THE STATE OF PLASMA HEMOSTASIS IN PATIENTS WITH HYPERTONIC DISEASE AND NON-ALCOHOLIC FAT-LIVER DISEASE UNDER CONDITIONS OF HYPERCHOLESTERINEMIA AND ASSOCIATED STATINOTHERAPY

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Abstract

Background. Non-alcoholic fatty liver disease (NAFLD) is a marker of metabolic and dyslipidemic disorders. Given the prevalence of atherogenic dyslipidemias and their proven effect on the development of thrombotic cardiovascular complications in patients with NAFLD, it is important to understand the role of hemostatic blood activity.

The objective: to increase the effectiveness of early diagnosis of thrombophilic blood changes in patients with hypertension (HT) combined with non-alcoholic fatty liver disease by determining the state of plasma hemostasis in conditions of hypercholesterolemia and concomitant statin therapy.

Materials and methods. 152 patients were examined. Groups of patients: I - 46 patients with stage II HT, II - 54 patients with NAFLD without HT, group III - 52 patients with stage II HT with concomitant NAFLD.
Results. The degree of spontaneous aggregation was significantly higher in patients with hypercholesterolemia - by 32.4% (p <0.05). The level of fibrinogen was higher by 13.5% (p <0.05) due to hypercholesterolemia, at the same time there was a decrease in antithrombin III by 8.7% (p <0.05) in patients with high cholesterol. Patients with comorbid HT and NAFLD on statin therapy had a 16.5% (p <0.05) lower degree of spontaneous aggregation than patients who did not receive this treatment. In the NAFLD group, patients receiving statins had a significantly lower degree of AC-induced aggregation against patients without lipid-lowering therapy (by 54.0%, p <0.001). However, the analysis of the general population of the examined revealed a decrease in the degree of collagen-induced aggregation by 38.7% (p <0.05) in a subgroup of patients receiving treatment. We observed a 23.4% (p <0.001) shortening of PTT in the NAFLD group and a 16.0% (p <0.05) shortening in the combined course of HT and NAFLD. In the absence of statin therapy in the NAFLD group, there was a significant decrease in INR - by 9.7% (p <0.05) - compared with patients receiving lipid-lowering therapy. A decrease in TT by 12.2% (p <0.05) was observed in the subgroup receiving statins among patients with NAFLD. In the general cohort, the use of statins increased the activity of AT III by 10.7% (p <0.01), but in the NAFLD group this difference was more significant - by 14.3% (p <0.001) AT III was more active in patients who received lipid-lowering therapy.

Conclusion. The results of the analysis proved that patients with hypercholesterolemia have procoagulant and prothrombogenic blood activity, but against the background of statin treatment there is a decrease in platelet aggregation, blood coagulation potential and increased activity of anticoagulant hemostasis.

Key words: non-alcoholic fatty liver disease; platelet aggregation, plasma hemostasis; fibrinolysis; coagulation hemostasis; hypertension; dyslipidemia, statin therapy.

Introduction
Non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of cardiovascular disease (CVD) and mortality. The debate over causation between NAFLD and CVD is ongoing today; however, NAFLD is at least a risk marker, and therefore attention and control of CVD risk factors are important. In addition, the existence of links between NAFLD and various stages of the atherosclerotic process and the structural and functional state of the cardiovascular system, including endothelial dysfunction and atherogenic dyslipidemia, has been proven [1, 2].
The liver plays an important role in the development of atherogenic dyslipidemia, as changes in lipid metabolism begin at the level of the hepatocyte. All statins inhibit the activity of HMG-CoA reductase, resulting in an increase in the number of LDL receptors in hepatocytes, increased LDL uptake and thus reduce the level of circulating LDL. In addition, it reduces the content of intracellular cholesterol in the liver, which provides a lipid-lowering effect of statins [3].

According to a recent retrospective analysis of the GREACE study, statins have a positive effect on aminotransferase levels and improve the prognosis of cardiovascular events in patients with elevated aminotransferases in NAFLD [4]. A retrospective analysis of the IDEAL study also revealed the benefits of using statins in patients with elevated ALT levels [5].

Although elevated aminotransferases are not uncommon in patients receiving lipid-lowering therapy, severe liver damage by statins is quite rare in clinical practice [6–8]. Therefore, the use of statins in NAFLD is not only safe, but also recommended by international societies for the study of liver disease [9, 10], in particular the American Association for the Study of Liver Diseases (AASLD) [1].

Given the prevalence of atherogenic dyslipidemias and their proven effect on the development of thrombotic cardiovascular complications in patients with NAFLD, it is important to understand the role of platelets and hemostatic activity in the blood. In addition, the production of peripheral platelets is regulated mainly by the glycoprotein hormone thrombopoietin, which is mainly synthesized in the liver. According to recent studies, people with NAFLD have a significantly increased risk of decreased platelet counts compared to those without NAFLD [11]. Therefore, the question of the nature of the high frequency of thrombotic complications in such patients and the possibility of their prevention remains open.

Due to the lack of a clear understanding in the modern scientific world of pathophysiological changes in this process, we analyzed the impact of changes in lipid profile and concomitant statin therapy on platelet-plasma hemostasis in patients with hypertension as a major risk factor for cardiovascular events and concomitant.

**Objective:** to increase the effectiveness of early diagnosis of thrombophilic blood changes in patients with hypertension combined with non-alcoholic fatty liver disease by determining the state of platelet-plasma hemostasis.

**Materials and methods.** The study was conducted on the clinical basis of the Department of Propaedeutics of Internal Medicine №1 National Medical University named
after O. O. Bogomolets of the Kyiv Clinical Hospital by Rail №2 Branch of the Health Care Center of PJSC Ukrainian Railways. 152 patients were examined: 72 men and 80 women. The majority of patients were women - 80 people (52.6%), men among the surveyed were 72 people (47.4%). Patients were divided into groups: Group I - patients with stage II HT without signs of liver damage (46 people, median and interquartile age range of the subjects was 58.00 [51.00; 63.00] years); Group II - patients with NAFLD without HT (54 individuals, median and interquartile range of the subjects were 54.00 [43.00; 58.00] years); Group III - patients with stage II HT with NAFLD (52 individuals, median and interquartile range of the subjects were 57.50 [48.00; 64.50] years).

The control group consisted of 15 practically healthy individuals comparable in age and sex (median and interquartile age range of the surveyed were 49.00 [42.00; 55.00] years, who underwent preventive examination).

To achieve this goal, a study of spontaneous and induced platelet aggregation was performed. Coagulation activity, anticoagulant and fibrinolytic potential of blood in the examined patients were also studied.

**Results**

Among 152 people, hypercholesterolemia (total cholesterol ≥5 mmol / l) was found in 44.7% (68 people), hypertriglyceridemia (triglycerides ≥1.7 mmol / l) in 26.4% (40 people), the majority were who had HT, and the combined course of HT. with NAFLD.

When analyzing the data of the lipid spectrum on the indicators of total cholesterol (General cholesterol), the level of triglycerides (TG), which in the group of patients with HT. the level of total cholesterol was the highest - 5.9 [5.2; 6.9] mmol / l, which was 11.3% (p <0.05) more than in patients with NAFLD (5.3 [4.3; 5.8] mmol / l), and 7.3% (p <0.05) exceeded the value in the combined course of these diseases (5.5 [4.7; 6.2]). The highest values of triglycerides were found in patients with comorbid course of HT. and NAFLD - 2.06 [1.36; 2.69] mmol / l, instead in patients with HT. and in the NAFLD group, they seemed to correspond to normal triglyceridemia levels (1.58 [1.22; 2.28] and 1.17 [0.84; 1.94], respectively). However, the distribution of obesity revealed hypertriglyceridemia in patients with HT and BMI ≥30 kg / m2 (1.87 [1.36; 3.1] mmol / L). Thus, the tendency to hypercholesterolemia was determined in all groups, while hypertriglyceridemia only in the presence of a combined course of hepatic steatosis and HT.

Analyzing the value of platelet hemostasis, there was no significant difference in the level of induced aggregation in patients with normal and elevated cholesterol levels. However, it should be emphasized that the degree of spontaneous aggregation was
significantly higher in patients with hypercholesterolemia - by 32.4% (p <0.05) (3.31 [2.10; 3.90] vs. 2.5 [1.75 ; 3.04]).

The next step was to assess plasma hemostasis depending on the presence of hypercholesterolemia.

During the evaluation of plasma hemostasis in patients with high and normal cholesterol levels, it was found that the level of fibrinogen was higher by 13.5% (p <0.05) precisely in the case of hypercholesterolemia [86.5 [78.1; 96.0] % and 94.0 [82.0; 108]%), while there was a decrease in antithrombin III by 8.7% (p <0.05) in patients with high cholesterol [86.5 [78.1; 96.0] % and 94.0 ([82.0; 108]%). Other indicators of plasma hemostasis did not show significant differences depending on the level of cholesterol. Therefore, hypercholesterolemia was accompanied by a decrease in anticoagulant activity, while promoting coagulation at the end links of blood clotting.

Given the influence of NAFLD on the development of atherogenic dyslipidemias, which further contributes to the occurrence of cardiovascular complications, we analyzed the effect of statin treatment on the state of platelet-plasma hemostasis. Among 152 examined patients, 34.2% (52 persons) received statin treatment.

![Figure 1. Comparison of platelet hemostasis in patients depending on the presence of concomitant statins in therapy](image)

Note. The degree of probability of indicators relative to the control group:

* - p <0.05; ** - p <0.01; *** - p <0.001
We conducted a comparative analysis of platelet-plasma hemostasis in view of the use of statin therapy in the treatment of the examined groups of patients.

When analyzing the degree of spontaneous platelet aggregation, it should be noted that in all patients it was significantly higher than the control values (0.75 [0.47; 1.14]), regardless of statin therapy. It was also found that there was no significant difference between the degree of spontaneous aggregation in patients receiving statins and those who did not receive this treatment in any of the groups except the cohort with comorbid HT and NAFLD, where patients on statin therapy had 16.5% (p <0.05) lower degree of spontaneous aggregation than patients who did not receive this treatment (2.85 [2.29; 3.54] and 3.32 [2.73; 3.92]). Thus, the results of the analysis proved that the use of statins significantly reduced the degree of spontaneous aggregation in patients with comorbid course of HT and NAFLD both in comparison with the subgroup without statin therapy and in patients with NAFLD without concomitant HT who received lipid-lowering therapy.

Analysis of the degree of ADP-induced platelet aggregation showed that significant differences from control (45.0 [36.5; 52.6]) were found only in patients with NAFLD, in groups II and III, and, it should be noted, there was an increase in aggregation in both patients taking statins and those not receiving this treatment.

Assessing the degree of AK-induced platelet aggregation, we noted significantly lower values in the NAFLD group and in patients with comorbid course of HT and NAFLD for control (36.70 [31.99; 42.6]). At the same time, this indicator significantly exceeded the indicators of healthy people in patients with HT both with the use of statin therapy and without its use. However, only in the NAFLD group did patients receiving statins have a significantly lower degree of AK-induced aggregation against patients without lipid-lowering therapy (by 54.0%, p <0.001) (17.2 [13.6; 20.71] and 26.48 [20.20; 36.50]).

Changes in the degree of collagen-induced aggregation relative to control were found only in patients with grade II HT combined with NAFLD, whose treatment regimen included statins. It was 82.0% (p <0.05) (12.8 [10.14; 16.25]) lower than the control values (23.30 [16.18; 25.1]). Nevertheless, differences in the degree of collagen-induced aggregation were not found in any of the studied groups of patients on statin therapy and without lipid-lowering treatment. However, the analysis of the general population of the examined revealed a decrease in the degree of collagen-induced aggregation by 38.7% (p <0.05) in the subgroup of patients treated (15.5 [11.4; 28.70] against 21.5 [16.4; 28.1]), which demonstrated the stabilizing effect of statins on the vascular wall. Thus, treatment with statins led to a significant decrease in the overall cohort of subjects.
Analysis of adrenaline-induced platelet aggregation showed that in all patients they were significantly higher than control values, regardless of the use of statins. However, as in the analysis of ADP-induced aggregation, the comparison did not reveal a significant difference between the values of the degree of adrenaline-induced aggregation when taking statins and in the absence of them in treatment.

When analyzing the rate of clot formation during PTH, it should be noted that it was significantly higher than the control values (19.25 [18.6; 19.9]) only in patients who did not receive concomitant statin therapy and had hepatic steatosis, in groups II and III. Thus, we observed a 23.4% (p <0.001) (15.6 [14.2; 16.8]) shortening of clot formation time in the NAFLD group and a 16.0% reduction (p <0.05) (16.6 [13.6; 19.4]) in the group of combined HT. and NAFLD. Determination of the effect of lipid-lowering therapy revealed that patients with NAFLD without statin treatment 19.2% (p <0.01) formed a clot faster than patients receiving lipid-lowering therapy [18.6 [16.3; 20.9] against 15.6 [14.2; 16.8]]. Analysis of the results of PTC of all examined patients showed a reduction in time by 13.3% (p <0.01) in patients who did not receive statin therapy [18.8 [16.3; 21.0] against 16.6 [14.2; 19.3]). Therefore, the presence of statins in the treatment regimen led to a prolongation of the clot formation time at the initial stage of blood coagulation by the external mechanism of coagulation in patients with NAFLD and in the general population of subjects.

The study of INR found that the values were significantly lower compared to the control (0.83 [0.79; 0.89]), only in patients with NAFLD in the absence of statin treatment - by 15.3% (p <0.001) (0.72 [0.68; 0.81]). It should be noted that in this subgroup there was a significant decrease in INR - by 9.7% (p <0.05) - compared with patients with NAFLD who received lipid-lowering therapy [0.79 [0.72; 0.93] against 0.72 [0.68; 0.81]). Thus, the value of INR was most affected in patients with NAFLD who were not treated with statins - these patients had the lowest INR.

Determining the difference in the duration of thrombin time (TT) showed a shortening of the clot formation time in both subgroups of the NAFLD group compared with the control values (10.8 [10.1; 11.2]). However, a decrease in TT by 12.2% (p <0.05) was observed in the subgroup receiving statins among patients with NAFLD (8.2 [7.70; 8.60] vs. 9.20 [8.40; 10, 10]).

Treatment with statins did not have a significant effect on other indicators of the coagulation of hemostasis: APTT, fibrinogen, RFMC, although there were significant differences in the values of these indicators against control, which reflected the general trend of groups of patients with HT, NAFLD and their combination, regardless of treatment statins.
Interestingly, during the analysis of the anticoagulant link of hemostasis, despite the absence of probable discrepancies with control, it was in the NAFLD group that the use of statins showed a difference in the effect on blood pressure III, and a similar difference was observed in the general population. Thus, in the general cohort, the use of statins increased the activity of blood pressure III by 10.7% (p <0.01) (98.5 [83.6; 106.0] against 89.0 [81.0; 95.0]), but in the NAFLD group this difference was more significant - by 14.3% (p <0.001) BP III was more active in patients receiving lipid-lowering therapy (104.0 [102.0; 106.0] vs. 91.0 [85, 5; 94.0]). Thus, statin treatment significantly increased the activity of the anticoagulant link of hemostasis in the general population of subjects and among patients with NAFLD.

Indicators of the fibrinolytic system had significant deviations from control values in all groups, showing a decrease in fibrinolysis activity, but the difference between subgroups depending on concomitant statin therapy did not become statistically significant.

Summarizing the results of our analysis, we can state that the atherogenic nature of the changes in the lipid profile was diagnosed both in patients with NAFLD and in their combined course. At the same time the most expressed dyslipidemic deviations were observed at a comorbid course of HT and NAFLD. Instead, in patients with independent HT, we observed only hypercholesterolemia at nonatherogenic triglycerides, LDL, and HDL.

Analyzing the value of platelet hemostasis in patients with normal and elevated cholesterol levels and we found that the degree of spontaneous aggregation was significantly higher in patients with hypercholesterolemia. In addition, hypercholesterolemia was associated with higher platelet function. That is, an increase in cholesterol levels can be seen as an additional risk factor for increased platelet aggregation. Changes in plasma hemostasis also had a shift to the prothrombogenic side: hypercholesterolemia was accompanied by a decrease in anticoagulant activity and activation of coagulation at the end links of blood coagulation.

After analyzing the effect of statin therapy on the state of platelet-plasma hemostasis, it was found that the use of statins significantly reduced the degree of spontaneous aggregation in patients with comorbid HT and NAFLD. Lipid-lowering therapy also decreased AK-induced aggregation in patients with self-NAFLD, indicating a decrease in platelet response to proinflammatory prostaglandins. Statin treatment also markedly reduced the degree of collagen-induced aggregation in patients with comorbid HT and NAFLD, and led to a significant decrease in this indicator in the general cohort of subjects, indicating a decrease in prothrombogenic response in case of damage to the vascular wall or
destabilization of atherosclerotic plaque. In addition, the use of statins in the treatment of dyslipidemia was accompanied by a decrease in the coagulation activity of hemostasis and an increase in the anticoagulant potential of the blood.

Thus, the results of the analysis proved that patients with hypercholesterolemia have procoagulant and prothrombogenic blood activity, but against the background of statin treatment there is a decrease in platelet aggregation, blood coagulation potential and increased activity of anticoagulant hemostasis.

Conclusions
1. Hypercholesterolemia is associated with higher platelet function, hyperfibrinogenemia, decreased blood anticoagulant potential, so such changes in the lipid profile can be considered as an additional risk factor for prothrombogenic blood changes.

2. The use of lipid-lowering therapy reduces the degree of spontaneous aggregation in the comorbid course of HT and NAFLD, leads to a significant decrease in collagen-induced aggregation in the total cohort of subjects and a combination of HT with NAFLD, as well as to reduce the degree of AK-induced aggregation in patients with NAFLD, which suggests the presence of pleiotropic antithrombotic effect of statins.

3. The use of statins in the treatment of dyslipidemia reduces the coagulation activity of hemostasis and enhances the anticoagulant potential of the blood, to a greater extent this effect is observed in patients with NAFLD and patients with HT, combined with NAFLD, which explains the prophylactic effect of statin therapy in cardiac thrombotic complications.

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