Soluble P-selectin concentration in patients with colorectal cancer

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During platelet activation, P-selectin is translocated onto the external platelet membrane. Surface exposure of P-selectin is temporary, and the molecule undergoes endocytosis or shedding to the circulation where it appears in a soluble form as sP-selectin.

The aim of the study was to assess platelet activation based on the level of soluble form of P-selectin (sP-selectin) in colorectal cancer patients.

The study involved 22 surgically treated patients, divided into two groups depending on histopathological malignancy grade: group I – patients with low malignancy grade (G2), group II – patients with high malignancy grade (G3). The examinations were carried out three times: before surgery (A0) and 3 (A1) and 12 days (A2) after the surgery. Control group (C) consisted of 20 healthy subjects. The sP-selectin level was determined in the plasma using the ELISA Kit (Human sP-selectin, R&D System).

In colorectal cancer patients sP-selectin concentration was statistically significantly higher (69.25 ng/ml in group I and 66.50 ng/ml in group II) as compared to healthy subjects (46.01 ng/ml) ($p<0.05$), irrespective of malignancy grade. The surgical procedure has a significant effect on the dynamics of changes in the level of sP-selectin. Initially, 3 days (A1) after the procedure, there is a decrease in the level of sP-selectin, but after 12 days (A2) a rise is noted again, the level being the highest in group I. It indicates that the surgical procedure does not totally eliminate the factors responsible for platelet activation and did not normalize platelet activation.

Key words: colorectal cancer, platelet activation, sP-selectin

P-selectin (CD 62P) is an adhesion molecule bound to the membrane of the platelet α granule and Weibel-Palade body, the endothelial cells organelle that stores the procoagulant von Willebrand factor [23, 29]. During platelet activation, P-selectin is translocated via the Open Canalicular System (OCS) onto the external platelet membrane [7]. According to some authors, surface exposure of P-selectin is temporary; the molecule undergoes endocytosis or shedding to the circulation where it appears in a soluble form (sP-selectin) [5]. MICHELSON et al showed that the elevated level of sP-selectin in the plasma was accompanied by a drop in CD 62P expression [25]. However, JOHNSTON et al found the soluble P-selectin level to be decreased compared to the membrane molecule due to the loss of the fragment containing transmembrane domains [18]. The soluble form of P-selectin has been described in healthy subjects and its elevated level, associated with platelet activation, has been observed in patients with atherosclerosis, thrombotic diseases, diabetes, smokers and in cancers [4, 6, 11, 17].

P-selectin, like L- and E-selectins, is a receptor for mucin-like PSGL-1 ligand that occurs on the surface of leukocytes and endothelium [21]. These selectins also play a part of mediators between cancer cells and platelets, leukocytes and endothelium, which can explain their involvement in the formation of neoplastic metastases and cancer spread in the body [30]. This has been confirmed by detection of the CD 24 receptor on the surface of cancer cells, which mediates binding of tumor cells with platelet surface P-selectin [2, 19].

Neoplastic disease is characterized by disorders in hemostasis and by vascular dysfunction, which is reflected in an increased risk of thrombosis, bleeding tendency and DIC [2]. The role of platelets in these disorders seems to be interesing. In patients with different types of cancer blood platelets exhibit both qualitative and quantitative changes, such as increased or decreased aggregation, attenuated adhesion and enhanced sensitivity to the action of various agonists [3, 12, 15]. The abnormalities also involve platelet count, so both thrombocytopenia and thrombocytosis as well

as normal platelet count may occur [12]. The neoplastic cells themselves are the source of many platelet-activating factors and substances, including ADP, TXA2, TF (Tissue Factor), neoplastic procoagulants and thrombin [16]. During platelet activation some morphological changes are observed both in the platelets and on their surface. Platelet granules release numerous proteins exhibiting a prothrombic effect, e.g. fibrinogen, ADP, vWf, fibronectin, PF 4, with P-selectin appearing on the external membrane [20].

The aim of the current study was to assess blood platelet activation based on the plasma level of soluble P-selectin in patients with colorectal cancer with regard to tumor malignancy grade and the effect of surgical procedure.

Material and methods

Patients. Twenty-two colorectal adenocarcinoma patients surgically treated in II Department of General Surgery, Teaching Hospital in Bialystok, were recruited to the study.

The diagnosis was based on clinical symptoms as well as radiographic and endoscopic findings in addition to histopathological analysis of the neoplastic lesion and local lymph node involvement.

The patients were divided into two subgroups according to histopathological malignancy grade:

Group I – 16 patients with low malignancy grade cancer (G2), 8 women and 8 men, aged 52–78, mean 66 years;

Group II – 6 patients with high malignancy grade cancer (G3), 2 women and 4 men, aged 56–68, mean 63 years.

Malignancy grade was verified based on histopathological assessment of cancerous lesions excised during surgery.

Blood for analysis was collected three times:

1. before surgical procedure (A0), 2. three days after surgical procedure (A1), 3. twelve days after surgical procedure (A2).

Low molecular weight heparin (LMWH) was administered to all the patients directly after the surgery once a day for 7–21 days.

Control group (C) consisted of 20 healthy subjects (10 women and 10 men; mean age 43 years), who had not taken aspirin, steroids or other antiplatelet drugs during the preceding week.

Material. 3.6 ml venous blood samples were collected to test-tubes containing 3.2% sodium citrate, then the blood was centrifuged for 30 min at 1000 x g. The plasma specimens so prepared were stored at –18 °C until the level of sP-selectin was determined.

Assay of soluble P- selectin. Soluble P-selectin was measured using a commercially available immunoenzymatic method with the human sP-selelctin kit (R&D System).

All samples were diluted at last 20-fold into Sample Diluent (15 µl sample + 285 µl Sample Diluent). To each well 100 µl of Standard or samples were added. The sP-selectin Standard was diluted with distilled water. Then 100 µl of sP-selectin conjugate was added. The plate was incubated for 1 hour at 37 °C, and washed 3 times using Wash Buffer. After washing substrate was added to each well, cover the plate and incubate at room temperature for 15 min. The enzyme reaction was stopped by the addition of 100 µl stop solution and the absorbance at 450 nm within 30 minutes was measured in a microplate reader (ALAB PLATE READER ELISA). The results obtained using natural human sP-selectin showed linear curves that were parallel to the standard curves obtained using the recombinant parameter kit standard.

Statistical analysis. Data were subjected to statistical analysis by means of Student t-test for pairs comparing the results between the groups of patients and healthy subjects, between the patients themselves and before and after the surgery. The differences were considered statistically significant for $p<0.05$.

Results

Platelet activation was found to be statistically significantly higher in colorectal carcinoma patients, compared to healthy subjects ($p<0.05$) (Fig. 1).

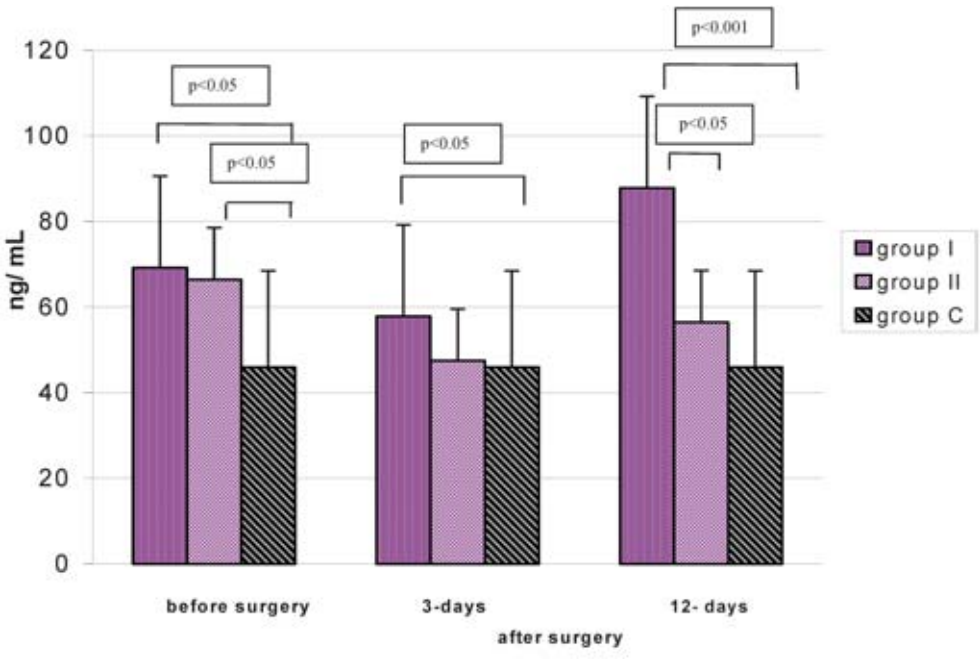

Figure 1. Soluble P-selectin concentration in patients with colorectal cancer.

The level of sP-selectin in low grade carcinoma patients (group I) was 69.25 ng/ml and was slightly higher than sP-selectin level in high grade patients (group II) (66.50 ng/ml). In both groups, the level of sP-selectin was significantly higher as compared to control group (46.01 ng/ml ($p<0.05$) (Fig. 1).

Three days after the surgery (A1) the level of sP-selectin was decreased as compared to that measured before the surgical procedure (A0). In group I, sP-selectin concentration was statistically significantly lower (57.88 ng/ml) ($p<0.05$ vs C), while in group II (47.50 ng/ml), it did not differ statistically.

Twelve days after the surgery (A2), a statistically significant increase was noted in the marker (87.88 ng/ml) compared to the level obtained three days (A1) after the procedure ($p<0.05$) and highly significant in comparison to that measured before the surgery (A0) ($p<0.001$). An increase in the level of sP-selectin was also observed in group II (56.50 ng/ml), but it was not statistically significant compared to the previous level (Fig. 1).

The statistical analysis revealed a highly significant difference in the level of sP-selectin between groups I and II in patients with colorectal carcinoma 12 days after the surgical procedure ($p<0.05$) (Fig. 1).

Discussion

As shown by anatomopathological studies, thromboembolic complications occur in nearly 50% of patients with neoplastic disease [12]. A significant role in their origin has been described to substances produced by neoplasms or due to a pathology or therapy-induced complication. Hypercoagulative states observed in cancer patients can also be related to increased platelet activation and aggregation.

The results of our study conducted on colorectal carcinoma patients showed increased intravascular platelet activation, reflected by statistically significantly higher sP-selectin concentration compared to healthy subjects. However, no differences were observed in sP-selectin concentration depending on tumor malignancy grade. Reports on the effect of tumor advancement or the presence of metastases on platelet activation and directly on sP-selectin are divergent.

MANTUR et al studying renal carcinoma stated, basing on CD62P platelet expression and sP-selectin level, that it was the renal cancer itself that induced platelet activation, while the presence or absence of metastases had no significant effect [24]. However, ROSELLI et al in non-small cell lung cancer patients found a significant, advancement-dependent increase in sP- and sE-selectins, compared to healthy subjects [27]. The authors believe that the increased expression of selectins, which was detected on endothelial cells in lung cancer, indicates their role in the chemotaxis for neoplastic cells. Moreover, they suggest that determination of soluble selectins in the plasma can be a useful marker in handling lung cancer patients [27].

CAINE et al found a significant increase in the level of sP-selectin in breast and prostate cancer, as compared to healthy subjects (respectively, women and men) [10]. Interesting is the fact that these authors noted no correlation between sP-selectin and markers of endothelial cell activation (VEGF), suggesting that most of the plasma sP-selectin, if not the whole, comes from blood platelets [10]. Other authors showed a correlation between platelet count and sP-selectin concentration, which in their opinion points at platelets as the major source of this marker in conditions in which endothelium is not damaged [28]. Platelet hyperactivation *in vivo* in colorectal cancer patients with sP-selectin of platelet origin can be confirmed by an increased plasma level of β-thromboglobulin (β-TG), another platelet activation marker, observed in our earlier studies [13]. Platelet count in colorectal cancer was significantly higher compared to healthy subjects, although there were no statistically significant differences in relation to clinical advancement [13].

Blood platelets facilitate spreading of neoplastic metastases and platelet count has been described a key role [9]. VARKI et al have demonstrated that blood platelets together with leukocytes form complexes that surround tumor cells in the circulation, facilitating their spread to distant organs [30]. However, experimental studies carried out on tumor cell cultures have revealed that the soluble form of P-selectin binds to neoplastic cells as efficiently as surface P-selectin [22].

Colorectal cancer is characterized by hemostatic disorders with higher predisposition to thrombosis and fibrinolysis attenuation. Both surgical procedure and general anesthesia induce hypercoagulation in these patients [26].

In the current study, in the initial period following the resection of tumor together with the adjacent lymph nodes we found decreased platelet activation *in vivo.* Three days after the procedure sP-selectin level was reduced in both groups of patients, but in group II was still higher than in control subjects. MODRAU has demonstrated that the reduction in sP-selectin level can be the result of the surgical procedure itself, elimination of the major source of platelet-activating factors and decreased platelet count due to intraoperative bleeding [26].

Directly after the procedure all the patients received heparin, which according to BORSING et al inhibits tumor growth and decreases the risk of metastasis formation [8]. Heparin is a potential P- and L-selectin inhibitor, and as reported by some authors, a single heparin injection significantly suppresses metastasis formation through the inhibition of P-selectin mediated inhibition of platelet interaction with neoplastic cells [8]. Either ABBASCIANO et al in patients with gastrointestinal tract carcinomas (gastric and colorectal cancer) have demonstrated that patients subjected to surgical procedures should receive higher doses of heparin to prevent thromboembolic complications and additionally reduce metastatic spread [1].

Twelve days after surgery in group I we observed a significant increase in sP-selectin level as compared both to the level noted 3 days after the procedure and to control values.

Interesting is the finding of statistically significant differences in sP-selectin concentration between the groups 12 days after the surgery, according to the tumor malignancy grades.

The increase in the level of sP-selectin and other markers of platelet activation in these patients can be associated with a significant rise in platelet count, which 12 days after the procedure exceeded the upper range of reference values for platelets. This could probably occur due to thrombopoiesis activation as the result of platelet count reduction observed on day 3 following surgery and can explain increased sP-selectin in these patients. FOLMAN et al have shown that the platelet count frequently noted after surgical procedure may be related to the level of circulating thrombopoietin (Tpo), the main thrombopoietic regulator [14].

Also trauma associated with extensive surgical procedure can be a powerful stimulus, sufficient to activate platelet production in the marrow. The new platelets are more active metabolically and more sensitive to the action of various activating factors. However, it should be remembered that patients with cancers show a high tendency to develop thrombosis, so the increased platelet count and the state of platelet hyperactivation are considered unfavorable.

Concluding, our study has revealed increased platelet activation irrespective of colorectal cancer malignancy grade. Although directly after surgical procedure a decrease was noted in sP-selectin concentration, yet later findings indicate that the radical surgical procedure did not eliminate the source of platelet activation in the patients.

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