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RISK FACTORS IN THE DEVELOPMENT OF THROMBO-HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Key Words: *myeloproliferative neoplasms, vascular complications, acute phase proteins, alpha-acid glycoprotein, fibronectin, matrix metalloproteinases, mutations V617F of the JAK2 gene.*

The article is devoted to the urgent problem of onchematology — the diagnos vention and treatment of thrombo-hemorrhagic complications (THC) in with Ph-negative myeloproliferative neoplasms (MNP). ***Aim:*** *the determ the state of disintegration processes in the system of hemostasis in the form THC in patients with Ph-negative MNP; the development of new algori the diagnosis and prevention of thrombotic conditions.* ***Object and metho*** *were examined 120 patients with MNP: 33 — with polycythemia vera (PV with primary myelofibrosis (PMF), 9 — with essential thrombocythemia. T parison group consisted of 95 patients with atherosclerotic lesions of the v the lower extremities. In the blood serum was determined the level and of proteins of the acute phase of inflammation, adhesive molecules, m talloproteinases (MMP), the presence of the V617F mutation in the JA* ***Results:*** *in 16 patients with MPN the V617F mutation in the JAK2 gen tected, in 11 JAK2-positive patients there were vascular complications nesis. In almost all cases of PMF, fragments with m.m. 84 and 126 kDa termined in the composition of alpha-acid glycoprotein (AAGP), repre polyantenic glycans with a high level of sialylation. In the blood serum patients with THC, the level of AAGP two-antenna glycans was signific creased. A distinctive feature of patients with PV was the presence of ter cose in the composition of O-glycans of fibronectin and the V617F mu the JAK2 gene. In case of MPN, a decrease in the content fibronectin in s accompanied by a decrease in its activity due to increased fragmentatio ments with m.m. from 15 to 200 kDa: 220–180 kDa, 165 and 58 kDa, 28 kDa, 19–15 kDa were determined, which is explained by changes in t ture of the molecule (branched glycans). The decrease in serum fibronec correlated with a decrease in its functional activity. In MPN patients wi there is a direct correlation between increased levels of AAGP and high of MMP-9, which confirms the role of activated neutrophils in the formati thrombotic state.* ***Conclusion:*** *the practical use of the laboratory diagnos rithm as an early marker for development of possible vascular complica allow timely to identify patients that requires specialized outpatient hemat fices the administration of hydroxyurea and/or interferon agents in, ana istration of disaggregants and agents for microcirculation improvement. with manifestations of microcirculatory disorders and corresponding ch laboratory parameters require a more thorough prognostic assessment, monitoring of clinical and laboratory status.*

Myeloproliferative Ph-negative neoplasia (MPN) — polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) are validated mainly at the age of 50–70 years with a benign and prolonged course of this pathology.

Due to excessive synthesis of cytokines and proteolytic enzymes with activation of IL6–JAK2–APRF–STAT signal cascade, regulating proliferation and diff tion of cells in increasing hypoxia, a specific m microenvironment is formed.

A cell that has entered the path of differe inevitably dies. Subsequent generations of cells sy their own proteins, some of which enter in the i lular matrix. The glycan components of proteins ıst sensitive to changes in the composition of the cellu-genome [1]. The specificity of the structure of glycans letermined by the microheterogeneity of proteins and ue to the presence of several molecular forms that dif-in the composition of carbohydrate chains and func-nal activity. The appearance of atypical branched gly-ıs is considered to be early specific biochemical mark-of development of malignant diseases [2].

The intercellular matrix is a complicated complex of cromolecules, collagen proteins, proteoglycans and coproteins. In the biochemical aspect, a high degree organization and ordering of the intercellular matrix xpressed by specific quantitative ratios of the biopoly-rs forming it. Any deviations from these specific ra-cannot but entail disorders in the structure and func-ıs of the connective tissue. A highly ordered three-di-nsional structure of the matrix, which includes type collagen — a protein from the group of glycoproteins ɔrmed by the interaction of glycoprotein binding cen-with adhesion proteins — laminin, fibronectin (FN) integrins. The nature of protein-carbohydrate bonds ermines the timing of cell attachment to the intracel-r matrix [3].

Collagen is synthesized by fibroblasts: translation and t-translational modification of polypeptide chains ur inside the cell, and extracellularly collagen «ma-s» — protein modification is completed with the par-pation of FN with the formation of collagen fibers [4].

Collagen type IV contains $1\alpha_1$ (IV) $2\alpha_2$ (IV) chains. After secretion by cells, they do undergo proteolytic modification and therefore re-the structure of the N- and C-terminal globular do-ns (NC_1, 7S and NC_2). If intracellular protein synthe-s disturbed, oligosaccharide part is absent in them, leads to increase in the activity of proteolysis pro-es — the leading post-translational mechanism for nitoring the state of extracellular matrix (ECM) [5].

FN is formed in hepatocytes, fibroblasts and neutro-ic granulocytes already at the stage of early myeloid uration [6]. Increase in the adhesive ability of leuko-s in MPN patients due to a significant stiffness and airment of the elasticity of the inner membrane [7] ably results from structural-functional disturbances ycane components of anomalous cells.

n the process of adhesion a FN molecule with one ain binds to the membrane receptor in with an-r one — with the corresponding centers on collagen cules in the ECM. In ECM, multimers «fibronec-platelet receptor GPIc-IIa + collagen» are located nd collagen fibers and endothelial cells [8]. The car-drate component of FN makes up 25% of the whole cule. In a state of physiological norm, the structure cans of cellular (leukocyte) FN is represented by O-ns and bi- or tri-antenna N-glycans (asparagine N-ues containing core fucose.

rotease-resistant domains of FN ligands have sites ding with heparin, fibrinogen, collagen, cell surface tors. In the plasma FN is present in several forms: — in the form of dimers, aggregated — consisting of multimers and complexes, degraded — represented by fragments formed during proteolysis [9, 10].

In MPN patients a significant decrease in the functional activity of FN with a decrease of its content in blood plasma and an increase in the level and activity of matrix metalloproteinase (MMP)-9 was established, which is interrelated — in the process of degradation of FN under the action of trypsin and chymotrypsin, the amount of fFN with m.m. 165 kDa and 58 kDa increases, namely they activate the synthesis of MMP-9 in neutrophils [11]. A disorder in the synthesis of fibronectin, especially in the progression of PMF occurs due to a defect structure of hepatocytes, the main source of plasma FN synthesis; at a leukocyte level of more than $20 \cdot 10^9$/l, functional activity of FN decrease significantly and the processes of degradation are enhanced due to the impairment of the structural features of its glycans (increased core fucosylation rate in their significant branching) and a significant increase in MMP activity, which proves the role of neutrophils of varying degree of maturity, including granulocytes of a pathological clone, in the synthesis of FN with an anomalous structure of glycans (branched hypersialized) and hyperproduction of MMP-9. It is the specific features of FN neutrophils (including hydrolyzed), the degree of their activation, migratory abilities and stimulation of adhesion of thrombocytes to collagen is determined [12]. Activation of proteolysis processes leads to disorder of adhesion processes — «cell-matrix».

The participation of C-reactive protein (CRP) in the expression of adhesive molecules, including FN, enhances the migration of leukocytes from the vasculature to the tissue and changes the transmission route of signals from receptors in the sequence: IL-1/TNFα/IL-6 → Ras → MAP kinase → NF/IL-6. Catabolism of cellular proteins and intracellular matrix is performed with participation of MMP. Expression of MMP is similar to the expression of acute phase proteins.

Under physiological conditions MMP are synthesized episodically in the form of non-glycosylated proenzymes on a specific area of the cellular membrane of fibroblasts, phagocytes, epithelial cells and lymphocytes. The source of MMP-9 synthesis is tertiary peroxidase-negative granules of activated myeloid cells — stab and segmentonuclear neutrophils in the form of pro-MMP-9 with m.m. 78.4 kDa. During subsequent glycosylation in the Golgi apparatus, a proenzyme with m.m. 92 kDa — MMP-9 is formed and secreted. Due to the presence of an additional site identical to fibronectin in the catalytic domain of the proenzyme, MMP is ensured with a high affinity to membrane components [10]. In tumor cells in a state of hypoxia, MMP are continuously synthesized by the entire surface of the membrane with the involvement of Src tyrosine kinase in the process [13].

Variations concomitant to *JAK2* mutations in 16 chromosome which encodes synthesis of metalloproteinases, bring epigenetic abnormalities in transcriptional regulation [14] and change ECM structure [15].

With the simultaneous exposure to several proteinases synthesized by tumor and/or stromal cells, disorganization of the ECM progresses [10, 16]. Activation of enzymes also occurs in response to changes in the state of components of the ECM itself [17–19]. Strengthening of synthesis and increase in activity of MMP, especially MMP-9 [20] contributes to the progression of stromal-vascular dystrophy with the formation of fibrosis in patients.

Elevated level and high activity of MMP and especially of MMP-9 contributes to progression of stromal-vascular dystrophy with formation of fibrotic changes in the bone marrow.

The impact of cytokine, MMP-2 and MMP-9 on FN molecule containing atypical glycans leads to their enhanced cascade degradation. FN and MMP are components of the ECM: FN provides cell adhesion to the substrate and opsonization of foreign material [21, 22], and MMP by means of proteolysis changes the structure of microenvironment proteins (collagen, fibronectin, laminin, and glycoproteins of the basement membrane) with matrix remodeling [23].

Therefore, presence of defective glycans in the composition of FN molecule as the most sensitive markers for myelofibrosis development and in the following of myeloproliferative syndrome, as well as products of their degradation, as markers of thrombo-hemorrhagic complications (THC), may be the earliest criteria when making a diagnosis and predicting the development of the leukemic process and defining risks of THC [24, 25].

In MPN patients an increased content of cellular elements with the impairment of the rheological properties of blood is followed by impairment of thomboresistance of capillaries. Rheologic disorders are caused by and enhanced spontaneous platelet aggregation and adhesion of leukocytes with formation of cellular conglomerates — phenomenon of leukocyte–platelet aggregation is realized. In disorders of endothelial-leukocyte interaction synthesis of MMP-2 and MMP-9 is enhanced with the increase in their activity with involvement of «acute phase» proteins (CRP), adhesive proteins (FN), and highly glycosylated alpha-acid glycoprotein (AAGP).

AAGP consists of five complex-type N-glycans with a different number of antennas; the biological activity of glycan «antennas» is determined by negatively charged sialic acids (12% of the molecule) and fucose components [26]. In conditions of physiological norm, the carbohydrate determinants of AAGP contain bi- and triantenic glycans of a complex type [27]. Cytokines (TNFα and IL-6) initiate the synthesis of AAGP with m.m. 42 kDa by hepatocytes. The source of alternative synthesis of AGP are polymorphonuclear neutrophils; its m.m. 50–64 kDa, and the structure is represented by a hypersalinated peptide glycoform with fucosylated glycans and polylactamase complexes.

Membrane glycoproteins of platelets, leukocytes, and endothelium belong to the products of gene families of receptors and have a similar structure. They determine the processes of proliferation, differentiation, intercellular contacts, and cell migration.

Taking into account specific features of formation of the stromal microenvironment of the bone marrow in the process of myelofibrosis development with subsequent myeloid metaplasia, complicated with vascular events, we judge it expedient to study the problem of THC in MPN in the aspect of membrane components of cellular elements at the level of glycans as the earliest markers of pathologic processe, including leukemic ones.

Aim: to determine the state of disintegration processes in the system of hemostasis in the formation of thrombotic complications in CNP patients, the development of new algorithms for the diagnosis of thrombotic conditions and antithrombotic prevention.

MATERIALS AND METHODS

Our sample included 120 patients with MPN (men — 66, women — 54) aged 22–75 years (average 60.1 ± 1.1 years), with established diagnoses: in 33 (27.5%) — PV; 78 (65.0%) — PMF; 9 (7.5%) — ET; 38 (31.7%) patients developed thrombohemorrhagic complications in the form of acute cerebrovascular accidents, myocardial infarction, episodes of thrombosis of arteries of the lower extremities. In the comparison group there were 95 patients with manifestations of lower limb ischemia of atherosclerotic origin (men — 70, women — 25) aged 55–75 years (average 63.4 ± 0.88 years), in 38 (40.0%) atherothrombosis has developed.

The examination of patients was carried out using modern methods: general-clinical, morphological, cytological, biochemical, hemostasiological in compliance with international standards according to the recommendations of [28], as well as specific methods for determining the level, structure and functional activity of AAGP, FN, fibronectin fragmentation (fFN), MMP and their glycan component.

The concentration of AAGP in plasma was determined by the method of rocket immunoelectrophoresis (RIEP), the lectin-binding ability of AAGP — by cross affinity immunoelectrophoresis (CAIEP) using lectins of different specificity [2].

Isolation of AAGP from human blood serum was performed in two ways: by hydrophobic extraction with phenol and by immunoaffinity chromatography on agarose with immobilized antibodies to this glycoprotein. Fractions enriched with AAGP were tested using immunodot and immunoblot methods. The concentration of FN was determined by immunodot analysis using polyclonal rabbit antibodies to FN and secondary antibodies conjugated with horseradish peroxidase (Bio-Rad, USA). GelProAnalyser 32 software was used to quantify the results of the analysis [29].

The functional activity of FN was determined according to the degree of its binding to heparin by the method of cold heparin precipitation. Proteolytic cleavage of FN in vitro was performed by incubating the commercial preparation of FN (Sigma, USA) with trypsin (3.4.21.4) (Sigma, USA), chymotrypsin (3.4.21.1) (Sigma, USA), collagenase (3.4.24.3) (Boehringer Mannheim, Nimechchina), MMP-2 (3.4.24.24) (Sigma, USA), MMP-9

Table 1

Findings of hemogram in [illegible] of the disease, M ± m (s)

Finding	PMF (n = 78)	ET (n = [illegible])	[illegible]	[illegible]	Group of comparison (n = 95)
Erythrocytes, 10^{12}/l	6.17 ± 0.21^2 (1.84)	5.99 ± 0.19^2 (0.54)	[illegible]	[illegible] $\pm 0.16^2$ (1.73)	4.28 ± 0.10 (0.95)
Hemoglobin, g/l	150.39 ± 4.00^2 (35.10)	148.01 ± 4.20 (11.88)	175.90 ± 5.81^2 (32.90)	[illegible] $\pm 3.53^2$ [illegible]	133.90 ± 2.84 (27.60)
MCV μ/m³	85.64 ± 8.28 (72.66)	77.43 ± 6.34 (17.93)	67.34 ± 5.76^1 (33.09)	[illegible]	83.23 ± 4.38 (42.47)
Hematocrit, %	47.21 ± 3.52 (30.89)	48.71 ± 4.62 (13.07)	53.17 ± 3.83 (21.66)	[illegible]	43.27 ± 3.32 (32.19)
Platelets, 10^9/l	671.31 ± 7.60^2 (66.69)	$989,69 \pm 8,66^2$ (24.49)	447.2 ± 13.48^2 (76.25)	[illegible]	252.2 ± 10.59 (102.7)
MPV μ/m³	7.92 ± 0.24 (2.11)	9.24 ± 0.82^1 (2.32)	8.22 ± 0.40 (2.26)	[illegible]	7.82 ± 0.17 (1.62)
Leukocytes, 10^9/l	20.34 ± 1.44^2 (12.64)	8.22 ± 1.41 (3.99)	11.57 ± 1.02^2 (5.82)	$17.16 \pm$ [illegible] (11.70)	9.03 ± 0.19 (1.89)
Granulocytes, ⩾ 50%	83.12 ± 5.34^2 (46.86)	63.75 ± 4.86 (13.75)	65.73 ± 4.64 (26.25)	76.74 ± 3.78^1 (41.24)	57.94 ± 5.34 (51.77)

Note: $^1p < 0.05$; $^2p < 0.001$ relative the to group of comparison.

(3.4.24.35) (Sigma, USA), thrombin (3.4.21.5) («Renam», Russia).

FN fragmentation was studied using electrophoresis in density gradient of 5–17.5% of polyacrylamide gel, using Laemmli method [30] and western blot analysis [31].

The microheterogeneity of AAGP and FN was determined by lectin-enzyme analysis using de-glycosylated with N-Glycosidase F (US Biological, USA) antibodies to this protein [32].

The conjugation of lectins with horseradish root peroxidase and lectin blot analysis was performed according to the recommendations of [33].

Determination of gelatinase activity (MMP-2 and MMP-9) in blood plasma was performed by gelatin-zymography with preliminary vertical electrophoresis of samples in 7.5% PAAG in the presence of 0.1% SDS and 1.0% gelatin (Sigma, USA).

Statistical processing of the research results was carried out using biostatistics methods, STATISTICA v.6.1 (license number AJAR909E415822FA). To build mathematical models for assessing the likelihood of developing thrombotic complications, we used the logistic function: $P(y) = 1/(1+\exp\{-y\})$, (y) is a linear combination of prognostically significant thrombus formation factors.

RESULTS AND DISCUSSION

The analysis of hemograms during the initial examination of patients showed that in MPN the level of cellular elements (erythrocytes, leukocytes and platelets) in relation to the group of patients with atherosclerotic lesions of the vessels of the lower extremities was significantly higher: in ET there was noted a significant increase in platelet levels (by 3.9 times; $p < 0.001$), in PMF — leukocytes and granulocytes (by 2.3 and 1.4 times, $p < 0.001$, respectively), in PV — the level of red blood cells and hemoglobin — by1.6 and 1.3 times, $p < 0.001$ (Table 1).

In trepanobioptates and aspirates of the bone marrow, exemplified by patients with PV, there was revealed an increased content of megakaryocytes and myeloid cells of various maturity and sizes with single atypical forms. The adipose tissue content was reduced; in the perivascular zones of the bone marrow there was determined the proliferation of fibroreticular elements with structures of framework spaces replaced by them.

Clinical evaluation of MPN patients revealed manifestations of plethoric syndrome in 59 (49.2%) patients, including 42.3% of patients with PMF and 78.8% of patients with PV. Splenomegaly was noted in 51 (42.5%) cases, of which 53.8% with PMF, 15.2% with PV and 4 of 9 (44.4%) with ET. Manifestations of hepatomegaly were noted in every third patient with MPN (38 patients — 31.7%), mainly in PMF (34 patients — 43.6%).

The trigger mechanism in the processes of thrombus formation is an increase in platelet activity [1]. In our studies, indicators of spontaneous platelet aggregation (39.96 ± 0.95%) and ADP-induced aggregation (66.12 ± 1.43%) in PV; ristomycin-induced platelet aggregation (77.46 ± 2.32% and 77.21 ± 2.03%), blood clot retraction (61.22 ± 1.17% and 58.41 ± 1.33%) in patients with PMF and ET significantly exceeded the corresponding findings of patients with atherosclerotic process (Table 2).

According to the results obtained, findings of spontaneous platelet aggregation (39.96 ± 0.95%) and ADP-induced aggregation (66.12 ± 1.43%) in PV; ristomycin-induced platelet aggregation (77.46 ± 2.32% and 77.21 ± 2.03%) in patients with PMF and ET significantly differed from the corresponding findings in patients with the atherosclerotic process, which indicates severe microcirculatory disorders due to the features of cell composition, especially with the level of leukocytes being more than 20 • 10^9/l.

In the study of routine laboratory findings, mathematical models for assessing the likelihood of developing thrombotic complications in MPN patients are proposed, with the inclusion of a set of hemogram and hemostasiogram findings in the diagnostic algorithm.

In patients with MPN and atherosclerotic vascular lesions, fragments with a small and medium molecular weight were included in the wide range of fFN. Herewith, in patients of the main groups with decrease in the

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Findings of hemogram in patients with [illegible] of the disease, $M \pm m$ (s)

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Erythrocytes, 10^{12}/l	6.17 ± 0.21^2 (1.84)	5.99 ± 0.19^2 (0.54)	[illegible]	[illegible] $\pm 0.16^2$ (1.73)	4.28 ± 0.10 (0.95)
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According to the results obtained, findings of spontaneous platelet aggregation (39.96 ± 0.95%) and ADP-induced aggregation (66.12 ± 1.43%) in PV; ristomycin-induced platelet aggregation (77.46 ± 2.32% and 77.21 ± 2.03%) in patients with PMF and ET significantly differed from the corresponding findings in patients with the atherosclerotic process, which indicates severe microcirculatory disorders due to the features of cell composition, especially with the level of leukocytes being more than $20 \cdot 10^9$/l.

In the study of routine laboratory findings, mathematical models for assessing the likelihood of developing thrombotic complications in MPN patients are proposed, with the inclusion of a set of hemogram and hemostasiogram findings in the diagnostic algorithm.

In patients with MPN and atherosclerotic vascular lesions, fragments with a small and medium molecular weight were included in the wide range of fFN. Herewith, in patients of the main groups with decrease in the

Hemostasiogram findings and some biochemical tests in MPN patients, M ± m (s)

Finding	PMF (n = 78)	ET (n = 9)	PV (n = 33)	MPN (n = 120)
Spontaneous platelets aggregation,%	33.09 ± 0.59[2] (5.19)	38.52 ± 0.83 (2.35)	39.96 ± 0.95[1] (5.37)	35.49 ± 0.55[1] (5.98)
Platelets adhesion, %	36.50 ± 1.41[1] (12.37)	30.52 ± 2.63 (7.43)	32.74 ± 2.28 (12.92)	35.08 ± 1.21 (13.18)
Blood clot retraction, %	61.22 ± 1.17[3] (10.27)	58.41 ± 1.33[1] (3.76)	58.10 ± 2.48[1] (14.05)	60.22 ± 1.04[3] (11.30)
Autocoagulation test (ACT), %	90.47 ± 1 03 (9.04)	85.43 ± 2.96 (8.37)	77.14 ± 3.95[2] (22.36)	86.66 ± 1.43 (15.44)
Antithrombin, %	92.01 ± 0.94[3] (8.25)	78.32 ± 1.34 (3.79)	77.26 ± 1.42 (8.05)	86.94 ± 0.97[3] (10.58)
X111-factor, %	93.86 ± 0.87 (7.63)	91.42 ± 1.53 (4.33)	91.65 ± 1.61 (9.11)	93.04 ± 073 (7.98)
Maximal activity of clotting (MA), %	85.24 ± 2.06 (18.08)	82.73 ± 2.47 (6.98)	82.88 ±3.24 (18.35)	84.47 ± 1.61 (17.58)
T1 – minimal time of achieving ½ MA, sec.	5,68±0,14 (1,23)	5,42±0,26 (0,74)	6,79±0,23[3] (1,28)	5,99±0,13[1] (1,37)
T2 – minimal time MA, sec.	10,72±0,18[3] (1,58)	10,34±0,37 (1,05)	11,56±0,32[3] (1,81)	10,95±0,16[3] (1,67)
Degree of ADP aggregation,%	54,18±0,78[3] (6,84)	57,12±1,51[3] (4,27)	66,12±1,43[3] (8,11)	57,68±0,83[3] (8,98)
Degree of aggregation with ristomycin,%	77,46±2,32[3] (20,36)	77,21±2,03[1] (5,74)	78,41±4,78[2] (27,05)	77,72±2,07[3] (22,54)
Time of aggregation, sec.	352,6±7,57[3] (66,4)	421,3±14,03[3] (39,68)	425,3±17,48[3] (98,9)	377,6±7,84[3] (85,4)
Total protein, g/l	63,44±4,24[1] (37,21)	69,32±3,53 (9,98)	67,83±3,32 (18,78)	65,12±3,21[1] (35,0)
C-reactive protein (CRP)	++	+	+	+
Cholesterol level, g/l	3,68±0,15[3] (1,32)	3,32±0,93[2] (2,63)	3,98±0,14[3] (0,80)	3,74±0,12[3] (1,28)
Lactate dehydrogenase (LDH), un/l	675,7±68,4[3] (600,2)	310,3±30,9 (87,4)	352,6±24,1[1] (136,3)	559,4±47,2[3] (514,8)

Note: [1]$p < 0.05$; [2]$p < 0.01$; [3]$p < 0.001$ relative to the comparison group; «+» – degree of reaction.

Structural and functional changes in FN in MPN, M ± m (%)

Group	Lectins				
	LABA	AAL	LCA	WGA	SNA
Norm	100	100	100	100	100
PV	182 ± 14,11[3] ↑	210 ± 24,3[3] ↑	159,6 ± 21,5[2] ↑	171,2 ± 15,3[3] ↑	101,5 ± 5 N
ET	75,13 ± 8,42[1] ↓	209,6 ± 11,2[3] ↑	134,6 ± 15,4[1] ↑	106,3 ± 3,41 N	98,1 ± 1 N
PMF	65,85 ± 5,92[2] ↓	230,1 ± 14,8[3] ↑	117,1 ± 11,8 N	167,8 ± 12,6[3] ↑	97,4 ± 1 N
Atherothrombosis	98,8 ± 8,65 N	44,7 ± 4,56[3] ↓	71,91 ± 5,94[1] ↓	387,0 ± 28,4[3] ↑↑↑	63,89 ± 3 ↓

Note: [1]$p < 0.05$; [2]$p < 0.01$; [3]$p < 0.001$ relative to the norm; ↓ – decrease; ↑ – increase; ↑↑↑ – significant increase; N – normal.

LABA – laburnum anagyroides agglutinin; AAL – aleuria aurantia lectin; LCA – lentil lectin; WGA – wheat germ agglutinin; SNA mannose binding lectin.

content of FN fragments with m.m. 175–160 and 155–150 kDa by 50% or more, the content of fFN with m.m. 98–90 (from 60% in PV to 87% in PMF) was significantly increased, which correlates with a high risk of thrombotic complications development.

Quantitative and qualitative composition of α-acid glycoprotein in MPN. In MPN patients in composition of AAGP, protein with m.m. 42 kDa (plasma) synthesized by hepatocytes prevailed; in patients with atherothrombosis and with vascular complications in patients with PV, peptides with m.m. 42 and 68 kDa were revealed; in PMF AAGP with m.m. 68, 84 and 126 kDa, due to the existence of additional sources of its synthesis, including leukemic clone cells was revealed.

The structure of glycans of A origin is different from those synth In MPN patients as part of AAC 126 kDa glycans contain residues o acid located in positions 2 → 6 and bosis, sialic acid was located in gly Activated neutrophils were the sou sis in both cases, but in MPN neu ing maturity, which may be an addi diagnosis of myeloproliferative ne

The lectin-binding activity of glycans consisted of branched st but the functional activity, which gree of glycosylation, is significan

Table 4

MMP activity and spectrum of FN fragments in blood plasma of patients of study groups, M ± m

Studied group/course		[illegible]			fFN, kDa
		Pro-MMP-9	MMP-9	MMP-2	
PV	uncomplicated (n = 20)	104.9 ± 4.1	264.6 ± 6.2³	113.4 ± 3.4	220–20
	complicated (n = 13)	123.0 ± 6.1	354.3 ± 7.8³	134.3 ± 6.5¹	
ET	uncomplicated (n = 5)	125.4 ± 9.3	262.0 ± 15.6³	376.7 ± 16.5	220–15
	complicated (n = 4)	144.2 ± 7.4¹	385.3 ± 27.9³	533.2 ± 15.4³	
PMF	uncomplicated (n = 54)	126.3 ± 5.2	436.0 ± 12.9³	137.9 ± 10.1¹	220–15
	complicated (n = 24)	143.1 ± 6.2³	674.3 ± 32.2³	145.2 ± 14.3¹	
Atherosclerosis	uncomplicated (n = 57)	103.9 ± 4.7	121.7 ± 10.9	96.5 ± 2.6	220–15
	complicated (n = 38)	100.3 ± 6.3	114.4 ± 16.0	156.2 ± 5.4³	
Control	n = 30	100	100	100	220–90

Note: ¹$p < 0.05$; ²$p < 0.01$; ³$p < 0.001$ relative to the control.

Table 5

Results laboratory test in MPN patients with *JAK2* gene mutation, M ± m

Finding	Presence of *JAK2* mutation		MPN without *JAK2* mutation
	PMF/ ET	PV	PMF/ ET / PV
Erythrocytes, 10^{12}/l	7.0 ± 0.1	7.3 ± 0.4	6.3 ± 0.2¹·²
Leukocytes, 10^9/l	17.6 ± 0.6	11.9 ± 1.1	9.3 ± 0.8¹·²
Platelets, 10^9/l	859.2 ± 118.9	568.6 ± 27.0	461.9 ± 17.1¹·²
LDH, units.	760.7 ± 98.4	358.6 ± 26.2	283.8 ± 8.4¹·²
AAGP, µg /l	0.95 ± 0.01	0.89 ± 0.01	0.85 ± 0.01¹·²
FN, µg /l	257.1 ± 3.9	224.6 ± 0.9	251.3 ± 1.9²
fFN, %	44.9 ± 1.1	48.2 ± 1.5	63.6 ± 0.6¹·²
MMP-2,%	138.9 ± 2.8	145.0 ± 5.7	125.0 ± 1.3¹·²
MMP-9,%	573.1 ± 17.8	336.0 ± 6.7	185.0 ± 7.5¹·²
Pro-MMP-9,%	141.3 ± 3.8	125.0 ± 2.9	108.8 ± 2.5¹·²

Note: ¹$p < 0.05$ in comparison with PMF/ET patients with *JAK2* mutation; ²$p < 0.05$ in comparison with PV patients with *JAK2* mutation.

In MPN patients low plasma FN with a decrease in FA is due to differences in its glycan component (the presence of terminal fucose as a part of *O-glycans* and core fucose as a part of F-N-glycans) with increased degradation with the formation of fFN in the range of m.m. from 200–19 kDa.

Incubation of FN with MMP-2 and MMP-9 showed a significant increase in the activity of gelatinases in MPN patients compared with the norm, especially in the complicated course of the disease (Table 4), which also indicates the involvement of activated leukocytes of varying maturity in the proteolysis processes, including neutrophils of a pathological clone. MMP-2 was determined in active form, and MMP-9 was also present in the form of zymogen (pro-MMP-9).

In the serum of all patients which have undergone thrombotic complications, there was a significant increase in the content of fFN with m.m. 98–90 kDa ⩾500% with a high total activity of MMP-2 and MMP-9 ⩾200%, which should be considered as markers of vascular catastrophes.

Mutation V617F of *JAK2* gene was detected in 16 of 24 patients with MPN (66.7%), of which 11 cases (68.8%) had a history of vascular complications — in 3 out of 4 patients with ET, in 5 out of 7 patients with PMF, in 3 out of 5 patients with PV. In 6 cases, episodes of vascular catastrophes repeated. In ailments in chronic kidney disease in the presence of a JAK2 mutation, a significant increase in the number of red blood cells, white blood cells and platelets in peripheral blood was established, as well as a significant increase in the level of LDH and MMP-9 with a decrease in the functional activity of FN (Table 5).

It is advisable for patients with an increased risk of developing thrombohemorrhagic complications in MPN to undergo elective courses of inpatient treatment to correct overall health against the background of cytoreductive therapy.

According to the results of the correlation analysis, it has been established that a direct correlation between the increased number of platelets and leukocytes in MPN patients ($r = 0.476$; $p < 0.001$) is a negative prognostic factor in the progression of myeloproliferative syndrome.

Thus, we monitored the mechanisms of the formation and realization of THC based on the assessment of the cellular and coagulation components of the blood composition of MPN patients and atherosclerotic vascular lesions (as a comparison group) with an in-depth study of the composition of glycans in patients' blood serum samples.

Thus, informative criteria for the likelihood of developing thrombotic and hemorrhagic events were established in MPN patients, and groups of increased risk of their development were formed, a mathematical model was compiled taking into account the levels of hemoglobin, leukocytes, spontaneous and ristomycin-induced platelet aggregation.

Additional, earlier, criteria specifying the mechanisms of the pathogenesis of the development of THC are: increased activity of MMP-2 and MMP-9 with enhanced fibronectin degradation in the presence of fFN with m.m. 90–98 kDa ⩾ 50% of the total amount of fFN; a high level of AAGP, due to an increase in the level of biennial glycans, the appearance of AAGP components with m.m. 126 and 84 kDa.

In the comparison group, in ischemic damage to the main vessels of the lower extremities of atherosclerotic genesis, significant risk criteria for the development of vascular events were high cholesterol, indicators of spontaneous and AAGP-induced platelet aggregation. The above changes were due to increased levels of AAGP, FN, increased functional activity of fibronectin, MMP-2 and MMP-9, a high degree of degradation of FN under the influence of MMP-2 and MMP-9 with a content of ffn with m.m. 90–98 kDa ⩾ 50% of the total amount of fFN.

Considering the gender factor in comorbidity with cardiovascular pathology (metabolic syndrome, hypertension, diabetes mellitus, ischemic heart disease), the risk of developing fatal outcomes is significantly increased. Taking into account the above factors, considering the individual characteristics of patients, will allow for an adequate assessment of risk factors in the development of THC in patients in cases of comorbid conditions, and therefore, to prevent them.

Since the mathematical model of the risk of developing THC in MPN patients and atherosclerotic vascular damage is compiled on the basis of routine findings of a general blood test and a hemostasiogram, it is possible to use it by family practitioners at the primary care level in order to early identify the high-risk population for possible THC and timely the administration of adequate special and preventive therapy.

An in-depth study of the glycan components of the blood serum of patients made it possible to clarify the sources of synthesis of the main components that affect the mechanisms of development of THC and to determine the leading role and sequence of action of activated cells — neutrophils and platelets in the initiation of thrombosis mechanisms in MPN and atherosclerotic process.

The regularity and sequence of pathological changes in MPN patients allows timely and purposeful correction of possible disorders in the hemostasis system by the integrated administration of hydroxyurea, interferon, disaggregants (ASA, clopidogrel, curantyl) in combination with drugs that improve the rheological and metabolic characteristics of the blood.

CONCLUSIONS

1. Identification of the probable risk factors for the development of vascular complications in patients of the study groups using a mathematical model with a set of laboratory screening indicators (in MPN patients a high level of platelets, hemoglobin with increased sponta[neous] platelet aggregation was determined) may precede [clin]ical manifestations of the disease and its complica[tions].

2. The practical use of the laboratory diagno[stic al]gorithm as an early marker for development of po[st-] vascular complications will allow rationally and ti[mely] (in the early stages) to identify patient populati[on] requiresthe treatment in specialized outpatient onc[ohe]matology offices and the administration of hydroxy[urea] and/or interferon agents in, and administration of di[sag]gregants and agents for microcirculation improvem[ent].

3. A study of the composition of glycans in the b[lood] serum of patients made it possible to clarify the sourc[es of] synthesis of the main components that affect the m[ech]anisms of development of THC in MPN and to d[eter]mine the leading role and sequence of action of activ[ated] cells-neutrophils and platelets in the initiation of b[lood] clotting mechanisms.

4. Progressing fibrosis formation in patients w[ith] MPN in the presence of concominant genetic de[fec]tions (mutation *V617F* in the *JAK2* gene) contribu[tes to] the initiation of «defective» clones of white blood c[ells], platelets, red blood cells, which ultimately may trans[form] into a leukemic process.

5. Patients with manifestations of microcircul[atory] disorders and corresponding changes in laboratory [pa]rameters require a more thorough prognostic assessm[ent], constant monitoring of clinical and laboratory status [and] correction of administrations.

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ФАКТОРИ РИЗИКУ РОЗВИТКУ ТРОМБО-ГЕМОРАГІЧНИХ УСКЛАДНЕНЬ У ПАЦІЄНТІВ З МІЄЛОПРОЛІФЕРАТИВНИМИ НОВОУТВОРЕННЯМИ

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Стаття присвячена актуальній проблемі онкогематології — діагностиці, профілактиці та лікуванню тромбо-геморагічних ускладнень (ТГУ) у пацієнтів з Ph-негативними мієлопроліферативними новоутвореннями (МПН). ***Мета:*** *визначення стану дезінтеграційних процесів у системі гемостазу при формуванні ТГУ у пацієнтів з МПН; розроблення нових алгоритмів діагностики і профілактики тромботичних станів.* ***Об'єкт і методи:*** *обстежено 120 пацієнтів із МПН: 33 — зі справжньою поліцитемією (СП), 78 — з первинним мієлофіброзом (ПМФ), 9 — з есенціальною тромбоцитемією. Групу порівняння становили 95 пацієнтів з атеросклеротичним ураженням судин нижніх кінцівок. У сироватці крові хворих визначали рівень і активність білків гострої фази запалення, адгезивних молекул, матриксних металопротеїназ (ММР), наявність мутації V617F в гені JAK2.* ***Результати:*** *у 16 пацієнтів із МПН виявлена мутація V617F в гені JAK2, у 11 JAK2-позитивних пацієнтів в анамнезі були судинні ускладнення. Майже у всіх пацієнтів з ПМФ у складі альфа-кислого глікопротеїну (АКГП) виявлені фрагменти з молекулярною масою 84 і 126 кДа, представлені поліантенними гліканами з високим рівнем сіалування. У сироватці крові пацієнтів із МПН, які мали геморагічні ускладнення, рівень біантенних гліканів був значно підвищений. Для пацієнтів з СП характерними були наявність термінальної фукози у складі О-гліканів фібронектину та мутація V617F в гені JAK2. При МПН зниження вмісту фібронектину у сироватці крові супроводжувалося зниженням його активності за рахунок посиленої фрагментації — фрагментів з м.м. від 15 до 200 кДа: 220–180 кДа, 165 і 58 кДа,* *190 і 28 кДа, 19–15 кДа, що пояснюється змінами у структурі молекули (розгалужені глікани). Зниження рівня фібронектину в сироватці крові корелювало зі зменшенням його функціональної активності. У хворих на МПН з ТГУ виявлений прямий кореляційний зв'язок між підвищенням рівня АКГП і високою активністю ММР-9, що підтверджує роль активованих нейтрофілів у формуванні тромботичного стану.* ***Висновок:*** *дослідження складу гліканів у сироватці крові хворих на МПН дозволило з'ясувати джерела синтезу основних компонентів, що впливають на механізми розвитку ТГК; визначити роль та послідовність дії активованих клітин-нейтрофілів та тромбоцитів у ініціації механізмів згортання крові. Пацієнти із проявами мікроциркуляторних порушень та відповідними змінами лабораторних показників потребують ретельного прогностичного оцінювання, пост го моніторингу клінічного та лабораторного ну з корекцією лікування.*

Ключові слова: мієлопроліферативні новоутворення, судинні ускладнення, білки гострої фази, альфа-кислий глікопротеїн, фібронектин, матриксні металопротеїнази, мутація V617F в гені *JAK2*.

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