Platelet activation in patients with advanced gastric cancer

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The aim of this study was to evaluate platelet activation in gastric cancer patients with regard to histopathological classification and the presence of distant metastases, by using platelet morphological parameters: MPV, L-PLT, MPC, as well as quantitative evaluation of surface receptor expression: CD41a, CD61, CD42b, CD62P, by flow cytometry at the resting state and after TRAP activation.

In gastric cancer patients higher values of MPV and LP, as well as decreased MPC values were determined. Quantitative evaluation of surface antigen expression also revealed higher number of CD41a, CD61 and CD62P molecules, as compared with the platelets in the control group.

Significant decrease of CD42b molecules' number after TRAP incubation, and the increased CD41a, CD61 and CD62P expression also point to the retained reactivation capacity of platelets.

Good correlation between morphological parameters and the number of CD62P molecules indicates the usefulness of routine tests in evaluation of platelet activation.

Key words: gastric cancer; platelets, CD41a, CD61, CD42b, CD62P, TRAP.

The increased susceptibility to spontaneous and postoperative thrombosis in cancer patients, described by Trousseau more than 100 years ago is confirmed by randomised clinical tests results. It is estimated that 15% of diagnosed cancer patients additionally suffer from thromboembolism. Highly increased risk of venous thrombosis can be observed as early as the first months after a diagnosis, especially in case of advanced cancer. The risk of thrombotic complications grows in presence of additional thrombosis risk factors, such as: surgery procedures, hyperviscosity syndrome, infections, immobilization or chemotherapy. Prevention and treatment of thrombosis, being the second incidence factor of death pose a true diagnostic challenge [1, 2, 3].

In the hypercoagulability mechanism in cancer the important role is played by interactions between cancer and regular host's cells, leading to platelet activation which is induced both by direct contact between a cancer cell with a platelet through adhesive molecules, and production and releasing of its own proinflammatory, prothrombotic and proangiogenic factors: ADP, thromboxane, thrombin, IL-1, TNF-α, VEGF [vascular endothelial growth factor], TF [tissue factor] and CP [cancer procoagulant] [1, 3, 4, 5, 6, 7]. Activated platelets change their shape and size, release cytoplasmatic granules content and change glycoprotein expression, located both in membrane and in its granules [4, 5, 8, 9]. Progress in blood morphology measurement and tests' automation give an opportunity to monitor these changes through the evaluation of platelet number, their volume, the granulation level, the presence of youngest platelets subpopulations – the most active in homeostasis, or platelet aggregates [6, 10, 11, 12, 13]. Activating signals also lead to biochemical platelet modifications, accompanied by the change in surface glycoprotein expression which is widely used in the evaluation of platelet state [4, 9]. The quantitative evaluation of glycoprotein platelet antigens can be performed by flow cytometry method, whose accessibility in routine diagnostics is yet limited by its high costs.

There are only few reports about complex evaluation of the number, morphological parameters and quantitative expression of platelet glycoproteins in patients with gastric cancer.

The aim of the study was the evaluation of platelet activation in patients with gastric cancer, with regard to cancer's histopathological type and presence of distant metastases. The evaluation covered the analysis of platelet number (PLT), their morphology: MPV - mean platelet volume, L-PLT – Large Platelets, MPC – Mean Platelet Component as well as platelet antigens’ expression CD41a (GPIIb/IIIa), CD61 (GPIIIa), CD42b (GPIb), CD62P (GMP140) at the resting state and after TRAP [Thrombin Receptor Activation Peptide] activation.
Materials and methods

Peripheral blood was taken before surgical treatment from 40 patients with gastric cancer (14 women and 26 men, aged 28 – 84), treated in II Department of General and Gastroenterology Surgery, was the research material. In all the gastric cancer patients adenocarcinoma in III and IV progression stage according to UICC/TNM classification was found. Histopathological gastric cancer classification according to Lauren revealed intestinal type cancer (LI) in 55% of the patients, and diffuse cancer (LII) in 45%. Lymph metastases were detected in 95% of the patients, and distant metastases – in 45% of the patients (M1). In 30% of the patients cancer’s grade was determined as G2, and in 70% - G3.

Control group were 25 healthy persons (10 women and 15 men), aged 25 – 85 years. These volunteer donors had not been on any medication including aspirin or non-steroid anti-inflammatory drugs during the previous two weeks.

The number and morphological parameters of platelets were measured with ADVIA 2120 Hematology System (Siemens). Whole blood was collected into potassium ethylenediaminetetraacetic acid (EDTA).

To evaluate platelet surface antigens whole blood collected into sodium citrate was used. Flow cytometry method was applied (FACS Calibur, Becton Dickinson). Platelet Gp Receptors Kit (Bicicytix, France) was used to evaluate the expression of the following platelet glycoproteins: GpIIb/IIIa (CD41a), GpIIia (CD61), GpIb (CD42b), GMP 140 (CD62P), All at the resting state (in native blood), and after TRAP (Thrombin Receptor Activation Peptide) in vitro activation. The mean fluorescence intensity was measured with flow cytometer after addition of a staining reagent to the sample and calibrator tubes. The usage of calibration beads (coated with defined increasing numbers of monoclonal antibody molecules) made it possible to prepare calibration curve (Microsoft Office Excel 2003) and calculate the absolute number of glycoprotein molecules presented on a single platelet based on mean fluorescence intensity.

Statistical analysis was based on Statistica 5.1 program. The differences between the gastric cancer patients subgroups and control subjects were evaluated using nonparametric Kolmogorov-Smirnov test. Spearman’s rank correlation coefficient test was used to calculate the correlation coefficient between number of CD62P molecules per platelet and the chosen morphological parameters. The differences were considered statistically significant at p<0.05.

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Results

Mean platelet number and platelet morphological parameters in individual research subgroups and in the control group were compared and presented in Table 1.

In all the subgroups of the patients with cancer, the platelet number (PTL) was comparable with the platelet number in the control group.

The number of large platelets (L-PLT) in the group of gastric cancer patients was significantly increased as compared with L-PLT in the control group in the subgroups: LI (p<0.025), LII (p<0.005), M0 (p<0.005) and G2 (p<0.01). L-PLT in G2 subgroup was significantly higher than in G3 (p<0.05).

Mean platelet volume (MPV) in cancer patients was significantly higher as compared with the healthy subgroups: LI (p<0.05), LII (p<0.05), M1 (p<0.05) and G2 (p<0.025).

Mean platelet component (MPC) in all the subgroups of patients was significantly lower as compared to the healthy group: LI (p<0.001), LII (p<0.001), M0 (p<0.001), M1 (p<0.001), G2 (p<0.005), G3 (p<0.001).

Mean receptor number on platelet surface in individual subgroups is presented in Table 2.

Mean molecule numbers of CD41a (GpIIb/IIIa) and CD61 (GpIIia) on platelet surface in patients with gastric cancer in all subgroups were significantly higher as compared to the control group (p<0.001). After TRAP activation mean CD41 receptor number increased considerably, and increase dynamics was similar in the tested subgroups (LII 1,5-fold; LI, M1 1,4-fold; M0, G2, G3 1,3-fold), and in the control group (1,5-fold) (Figure 1). Similar results were obtained in the evaluation of CD61 after TRAP activation (LI, LII, M0 – the increase 1,4-fold; M1, G3 1,3-fold, G2 1,2-fold (Figure 2).

CD42b (GpIb) in patients with gastric cancer was comparable with the control group. After TRAP activation the number of CD42 receptors on the platelet significantly decreased in LI, M0, M1, G2, G3 subgroups up to 50% of the value before the activation. In LII subgroup the GpIb receptors expression decreased to 50% of the initial value, which indicates more

Table 1. Comparison of platelet number and platelet morphological parameters values in gastric cancer patients subgroups and in the control group

<table>
<thead>
<tr>
<th>Group/Subgroup</th>
<th>PLT x 10^3/µl</th>
<th>L-PLT x 10^3/µl</th>
<th>MPV fl</th>
<th>MPC g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI</td>
<td>249±93</td>
<td>9.4±6.8</td>
<td>9.8±1.2</td>
<td>23.5±2.3</td>
</tr>
<tr>
<td>LII</td>
<td>258±109</td>
<td>6.1±2.2</td>
<td>9.5±0.9</td>
<td>23.7±1.7</td>
</tr>
<tr>
<td>M0</td>
<td>248±75</td>
<td>6.8±4.5</td>
<td>9.1±1</td>
<td>24.4±1.9</td>
</tr>
<tr>
<td>M1</td>
<td>292±122</td>
<td>8.5±5.4</td>
<td>9.8±1.4</td>
<td>22.9±2.7</td>
</tr>
<tr>
<td>G2</td>
<td>293±129</td>
<td>13±7.0</td>
<td>10.2±1.6</td>
<td>24.6±2.4</td>
</tr>
<tr>
<td>G3</td>
<td>239±80</td>
<td>5.2±2.8</td>
<td>8.9±0.8</td>
<td>23.9±1.8</td>
</tr>
<tr>
<td>Control Group</td>
<td>229±59</td>
<td>4.5±1.7</td>
<td>8.4±0.8</td>
<td>28.1±1.6</td>
</tr>
</tbody>
</table>

*p<0.05 difference between gastric cancer patients and control group
**p<0.05 difference between gastric cancer patients subgroups (LI vs LII; M0 vs M1; G2 vs G3)
Table 2. Comparison of receptor number on platelet surface in gastric cancer patients subgroups and in the control group.

<table>
<thead>
<tr>
<th>Group/Subgroup</th>
<th>CD41a before activation</th>
<th>CD61 before activation</th>
<th>CD42b before activation</th>
<th>CD62P before activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI</td>
<td>71034 ±11240</td>
<td>97677 ±18418</td>
<td>90551 ±10440</td>
<td>22481 ±18021</td>
</tr>
<tr>
<td>LII</td>
<td>72415 ±14323</td>
<td>105470 ±17931</td>
<td>104403 ±36832</td>
<td>18021 ±8702</td>
</tr>
<tr>
<td>M0</td>
<td>66226 ±10372</td>
<td>89385 ±21741</td>
<td>97186 ±22141</td>
<td>23264 ±7830</td>
</tr>
<tr>
<td>M1</td>
<td>74742 ±15960</td>
<td>104337 ±21741</td>
<td>103053 ±22141</td>
<td>21892 ±7830</td>
</tr>
<tr>
<td>G2</td>
<td>66265 ±13639</td>
<td>90505 ±71146</td>
<td>82777 ±36339</td>
<td>21515 ±11801</td>
</tr>
<tr>
<td>G3</td>
<td>63282 ±13182</td>
<td>81470 ±7605</td>
<td>8921 ±5735</td>
<td>4865 ±495</td>
</tr>
<tr>
<td>Control Group</td>
<td>47150 ±7067</td>
<td>48940 ±72870</td>
<td>35581 ±6098</td>
<td>18254 ±573</td>
</tr>
</tbody>
</table>

*p<0.05 difference between gastric cancer patients and control group
**p<0.05 difference between gastric cancer patients subgroups (LI vs LII; M0 vs M1; G2 vs G3)

Figure 1. Mean number of CD41a molecules on platelet surface at the resting state and after TRAP activation in gastric cancer patients subgroups and in control group.
*p<0.05 difference between gastric cancer patients and control group
**p<0.05 difference between gastric cancer patients subgroups (LI vs LII; M0 vs M1; G2 vs G3)

Mean CD62P molecule number (GMP140) on platelet surface was significantly higher in the patients with gastric cancer in relation to the control group (LI, LII p<0.005; M0 p<0.005; M1 p<0.001; G2 p<0.001; G3 p<0.005). After TRAP activation the number of CD62P receptors on the platelet in the patients’ subgroups increased more dynamically (LI 6-fold, LII 11-fold, M0 7-fold, M1 12-fold, G2 7-fold, G3 9-fold) in comparison with the control group. Statistically significant changes were found in GD62P number of molecules per platelet between the group M0 and M1 (p<0.05), G2 and G3 (p<0.005) (Figure 4).

The results of CD62P correlation, the well-defined platelet activation indicator, with the chosen morphological parameters were presented in Table 3.
Figure 2. Mean number of CD61 molecules on platelet surface at the resting state and after TRAP activation in gastric cancer patients subgroups and in control group.
* p<0.05 difference between gastric cancer patients and control group
** p<0.05 difference between gastric cancer patients subgroups (LI vs LII; M0 vs M1; G2 vs G3)

Figure 3. Mean number of CD42b molecules on platelet surface at the resting state and after TRAP activation in gastric cancer patients subgroups and in control group.

Figure 4. Mean number of CD62P molecules on platelet surface at the resting state and after TRAP activation in gastric cancer patients subgroups and in control group.
* p<0.05 difference between gastric cancer patients and control group
** p<0.05 difference between gastric cancer patients subgroups (LI vs LII; M0 vs M1; G2 vs G3)
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Table 3. The results of CD62P molecules number correlation with morphological parameters of platelet.

<table>
<thead>
<tr>
<th></th>
<th>R Spearman</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC vs CD62P</td>
<td>-0.685</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LP vs CD62P</td>
<td>0.452</td>
<td>0.018</td>
</tr>
<tr>
<td>MPV vs CD62P</td>
<td>0.136</td>
<td>0.499</td>
</tr>
</tbody>
</table>

Discussion

Credible evaluation of hypercoagulability risk factors in patients with gastric cancer is indispensable for the improvement of treatment effects and the increase in survival rate of this group.

The increased number of platelets noted in 30 – 60% of patients with cancer is acknowledged as an independent, negative prognostic factor inter alia in gastrointestinal cancer [2,14,15]. Thrombocytopenia in patients without chemotherapy can be the first signal of disseminated intravascular coagulation process, bone marrow metastases, or accompanying megaloblastic anemia [5]. The author's research conducted on patients with advanced gastric cancer did not reveal significant deviation from normal in platelet number.

What could be observed were higher PLT values in patients with distant metastases (M1) and in the group with medium grade of the disease (G2). Thus, although evaluation of platelet number should be part of routine tests, PLT seems to be not very useful as independent parameter in diagnosing hypercoagulability risk in patients with gastric cancer.

Literature of the subject confirms the relationship between intensified platelet activation and hypercoagulability risk in patients with cancer [2, 3, 5, 6]. In the tested patients with gastric cancer intensified platelet activation was estimated by their morphological parameters' analysis. Significantly higher mean platelet volume (MPV) and large platelet number (L-PLT) indicates the presence of subpopulations of the most active platelets in homeostasis processes [10, 11, 13]. MPC value, significantly lower in patients with gastric cancer, indirectly points to degranulation and platelet granules release, connected with the last stage of platelet activation [12, 13]. Dislocation of glycoproteins contained in granulation membrane α on platelet surface is another degranulation result. Dislocation manifests itself in the increased surface expression of CD62P antigens. In patients with gastric cancer mean number of C62P molecules on platelets' surface was significantly higher as compared to the healthy group. CD62P antigen, called P-selectin, is not found on resting platelets, and along with PF-4 and β TG is the main marker of platelet activation and release reaction. Exposed on platelet membrane as functionally active surface receptor it is a factor which mediates between adhesion of active platelets to leucocytes, endothelial cells and cancer cells. Increased platelet activation in patients with gastric cancer also manifests itself in the increased expression of antigens' CD41a and CD61. Most of CD41a (GPIIb/IIIa) molecules are bound with platelets' cell membrane but the remaining part is found in a granulation membranes. At the moment of platelet activation on their surface expression of GPIIb/IIIa grows, as the result of intracellular pool's displacement [1, 4, 10]. GPIIb/IIIa complex is one of the better investigated and most important receptors for platelets' function. It plays the main role in platelet aggregation, being a receptor for fibrinogen. CD41a is also capable of binding adhesive proteins of cell walls, such as von Willebrand's factor, fibronectin, vitronectin, thrombospondin, lamin or collagen [4, 8, 16].

The increase of platelet glycoprotein number can be a bad prognostic factor [1, 2, 3, 8, 16, 17]. It intensifies platelets' adhesion and aggregation ability, and in consequence, it increases the risk of thromboembolic changes, fosters tumor cells' proliferation, angiogenesis, distant metastases growth and a disease progression. It has been demonstrated that monoclonal antibodies against CD41a considerably inhibit experimental metastases by hematogenous tract [4, 8, 16, 17]. Then detecting early symptoms of platelet activation is an important diagnostic challenge. The research revealed good correlation between MPC and LP values, and CD62P expression (CD62P is an acknowledged platelet activation marker), which confirms the usefulness of the mentioned morphological parameters in the evaluation of platelet activation extent in patients with gastric cancer.

After TRAP activation in patients with gastric cancer, further increase of CD62P, CD41α and CD61 receptors' expression, as well as CD42b receptor's internalization can be noticed, which testifies to the retained activation potential of the platelets. Unlike in other tested molecules, CD42b (GPIb) is an internalized receptor, and its expression on platelet surface decreases with activation. CD42P plays a vital role in keeping negative value of platelet surface charge. The interaction between GPIb and von Willebrand's factor causes platelets' adhesion to the damaged vessel wall, and is responsible for shear-induced platelet aggregation. Decreased expression of this antigen corresponds to increased adhesion and aggregation, which may induce thrombosis [4, 8]. Interestingly, in patients with gastric cancer decreased CD42b expression in resting conditions was not observed.

The number of CD62P antigens on platelet surface after TRAP activation increased 6 to 12-fold in patients with gastric cancer, and only 3-fold in the control group. Additionally, the tests revealed intensified reactivation capacity of platelets in patients with diffused type according to Lauren (LII) as compared to intestinal type (LI), and with distant metastases (M1) in comparison with the group of patients without metastases. The expression changes of CD61, CD41a, CD42b antigens, corresponding with platelet activation, reaches up to ± 50% of the initial values and were comparable in the research and control groups.

The high number of surface glycoproteins observed in the presented study in patients with gastric cancer, both in rest-
ing state and after in vitro activation, in the light of literature indicates susceptibility to thrombosis complications, especially in advanced stage of the disease [4, 7, 8, 9, 11]. Hence thorough evaluation of platelets parameters in patients with gastric cancer who qualify for radical surgery can have a vital importance in prognosis of thrombotic complications.

In conclusion – 1, in patients with gastric cancer increased platelets’ activity with their normal number can be observed, 2, changes in the expression of surface glycoproteins after TRAP activation indicate platelets’ high activation potential in patients with gastric cancer, especially in patients with LII, G3 and M1 types, and 3, MPC decrease and LP increase are a useful routine indicator of hypercoagulability in patients with gastric cancer.

References