Evaluation of PDGF-AB and sP-selectin concentrations in relation to platelet count in patients with colorectal cancer before and after surgical treatment

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Abstract: **Introduction.** Platelet-derived growth factor (PDGF) and P-selectin, the low-molecular weight proteins located mainly in the platelet α-granules, are considered to be biologically active markers of platelet (PLT) activation. **Objectives.** The study objective was to assess levels of PDGF-AB and sP-selectin in relation to PLT blood count in patients with colorectal cancer (CRC) who were examined before and after radical surgical treatment of the cancer. **Patients and methods.** The study involved 38 CRC patients including B1 – 20 patients ($T_{2-3}N_1M_0$), B2 –18 patients ($T_{2-3}N_2M_0$) and 24 age and sex-matched healthy subjects (the control group). Blood samples were collected from the antecubital vein prior to and 3 months after the radical surgery. PDGF-AB and souble (s)P-selectin, the markers of PLT activation, were determined by the immunoenzymatic methods. **Results.** In CRC patients, the levels of PDGF-AB and sP-selectin were a few times higher, whereas the PLT count was lower as compared to the control group. Moreover, these levels were statistically much higher before, compared to those after the surgery, in patients with a higher grade of clinical and histological differentiation ($p < 0.05$) as well. However, no positive correlation was found between the PLT count and the PDGF-AB and sP-selectin levels. **Conclusions.** High levels of PDGF-AB and sP-selectin, the sensitive markers of PLT activation prior to surgical treatment seem to indicate cancer tissue as the source of both PDGF and sP-selectin. For this reason, PDGF-AB and sP-selectin determination may help in early non-invasive CRC evaluation in the future.

Key words: colorectal cancer, PDGF-AB, sP-selectin

INTRODUCTION

Interaction between the cancer cells and platelets (PLT) has been analyzed for many years; however not all the mechanisms are well known. It is assumed that PLT take part in cancer development, by their participation in neoangiogenesis and distant metastasis formation [1,2]. On the other hand, cancer cells from cancer procoagulant secretion induce PLT activation, resulting in excessive adhesion and aggregation (tumour cell induced platelets aggregation). Activated PLT are a cause of thrombotic disorders, not only in cancer tissue, but also in the tumor stroma. Thrombotic episodes favor the release of the mitogenic growth factor platelet-derived growth factor (PDGF) and P-selectin (CD62P) from the platelet α-granules, small molecules recognized as PLT activation markers [1,3].

Platelet-derived growth factor is a non-homogeneous molecule and comprises proteins forming 4 types of chains: PDGF-A, PDGF-B, PDGF-C and PDGF-D, observed in an active form as the dimers. The first two isoforms can form both homo- and heterodimers; however PDGF-C and PDGF-D chains can form homodimers only [4-6]. The most commonly assayed form in blood serum is PDGF-AB. It has been proven that the platelet-derived growth factor is a strong mitogen for cancer cells. Interaction of PDGF on the cells is executed by two structurally similar receptors, α and β [7-9]. Both receptors are tyrosine kinases of different affinity to PDGF [10-12].

P-selectin is a cellular adhesive molecule, located on cellular membrane of PLT as the CD62P receptor, and in blood serum as soluble form (sP-selectin). Both forms take part in PLT ad-

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hesion to the leukocytes, and sP-selectin blood concentration increases in inflammatory and carcinogenic processes [13,14]. PDGF-AB and P-selectin are the PLT activation markers, whose role in cancer development is widely discussed [15-17]. However, the scarce data on the dynamics of PDGF-AB and P-selectin level changes in patients with cancer promoted us to undertake our study.

The aim of our study was to evaluate PDGF-AB and P-selectin concentrations as biologically active PLT activation markers, which are of crucial importance in cancer development. Because colorectal cancer (CRC) is one of the most frequently observed cancers in countries with a high standard of living, such as Poland, patients with CRC and variable involvement of regional lymph nodes with no evidence of distant metastasis were included in the study. The impact of surgical treatment on CRC on PDGF-AB and sP-selectin levels was also evaluated.

PATIENTS AND METHODS

Thirty-eight patients with CRC (20 women, 18 men, aged 48–69 years; average age 62.4) and 24 healthy individuals (10 women, 14 men, aged 46–65 years; average age 60.8) were analyzed. The histological CRC classification according to WHO was *Adenocarcinoma*, a macroscopic classification: *polyp-like, localised low in retroperitoneal part of the rectum*. Patients were treated surgically with complete intestine amputation using the abdominal-perineal method. Diagnostic tests were performed before the operation in all patients outside of clinical research: ultrasound examinations of the abdominal cavity and colonoscopy with specimen collection and histological evaluation. Patients were divided into two groups, according to their clinical-pathomorphological TNM classification [18]; the B1 group consisted of 20 patients ($T_{2\text{-}3}N_1M_0$ i.e. metastasis was found in 1, 2 or 3 regional lymph nodes), and the B2 group of 18 patients ($T_{2\text{-}3}N_2M_0$ – metastasis in 4 or more regional lymph nodes).

All patients obtained standard antithrombotic prophylaxis in the form of low-molecular-weight heparin after the operation. The results of basic laboratory tests such as glucose, urea and creatinine were within the norms. Patients with renal failure, diabetes and circulatory system diseases were excluded from the study. Patients did not obtain antiaggregation and antiplatelets medications and were not treated with aminoglycosides for 7 days prior to blood sampling. None of the patients prior to and 3 months after the operation underwent chemo- or radiotherapy. Moreover, habitual smokers were excluded from the study and control group.

Material for the analysis, venal blood, was sampled twice; two days prior to and 3 months after the operation. The PLT count was established with haematological analyser and PLT activation markers were established by ELISA method (in duplicates) with the ELISA-Kit Quantikine Immunoassay R&D reagents sets (PDGF-AB cat. no DHDOOB; sP-selectin cat. no BBE6). The reagents sets were destined for scientific studies performed in cellular cultures, blood serum and plasma. Permission was obtained from the Bioethical Committee to perform the study (R-I-003/325/2004).

Results of the study were presented as average ± standard deviation values. The occurrence of the normal distribution of the analyzed data with the Shapiro-Wilk test and classic χ^2 test were evaluated. Taking into consideration that the number of patients was below 40, the hypothesis of the identity of the 2 averages was suggested, which was verified by the classic non-parametric Mann-Whitney U test. The results were analyzed with Spearmann's correlation. A statistical analysis was performed with the STATISTICA 6.0 program. Statistically significant differences were considered for $p < 0.05$.

RESULTS

In all patients with CRC before the operation the blood concentration of PDGF-AB was statistically significantly higher than in the control group (Tab. 1). The average PDGF-AB concentration in the control group was 34.3 ng/ml. In the B1 group the concentration was about 6 times higher and reached 208.1 ng/ml, but in the B2 group it was about 11 times larger compared to control group, reach-

Table 1. PDGF-AB and sP-selectin blood concentrations in CRC patients compared to the control group

Analyzed parameter	Time of blood sampling	The whole group (B) n = 38	Control group (K) n = 24	p
		x ±SD	x ±SD	
PDGF-AB (ng/ml)	Before operation	293.5 ±61.5	34.3 ±9.2	B vs. K**
	After operation	161.6 ±39.8	34.3 ±9.2	B vs. K**
sP-selectin (ng/ml)	Before operation	177.3 ±36.2	20.1 ±3.9	B vs. K**
	After operation	127.9 ±27.8	20.1 ±3.9	B vs. K*

*p <0.01, **p <0.001

B – the whole analyzed group – ($T_{2\text{-}3}N_{1\text{-}2}M_0$)

Abbreviations: CRC – colorectal cancer, PDGF-AB – platelet-derived growth factor, SD – standard deviation

Table 2. PDGF-AB and sP-selectin blood concentrations in CRC patients in relation to TNM classification

Analyzed parameter	Time of blood sampling	Analyzed group		p
		B1 (n = 20)	B2 (n = 18)	
		x ±SD	x ±SD	
PDGF-AB (ng/ml)	Before operation	208.1 ±82.4	379.0 ±121.0	B1 vs. B2*
	After operation	78.9 ±30.4	244.3 ±69.8	B1 vs. B2**
sP-selectin (ng/ml)	Before operation	111.6 ±27.6	243.0 ±57.1	B1 vs. B2**
	After operation	91.3 ±25.8	164.0 ±29.8	B1 vs. B2*

*p < 0.05, **p<0.01
Analyzed group (B1 – $T_{2\text{-}3}N_1M_0$) (B2 – $T_{2\text{-}3}N_2M_0$)
Abbreviations – see Table 1

ing 379.0 ng/ml (Tab. 2). During the analysis of PDGF concentration in both groups it was observed that the average PDGF-AB concentration prior to the operation was higher in the B2 group than the B1 group. Comparing the results of PDGF concentration prior to and 3 months after the operation, a significant decrease in PDGF-AB concentration in both analyzed groups was observed; however, the decrease in patients with a lower clinical grade was more significant and in the B1 group was equal to 78.9 ng/ml, while in the B2 group the concentration was a few times higher – 244.3 ng/ml (Tab. 2). PDGF-AB level dependence on age and sex were not observed.

Analysis of sP-selectin concentrations in CRC patients showed that they were statistically significantly higher than in the control group (Tab. 1). In the B1 group the average sP-selectin value was 111.6 ng/ml before the surgery, and in the B2 group – 243.0 ng/ml; however, in the control group of healthy people it was only 20,1 ng/ml. Thus, the average sP-selectin concentration before the surgery in the B1 group was about five times, and in the B2 group over ten times higher than in the control group (Tab. 1 and 2). Comparing average sP-selectin concentration in both analyzed groups before the procedure, higher values were observed in the B2 group (Tab. 2), but after the surgery, the concentration of this cytokine decreased (Tab. 2). Moreover, sP-selectin concentration, like PDGF-AB concentration, was independent of age and sex.

The analysis confirmed higher PLT count in CRC patients before the treatment in the B1 group, and lower in the B2 group than in the control group p <0.05 (Tab. 3). A decrease in the PLT count to the value of 246.52 × 10^3/µl was observed in the B1 group three months after the radical CRC excision, but in the B2 group no significant change was observed (Tab. 3).

DISCUSSION

Results of the study show that not only CRC presence, but also the grade of clinical advance have significant impact on PDGF-AB and sP-selectin levels. However, with complete CRC amputation the abdominal-perineal method caused a decrease in PDGF-AB and sP-selectin concentration, even though the level was significantly higher than in the control group, which might be evidence of autocrine and paracrine secretion of both analyzed parameters by CRC and PLT, which take part in thrombotic events in cancer tissue.

Table 3. Blood platelet (PLT) count in patients prior to and after radical CRC excision compared to the control group

Analyzed parameter	Time of blood sampling	Analyzed group		Control group	p
		B1 (n = 20)	B2 (n = 18)	K (n = 20)	
		x ±SD	x ±SD	x ±SD	
Blood PLT count PLT × 10^3/µl	Prior to operation	280.70 ±49.52	195. 73 ±22.16	221.24 ±24.06	B1 vs. B2***, B1 vs. K***, B2 vs. K**
	After operation	246.52 ±38.45	193.07 ±34.39	221.24 ±24.06	B1 vs. B2*, B1 vs. K*, B2 vs. K**

*p <0.05, **p <0.01, ***p <0.001
Analyzed B1 group – $T_{2\text{-}3}N_1M_0$
Analyzed B2 group – $T_{2\text{-}3}N_2M_0$
Abbreviations – see Table 1

PDGF-AB and sP-selectin are considered sensitive markers of PLT activation, which plays an important role in cancer development. PDGF-AB is an important factor regulating the migration and proliferation of many cells, including CRC cells [19]. The interaction of PDGF on the cells is caused by two structurally similar receptors – α and β. Both receptors are tyrosine kinases, which have variable affinities to PDGF-AB, a strong mitogen for cancer cells [8]. PDGF is not observed in blood plasma; it is, however, released by PLT to blood serum during thrombotic disorders, observed in cancer tissue and the tumour stroma, a source rich in CP (cancer procoagulant) [20]. The results of our study seem to confirm the hypothesis that cancer tissue is, beside PLT, a PDGF-AB source too; PDGF-AB concentration in all CRC patients was statistically significantly higher compared to the control group. Similar results were also obtained by Filiberti et al. [21], who presented about two-fold higher PDGF concentration in an analyzed group (45.8 ng/ml) compared to the control group (26.8 ng/ml) in a study on lung cancer.

The analysis of the impact of the surgical excision of cancer lesions on PDGF-AB levels showed about half the concentration after the procedure. Such results indicate the favourable impact of radical CRC excision on PDGF concentration decreases. Such a phenomenon can be explained by the removal of cancer tissue in places of PLT activation, a rich source of PDGF.

Yu et al. [22] showed that PDGF correlates with cancer grading and plays an important role in metastasis formation. We obtained similar results in our own studies. Comparing PDGF-AB concentrations as dependent on clinical-pathomorphological TNM classification, we observed higher PDGF concentrations in patients with higher grading, i.e. with metastases in larger numbers of regional lymph nodes. Thus, the results of our studies are similar to results obtained by other authors [22], indicating strong PDGF-AB impact on CRC cells, from which it is subsequently secreted to the blood stream. Analysis of PDGF-AB blood concentration allows us to trace the dynamics of how benign changes become malignant changes. Moreover, the advantage of tracing the changes in dynamics of PDGF-AB blood concentration is the observation that venal blood is a biological material easier to obtain than biopunctates or tumour specimens, and its sampling is less invasive.

During PLT activation, the fusion of α-granules with the cellular membrane of platelets was observed. As a result, surface P-selectin is expressed in the form of membrane receptor CD62P. However, extracellular domain of this receptor can be released to the blood, where it is observed in the form of a soluble fragment (soluble P-selectin). According to Małyszko et al. [23]. PLT-related thrombotic diseases in kidney diseases are related to a massive expression of P-selectin; our previous study on renal cancer showed, however, that both forms of P-selectin (both CD62P receptor and sP-selectin) interact with cancer cells by sialoglycoprotein, a specific ligand for P-selectin [13]. Integrin $\alpha_{IIb}\beta_3$ receptors for fibrinogen are responsible for platelet-platelet interaction, but E-selectin takes part in platelet-endothelium interaction. As a result of the interaction of PLT with cancer cells, the tumour is surrounded by activated PLT, which releases many compounds, including the previously discussed PDGF-AB.

In own studies, a higher sP-selectin concentration than in the control group was observed in all CRC patients, both before and after the operation. Similar results were presented by Dymicka-Piekarska et al. [24], who showed higher sP-selectin concentrations in their analyzed groups (74.22 ng/ml and 70.33 ng/ml) of CRC patients in comparison to their control groups (41.01 ng/ml). Each neoplastic process is accompanied by intensified granulocytes and macrophages migration to the tumour stroma whic causes leukocyte infiltration in pericancer tissue. P-selectin is responsible for mutual platelet-leukocyte interaction [25]. This fact can additionally explain the higher sP-selectin concentration in the blood serum of the CRC patients. An analysis of the influence of clinical-pathomorphological grades on sP-selectin levels showed higher concentrations in patients with higher clinical grades of tumours, i.e. with larger numbers of lymph nodes involved. As a result, it can be assumed that patients without metastasis to the lymphatic system may present other results, but this was beyond the scape of this study.

Analysis of PLT count with reference to the parameters did not reveal a positive correlation between PLT count and PDGF-AB and sP-selectin concentration as markers of PLT activation. However, lower PLT count in patients at more advanced stages may confirm our earlier observation that cancer tissue, in addition to PLT, can be a PDGF-AB and sP-selectin source as well.

Because CRC is situated second with regard to frequency of malignant tumors in Poland, more refined and sensitive biochemical markers and less invasive methods of early CRC diagnostics are being investigated. The results of our study show that establishing PDGF-AB and sP-selectin in the patient blood is of diagnostic importance. Colorectal cancer recurs with high frequency. For this reason a high concentration of both analyzed parameters might be an important indicator of the recurrence of the disease. On the other hand, analysis of the dynamics of changes in PDGF-AB and sP-selectin levels in time can play an important role in evaluating the transformation of benign lesions into malignant lesions. Supression of PDGF-AB and sP-selectin activity by blocking receptors for these factors will probably be new therapeutic strategy in the future; however further studies are necessary to implement these solutions.

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