Changes in PDGF concentration in surgically treated colorectal carcinoma

Mantur M1*, Snarska J2, Sidorska A3, Ostrowska H4, Kruszewska-Wnorowska K1, Wojszel J1

1 Department of Clinical Laboratory Diagnostics, Medical University of Bialystok
2 Department of General Surgery and Endocrinology, Medical University of Bialystok
3 Department of General Surgery Sniadecki’s Hospital of Bialystok
4 Department of Biology, Medical University of Bialystok

ABSTRACT

Purpose: The aim of the present study was to assess the effect of tumor advancement, differentiation grade and surgery treatment on PDGF-AB level and platelet (PLT) count depending on the site of blood collection.

Material and methods: The study included 38 patients submitted to surgical treatment due to diagnosis of colorectal cancer (CRC) without remote metastasis: G2- 20 patients and group G3- 18 patients. The control group consisted of 24 healthy subjects. In CRC patients the blood samples was collected three times: 1) before surgery, 2) intrasurgically and 3) 90 days after surgery. Serum PDGF-AB concentration was determined by ELISA-Kit reagents.

Results: PDGF concentration in all the patients was several times higher than in the control group, irrespective of tumor differentiation grade and the site of blood collection. However the level of PDGF-AB in intraoperatively collected arterial blood and venous blood in group G3 (arterial blood- 379.9±12.1ng/ml; venous blood- 398.4±13.2 ng/ml) was significantly higher than in group G2 (arterial blood- 169.4 ±88.6 ng/ml; venous blood- 194.2±84.0 ng/ml). No significant differences were observed between venous and arterial blood. No correlation was found between the PLT count and PDGF-AB concentration.

Conclusion: High blood PDGF-AB concentration in CRC patients but no significant positive correlation observed between the PLT count and PDGF-AB concentration suggests its neoplastic origin beside PLT. Determination of this factor in blood serum may have an important implication in early diagnosis of CRC, which is the second most common malignant neoplasm with high recurrence rates.

Key words: colorectal carcinoma (CRC), PDGF-AB (platelet-derived growth factor-AB), platelets (PLT)

INTRODUCTION

The platelet-derived growth factor (PDGF), a micromolecular protein secreted from numerous cells, is found in cells and blood serum but not in plasma [1]. It was first identified and described as a mitogenic factor secreted from the alpha granules of platelets during blood coagulation [2,3]. Subsequent studies have shown its presence in vascular endothelial cells, fibroblasts, smooth muscle cells and in great amounts in tumor tissue [4-7]. Histopathological studies have revealed that tumor cells and fibroblasts in the tumor stroma resistant to PDGF action increase the autocrine production of this factor [8-9].

PDGF is not a single molecule, but constitutes a group of proteins that build up 4 types of chains, i.e. PDGF-A, PDGF-B, PDGF-C and PDGF-D, which in the active form occur as dimers. The PDGF-A and PDGF-B chains may give rise to 3 isoforms that form homodimers AA and BB or heterodimer AB, differently expressed in various cell types. The homodimers CC and DD originate from PDGF-C and PDGF-D chains [8,10-12].

The biological role of PDGF involves the regulation of cell growth and differentiation, and is associated with its chemotactic properties toward neutrophils and monocytes. PDGF participates in angiogenesis and neoangiogenesis, and in wound healing it stimulates the synthesis of collagen I and III and glycosaminoglycans [1].
PDGF exerts its effect on cells through two structurally related receptors α and β [13,14]. Fibroblasts and smooth muscle cells possess the two receptors, blood platelets have only one receptor α and macrophages only one receptor β [15]. The receptors are tyrosine kinases showing different affinity for PDGF. The receptor α (170 kDa) binds all PDGF isoforms while the receptor β (180 kDa) binds only isoforms containing the PDGF-B chain. Interesting is the fact that different cells show different expression of these two receptors, depending on their activation and proliferation. For instance, the number of β receptors on connective tissue cells is small, but increases during the inflammatory process and neoplastic transformation [8,16].

Colorectal carcinoma (CRC) is one of the most common malignancies in Poland, with the second highest incidence irrespective of age. The prevalence rate increases with age, particularly over the age of 50. The risk of recurrence of the so called metachronous adenocarcinoma (even after 10 years) refers to approximately 4% of patients considered surgically cured [1]. As the progression of benign lesions to malignant tumors is a long process (approximately 10 years) there is a constant search for noninvasive diagnostic methods that would facilitate monitoring of its dynamics. This would allow implementation of pharmacological therapy to delay neoplastic progression whenever surgical treatment cannot be applied. According to literature survey, PDGF is an early marker of colorectal carcinoma growth. As reported by some researchers, an increased PDGF level stimulates both proliferation and dissemination of tumor cells [12].

The lack of unequivocal data evaluating blood serum PDGF levels before and after surgery inspired us to undertake further investigations in this field in relation to differentiation malignancy grade.

The aim of the present study was to assess the surgery treatment and the effect of tumor advancement, malignancy grade on the PDGF level and platelet count depending on the site of blood collection.

### MATERIALS AND METHODS

The protocol was approved by the Medical University of Bialystok Bioethics Commititee, according to Guidelines for Good Clinical Practice (GCP) (R-I-003/325/2004). Only patients who gave their informed written consent were enrolled in the study.

The study included 38 colorectal cancer patients (CRC) without remote metastasis (20 men and 18 women; aged 42 - 68 years) diagnosed and operated in the Department of General Surgery at the J. Sniadecki Hospital in Bialystok and 24 healthy subjects (control group) (10 men and 14 women; aged 40 - 64 years).

The diagnosis of colorectal cancer was based on clinical symptoms, abdominal ultrasound scanning, and colonoscopy. The patients undergone surgical procedure of tumor resection with subsequent regional lymphadenectomy. During surgery, samples (primary tumor, lymph node involvement) were collected and neoplastic lesions were histopathologically verified. All patients had CRC. None of the patients received chemotherapy or radiation therapy before and post surgical intervention.

The colorectal cancer patients qualified for our study did not have any other ailment that could affect the immune response. Those patients who had a recent inflammatory or any acute infection were excluded from this study. Clinical advancement was evaluated based on TNM classification by Hutter and Sobin [17], while tumor type and malignancy grade were determined by histopathological analysis.

CRS patients were divided into two groups: Group G2 - 20 patients (T2, N1, M0, malignancy differentiation grade G2) and Group G3 - 18 patients (T2, N1, M0, malignancy differentiation grade G3). The control group consisted of 24 healthy subjects.

Blood collected in clot tubes was the material used for determination of PDGF-AB and in EDTA-K2 tubes – to assess the PLT count. Blood samples were collected three times: 1) prior to surgery (peripheral blood), 2) intrasurgically (from the afferent and efferent vessels within the region of tumor mass) and 3) 3 months after surgery (peripheral blood from the ulnar vein).

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**Table 1. PDGF (ng/ml) levels in colorectal adenocarcinoma patients; before - before resection of colorectal adenocarcinoma, after 3 months after surgery, in intraoperatively collected arterial (aa) and venous (vv) blood, and in the control group.**

<table>
<thead>
<tr>
<th>Time of collected blood</th>
<th>PDGF concentration (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group (G2) (N = 20)</td>
</tr>
<tr>
<td></td>
<td>x ± SD</td>
</tr>
<tr>
<td>Before surgery</td>
<td>208.1 ± 82.4</td>
</tr>
<tr>
<td>Post surgery</td>
<td>78.9 ± 30.4</td>
</tr>
<tr>
<td>Intrasurgically (aa)</td>
<td>169.3 ± 88.5</td>
</tr>
<tr>
<td>Intrasurgically (vv)</td>
<td>194.2 ± 84.0</td>
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</tbody>
</table>

Group G2 - (T2, N1, M0, histopathological type G2), Group G3 - (T2, N1, M0, histopathological type G3), *p<0.05; ** p<0.01; *** p<0.001.
Figure 1. PLT count in colorectal adenocarcinoma patients before resection of colorectal adenocarcinoma (before), 3 months after surgery (after), in intraoperatively collected arterial (aa) and venous (vv) blood, and in the control group.

PDGF-AB was measured in blood serum using commercially available immunoenzymatic method with the human PDGF-AB ELISA-Kit (Bender MedSystem, Viena, Austria), while PLT count - using a hematological analyzer SYSMEX.

Statistical analysis
Nonparametric U Mann-Whitney test and Spearman correlation test were used for statistical analysis of the results. The program STATISTICA 6.0 for Windows was applied. Differences were considered statistically significant for p<0.05.

RESULTS
In all the study patients, the PDGF-AB concentration in peripheral venous blood was several times higher than in the control group, both before and after surgery (p<0.001) (Tab. 1). However, statistically significantly higher values were found in group G3: before treatment (379.0±121.0 ng/ml), after treatment (244.4±69.8 ng/ml) (p<0.05) as compared to group G2: before treatment (208.1±82.4 ng/ml), after treatment (78.9±30.4 ng/ml) (p<0.01) (Tab. 1). According to the Spearmann’s coefficient, group G2 showed a positive correlation between PDGF level before and after surgery (r = 0.78 p<0.05). No such correlation was found in group G3 level before and after surgery (r = 0.14 p > 0.05).

A comparison of PDGF values between venous and arterial blood collected during surgery from the region of tumor mass revealed no statistically significant differences (p<0.05) (Tab. 1) However, the PDGF level in group G3 was found to be approximately twice as high as in group G2, both in venous and arterial blood (Tab. 1). (G2 vs G3 p<0.01) (Tab. 1). According to Spearman’s coefficient, the analysis showed a positive correlation between PDGF in arterial and venous blood collected intrasurgically in both study group G2 (r = 0.60 p = 0.024) and G3 (r = 0.74 p = 0.035).

The PLT count was higher before and after surgery when compared to the control group (Fig. 1). The analysis of PLT count in venous and arterial blood collected during surgery from the region of the tumor mass showed higher PLT count in arterial blood in both groups (p<0.001). However, the PLT count was found to be lower in group G3 as compared to group G2 (Fig. 1). In the analysis of Spearmann’s correlation coefficients the PDGF level did not correlate with PLT count prior to surgery and intrasurgically. However, 3 months after tumor resection, both PDGF concentration and PLT count became reduced, although they were still significantly elevated but no showed positive correlation (r = 0.38 p>0.05).

DISCUSSION
As neoplastic progression is known to be accompanied by thromboembolic disturbances associated with enhanced activation, adhesion and platelet aggregation, we determined the PLT count, which in all our study patients was statistically significantly higher before than after surgery, irrespective of tumor malignancy grade.

Our studies shown that the PLT count was higher before and after surgery compared to the control group. The analysis of PLT count in venous and arterial blood collected during surgery from the region of the tumor mass showed higher PLT count in arterial blood in both groups. However, the PLT count was found to be lower in group G3 as compared to group G2. Colorectal carcinoma frequently predisposes to thromboembolic complications, which are the second cause of mortality in these patients. Blood platelets actively participate in the pathogenesis of thrombosis promoted by the inflammatory process. Inflammation is characterized by mutual interactions between PLT, leukocytes and endothelial cells, with a significant role of active platelet surface receptor CD62P [18]. Platelet activation is accompanied by a release of many
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Smooth muscle cells of the vascular wall, vascular endothelial cells, fibroblasts, smooth muscle cells and in great amounts in tumor tissue [7]. Histopathological studies have revealed that tumor cells and fibroblasts in the tumor stroma resistant to PDGF action increase the autocrine production of this factor [9]. The PDGF is an important factor that regulates migration and proliferation of a number of cells. As a mitogenic factor, it exerts an effect on cells through specific surface receptors. PDGF does not occur in plasma and is released to blood serum during thromboembolic disorders that constantly occur in the neoplastic tissue due to the dense vasculature and an abundant source of cancer procoagulant (CP) [11].

The results obtained in our study revealed a significantly increased concentration of PDGF in colorectal cancer patients as compared to the healthy population, which confirms platelet activation in cancer patients. The finding of a statistically significant correlation between level and histopathological type is interesting. We obtained higher concentration of PDGF in patients with higher histopathological differentiation G1 (379.0 ng/ml), as compared to lower differentiation G2 (169.3ng/ul). The correlation between PDGF and differentiation type may indicate that the increase in PDGF level as a marker of platelet activation in vivo is associated with the presence of tumor. The positive correlation observed between the PLT count and PDGF concentration shows its neoplastic origin beside PLT.

We have shown an important effect of tumor differentiation malignancy grade on the level of PDGF in blood serum. This factor released from neoplastic cells is a chemotactic and mitogenic factor for mesenchymal cells. It can be thus assumed that PDGF plays an essential role in neoplastic metastases, in correlation with differentiation malignancy grade.

There is an evidence that in neoplastic tissue PDGF is an abundant source of cancer procoagulant (CP) [11]. Moreover, platelets interact with neoplastic cells facilitate formation of metastases and participate in microvascularization of tumors.

The platelet-derived growth factor (PDGF) was first identified and described as a mitogenic factor secreted from the alpha granules of platelets during blood coagulation [2]. Subsequent studies have shown its presence in vascular endothelial cells, fibroblasts, smooth muscle cells and in great amounts in tumor tissue [7]. Histopathological studies have revealed that tumor cells and fibroblasts in the tumor stroma resistant to PDGF action increase the autocrine production of this factor [9]. The PDGF is an important factor that regulates migration and proliferation of a number of cells. As a mitogenic factor, it exerts an effect on cells through specific surface receptors. PDGF does not occur in plasma and is released to blood serum during thromboembolic disorders that constantly occur in the neoplastic tissue due to the dense vasculature and an abundant source of cancer procoagulant (CP) [11].

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and allow treatment monitoring. Since this CRC is known to give frequent recurrences, the assessment of PDGF level can serve as a pivotal index of the disease relapse. The investigation of PDGF change dynamics may play an important role in the evaluation of malignant conversion.

PDGF concentration was higher in all CRC patients irrespective of differentiation malignancy grade and site of blood collection as compared to the control group. Prior to CRC resection, PDGF concentration was higher than after surgery, and still significantly higher as compared to healthy subjects. The sources of PDGF serum level were not only PLT but also tumor tissue.

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