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ACTIVATION OF EXTRINSIC COAGULATION PATHWAY IN MYELOPROLIFERATIVE NEOPLASMS – PRELIMINARY REPORT

AKTYWACJA UKŁADU KRZEPNIĘCIA KRWI DROGĄ ZEWNĄTRZPOCHODNĄ W NOWOTWORACH MIELOPROLIFERACYJNYCH – DONIESIENIE WSTĘPNE

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Summary

The aim of the study was to assess the activation of extrinsic blood coagulation pathway, based on the TF and TFPI measurements in patients with MPNs.

The study group consisted of 17 patients with MPNs (mean age 59 years). The control group was made of 30 healthy volunteers (mean age 52 years). In blood samples tissue factor TF, tissue factor pathway inhibitor (TFPI), thrombin–antithrombin complexes (TAT) concentration and antithrombin (AT) activity were determined.

The present study showed a significantly higher level of tissue factor and decreased TFPI concentration in patients with polycythemia vera, essential thrombocythemia and primary myelofibrosis.

Conclusions:

1. The results showed that in all groups of patients with MPNs an extrinsic activation of coagulation, resulting in a high concentration of TF in the blood of these patients, occurs.

2. It seems that in patients with ET, PMF and PV, an extrinsic activation of coagulation is inhibited by TFPI inhibitor as evidenced by its reduced concentration observed in the blood of these patients (the result of extrinsic consumption in the track).

Streszczenie

Celem pracy była ocena aktywności krzepnięcia krwi drogą zewnątrzpochodną u chorych na przewlekłe nowotwory mieloproliferacyjne (PZM), na podstawie oceny stężenia czynnika tkankowego (TF) i inhibitora drogi zewnątrzpochodnej układu krzepnięcia (TFPI).

Badaniami objęto grupę 17 chorych na PZM (kobiet i mężczyzn w średnim wieku 59 lat). Grupę kontrolną stanowiło 30 zdrowych ochotników, kobiet i mężczyzn w średnim wieku 52,4 lat. W cytrynianowej krwi żylnej oznaczono stężenie TF, całkowitej puli TFPI, stężenie kompleksów TAT oraz aktywności AT.

Stwierdzono we krwi chorych na PZM istotnie podwyższone stężenie TF, a obniżone stężenie TFPI u chorych na CzP, NS i MF.

Wnioski:

1. Przeprowadzone badania wykazały, że we wszystkich grupach chorych z PZM występuje aktywacja krzepnięcia drogą zewnątrzpochodną, czego wyrazem jest wysokie stężenie TF we krwi tych chorych.

2. Wydaje się, że u chorych na NS, MF i CzP aktywacja układu krzepnięcia drogą zewnątrzpochodną jest hamowana przez inhibitor TFPI, o czym może świadczyć obniżone jego

stężenie obserwowane we krwi tych chorych (wynik zużycia w torze zewnątrzpochodnym).

Key words: myeloproliferative neoplasms, tissue factor, tissue factor pathway inhibitor
Słowa kluczowe: nowotwory mieloproliferacyjne, czynnik tkankowy, inhibitor zewnątrzpochodnej drogi krzepnięcia

INTRODUCTION

The first formal classification of myeloproliferative diseases was proposed in 1951 by William Dameshek. Dameshek's concept assumed that these diseases result from the disturbances of mechanisms regulating normal hematopoiesis.

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders, characterized by proliferation in the bone marrow of one or more of the myeloid (granulocytic, erythroid, mast cell and megakaryocytic) lineages [1].

MPNs are now classified into eight categories:

1) chronic myeloid leukemia (CML)
2) polycythemia vera (PV)
3) essential thrombocythemia (ET)
4) primary myelofibrosis (PMF)
5) systemic mastocytosis (SM)
6) chronic eosinophilic leukemia (CEL) not otherwise specified
7) chronic neutrophilic leukemia (CNL)
8) unclassifiable MPNs.

MPN-unclassifiable is a group of MPN-like disorders that do not meet the diagnostic criteria for either the classic or non-classic MPNs.

MPNs have a number of common features in the etiology, pathogenesis, clinical presentation, and treatment. In myeloproliferative neoplasms occurs clonal proliferation of all hemopoietic cell lines, of which the dominant one determines the type of disease.

Clonal nature of hematopoiesis in MPNs was confirmed by the results of the studies concerning the isoenzymes of glucose-6-phosphate dehydrogenase (G-6-PD). In heterozygous patients with myeloproliferative neoplasms in all somatic cells were observed two isoenzymes of G-6-PD (A and B), but in hematopoietic cells only one of them (type A or B). The loss of one isoenzyme started in multipotent stem cells, and then as a result of proliferation took up all the hematopoietic cells. Moreover, cytogenetic and molecular studies in chronic myeloid leukemia (CML) confirm the presence of the Ph chromosome and BCR-ABL fusion gene, which encodes a P 210 protein responsible for the malignant transformation [2]. Recent studies indicate the presence of a JAK2 mutation in patients with PV, ET and mutations in gene 10 (on chromosome X) as well as 11-13 in patients with PMF [3, 4].

In patients with MPNs are observed frequent hemostatic disturbances such as thrombotic disease and bleeding disorder.

Recent reports indicate that the major and the most important pathway of coagulation is the extrinsic coagulation pathway, in which the key role plays a tissue factor (TF) and factor VII [5]. Data on the TF concentration in cancer are divergent, but there is single report, which indicates increased level of TF in patients with myeloproliferative diseases [6].

The major endogenous regulator of coagulation activation dependent on TF is tissue factor pathway inhibitor (TFPI). TFPI inhibits the activity of factor Xa and the complex of factor VIIa / TF. In neoplasms the concentration of coagulation inhibitors such as TFPI may be normal or elevated with reduced its activity at the same time.

Thromboembolism and bleeding are one of the most common causes of death in patients with proliferative diseases.

Experimental studies on animals as well as clinical researches provide information about relationship between blood coagulation system and tumor growth. It is well known that hemostatic disturbances are secondary to the cancer and usually develop as the disease progresses. There are no ideal parameters, which allow assessing the risk of thrombotic disease and bleeding disorder. Well established laboratory tests have poor diagnostic and prognostic value. For this reason, hemostatic disturbances observed in cancer patients are still important clinical problem.

The aim of the study was to assess the activation of extrinsic blood coagulation pathway, based on the TF and TFPI measurements in patients with MPNs.

MATERIAL AND METHODS

The study group consisted of 17 patients with myeloproliferative neoplasms (mean age 59), treated in the Clinic of Haematology, Dr Jan Biziel University Hospital No. 2 in Bydgoszcz. Based on medical interview, physical examination and additional tests (complete blood count with peripheral blood smear, selected parameters of coagulation system, bone

marrow biopsy with histopathological evaluation and cytogenetic) the diagnosis of MPNs was made. None of the patients had clinical symptoms of thrombotic disease and hemorrhagic diathesis at the time of blood collection.

The control group consisted of 30 healthy volunteers, age- and sex- matched (mean age 52). The study obtained the approval of the local Ethics Committee. Each studied person was informed about the purpose of the research and gave written consent.

Blood samples were drawn from an antecubital vein (in the morning) into a plastic tube containing 3.2% sodium citrate (anticoagulant: blood - 1:9). Then the samples were centrifuged at 3000 rev/min for 15 minutes at 4°C. The obtained plasma was divided into aliquots and stored at -80°C until analysis.

In the blood collection day the following parameters were determined:

1. complete blood count with peripheral blood smear
2. In the platelet-poor plasma the following parameters were measured using ELISA technique:
3. tissue factor concentration (TF)
4. tissue factor pathway inhibitor (TFPI)
5. level of thrombin-antithrombin complexes (TAT) with American Diagnostica kits
6. antithrombin activity (AT) (Dade Behring kit) using chromogenic method.

Analysed variables were examined statistically (using STATISTICA for Windows) on account of the compliance with the normal distribution. Kolmogorov-Smirnov test was used to assess the normality of the distribution. Data are presented as mean and standard deviation for normally distributed continuous variables and were measured by Student's t-test. Values of $p<0.05$ were considered to be statistically significant.

RESULTS

Table I shows the mean and standard deviation of TF and TFPI concentration in patients with neoplasms and in the control group. As shown, in all myeloproliferative neoplasms was significantly increased TF concentration in comparison to the controls. TFPI concentration was significantly elevated in patients with CML, and decreased in the other MPNs.

Table I. *Plasma levels of TF and* TFPI in patients *with myeloproliferative neoplasms in comparison to the control group*

Tabela I. *Stężenie TF i TFPI w osoczu chorych na PZM w porównaniu grupą kontrolną*

Parametr Parameter	**Grupa badana Study group (N=17)**				**Grupa kontrolna Control group (N=30) (M;SD)**	**p**
	CML (N=3) (M;SD)	**PV (N=4) (M;SD)**	**ET (N=7) (M;SD)**	**PMF (N=3) (M;SD)**		
TF [pg/ml]	691.86; 23.55	1126.51; 210.82	1526.43; 2134.09	856.35; 866.33	174.43; 76.11	**0.0001**
TFPI [ng/ml]	157.90; 188.71	33.74; 8.10	58.38; 27.10	53.25; 11.58	86.71; 23.22	**0.0063**

Table II. *Concentration of TAT complexes and AT activity in patients with myeloproliferative neoplasms in comparison to the control group*

Tabela II. *Stężenie kompleksów TAT i aktywności AT w osoczu chorych na PZM w porównaniu z grupą kontrolną*

Parametr Parameter	**Grupa badana Study group (N=17)**				**Grupa kontrolna Control group (N=30) M;SD**	**p**
	CML (N=3) M;SD	**PV (N=4) M;SD**	**ET (N=7) M;SD**	**PMF (N=3) M;SD**		
TAT [μg/l]	3.74 2.12	45.11 87.91	3.91 6.28	10.21 15.03	3.23 2.32	0.2369
AT [%]	113.5 19.09	117.0 14.90	122.29 25.20	113.00 20.42	114.20 17.14	0.3174

Table II contains the average value and the standard deviation of antithrombin (AT) and thrombin-antithrombin complexes (TAT) in the study group and controls. The highest average value of TAT concentration was observed in patients with PV and PMF, but the differences were not statistically significant.

DISCUSSION

Present study has shown that in patients with ET, PV there is a significant increase of TF concentration as well as a decrease in TFPI level in comparison to controls. However, clear interpretation in CML and PMF patients is impossible due to the small size of the group.

Tissue factor, also known as a thromboplastin is a cellular receptor of factor VII. The extrinsic coagulation pathway begins by the binding of factor VII to TF produced by subendothelial cells. The resulting complex (TF/VIIa) activates factor X, which

leads to thrombin generation and, ultimately, initiates the process of fibrin formation [7]. In thrombotic process in patients with PV and ET are involved: JAK2 mutation, tissue factor expression, circulating microparticles, increased blood viscosity, leucocyte activation and their interactions with platelets and endothelial cells [8].

In the available literature there were not any reports concerning TF in myeloproliferative neoplasms. Takahashii et al. showed increased levels of TF in proliferative diseases of the hematopoietic system [9]. High TF concentration in patients with acute myeloid leukemia may reflect an increased procoagulant readiness [10]. Other studies have demonstrated that changes in TF concentration in patients with acute promyelocytic leukemia were not statistically significant; furthermore there was no correlation between the concentration and activity of TF [6,11].

The next parameter evaluated in patients with MPNs was TFPI concentration. TFPI is a specific inhibitor of coagulation pathway initiated by TF. This inhibitor is bound to either endothelial cells or lipoproteins and it can also exist as a free TFPI fraction [12]. TFPI which consists of 276 amino acides, can effectively inhibit TF/FVIIa complexes, as well as factor Xa (FXa). TFPI is synthesized mainly by endothelial cells and for this reason contributes to the antithrombotic potential of the vascular endothelium [7].

In our study it was found that in patients with ET, PMF and PV the TFPI concentration is lowered. This may be a result of its consumption in the coagulation pathway.

The analysis of literature shows that so far there has been only sporadic research concerning TFPI concentration in patients with hematologic malignancies. Mazgajska et al. and Radziwon et al. showed elevated level of TFPI in patients with hematologic malignancies [6, 13]. In patients with acute myeloid leukemia with a course complicated by disseminated intravascular coagulation there was observed a low concentration and activity of TFPI [6, 14].

Antithrombin is a plasmin coagulation inhibitor, which inactivates thrombin - a key enzyme in the coagulation process. The consequence of this process is the formation of inactive thrombin-antithrombin complexes (TAT). Concentration of TAT complexes shows intravascular thrombinogenesis. Research indicates that an increase in TAT concentration is a sensitive and authoritative indicator of ongoing thrombotic process.

The study showed that in patients with myeloproliferative neoplasms was higher mean TAT concentration compared to the control group, however due to the wide range of individual values and small number of patients, the difference was not statistically significant. The mean AT activities in patients with ET and PV were higher than in the control group, but the differences were not statistically significant. Research is being continued on a larger number of patients with MPNs and the current results are considered preliminary.

CONCLUSIONS

1. The results showed that in all groups of patients with MPNs an extrinsic activation of coagulation, resulting in a high concentration of TF in the blood of these patients occurs.

2. It seems that in patients with ET, PMF and PV, an extrinsic activation of coagulation is inhibited by TFPI inhibitor as evidenced by its reduced concentration observed in the blood of these patients (the result of extrinsic consumption in the track).

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