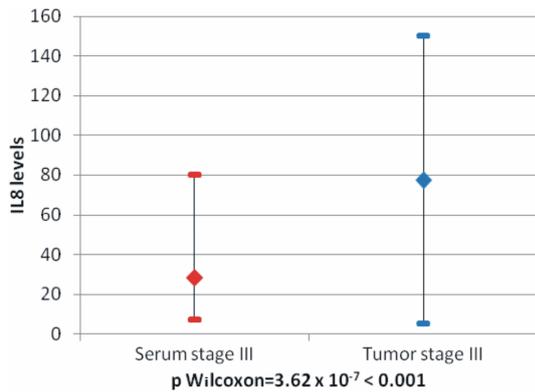
IL-8 values for patients with stage III TNM colorectal cancer presented an average of 28.4  $\text{pg/mL}$  in serum and an average of 77.25  $\text{pg/mL}$  in tumor supernatant. These values are higher than the ones found at patients in stage II TNM. There is also a significant difference between the serum IL-8 values and the tumor supernatant IL-8 values at patients in stage III TNM (Figure 2).

### IL-8 values for patients with stage IV TNM colorectal cancer

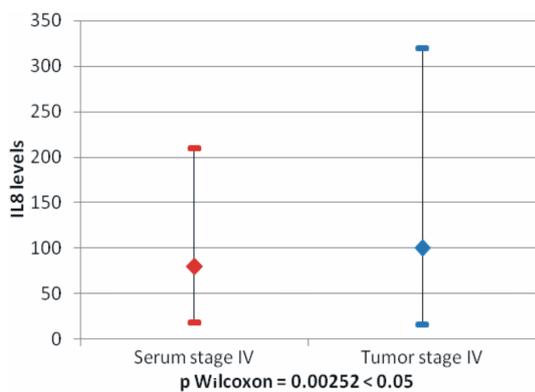
IL-8 values for patients with stage IV TNM were the most increased, with an average of 79.8  $\text{pg/mL}$  in serum and of 99.95  $\text{pg/mL}$  in tumor supernatant (Figure 3).

We performed the Kruskal–Wallis test to assess the influence of the stage on the IL-8 levels for serum samples and tumor samples, respectively. The *p*-value resulted from computing both Kruskal–Wallis tests was less than 0.001, which means there are highly significant statistical

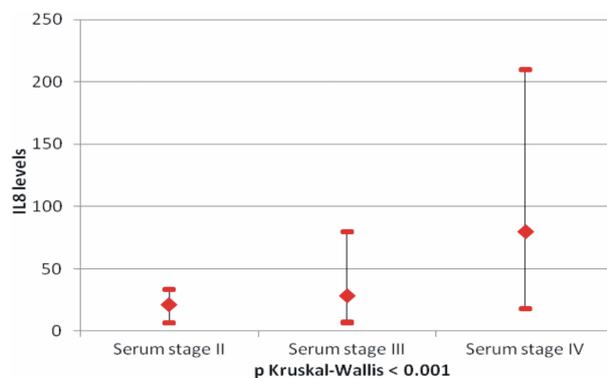
differences between the ranks of the IL-8 levels found for each tumor stage (Figures 4 and 5).



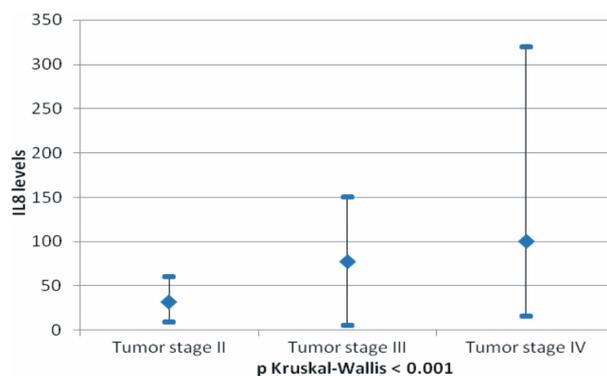
**Figure 2 – IL-8 levels for patients with stage III TNM colorectal cancer.**



**Figure 3 – IL-8 levels for patients with stage IV TNM colorectal cancer.**



**Figure 4 – IL-8 serum values for patients with colorectal cancer.**



**Figure 5 – IL-8 tumor supernatant values for patients with colorectal cancer.**

## Discussion

IL-8 is an alpha chemokine (CXC chemokine) produced by a large number of cells: monocytes, neutrophils, endothelial cells, epithelial cells, fibroblasts, tissular macrophages of T-lymphocytes.

IL-8 possesses proinflammatory effects such as neutrophil degranulation, oxidative metabolism stimulation, neutrophil adherence to endothelial cells and to proteins of the subendothelial matrix. IL-8 is also a powerful chemo-attractant and activator for basophils and histamine release [15].

IL-8 is produced by some malignant cells, including the colorectal cancer cells. IL-8 has tumorigenic and proangiogenic effects not only *in vivo*, but also *in vitro*, on colorectal cancer cell lines [16].

IL-8 tumor supernatant values were higher than IL-8 serum values fact which proves that in the tumor microenvironment IL-8 is produced by both malignant and normal cells (endothelial cells, neutrophils and macrophages) [17].

IL-8 represents a tumor microenvironment regulator, which can contribute to tumor progression [18–20]. IL-8 binding to its receptor CXCR2 at tumor surface produces signals inside the malignant cells, signals that activate transcription factors like NF-KB or AP-1. These factors determine growth and survival of the malignant cells [21–23].

The fact that the most significant IL-8 values were obtained in stage IV TNM of colorectal cancer (both in serum and in tumor microenvironment) proves that this cytokine correlates with tumor progression and with metastasis potential. IL-8 is also a predictor for poor prognosis.

The reduction of IL-8 expression in colorectal cancer using therapeutic methods that block IL-8 production could improve the disease evolution and prognosis. Most of these methods present high risks for the patients because they can interact with the unpredictable human immune system. Only the nanoparticle method can reduce IL-8 expression in colorectal cancer, this way reducing the risk of tumor growth [18, 21].

## Conclusions

Both serum and tumor microenvironment IL-8 values were significantly increased and correlated with the disease TNM stage. For colorectal cancer, IL-8 represents a factor of poor prognosis. In colorectal cancer, IL-8 levels must be monitored; increased levels suggest unfavorable evolution. IL-8 represents an important therapeutic target in colorectal cancer. Methods used for reduction of IL-8 expression can improve the patient evolution.

## Author contribution

All authors equally contributed to this paper.

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Received: January 22, 2014

Accepted: July 1, 2014