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Colorectal carcinoma: evaluation of systemic values of interleukin-1 and interleukin-33 in patients with and without thrombocytosis

Kolorektalni karcinom: procena sistemskih vrednosti interleukina-1 i interleukina-33 kod bolesnika sa i bez trombocitoze

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Abstract

Background/Aim. Reactive thrombocytosis, as a paraneoplastic syndrome, is often observed in cancer patients. A variety of tumor-related humoral factors and cytokines contribute to tumor-stimulated thrombopoiesis. However, the exact role of these cytokines in the pathogenesis of thrombocytosis remains unclear. The aim of this study was to analyze systemic values of cytokines and clinical-pathological characteristics in colorectal carcinoma (CRC) patients with and without thrombocytosis. **Methods.** Fifty nine CRC patients were involved in this study and divided into two groups according to the number of platelets. We recorded and analyzed the data about: age, gender, size of the cancer, localization, metastasis, vascular or lymph vessel invasion, nuclear grade, histological differentiation rate, tumor, nodus, metastasis (TNM) stage and concentration of cytokines [interleukin (IL)-1, IL-33, IL-12, IL-17 and interferon (IFN)-γ] in both groups. **Results.** CRC patients with thrombocytosis had significantly higher nuclear grade of the cancer ($p = 0.002$); higher percentage of detectable metastatic lesions in the liver ($p = 0.002$), lung ($p = 0.001$), peritoneal carcinomatosis ($p = 0.001$), detectable invasion of blood ($p = 0.012$) and lymph vessels ($p = 0.010$). Concentrations of tumor markers [alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA19-9)] and serum values of IL-1 and IL-33 were significantly higher in CRC patients with thrombocytosis. IL-1/IL-12 ($p = 0.016$), IL-1/IFN-γ ($p = 0.007$), IL-1/IL-17 ($p = 0.006$), IL-33/IL-12 ($p = 0.001$), IL-33/IFN-γ ($p = 0.001$), IL-33/IL-17 ($p = 0.002$), and IL-33/IL-1 ($p = 0.006$) ratios were significantly higher in CRC patients with thrombocytosis in comparison to CRC patients without thrombocytosis. Analysis of Receiver Operating Characteristic (ROC) curves showed that values of IL-1 [area under curve (AUC) = 0.718; 95% confidence interval (CI): 0.567–0.868; sensitivity 69.2%, specificity 62.9%] and IL-33 (AUC = 0.763; 95% CI: 0.614–0.911; sensitivity 84.6%, specificity 65.7%)], could be serve as possible markers for paraneoplastic thrombocytosis in CRC patients. **Conclusion.** IL-1 and IL-33 significantly correlated to high thrombocyte number in patients with more aggressive CRC.

Key words:
colorectal neoplasms; thrombocytosis; cytokines; interleukins.

Apstrakt

Uvod/Cilj. Reaktivna trombocitoza, kao paraneoplastični sindrom, često se sreće kod obolelih od karcinoma. Različiti humoralni faktori i citokini povezani sa tumorom doprinose povećanom stvaranju trombocita. Međutim, tačna uloga ovih citokina u patogenezi trombocitoze nije potpuno jasna. Cilj studije je bio da se analiziraju sistemske vrednosti citokina i kliničko-patološke karakteristike kod obolelih od kolorektalnog karcinoma (CRC) sa i bez trombocitoze. **Metode**. U istraživanje je bilo uključeno 59 bolesnika sa CRC, podeljenih u dve grupe u zavisnosti od broja trombocita. Analizirani su podaci o: starosti, polu, veličini tumora, lokalizaciji, metastazama, invaziji krvnih ili limfnih sudova, nuklearnom

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gradusu, stepenu histološke diferencijacije, tumor, nodus, metastaza (TNM) stadijumu i serumskim koncentracijama citokina [interleukina (IL)-1, IL-33, IL-12, IL-17 i interferona (IFN)-γ] kod obe grupe ispitanika. **Rezultati**. Oboleli od CRC sa trombocitozom imali su značajno veći nuklearni gradus karcinoma ($p = 0,002$); veći procenat detektabilnih metastatskih lezija u jetri ($p = 0,002$), plućima ($p = 0,001$), karcinomatoza peritoneuma ($p = 0,001$), detektibilnih invazija krvnih ($p = 0,012$) i limfnih sudova ($p = 0,010$). Takođe, kod obolelih od CRC sa trombocitozom zabeležene su veće koncentracije tumorskih markera [alfafetoproteina (AFP), karcinoembrionalnog antigena (CEA) i karcinomskog antigena 19-9 (CA 19-9)] i serumskih vrednosti IL-1 i IL-33. IL-1/IL-12 ($p = 0,016$), IL-1/IFN-γ ($p = 0,007$), IL-1/IL-17 ($p = 0,006$), IL-33/IL-12 ($p = 0,001$), IL-33/IFN-γ ($p = 0,001$), IL-33/IL-17 ($p = 0,002$), and IL-33/IL-1 ($p = 0,006$) odnosi bili su značajno veći kod obolelih od CRC sa trombocitozom u odnosu na obolele od CRC bez trombocitoze. Analiza *Receiver Operating Characteristic* (ROC) krivulje pokazuje da se IL-1 (AUC = 0,718; 95% Cl: 0,567–0,868); osetljivost 69,2%, specifičnost 62,9% i IL-33 (AUC = 0,763; 95% Cl: 0,614–0,911); osetljivost 84,6%, specifičnost 65,7%, mogu koristiti kao potencijalni markeri paraneoplastične trombocitoze kod obolelih od CRC. **Zaključak.** IL-1 i IL-33 značajno koreliraju sa brojem trombocita kod bolesnika sa agresivnijom formom kolorektalnog karcinoma.

Ključne reči:
kolorektalne neoplazme; trombocitoza; citokini; interleukini.

Introduction

Colorectal cancer (CRC) is among the leading causes of mortality and morbidity throughout the world [1]. Overall, CRC ranks third in terms of incidence but second in terms of mortality [2]. The incidence of CRC is increasing due to ageing and unhealthy life style [3]. Although the distribution of CRC varies widely (worldwide incidence and mortality, 10.2% and 9.2% of all cancers, respectively), more than two-thirds of all cases and more than half of all deaths happen in countries with high human development index (HDI) [2]. Consumption of red or processed meat, alcohol drinks, and body fatness frequently increase the risk of CRC, whereas physical activity is protective [4–6].

Thrombocytosis, a paraneoplastic syndrome, frequently accompanies cancer growth and metastatic dissemination, and is observed in as many as 10–57% of cancer patients [7, 8]. A pathogenic feedback loop may be operative between platelets and tumor cells, with reciprocal interactions between tumor growth/metastasis and thrombocytosis/platelet activation [7]. A variety of tumor-related humoral factors and cytokines such as granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GMCSF), thrombopoietin (TPO) [8], interleukins (IL)-1, IL-3, IL-6, IL-11 influence thrombopoiesis in cancer and contribute to tumor-stimulated thrombopoiesis [8, 9].

Several recent studies have reported that thrombocytosis may be associated with the poor prognosis of CRC [10]. Thrombocytosis is associated with shorter overall, disease-free and cancer-specific survival. Overall survival is reduced in patients with thrombocytosis regardless of their clinical tumor stage, and ethnicity. Also, thrombocytosis is significantly related to female patients, colon tumor location, T3–4 stage, lymph node positivity, metastasis, undifferentiated histology and lymphatic involvement [11, 12].

IL-33 and IL-1 family member play an essential role in the regulation of immune response after cellular stress or damage [13, 14]. A recent study revealed that IL-33 by biding to its receptor ST2 inhibits host anti-tumor immunity, remodels tumor stroma and enhances angiogenesis, thereby promoting the development of CRC [15]. Patients with metastatic CRC, with higher expression of IL-33 in cancer tissues were significantly associated with poorer survival [14, 16]. IL-1 is secreted by different cell types, such as myeloid cells. Previous studies confirmed the importance of IL-1 in different processes, such as tumorigenesis, invasiveness and progression of tumor cells, as well as invasiveness of CRC, activation or inhibition of anti-tumor immune response [17]. However, the exact role of these cytokines in the pathogenesis of reactive paraneoplastic thrombocytosis remains unclear.

The aim of this study was to analyze clinical-pathological characteristics of the disease and systemic values of IL-1 and IL-33, as well as their correlation with pro-inflammatory cytokines [IL-12, interferon (IFN)-γ and IL-17], in CRC patients with and without thrombocytosis.

Methods

Patients

Fifty nine CRC patients were involved in this study, after confirmed diagnosis of CRC by means of endoscopic and histopathological examination. Exclusion criteria for patients were: not well-defined pathology, inadequate clinical document available or diagnosed CRC, previously treated with chemotherapy or radiation, active bleeding. After being included, all patients signed informed consent. In the study, we recorded and analyzed data about: age, gender, size of the cancer, localization, metastasis, vascular or lymph vessel invasion and clinical tumor, nodus, metastasis (TNM) stage [17]. In addition, pathological features (nuclear grade and histological differentiation rate) were analyzed in accordance with the American Joint Committee on Cancer (AJCC, 2010) classification. Thrombocytosis was defined as platelet count more or equal than 450×10^9/L, similar to the related study [18].

Ethical approvals

Study was performed at the Center for Gastroenterology, Clinical Center of Kragujevac and Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia. The researches were allowed by both institutions by obtaining ethical approvals

and made according to the Declaration of Helsinki and the Principle of Good Clinical Practice.

Measurement of cytokine concentration in serum

One blood sample in the volume of 10 mL was taken preoperatively, before surgery, from each patient. Serum was separated by centrifugation and stored at -80 °C until testing. The test was performed using commercial sensitive enzyme-linked immunosorbent assay kits (ELISA, R&D Systems, Minneapolis, MN, USA) particularly for human cytokines, such as IL-1, IL-33, IL-12, IL-17 and IFN-γ. The micro titer plates (MTP), with 96-wells, were coated with 100 μL of capture antibody in carbonate/bicarbonate buffer (pH 9.6) at the recommended concentrations, overnight. MTP were washed with washing buffer (0.05% Tween-20 in phosphate buffered saline – PBS). Briefly, standard recombinant IL-1, IL-12, IL-17, IL-33, IFN-γ or serum samples were incubated in plates for 2 hours before adding 100 μL of detection antibody and incubating for 1 hour at room temperature covered with an adhesive sealing film. We washed MTP and added 100 μL of streptavidin peroxidase for 1 hour, and then 100 μL of substrate reagent (prepared by mixing equal volumes of Color A and Color B reagents) for 20 minutes. The reaction was stopped with 50 μL of stop solution (4 mol/L sulfuric acid) Using a microplate reader (Biochrom, Anthos Zenyth 200, UK), we read the absorbance at 450 nm. As stated in the instructions of the manufacturer, concentrations of cytokines were estimated by interpolation of a standard curve (made from a series of previously established concentrations of cytokine) and were presented as pg/mL of sera. All measurements were performed in duplicate and in accordance with the manufacturer's recommendations.

Statistical analysis

SPSS software version 20.0 was applied for statistical analyses. All values were reported as means ± standard error of the means (SEM). The Student's *t*-test, Mann-Whitney *U*-test or Kruskal-Wallis test were used in order to assess statistical significance of differences between the means of two groups. Associations between thrombocytosis and tumor characteristics were evaluated using the chi-square (χ^2) test. Possible correlation between the markers of interest and thrombocytosis in CRC patients were analyzed with the Pearson's or Spearman's tests, as appropriate, and were determined as weak, moderate or strong (0.1–0.3, 0.3–0.5, 0.5–1.0, respectively), positive or negative. *P*-value ≤ 0.05 was considered significant.

Results

Fifty nine adult patients (n = 59) with diagnosed CRC were recruited in this study. Patients with diagnosed CRC were divided into two groups according to the number of platelets. The first group includes 35 CRC patients with the number of platelets < 450 × 10^9/L (group without thrombocytosis), while the second group included 24 CRC patients with the number of platelets ≥ 450 × 10^9/L [18, 19] (group with thrombocytosis). Clinical and pathologic characteristics of these patients are presented in Table 1.

There were no differences in age or gender distribution between two groups. Concentrations of tumor markers, alpha fetoprotein (AFP), carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9) were significantly higher in CRC patients with thrombocytosis compared to CRC patients without thrombocytosis (Table 1).

Evaluation of nuclear grade of CRC was also made in patients with the presence or absence of thrombocytosis. Nuclear grade was based on evaluation of the size and shape of the nucleus in cells of colorectal cancer and the percentage of tumor cells that are in the process of dividing or growing [20]. Results revealed that CRC patients with thrombocytosis had significantly higher nuclear grade of colorectal cancer in comparison to patients with normal number of platelets (*p* = 0.002; Figure 1A). Next, CRC patients with/without thrombocytosis were analyzed regarded detection of lymph and blood vessels invasion. Significantly higher percentage of CRC patients with diagnosed thrombocytosis had detectable invasion of blood

Table 1

Demographic, clinical and pathological characteristics of patients with colorectal carcinoma (CRC)

Characteristics	CRC patients		*p*
	without thrombocytosis	with thrombocytosis	
Gender (male/female), n (%)	23/12 (65.71/34.29)	12/12 (50/50)	0.487
Age (years), mean (range)	64.82 (50–82)	65.14 (56–80)	0.938
Platelets (× 10^9/L), mean ± SD	307.14 ± 12.36	555.28 ± 27.13	0.001
Histological differentiation rate, (well/moderate), n (%)	10/25 (28.57/71.43)	8/16 (33.34/66.67)	0.568
AFP (ug/mL), mean ± SD	4.97 ± 5.56	402.83 ± 212.03	0.047
CEA (ug/mL), mean ± SD	16.89 ± 5.19	402.18 ± 178.54	0.002
CA 19-9 (U/mL), mean ± SD	12.71 ± 2.27	814.26 ± 306.96	0.001

AFP – alpha fetoprotein; CEA – carcinoembryonic antigen; CA – cancer antigen; SD – standard deviation.

and lymph vessels in comparison to CRC patients without thrombocytosis (p = 0.012 and p = 0.010; Figures 1B and 1C, respectively). CRC patients with thrombocytosis had advanced TNM stage (III or IV), while CRC patients without thrombocytosis mostly had TNM stage I or II, but this difference did not reach statistical significance (data not shown). Further, CRC patients with/without thrombocytosis were analyzed on the basis of detection metastatic lesions in the liver and lung as well as peritoneal carcinomatosis. Significantly higher percentage of CRC patients with thrombocytosis had detectable metastatic lesions in the liver and lung as well as peritoneal carcinomatosis, in comparison to CRC patients without thrombocytosis (p = 0.002, p = 0.001, and p = 0.001, respectively; Figures 1D, 1E, and 1F, respectively).

Serum concentrations of cytokines of interest were measured in CRC patients with/without thrombocytosis. Results revealed that CRC patients with diagnosed thrombocytosis had significantly higher serum concentration of IL-1 in comparison to CRC patients with normal number of platelets (p = 0.022) (Figure 2A). Serum levels of IL-33 were significantly increased in CRC patients with diagnosed thrombocytosis compared to CRC patients without thrombocytosis (p = 0.001) (Figure 2B). Moreover, strong positive correlation was detected between serum values of IL-1 and IL-33 (r = 0.879, p = 0.001) (Figure 2C).

Further, we analyzed ratios of IL-1 and IL-33 and different pro-inflammatory cytokines. IL-1/IL-12 (p = 0.016), IL-1/IFN-γ (p = 0.007) and IL-1/IL-17 (p = 0.006) ratios were significantly higher in CRC patients with thrombocytosis compared to CRC group of patients with normal number of platelets (Figures 3A, 3B, and 3C, respectively). CRC patients with thrombocytosis had significantly higher ratios of IL-33/IL-12 (p = 0.001), IL-33/IFN-γ (p = 0.001) as well as IL-33/IL-17 (p = 0.002), in comparison to CRC group of patients without thrombocytosis (Figures 3D, 3E, and 3F, respectively). Additionally, IL-33/IL-1 ratio was significantly increased in CRC patients with thrombocytosis compared to CRC group of patients with the normal number of platelets (p = 0.006) (Figure 3G).

Finally, we analyzed the sensitivity and specificity of IL-1 and IL-33 in order to confirm whether these cytokines could predict paraneoplastic thrombocytosis in CRC patients. Analysis of receiver operating characteristic (ROC) curves showed that IL-1 [area under curve (AUC) = 0.718; 95% confidence interval (CI): 0.567–0.868; sensitivity 69.2%, specificity 62.9%] and IL-33 (AUC = 0.763; 95% CI: 0.614–0.911; sensitivity 84.6%, specificity 65.7%), could serve as possible markers for diagnosing paraneoplastic thrombocytosis in CRC patients (Figures 4A an, 4B, rspectively). The optimal cut-off values estimated for detection of paraneoplastic thrombocytosis in

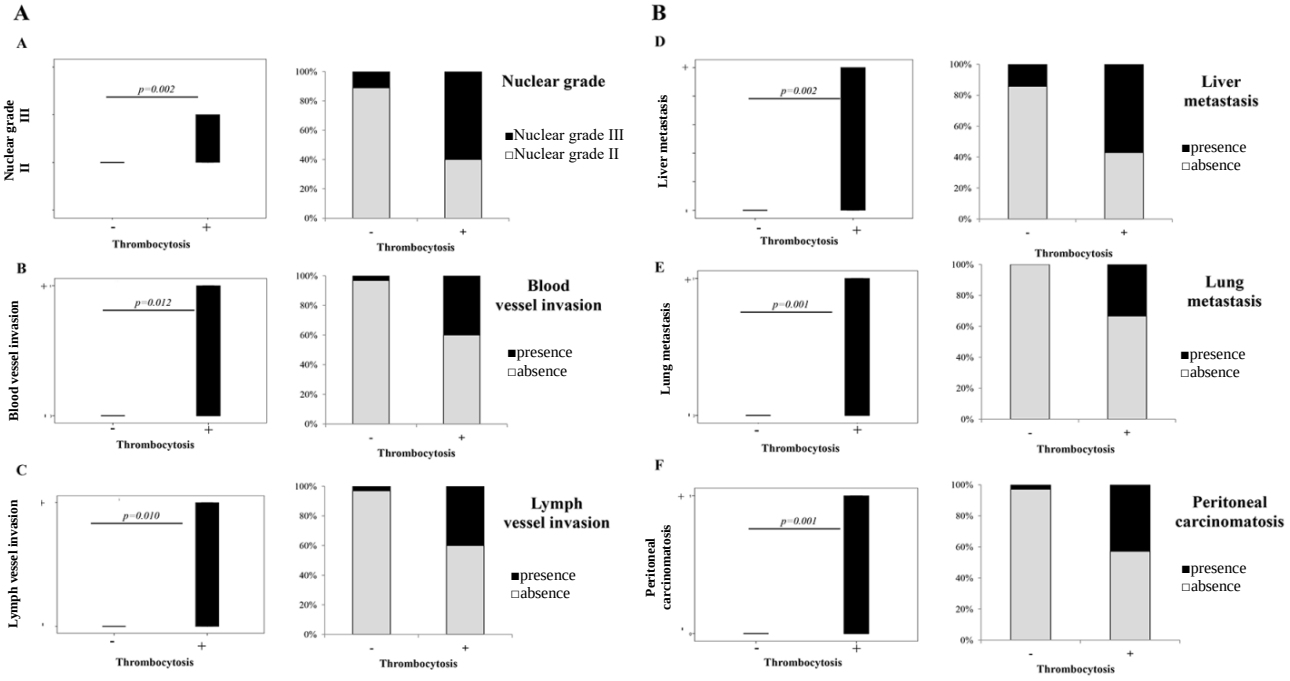

Fig. 1 – Colorectal cancer (CRC) patients with thrombocytosis have severe and advanced disease.

Panel A: Higher nuclear grade of CRC in patients with thrombocytosis in comparison to CRC patients without thrombocytosis (p = 0.002); Significantly higher percentage of CRC patients with diagnosed thrombocytosis had detectable invasion of blood (p = 0.012) and lymph vessels (p = 0.010) in comparison to CRC patients without thrombocytosis.

Panel B: Higher percentage of CRC patients with thrombocytosis had detectable metastatic lesions in the liver (p = 0.002) and the lung (p = 0.001) as well as peritoneal carcinomatosis (p = 0.001) in comparison to CRC patients without thrombocytosis.

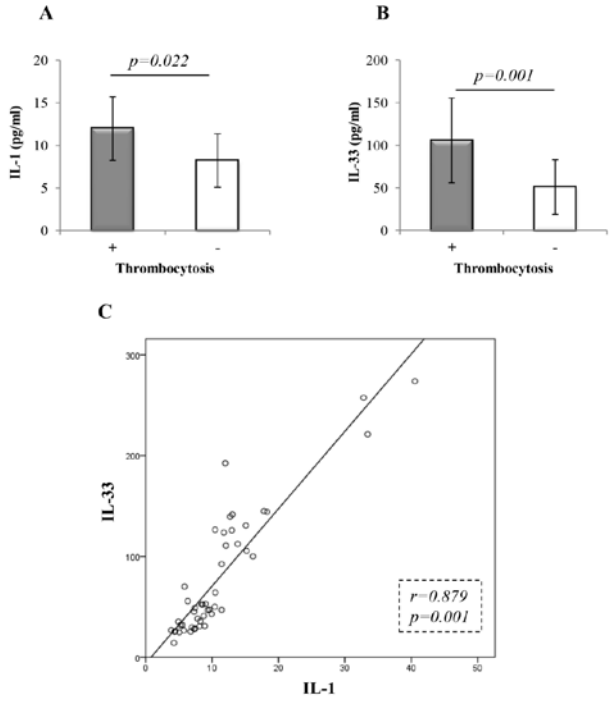

Fig. 2 – Increased serum values of interleukin (IL)-33 and IL-1 in colorectal carcinoma (CRC) patients with thrombocytosis.

(A) CRC patients with diagnosed thrombocytosis (+) had significantly higher serum concentration of IL-1 in comparison to CRC patients with normal number platelets count (–) (p = 0.022);

(B) Serum levels of IL-33 were also significantly increased in CRC patients with diagnosed thrombocytosis (+) compared to CRC patients without thrombocytosis (–) (p = 0.001);

(C) Strong positive correlation was detected between serum values of IL-1 and IL-33 (r = 0.879; p = 0.001).

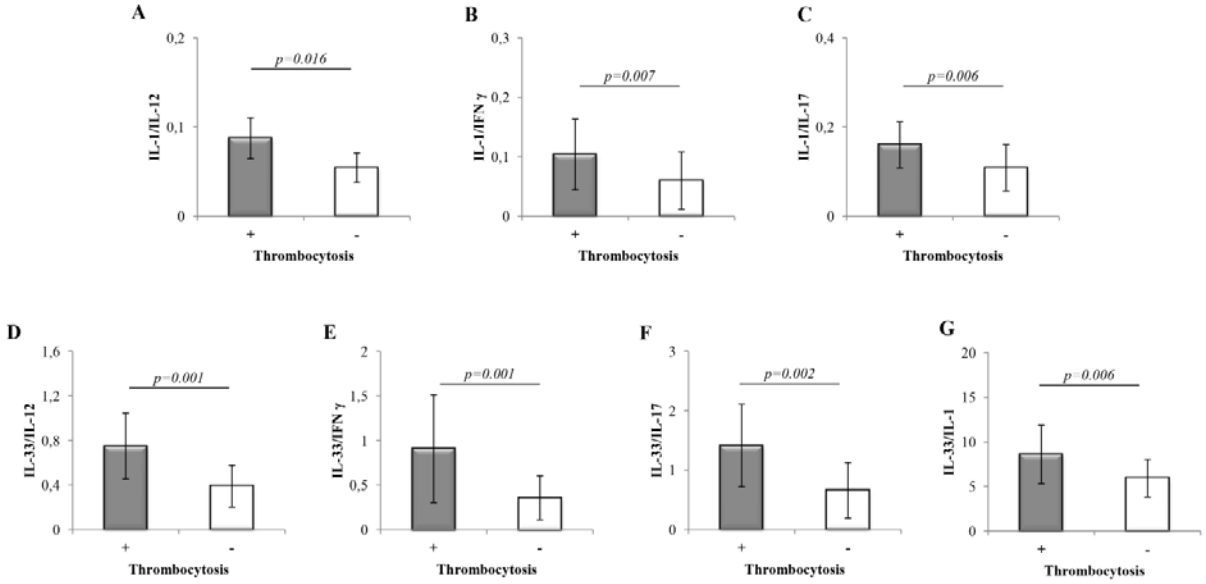

Fig. 3 – Predomination of interleukin (IL)-1 and IL-33 over IL-12, interferon (IFN)-γ and IL-17 in serum of colorectal carcinoma (CRC) patients with thrombocytosis.

CRC patients with thrombocytosis (+) compared to CRC group of patients with normal number of platelets (–) had significantly higher ratios of: (A) IL-1/IL-12 (p = 0.016); (B) IL-1/IFN-γ (p = 0.007); (C) IL-1/IL-17 (p = 0.006). CRC patients with thrombocytosis (+) in comparison to CRC group of patients without thrombocytosis (–) had significantly higher ratios of: (D) IL-33/IL-12 (p = 0.001); (E) IL-33/IFN-γ (p = 0.001); (F) IL-33/IL-17 (p = 0.002). (G) IL-33/IL-1 ratio was significantly increased in CRC patients with thrombocytosis (+) compared to CRC patients without thrombocytosis (–) (p = 0.006).

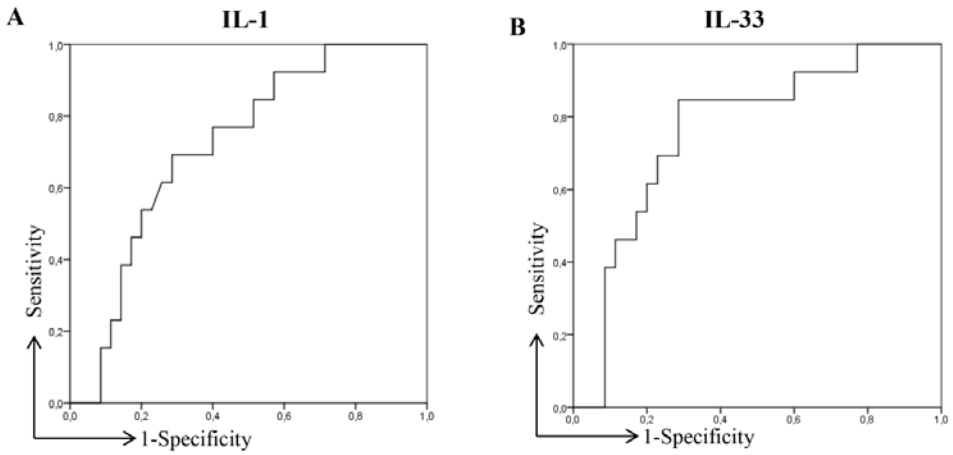

Fig. 4 – Receiver operating characteristic (ROC) curves analyses of serum interleukin (IL)-1 and IL-33 levels for prediction of paraneoplastic thrombocytosis in colorectal carcinoma (CRC) patients.
Sensitivity and specificity of IL-1 and IL-33 as possible markers for conformation of paraneoplastic thrombocytosis in CRC patients: Analysis of ROC curves illustrate sensitivity and specificity for (A) IL-1 [area under curve (AUC) = 0.718; 95% confidence interval (CI): 0.567–0.868, sensitivity 69.2%; specificity 62.9%], and (B) IL-33 (AUC = 0.763; 95% CI: 0.614–0.911; sensitivity 84.6%, specificity 65.7%).

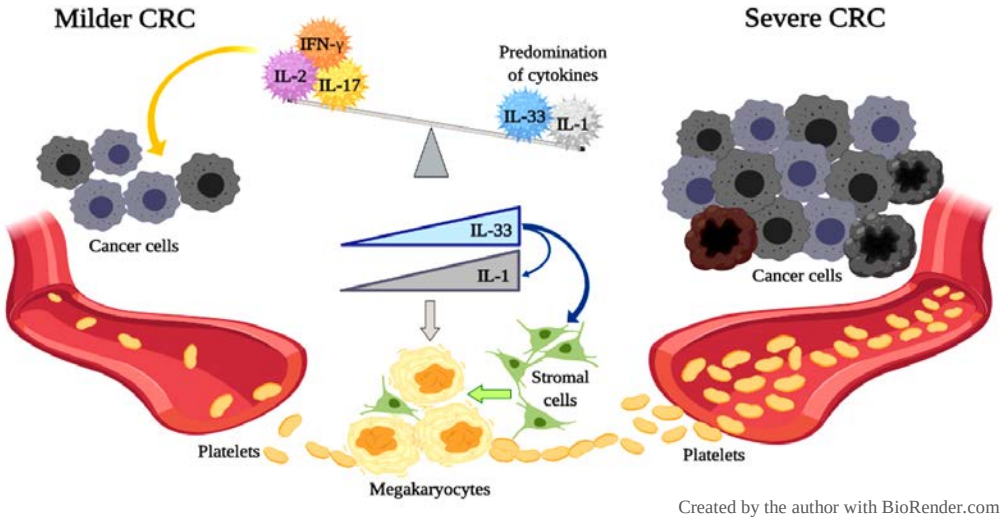

Fig. 5 – Potential united effect of interleukin (IL)-1 and IL-33 in pathogenesis of thrombocytosis in colorectal carcinoma (CRC) patients.
Schematic diagram showing direct stimulative effect of IL-1 on megakaryocytes, as well as, indirect stimulative effect of IL-33 on megakaryocytes through the stromal cells in bone marrow. Both cytokines (IL-1 and IL-33) play a potential simultaneous role in pathogenesis of thrombocytosis in CRC patients. Moreover, it is shown predomination of IL-1 and IL-33 over pro-inflammatory cytokines [IL-12, IL-17 and interferon (IFN)-γ] in severe form of CRC.

CRC patients were 9.47 pg/mL for IL-1 and 49.5 pg/mL for IL-33.

Potential united effect of IL-1 and IL-33 in pathogenesis of thrombocytosis in CRC patients is given in Figure 5.

Discussion

In the present study, we showed that CRC patients with diagnosed thrombocytosis had significantly higher nuclear grade compared to CRC patients with normal number of platelets. Significantly higher percentage of CRC patients with thrombocytosis had detectable metastatic lesions in the lung and liver and peritoneal carcinomatosis, as well as blood and lymph vessels invasion compared to CRC patients without thrombocytosis. Higher concentrations of tumor markers, AFP, CEA, CA 19-9, were detected in CRC patients with diagnosed thrombocytosis. In line with the revelation of previous studies that increased concentrations of tumor markers in patients with CRC indicate on more aggressive type of cancer and can be treated as poor prognostic factor, presented data indicate on more severe form of CRC in patients with thrombocytosis [21].

Reactive thrombocytosis is an elevated platelet count ($\geq 450 \times 10^9$/L) develops secondary to another disorder [19]. Previous studies investigated relation between cancer and thrombocytosis [10–12, 20–24]. Sasaki et al. [22] have shown that cancer-specific survival of CRC patients with thrombocyto-

sis was significantly shorter compared to CRC patients without diagnosed thrombocytosis. Moreover, thrombocytosis indicates adverse prognosis in CRC and also may serve as clinically useful, cost-effective, noninvasive marker to facilitate risk assessment and guide postoperative management [11, 12]. The platelet count is also treated as valuable prognostic marker for the survival in patients with metastasis of colorectal cancer [23]. Furthermore, pretreatment hematologic abnormalities, such as anemia and thrombocytosis, can also be considered as useful prognostic markers in patients with colorectal cancer [24]. In line with these studies, the presented data implicate on severe and more progressive form of CRC in patients with diagnosed thrombocytosis.

Several factors such as iron deficiency, acute infection and chronic inflammatory disorders can be causes of reactive thrombocytosis [19]. Lately, cancer is more often associated with paraneoplastic thrombocytosis [7]. A variety of tumor-related humoral factors and cytokines influence thrombopoiesis in patients with tumor and contribute to tumor-stimulated thrombopoiesis [8, 9]. In order to investigate the potential role of soluble molecules on thrombocytosis development, we further analyzed systemic concentrations of cytokines of interest. IL-1 is potent cytokine involved in variety of pro-inflammatory processes, but also in tumorigenesis and tumor progression [25]. Besides direct stimulating effect on tumor cell proliferation, it has also been shown that colon cancer cell–derived IL-1α may upregulate angiogenesis by modulating stromal cells within the tumor microenvironment [15]. In this study, significantly higher systemic level of IL-1 was measured in CRC patients with diagnosed thrombocytosis compared to CRC patients with normal number of platelets. Previous studies investigate the importance of IL-1 in thrombocytopoiesis [26–28]. Yang et al. [26] revealed a stimulative effect of IL-1β on megakaryocyte colony forming units production. Moreover, they confirmed that megakaryocytic cells have IL-1 receptors on their surface. The other study has shown that a single dose of IL-1 was able to stimulate an increase in platelet production for 3 weeks [27]. Also, administration of recombinant human IL-1β to C57B1/6 male mice consequently induced a remarkable thrombocytosis, about 2.3 times higher in IL-1β treated mice than in control mice [28]. These results are in line with our finding, suggesting an important role of IL-1 in thrombocytosis development.

IL-33, member of IL-1 family is mostly expressed by mucosal epithelial cells [29]. Previous studies confirmed important role of IL-33 in CRC pathogenesis [13, 14]. It is known that IL-33 activates core stem cell genes, recruits macrophages into the cancer microenvironment and stimulates them to produce prostaglandin E2, which all promote carcinogenesis of CRC and metastasis of cancers [16, 30]. To our knowledge, this is the first study describing significant increment of IL-33 in sera of CRC patients with diagnosed thrombocytosis compared to CRC patients without thrombocytosis. In line with our result are different animal and clinical studies indicating the importance of IL-33 in pathogenesis of thrombocytosis. Talabot-Ayer et al. [31] described that CMV/IL33 mice with IL-33 overexpression, characterized as increased local or systemic levels of pro–inflammatory mediators such as IL-1b, C-X-C Motof Chemokine hig and 1 (Cxcl-1), granulocyte colony – stimulating factor (G-CSF), and IL-6, also suffer from anemia, thrombocytosis, and dysregulation of myelopoiesis [31]. The other study has shown that ST2, membrane receptor for IL-33 is expressed in bone marrow mainly on endothelial, mesenchymal, and early myeloid cells. Activation of IL-33/ST2 signaling pathway on these cells stimulates the production of different soluble molecules that further promote development and proliferation of myeloid cells [32]. Previous studies have confirmed that thrombocytosis correlates with a shorter overall survival (OS) and poorer disease free survival (DFS) [33, 34]. Except platelet number, other platelet-associated indicators, such as plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW), may also correlate with poorer OS of CRC patient and can be used as potentional prognostic factors [35]. To our knowledge, some studies revealed that development and progression of CRC is associated with alterations in serum IL-1 level but its significance is not well defined [36, 37], while the significance of serum IL-33 as marker of CRC survival has not been explored yet.

Moreover, our study showed predomination of IL-1 and IL-33 over pro-inflammatory cytokines IL-12, IFN-γ and IL-17, known for their crucial role in antitumor immune response [38]. Interesting fact is that IL-33 also predominates over IL-1 in sera of CRC patients with thrombocytosis. Predomination of IL-1 and IL-33 over mediators of potent antitumor immunity in patients with thrombocytosis can partially explain more severe and progressive disease diagnosed in these patients.

Strong positive correlation detected between serum values of IL-1 and IL-33 indicated a potentially united effect of these cytokines in pathogenesis of thrombocytosis in CRC patients. This simultaneous effect can be realized in at least two manners: 1) IL-1 may directly affect megakaryocytes and stimulate production of platelets; 2) IL-33 may indirectly stimulate stromal cells in the bone marrow to produce mediators that can further stimulate thrombopoiesis in CRC patients.

This proposed mechanism was supported by analysis of ROC curves of IL-33 and IL-1 that revealed that these cytokines could be used as possible markers of paraneoplastic thrombocytosis in CRC patients.

Conclusion

Presented data revealed that IL-1 and IL-33 significantly correlated to high thrombocyte number in patients with more aggressive CRC.

Conflict of interest

The authors declare that they have no competing interests.

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