

## THE ROLE OF $\alpha$ -ACIDIC GLYCOPROTEIN IN FORMATION OF BLEEDING ABNORMALITIES IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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**Aim:** To compare glycoforms of alpha-acid glycoprotein (AGP) in myeloproliferative neoplasms (MPN) with hepatic lesions. **Materials and Methods:** 110 patients with MPN (31 with polycythaemia vera (PV), 75 with primary myelofibrosis (PMF), 4 with essential thrombocythemia (ET)) were examined. 92 patients with atherosclerotic lesions of lower extremities and 10 healthy people comprised the control. AGP concentration in blood serum was determined by rocket electrophoresis and affinity chromatography. The carbohydrate moiety of AGP was studied by lectin blotting with panel comprising eight lectins. The total content of sialic acids and the type of chemical linkage with galactose were determined. **Results:** High-molecular fragments of AGP detected in MPN (MM 68, 84, and 126 kDa) have been shown as deriving from leukocytes with glycan moiety identified as neutrophilic component. In MPN with thrombotic complications, AGP fragments with MM 84 and 126 kDa prevailed with hypersialic components suggesting the leukocyte component (originated from polymorphonuclear neutrophils) as the principal element in the development of thrombotic complications. Furthermore, in MPN with thrombotic complications, strong direct correlation was established between high levels of lactate dehydrogenase (LDH) and C-reactive protein on the one hand and high-molecular fragments of AGP with MM 84 and 126 kDa on the other hand. AGP level depended on primary diagnosis: in PV AGP level decreased, PMF (leukocytosis > 20 G/l) was characterized by normal level of AGP due to neutrophilic component. In MPN, glycosylation of AGP was reduced due to the N-glycans. Increase in the amount of branched glycans and sialylation of AGP in MPN seemed to originate from the high activity of neutrophils. **Conclusion:** It is determined that the deficiency of platelets and leukocytes function plays an important role in progression of myeloproliferative syndrome with myelofibrosis formation.

**Key Words:**  $\alpha$ -acidic glycoprotein, myeloproliferative neoplasms, polymorphonuclear neutrophils, thrombohemorrhagic complications.

The issue concerning pathogenesis of vascular complications in patients with myeloproliferative neoplasms (MPN) is considered to be extremely complicated and requires determination [1, 2]. Today it is clearly determined that qualitative deficiency of platelets and leukocytes as well as dysfunction of vessels' endothelial tissue play important role in progression of myeloproliferative syndrome with myelofibrosis formation and severe thrombohemorrhagic catastrophes development [3, 4].

It is known that with the help of hemopoiesis humoral factors and growth factors, intercellular space of bone marrow matrix regulates the mechanisms of adhesion and cells' migration, controls the processes of their differentiation and proliferation, participates in collagen exchange.

Myelofibrosis of different intensity is being observed for all MPN and considered to be their integral part. The most investigated are changes of microenvironment in disease progression. MPN are known to be characterized by clonal hyperplasia of dysplastic cells, referred to myeloid series [5], in consequence develops reactive collagen bone marrow fibrosis with deficiency of bone marrow hemopoiesis and appearance of extramedullary hematopoiesis lesions in liver

and spleen that leads to enlargement of their mass and sizes. Processes of inflammation, regeneration, malignant transformation have similar features with changes in structure of acute phase abiotic polymerization-CRP, ceruloplasmin,  $\alpha$ -2-microglobulin, fibrinogen,  $\alpha$ -acidic glycoprotein. General functional characteristic of proteins in acute phase is their ability to change blood coagulation, activate the system of complement and kinins, optimize chemotaxis, phagocytosis, as well as reactions of microcirculatory bloodstream. Proteins of acute phase limit the processes of tissue destruction and remove fragments of damaged cells and products of their decay from organism.

$\alpha$ -1-Acidic-glycoprotein (AGP) with m.m. from 35 to 43 kDa — is one of the least investigated component of hemostasis system. It is one of the most heterogenetic blood plasma glycoproteins, containing five complex type N-glycans, attached to asparagine residues. They involve 41–45% of total AGP molecular mass. AGP functions depend on carbohydrate component structure and its synthesis location. Thus, its change in protein structure could have other functional characteristics. At change of proteins' physicochemical characteristics, the cytoplasm hydration is observed and happens the damage of connection between proteins and lipids, which leads to destruction of cells' membrane structures and protein chemical modification.

According to the latest data, AGP is synthesized mainly by hepatocytes, the alternative source of AGP is blood cells, in particular, activated polymorphonuclear neutrophils (PMN), and AGP evolves in response for their stimulation. It is determined that AGP in PMN

Submitted: February 14, 2014.

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**Abbreviations used:** AGP —  $\alpha$ -1-acidic-glycoprotein; Con A — concanavalines A; ET — essential thrombocythaemia; LABA — *Laburnum anagiroidus*; LcL — *Lens culinaris*; MAA — *Maackia amurensis* agglutinin; MPN — myeloproliferative neoplasms; PMF — primary myelofibrosis; PMN — polymorphonuclear neutrophils; PV — polycythaemia vera; PHA-L — phytohemagglutinin; SNA — *Sumbucus nigra*.

presented via hypersialylated peptide glycoform, contained in secondary neutrophils' granulas.

In studies, concerning biochemical markers of neoplasms, reviewed the diagnostic significance of oncofetal proteins the most essential changes were defined during proteinoses — cytoplasm proteins closely connected with lipids, forming lipoprotein complexes, which are the basis of mitochondria membrane, endoplasmatic reticulum, Golgi complex.

The attachment of carbohydrate structures to proteins is the most common chemical modification. The system of protein-carbohydrate recognition is reviewed as additional to genetic code.

Glycosylation, as one of the regulatory mechanisms of cells' activity, defines intracellular transport and processes of intercellular interaction, enhance the stability of conformational protein structure. There is direct association between glycans' structure and proliferative cell index. As glycosylation occurs more than in 50% of proteins, the damage of their structure is more widely spread than expression of separate oncofetal proteins and, thus, it may define significantly earlier and considered as additional to protein — carbohydrate recognition system of human genetic code.

More than half of human proteins are proteoglycans, mucoproteins, glycoproteins. Glycoproteins — structural components of cellular membranes, collagenic, elastinic, fibrine meshes of matrix are connected with carbohydrate component and called protein-carbohydrate complexes (PCC). The formation of glycane component for surface glycoconjugates occurs in the process of obtaining specific characteristics of the cell and has stage-by-stage approach as for chronological sequence and for structural aspect.

The biological activity of glycoproteins implements only in condition of saving the spatial organization of microenvironment.

Glycan part of glycoproteins' molecules synthesizes influenced by cytokines in extracellular matrix. Structural variety of glycanes depends on tissue specificity of genes' regulation of glycotransferases. Significant part of glycoproteins consists of 2-antenna glycans, 3 and 4-antenna glycans normally synthesized rarely.

Malignant transformation is followed by structure-functional changes of surface and intracellular glycoconjugates, and especially, their carbohydrate component. During inflammatory processes the number of biantenna glycans increases, during oncotransformation — preveil three- and tetraantenna carbohydrate components. In the presence of changes in conformational structure of glycoproteins, the abnormalities in hemostasiological status are developed.

Therefore, the aim of this work was to compare AGP glycoforms in case of onco-hematological diseases with liver affection and involvement of hematopoiesis leukocytic component at MPN, which may provide additional information as of its origin for these patients, evaluate the type of damaged cells and define their role in development of vascular abnormalities at thrombotic complications.

## MATERIALS AND METHODS

The study was based on analysis of characteristics of clinical presentations among 110 patients with MPN. All patient samples were obtained under Research Ethics Board approval with informed consent. Clinical-laboratory characteristics: complete blood count (hemoglobin, RBC, WBC, differential leukocyte count, platelets), biochemical values, proteins of acute phase (C-reactive protein), LDH (lactate dehydrogenase), complete hemostasiogramm, results of trepan-biopsy samples from iliac bone, abdominal ultrasound) had been investigated at the moment of initial diagnostics of MPN. Demographic (age, sex), anamnestic (concomitant pathologies, episodes of vascular complications) and clinical data (general condition per WHO scale, presence of spleno- and hepatomegaly, plethora signs). Among 110 patients, for 31 — polycythaemia vera (PV) was diagnosed, for 75 — primary myelofibrosis (PMF), for 4 — essential thrombocythaemia (ET). Control group consisted of 92 patients with atherothrombosis.

For evaluation of probability of thrombotic complications development, it was defined content in blood serum of AGP as risk factor in development of thrombohemorrhagic syndrome.

$\alpha$ -Acidic glycoprotein was determined via electroimmunodiffusion in 1% agarose gel, containing monospecific polyclonal antiserum to this glycoprotein. The control was performed via affinity chromatography on sepharose with immobilized antibodies to human AGP [6].

As a source of AGP it was used the pool of 10 blood sera from almost healthy donors and analogically prepared blood sera of patients with MPN.

The control of received antibodies specificity was performed via immunoelectrophoresis and immunoblotting with lectins concanavalines A (Con A) and phytohemagglutinin (PHA-L) [7].

For determination the qualitative characteristics of composition of AGP carbohydrate part it was applied the lectin blot with the use of horseradish peroxide-conjugated lectins' panel [8]: common elder — *Sambucus nigra* (SNA), bean tree bark — *Laburnum anagiroides* (LABA) and lentil — *Lens culinaris* (LcL). Lectin SNA is specific to sialic (N-acetylneuraminic) acid, which joined to end galactose of glycoprotein carbohydrate antenna in position 2→6. Lectin of bean tree bark is similar to terminal L-fucosa and is not interacted with L-fucosa, which is in the middle of carbohydrate chain.

Lentil lectin is referred to mannosospecific lectin and requires the presence of core fucosa as part of glycoconjugates.

Almost all studies regarding AGP fucosylation and sialylation are related with the use of lectins and antibodies, specific to sialyl — Lewis-antigenes A and X, in particular, substantial composition of fucosa and sialic acids as a part of AGP.

The study of sialylation was implemented via determination of total content of sialic acids, or type of their connections with galactose, where 2 types of lectins

were used: *Maackia amurensis* agglutinin (MAA), which specifically connected with N-acetylneuraminic acid, joined to core galactose in position 2→3 and lectin *Sambucus nigra* (SNA), interacting with c N-acetylneuraminic acid in position 2 → 6 [9].

**RESULTS**

Among the patients with MPN prevailed men — 69 patients. Patients' age varied from 22 to 75 years.

Negative prognostic factors of MPN course were considered significantly enlarged liver and spleen with progressing development of myeloproliferative syndrome, hyperthrombocytosis, presence of vascular complication in anamnesis, concomitant cardio-vascular pathology, metabolic syndrome, age over 60 years.

The laboratory results for patients with MPN at disease primary diagnosis indicated in Table 1.

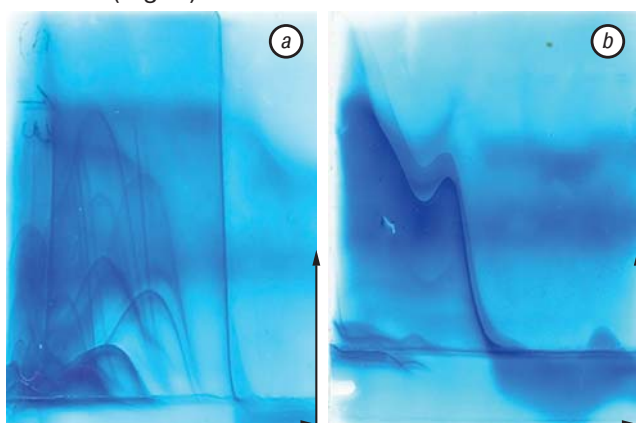
**Table 1.** Initial values of peripheral blood for patients with MPN

Value	Control group (n = 92)	PMF (n = 75)	PP (n = 31)	ET (n = 9)	Atherosclerosis (n = 92)
RBC (T/l)	4.10 ± 0.8	6.17 ± 1.98	6.73 ± 1.52	5.6 ± 1.34	4.27 ± 0.9
Hemoglobin (g/l)	130.4 ± 23.6	150.4 ± 38.3	176.1 ± 33.0	138.5 ± 34.4	134.8 ± 27.9
Platelets (g/l)	235.9 ± 97.3	651.3 ± 41.9	448.7 ± 76.3	875.20 ± 143.1	251.8 ± 103.0
WBC (g/l)	7.0 ± 2.2	20.34 ± 12.9	11.6 ± 5.8	10.0 ± 3.2	9.01 ± 1.9

LDH value for patients with varied from 250 g/l till 640 g/l (at average — 434 g/l, in control group — 265 g/l). CRP level at high LDH level the qualitative test showed ++++.

At PMF and essential thrombocythemia (ET) in bone trepan biopsy samples prevailed reticular fibrosis with hyperplasia of megakaryocytic lineage, and at PV — reactive changes in bone marrow characterized by increased erythroid and myeloid proliferations.

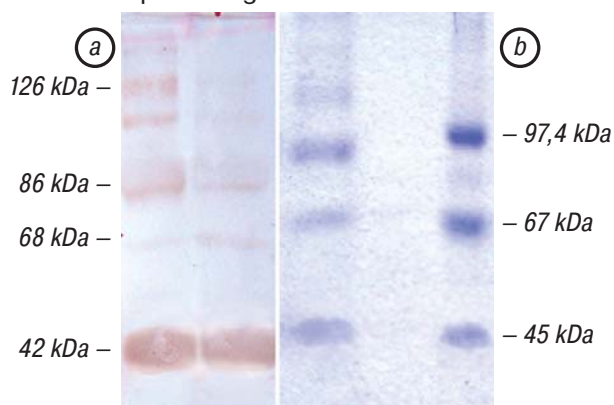
Phenolic extract of blood serum of patient with MPN contained AGP polypeptides with m.m. 42; 68; 84 and 126 kDa (Fig. 1).



**Fig. 1.** Crossed affine immunoelectrophoresis with the use of antiserum to human blood serum: a — serum of patients with MPN; b — blood serum of donors. ↑→ direction of electrophoresis (cathode — anode)

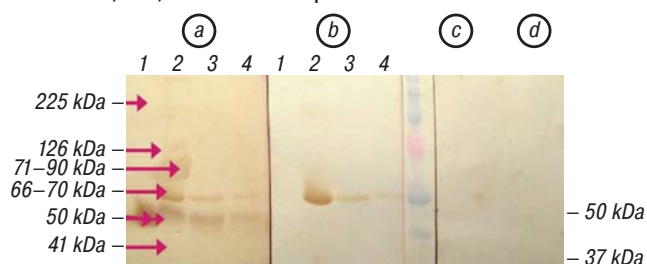
The control of electrophoresis results were performed via immunoblotting (Fig. 2). Upon that, the protein with molecular mass 42 kDa prevailed, which corresponds to plasma AGP of hepatic origin. Additionally high-molecular zones corresponding to molecular masses 68; 84 and 126 kD were defined, which may be determined by the presence of dimeric and trimeric

AGP forms. It is possible that high-molecular AGP may have neutrophilic origin.



**Fig. 2.** Blotogram (a) and electrophoretogram (b) of fractions, received via hydrophobic extraction by phenylic acid: 1 — phenylic extract of the blood sera pool of patients with MPN; 2 — phenylic extract of the blood sera pool of healthy donors; 3 — marker protein

On Fig. 3 the study results of AGP fragments with m.m. 68, 86, 126 kDa are presented.



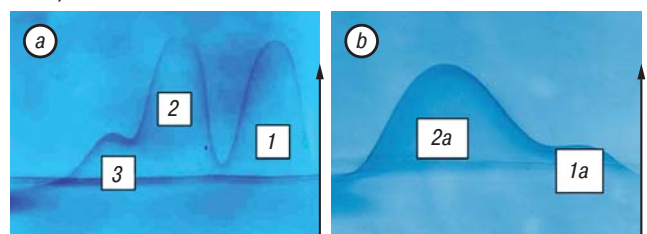
**Fig. 3.** Immunoblotting of AGP fraction, received via afinne chromatography on immunoabsorbent: a — interaction with antibodies to human AGP; b — interaction with antibodies to human IgG; c — marker protein; d — interaction with antibodies to crawl IgG. 1 — standard AGP solution; 2 — serum of healthy donor; 3 — AGP, received from the pool of healthy donors' blood sera; 4 — AGP, received from the pool of patients with MPN blood sera

Staining in zones 225; 110; 66–70 kDa corresponds to AGP complexes with ligands, which is transported (sialic acids).

There are single evidences in literature [10] that AGP with molecular mass 50–60 kDa synthesized by secondary granules of PMN and discharged in plasma at their stimulation, while plasma AGP, synthesized by hepatocytes, has molecular mass 42–45 kDa. PMN-AGP is hypersialylated glycoform of AGP.

Neutrophilic leukocytes at various MPN forms referred to several clones, which may indicate different degree of their maturity and activity.

At interaction of AGP with Con A 3 peak point appear, PHA-L two peak point appear (Fig. 4): AGP-1a — fraction, which is not interlinked and AGP-2a — fraction, which is interlinked with lectin.



**Fig. 4.** Crossed affine immunoelectrophoresis of AGP blood serum of healthy donors. ↑→ direction of electrophoresis (cathode — anode)



Fraction AGP-1a contains AGP glycoforms only with biantenna glycans, AGP-2a — glycoforms, containing polyantenna N-glycans.

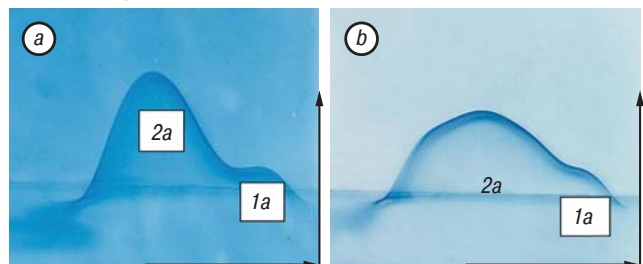
According to our results, the content of AGP in healthy donors' blood serum varied from 0.54 to 0.84 g/l and implied in average  $0.77 \pm 0.026$  g/l. It coincides the data of other studies, where AGP varies from 0.55 to 1.4 g/l.

Data, presented in Table 2, coincides with other studies results [11], which related to affinity to concanavalin (Con A) [12] and phytohemagglutinin PHA-L.

**Table 2.** Correlation of lectin-binding AGP fractions in healthy donors blood serum

Correlation of Con A-binding AGP fractions, %			Correlation PHA-L Binding AGP fractions, %	
AGP-1	AGP-2	AGP-3	AGP-1a	AGP-2a
42.54 ± 0.98	49.32 ± 0.77	8.15 ± 0.38	13.44 ± 0.77	86.56 ± 0.77

**The level of expression and microheterogeneity of  $\alpha$ -acidic glycoprotein at MPN.** In patients with MPN observed unclear separation of AGP fractions, which are not interlinked with PHA-L (Fig. 5). These qualitative characteristics of lectin-binding ability of AGP may indicate on more essential abnormalities in glycosylated AGP, which is directly related to glycoprotein fucosylation and sialylation.



**Fig. 5.** Crossed affine immunoelectrophoresis of AGP blood serum for human normal (a) and in MPN (b): 1a, 2a — fractions of AGP-1a, AGP-2a at interaction with PHA-L.  $\uparrow$  — direction of electrophoresis (cathode — anode)

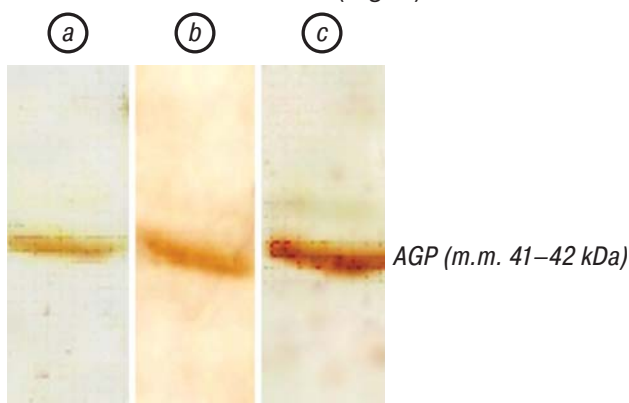
**The investigation of AGP concentration in blood serum in patients with MPN.** The AGP concentration in blood serum in patients with MPN firmly decreases at PV till  $0.60 \pm 0.025$  g/l, maybe due to decrease of AGP production by hepatocytes.

During PMF the AGP content in blood serum is just higher the values of patients from control group —  $0.85 \pm 0.017$  g/l. It may be the consequence of involvement in the process the leukocytic component, as AGP is synthesized also by PMN in response for their stimulation [12], that leads to increase of AGP and PMF development.

The increase of AGP level is observed for patients with MPN, who had thrombotic complications after 3–4 weeks since vascular catastrophe and implies  $0.97 \pm 0.03$  g/l ( $p < 0.01$ ). Taking into account lectins' specificity, it may affirm, that absolute content of bi-antenna glycans increase at thrombotic complications or the possibility of their occurrence. It is confirmed by the increase of values for acute phase proteins.

According to lectin blot as well as lectins' specificity, it may conclude as of the presence in the content of AGP blood serum for healthy donors core and terminal fucosa and sialic acids in position 2→6, as indi-

cated by intensive electrophoretic component during interaction of AGP with LcL (Fig. 6).

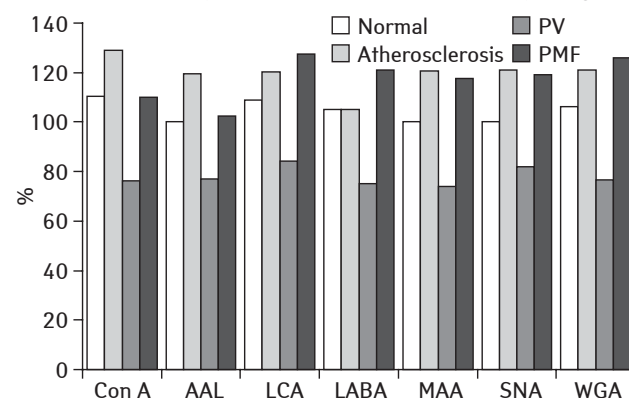


**Fig. 6.** Lectin-blot analysis of human AGP blood serum: a — interaction with SNA, b — interaction with LABA, c — interaction with LcL

Thus, taking into account different variants of lectin analysis, it was proved the microheterogeneity of AGP blood serum for healthy donors, which is characterized by: 1 — the presence of several AGP forms with different grades of glycans' branching: bi-, tri- and tetraantenna; 2 — different glycans' affinity to lectins Con A and PHA-L: interaction AGP with Con A showed by development of three glycoforms, at interaction with PHA-L — two; 3 — presence in the content of some AGP glycoforms terminal and core fucosa, as well as N-acetylneuraminic acid in position 2→6.

**Level of expression and  $\alpha$ -acidic glycoprotein microheterogeneity in blood serum for patients with MPN.** According to lectinenzyme method of analysis, with the use of lectin SNA, wide variation of sialic acids content in AGP is registered.

At MPN, it is firmly decreases the binding degree of AGP carbohydrate component with all lectins, which proves the decrease of glycoprotein's glycation. (Table 3, Fig. 7), consequently, the change of PMN qualitative characteristics with disturbance of activity due to different maturity degree.



**Fig. 7.** Degree of AGP binding with lectins for patients with MPN, who have atherothrombosis

AGP binding with LCA increased at PMF for those patients, who recovered from thrombotic complications, which indicates on increase of cortex fucosation processes. Comparison of AGP binding degree with LCA and AAL demonstrated that the first lectin had less affinity to AGP than AAL. Enhancement of AAL and AGP interaction may be related to appearance

of posicia a 1→3 fucosa in the content of AGP oligasaccharide chain.

**Table 3.** Nature of change of AGP carbohydrate component in patients with MPN

Parameters	Normal	PV	PMF	Complicated MPN	Atherothrombosis
AGP level, g/l	0.77±0.026	0.60±0.025	0.85±0.01	0.97±0.03	1.1 ± 0.05
AGP + Con A, %	100	80–90	↑100–110	↑120–130	↑130–150
AGP + LCA, %	100	80–90	↑120–130	↑150–170	↑150–170
AGP + AAL, %	100	75–80	↑120–130	↑150–170	100
AGP + LABA, %	100	75–80	↑120–130	↑120–130	90–100
AGP + SNA, %	100	80–90	↑100–120	↑150–170	100–120
AGP + MAA, %	100	70–80	↑100–120	↑120–130	90–100
AGP + WGA, %	100	80–90	↑100–120	↑100–120	90–100

The interaction of AGP with lectin LABA discovered the presence of terminal fucosa in N-glycans. According to our data, the AGP binding degree with LABA increases at PMF, probably, due to neutrophilic component of leukocytes. For the rest groups, this index was almost similar to normal ranges.

Thus, the appearance of terminal fucosa in glycans at AGP and LABA interaction may serve as additional criterion for diagnosing and progression of myeloproliferative processes for patients with MPN.

AGP binding degree sialospecific lectin SNA-1 increases at MPN. This lectin interacts with sialic acids of biantenna N-glycans and our data proved that for patients with MPN, who had thrombotic complications, increase the content of biantenna glycans with terminal N-acetylneuraminic acid. It coincides with the results of AGP interaction with another sialospecific lectin — MAA-II, affinated to sialic acids of polyantenna glycans, which connected with glycosidic binding. The binding with MAA-II increased at PMF due to leukocytic component; decreased at PV.

At AGP interaction with WGA, which is also specific to N-acetylneuraminic acid, increase of interaction defined for patients with PMF and myeloproliferative syndrome, mostly, — for patients, who had vascular complications. Lectin of wheat germs may be bound with polyantenna structures, increase of mentioned lectin's binding degree with AGP carbohydrate component regarding SNA-1 indicates on increase of polyantenna glycans in glycoprotein content. When compared the AGP binding with Con A and WGA, it was discovered that decrease of interaction with Con A at PFM, which proved the increase of N-glycans branching in glycoprotein content at MPN and indicated on the existence of malignantly transformed leukocyte clone. At normal ranges, AGP plasma contains insignificant number of such structures, and they are more characteristic for fetal alfa-acidic glycoprotein [11] at malignant cells' transformation. Their development may serve as additional marker at diagnostics of myeloproliferative neoplasma and be the criterion for myeloproliferative disease progression.

In conclusion, defined high-molecular AGP fragments with m.m. 68,84 and 126 kDa for patients with MPN are leukocytic derivatives, as AGP is proved to be synthesized by neutrophils, unlike its analog of hepatocytic origin, has heavy molecular mass due

to their different degree of maturity and activity. High-molecular AGP fragments at MPN consist of hypersialic components and polyantenna fucosated glycans. AGP binding with lectins Con A and PHA in cohort of patients with MPN didn't demonstrated accurate data as of presence of bi- and polyantenna glycans, which indicates on change of AGP plasma glycan component due to neutrophilic component. In blood serum of patients with MPN, who had thrombotic complications, direct correlation between high LDH and CRP with high-molecular AGP fragments (m.m. 68, 84 and 126 kDa) was established. Levels of AGP in patients with PV are decreased due to development of fibrotic processes in liver and abnormality of its synthesis by hepatocytes. In patients with PMF at leukocytosis more 20 G/l normal AGP content was registered in blood serum, which determined by neutrophilic component. Binding of AGP biantenna glycans with lectins Con A at MPN corresponded to control measure, and with other lectins' groups decreased, which indicated on decrease of AGP glycation due to N-glycans. Increase of glycans with branching and AGP syalilation for patients with MPN occurred due to high neutrophils' activity. AGP blood serum of patients with MPN was defined to contain N-glycans with terminal remains a posicia 1→2 of fucosa both at normal and in at pathology, and also N-glycans flucosation degree depends on type of pathological process and may serve additional criterion in MPN diagnostics. Multidirectional changes of AGP N-glycans syalilation in blood serum of patients depend on pathology type: at atherothrombosis observed the increase of syalilation on a 2→6N-acetylneuraminic acid remains, and at MPN the syalilation degree increases due to 2→6 and 2→3 N-acetylneuraminic acid remains. In patients with thrombotic complications at MPN prevailed fragments with m.m. 84 and 126 kDa, mainly with hypersialic components, that characterize leukocytic component (polymorphonuclear neutrophil) as the main activating element in development of thrombotic complications.

## REFERENCES

- Demidova AV. Polycythemia vera. In: Clinical Oncohematology. *MA Volkova*, ed. M: Medicine, 2011: 272–89 (in Russian).
- Kotsiubynsky NI, Maksimov AG. Oncohematological diseases. In: Clinical Hematology. *AN Bogdanov*, ed. St-Petersburg: Foliont, 2008: 307–21 (in Russian).
- Voznyuk VP. Shear stress and endothelial mechanosensation in patients with polycythemia vera. *Ukr J Hematol Transfusiol* 2008; **2**: 27–30 (in Russian).
- Kurmyshov OV, Skripets OV, Guseva SA, Goncharov YaP. Dependence of functional activity of blood neutrophils and the rate of microcirculation malfunction in polycythemia. In: Col Issue Gematologiya Blood Transfusion. Kyiv: 2002; 45–51 (in Ukrainian).
- Tefferi A. Classification diagnosis and management of myeloproliferative disorders in the JAK2 V617F. *Era Hematol* 2006; **92**: 674–7.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–5.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose

sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**: 4350–4.

**8.** Lutsik AD, Detjuk ES, Lutsik MD. Lectins in histochemistry. *EN Panasjuk*, ed. Lvov: Lvov University edition, 1989; 144 p. (in Russian).

**9.** Starodub NF, Artyukh VP, Nazarenko VI, Kolo-miets LI. Protein immunoblot and immunodot in biochemical studies. *Ukr Biochem J* 1987; **59**: 108–20 (in Russian).

**10.** Rahman MM, Miranda-Ribera A, Lecchi C, *et al.* Alpha(1)-acid glycoprotein is contained in bovine neutrophil

granules and released after activation. *Vet Immunol Immunopathol* 2008; **21**: 124–31.

**11.** Orczyk-Pawiłowicz M, Hirnle L, Kałtnik-Prastowska I. Alterations of N-glycan branching and expression of sialic acid on amniotic fluid alpha-1-acid glycoprotein derived from second and third trimesters of normal and prolonged pregnancies. *Clin Chim Acta* 2006; **367**: 86–92.

**12.** Brazaluk O, Maslak G, Tokarev D. Microheterogeneity of proteins in children with high levels of stigmatization. *Ann Univer Mariae Curie-Sklodowska Lublin-Polonia* 2006; **19**: 195–7.