Effect of tumor stage and nephrectomy on CD62P expression and sP-selectin concentration in renal cancer

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The CD62P receptor and its soluble form sP-selectin is a marker of platelet (PLT) activation, and constitutes a ligand for CD24 antigen of neoplastic cells and tumor stroma components.

The aim of the study was to evaluate the relationship dynamics of the percentage of CD62P+ platelets, the level of the receptor expression and the concentration of soluble form of sP-selectin in renal cancer. Examinations were performed before and after nephrectomy in patients with renal cancer (group A – 25, $T_2N_0M_0$; group B – 27, $T_2N_{1\text{-}2}M_0$) and in control group (C – 24 subjects).

The two groups A and B showed an increased subpopulation of CD62P+ platelets ($p<0.01$) and elevated sP-selectin concentration ($p<0.001$) before and after nephrectomy. Although following nephrectomy sP-selectin concentration decreased markedly, it was still higher 3 months after the procedure compared to control group ($p<0.05$). Following nephrectomy, however, no statistically significant differences were found in the % of CD62P+ platelets and the receptor expression. Greater dynamics of changes before and after nephrectomy in the percentage of CD62P+ platelets (B1:B2 $p<0.05$) and the receptor expression (B1:B3 $p<0.001$) was observed in patients with local lymph node involvement (group B) while sP-selectin concentration was similar in both groups.

Nephrectomy did not normalize intravascular activation of PLT and TNM had no significant effect on the expression of CD62P and concentration of sP-selectin.

Key words: sP-selectin, CD62P, platelet activation, nephrectomy, renal cell carcinoma.

Recent literature data frequently point at P-selectin (GMP140; CD62P) as a ligand for CD24 receptor of neoplastic cells [1,2, 4, 8]. CD62P has been recognised as the major marker of platelet (PLT) activation. CD62P receptor of activated platelets become involved in the interaction with peripheral blood leukocytes [5, 12, 13], monocytes [3], and elements of tumor stroma [11].

Activated blood platelets show not only enhanced expression of surface receptors, but also elevated levels of their forms soluble in plasma. CD62P expression on the cell membrane is short-lasting and after 2–4 hours, the supracytoplasmic domain of the receptor becomes "washed out" to the blood, where it functions as sP-selectin [6].

In neoplastic disease, blood platelets are capable of spontaneous activation. The main site of PLT activation in neoplasms is a dense network of blood vessels that drain the tumor. The major factors of this activation include tissue factor (t-PF), enhanced thrombinogenesis, blood turbulence and increased effect of clotting forces [17]. As there are no literature data available on the effect of renal cancer clinical stage and nephrectomy on CD62P expression and concentration, we decided to undertake the present study.

The aim of the study was to evaluate the activation of blood platelets *in vivo* in renal cancer growth. PLT activation was evaluated based on CD62P expression on blood platelets and plasma concentration of sP-selectin. The study was performed before and after nephrectomy, and the findings were analyzed according to the clinical stage of neoplasms.

Material and methods

The study involved 52 patients (16 women and 36 men)

Table 1. Clinical and laboratory characteristics of patients with renal cell carcinoma and control group

Clinical stage according to TNM	GROUP (A) $T_2N_0M_0$	(B) $T_2N_{1-2}M_0$	Control
Parameters (n)	25	27	24
Sex	16 M/9 F	20 M/7 F	16 M/8 F
Age (years)	(36–68/mean 62)	(42–72/mean 65)	(22–62/mean 57)
Creatinine (mg/dl)	0.76–0.98 (mean 0.85)	0.87–1.18 (mean 0.92)	0.42–0.79 (mean 0.66)
Urea (mg/dl)	24–30 (mean 26)	28–36 (mean 32)	0.18–28 (mean 24)
Creatinine clearance (ml/min)	normal	normal	normal
Albumin (mg/day)	<300	<300	<150
Erythrocytes >10/μl (n)	11	19	not found
Histopathological form	renal cell carcinoma	renal cell carcinoma	(–)

M – male, F – female

aged 36–68, with primary renal cell carcinoma and 24 control healthy subjects matched in age (Tab. 1).

The study group A consisted of 25 patients with tumor limited to the organ ($T_2N_0M_0$), while the study group B had 27 patients with local lymph node involvement, but with no distant metastases ($T_2N_{1-2}M_0$). All patients underwent radical nephrectomy and were examined three times: prior to nephrectomy (A1, B1), as well as three weeks and three months after the procedure (A2, B2; A3, B3, respectively). The patients were qualified to the groups based on pathological stage of the tumor, according to TNM classification [10, 14].

Before and after nephrectomy, the patients were not treated with cytostatics, received no radiation or immunotherapy. Ten days before blood was collected, they were not given antiaggregate drugs, antiplatelet drugs or aminoglycozides.

Material for analysis included venous blood collected without stasis with a 0.9 mm needle directly to test-tubes Diatube H Diagnostica Stago on anticoagulat CTAD (citrate, theophylline, adenine, dipiridamol).

CD62P expression on blood platelets was determined by flow cytometry method, using monoclonal anti-CD62P antibodies conjugated with fluorochrom. PLT activation was evaluated with cytofluorimetry method based on the percentage of CD62P-positive platelets (% of CD62P+) and on the mean fluorescence intensity (MIF), which reflects receptor density on blood platelets. The study was conducted using a flow cytometer EPICS XL, Coulter, according to the protocol developed by the European Committee for the study on blood platelets by flow cytometry [15].

The concentration of the receptor soluble form was assayed in low platelet plasma using specific monoclonal antibodies, which recognize a particular fragment (epitope) of tertiary structure of this glycoprotein. The study was done with ELISA Kit PARAMETER human sP-Selectin, R&D.

Statistical calculations were performed using the Statistica 5.0 programme. Shapiro-Wilk and Kolmogorow-Smirnow tests was used to evaluate distribution of variables, while F. Cochran-Cox test to estimate variance homogeneity. Non-parametric U Mann- Whitney test was used to verify zero hypothesis (Ho) for non-related variables. Wilcoxon test of pair order was performed for dependent variables. Differences were considered statistically significant at $p<0.05$.

Results

In primary renal cancer, the subpopulation of CD62P-positive platelets in both groups under study was higher than in control group. The percentage of CD62P+ platelets in the group (A1) was 4.84% and in group (B1) – 4.16%, compared to control (C) – 2.71% (Tab. 2). No statistically significant differences were found in the mean intensity of fluorescence (MIF), reflecting the receptor density on single blood platelets (Tab. 2).

Following nephrectomy, the subpopulation of CD62P platelets in both groups was still higher compared to control. However, based on MIF, we found the enhanced expression of the CD62P receptor (3.22 in group A3 and 3.10 in group B3), which was statistically significantly higher than in control group C (2.58) (Tab. 2) ($p<0.05$).

The analysis of the dynamics of changes between the percentage of CD62P+ platelets and MIF according to clinical stage showed no statistically significant differences before and after nephrectomy. No correlation was found between the parameters examined ($r=0.22$, $p>0.05$). No dynamic change was observed before and after nephrectomy in PLT activation, since following nephrectomy the percentage of CD62P+ platelets was maintained on the same level and the receptor expression increased only three months after the procedure.

The washing out of sP-selectin was excessive prior to

Table 2. PLT activation evaluated on the basis of expression and concentration of platelet CD62P receptor

Examination time			Before nephrectomy		3 weeks after nephrectomy		3 months after nephrectomy		
Parameter	Group	(n)		X ± SD		X ± SD		X ± SD	p
(%)	A	(25)	A_1	$4.84 \pm 1.05^{**}$	A_2	$5.32 \pm 0.93^{**}$	A_3	$5.14 \pm 1.26^{***}$	
CD62P+	B	(27)	B_1	$4.16 \pm 1.25^{**}$	B_2	$5.27 \pm 0.96^{**}$	B_3	$4.47 \pm 1.44^{***}$	B_1:B_2^{*}
platelets	C	(24)	Cl	2.71 ± 1.14	C	$2.71 \pm 1.l4$	C	2.71 ± 1.14	
	A	(25)	A_1	3.05 ± 1.74	A_2	3.19 ± 1.87	A_3	$3.22 \pm 1.34^{*}$	
MIF	B	(27)	B_1	2.93 ± 2.87	B_2	2.91 ± 1.91	B_3	$3.10 \pm 1.96^{*}$	B_2:B_3^{***}
	C	(24)	C	2.58 ± 0.35	C	2.58 ± 0.35	C	2.58 ± 0.35	
	A	(25)	A_1	$87.3 \pm 54.2^{***}$	A_2	$42.7 \pm 15.2^{**}$	A_3	$28.7 \pm 8.3^{**}$	A_1:A_2^{***}
(μg/ml)									A_2:A_3^{***}
sP-selectin	B	(27)	B_1	$72.2 \pm 29.5^{***}$	B_2	$36.4 \pm 10.6^{**}$	B_3	$23.2 \pm 6.7^{*}$	B_1:B_2^{***}
concentration									B_2:B_3^{***}
	C	(24)	C	16.0 ± 4.7	C	16.0 ± 4.7	C	16.0 ± 4.7	

Results are expressed as mean ± SD. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$. Group (A) – $T_2N_0M_0$, Group (B) – $T_2N_{1\text{-}2}M_0$, Control group – (C), MIF – mean intensity of fluorescence.

nephrectomy. Its concentration in both study groups was five times higher than in control (A1 – 87.3 ng/ml, B1 – 72.2 ng/ml, C – 16.2%). Three weeks after nephrectomy, the concentration in the tumor groups had a falling tendency (group A2 – 42.7 ng/ml, group B2 – 36.4 ng/ml), but it was still higher than in control group (Tab. 2). With time that passed after nephrectomy the plasma concentration of sP-selectin still showed a falling tendency. After 3 months, it decreased to 28.7 ng/ml in group A3 and to 23.7 ng/ml in group B3, although it was still higher than in controls ($p<0.01$).

Discussion

The present study performed on patients with renal cancer showed intravascular platelet activation, which was maintained almost on the same level before and after nephrectomy and was not affected by clinical stage of the neoplasm. It was characterized by twice as high percentage of CD62P+ platelets in both study groups, compared to control and a lack of statistically significant differences in CD62P expression between the study groups.

However, intravascular activation found in renal cancer was lower, compared to the activation of platelets observed in lung cancer [8], breast cancer [7] or circulatory diseases [9]. This may result from the presence of endogenic, organ-specific platelet agonists, which are not produced by the kidney.

The receptor expression reflects its density on platelet membrane [15]. In patients with renal cancer before nephrectomy, the expression of CD62P was normal. However, it increased on activated platelets after nephrectomy and was statistically higher than in controls. This can be explained by enhanced thrombocytopoiesis after nephrectomy and release of young, metabolically active blood platelets, which are characterized by high expression of surface receptors.

Certain platelet receptors have the so called soluble form, which gets to the plasma via "shedding"[6]. Experiments conducted on neoplastic cell cultures have revealed that the soluble form of sP-selectin is able to combine with neoplastic cells as effectively as P-selectin in the form of CD62P on platelets. We also observed intensified "shedding" of the receptor from platelet membrane to the plasma, which was indicated by a reduced PLT count, elevated percentage of platelets with CD62P expression and five-fold higher concentration of sP-selectin in blood plasma.

The neoplastic process causes depolarization of cellular membranes and increases the fluidity of cytoplasmic membranes [14, 16]. These abnormalities may lead to the enhanced "shedding". Therefore, depolarization and fluidity of cytoplasmic membranes in the primary renal cancer may be accounted for high sP-selectin concentration prior to nephrectomy and its rapid drop after the procedure.

In renal cancer patients, PLT count was reduced, but thrombocytopenia was not found. Since in the group A2, PLT count after nephrectomy increased by approximately 25%, and in the group B2 by over 20%, the hypothesis of inhibited thrombocytopoiesis can be excluded. Instead, enhanced activation and damage to platelets in tumor-draining vessels should be considered. This seems to be supported by over two-fold higher percentage of activated CD62P+ platelets, compared to control and very high plasma sP-selectin concentration.

Based on CD62P expression and the concentration of sP-selectin, it can be stated that renal cancer causes intravascular activation of blood platelets, while local lymph node

involvement has no significant effect on their activation. It can be also observed that radical nephrectomy does not eliminate completely the source of PLT activation *in vivo*, as it is not caused by the neoplasm only, but can be influenced by clotting forces and endogenic platelet agonists spontaneously released in renal cancer.

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