Features of hemostasis disorders in patients with CKD VD stage and their relationship with the course of the disease
Storozhuk O. B., Shevchuk S. V., Storozhuk L. O., Dovgalyuk T. V., Storozhuk B. G.

Disorders in the hemostatic system leading to the development of thrombosis are one of the main complications in patients with chronic kidney disease (CKD) stage VD who are on program hemodialysis. The development of thrombophilic syndrome as a process of intravascular coagulation is characterized by systemic activation of procoagulative mechanisms, which are not always compensated by internal natural anticoagulant systems. Determining the early predictors of possible thrombogenesis in the studied category of patients causes significant difficulties. The goal is to study the features of hemostatic system disorders in patients with CKD VD stage who are on programmed hemodialysis, and to assess their relationship with the characteristics of the course of the disease. In 88 patients with CKD of the VD stage who are on program hemodialysis (52 men and 36 women), the features of hemostasis disorders depending on the clinical characteristics of the patients were studied. The indicators of prethrombosis (soluble fibrin (sF), fibrinogen (Fg), functionally inactive forms of prothrombin (FIFP)), postthrombosis (D-dimer (D-d)) and anticoagulation (protein C (pC)) were determined depending on gender, hemodialysis experience, age, the presence of anemia, arterial hypertension (AH) and preserved residual renal function (RRF). Statistical processing of materials was carried out using methods of variation statistics using t-student criterion. It was found that violations in the hemostatic system are detected in almost all patients. At the same time, hyperfibrinogenemia, an increase in sF concentration, a deficiency of pC, less often an accumulation of FIFP and high levels of D-d are most often observed. These disorders of hemostasis are more often found in patients with absent RRF, a longer experience of hemodialysis, the presence of arterial hypertension and are less associated with gender, age of patients and are little dependent on the degree of anemia.

Keywords: CKD stage VD, hemodialysis, hemostasis, soluble fibrin, D-dimer, fibrinogen, protein C, forms of prothrombin are functionally inactive.
clotting inhibitor, which is protein C (pC) [20] and the accumulation index of functionally inactive forms of prothrombin (FIFP) [19]. To determine the potential suitability of activators of inhibitors of thrombogenesis as markers of thrombophilia, a study of markers of prethrombosis - Fg, sF, FIFP, postthrombosis - D-d and natural anticoagulant - protein C in different groups of patients with CKD stage VD treated by program hemodialysis.

The aim of the work is to study the features of hemostasis disorders in patients with CKD stage VD who are on program hemodialysis and to evaluate their connection with the peculiarities of the disease.

Materials and methods

In accordance with the principles of biomedical ethics, on the basis of informed consent, we examined 88 patients with CKD stage VD who receive renal replacement therapy by programmed hemodialysis (52 men and 36 women). 20 practically healthy persons served as control. Blood samples of examined patients and practically healthy individuals for plasma production were taken on an empty stomach from a vein, without the use of a tourniquet, in a vacutainer with 3.8 % sodium citrate solution and mixed in a ratio of 1:9. The deposition of formed blood elements was performed by centrifugation for 20 minutes with an acceleration of 1200-1400g.

The concentration of sF (marker of activation of the blood coagulation system) was determined using the method of bi-site ELISA for the cleavage of fibrin plasmin cleavage products [6]. Determination of the level of D was performed by ELISA assay using monoclonal antibodies to epitopes of D-dimers, which are formed only by cleavage of insoluble fibrin by plasmin [8]. The activity of protein C in blood plasma was determined spectrophotometrically, activating it with the venom of the Gloydius halys (Agkistrodon halus halus) [2]. Determination of fibrinogen content in blood plasma was performed using the thrombin-like enzyme ancistron- H, obtained from the venom of the Gloydius halys (Agkistrodon halus halus) by spectrophotometric method [14]. Calculations were performed according to the formula: Fg = (E280 - E320) x 255/15.06, where Fg is the concentration of fibrinogen in blood plasma in g/l; 255 - absorption extinction coefficient of 1 % solution of fibrin in a volume of the sample to its concentration in plasma; 15.06 - coefficient for converting the content of fibrinogen in the concentration of fibrinogen in blood plasma in g/l; 255 - absorption extinction coefficient of 1 % solution of fibrin in an acidic environment at a wavelength of 280 nm [8].

Thromboplastin was used to determine the prothrombin time (external pathway of the blood coagulation system), and the prothrombin index was calculated as the ratio of the prothrombin time of the studied plasma to the prothrombin time of healthy donors [17].

To determine the ecamulin time used ecamulin - the enzyme activator of prothrombin, isolated from the venom of Echis multisquamatus. The ecamulin index was defined as the ratio of the ecamulin time of the studied plasma to the ecamulin time of healthy donors [17].

The accumulation index of decarboxylated FIFP was calculated as the ratio between ecamulin and prothrombin indices based on predetermined quantitative patterns of FIFP content [19].

Statistical processing of materials was performed using the methods of variation statistics using Student's t-test.

Results

It was found that in patients with CKD stage VD with a high (95.5 %) frequency, disorders in the hemostasis system were registered, which was characterized by increased levels of fibrinogen, soluble fibrin, D-dimer, accumulation of FIFP and decreased protein C. In the control group revealed disorders in the hemostasis system were observed only in 45.0 % of cases (Table 1). In particular, the average level of fibrinogen in the studied patients was 89.0 % higher compared to the control, and the share of persons with hyperfibrinogenemia was more than 13 times higher. Many patients showed signs of activation of the blood coagulation system, which was manifested in an increase in average concentrations of soluble fibrin by 43.0 % and more than a 2-fold increase in the number of individuals with high levels of the studied indicator. High serum levels of D-dimer were found in 20.5 % of patients, while in the control group there were only 5.0 %. Similar trends are observed in patients with FIFP, in whom this indicator was elevated in 45.5 %, while in the control group such cases were not observed. Compared with healthy individuals, there was a significant decrease in the activity of pC <85 %

Table 1. Indices of hemostasis in patients with CKD stage VD and controls.

| Indices of hemostasis | Control group n=20 | Patients with CKD stage VD n=88 |
|----------------------|-------------------|---------------------------------|
| sF, µg/ml            | 2.60±0.312        | 3.72±0.190*                     |
| Proportion of persons with sF >3.0 µg/ml | 6 (30.0 %) | 60 (66.2 %)* |
| D-d, ng/ml           | 70.04±9.10        | 75.33±7.31                      |
| Proportion of persons with D-d >105 ng/ml | 1 (5.0 %) | 18 (20.5 %)* |
| Fg, mg/ml            | 2.20±0.251        | 4.16±0.121*                     |
| Proportion of persons with Fg >2.6 mg/ml | 1 (5.0 %) | 82 (93.2 %)* |
| Proportion of persons with Fg >3.5 mg/ml | 1 (5.0 %) | 57 (64.7 %)* |
| pC, %                | 98.90±7.88        | 81.8±1.67*                      |
| Proportion of people with deficits of pC <85 % | 7 (35.0 %) | 50 (56.8 %)* |
| FIFP, %              | 99.51±2.03        | 109.4±2.2*                     |
| Proportion of persons with level FIFP >110 % | - | 40 (45.5 %) |
| No violations         | 11 (55.0 %)       | 4 (4.5%)*                       |

Notes: * - probable differences in the control group on average values; * - the significance of the differences, calculated according to Fisher’s exact method for the control group.
of the natural anticoagulant protein C (by 20.9 %). The share of persons with its deficit in the main group was 56.8 %, in the control group - only 35.0 %.

In the next part of the study, an analysis of the identified disorders in the hemostasis system depending on the course of the disease was performed (Table 2). The study did not reveal sex differences in the frequency of disorders and the average values of the analyzed indicators of the hemostasis system. With regard to age, in the youngest age group, as in middle-aged and older patients, approximately the same values of soluble fibrin, fibrinogen, FIFP, D-dimer and protein C were observed. Disturbances in the hemostasis system in patients with CKD stage VD were associated over time on hemodialysis. In parallel with its increase in serum levels, FIFP levels increased significantly and protein C levels decreased. D-dimer, fibrinogen and soluble fibrin levels changed to a lesser extent, depending on the time spent on hemodialysis. According to these indicators, patients who were on hemodialysis for more than 15 years differed from those who were on hemodialysis for up to 5 years by 13-40 %.

It was found that the lack of residual renal function also significantly affected the severity of disorders in the hemostasis system. It was found that in persons with no residual renal function compared with the presence of RRF probably increased levels of fibrinogen, soluble fibrin, D-dimer and FIFP and decreased levels of protein C. Analysis of disorders in the hemostasis system depending on the presence of AH showed that levels of soluble fibrin and D-dimers are clearly associated with its presence, ie their concentrations were 13-58 % higher than in individuals without AH. According to other indicators in the hemostasis system, no correlations were found with the presence of AH.

### Discussion

Thus, the study found that disorders of the hemostasis system in patients with CKD stage VD are detected in 95.5 % of cases, while in the control group - only in 45.0 %. Regarding the frequency of disorders in the hemostasis system, the most frequent were high levels of sF - 68.2 % and hyperfibrinogenemia - 64.7 %, less often - protein C deficiency - 56.8 % and the presence of FIFP - 45.5 %. High levels of D-dimer were found in 20.5 % of people.

Table 2. Relationship of disorders in the hemostasis system in patients with CKD stage VD with the course of the disease.

| Groups of patients | n   | sF, μg/ml | D-d, ng/ml | Fg, mg/ml | pC, % | FIFP, % |
|--------------------|-----|-----------|------------|-----------|-------|---------|
| Control            | 20  | 2,603±0.312 | 70,04±9,10 | 2,202±0,251 | 98,90±7,88 | 99,51±2,03 |
| Patients with CKD stage VD | 88 | 3.721±0.190* | 75,33±7,31 | 4.162±0,121* | 81,81±1,67* | 109,4±2,2* |

**Notes:** sF - soluble fibrin; D-d - D-dimer; Fg - fibrinogen; pC - protein C; FIFP - functionally inactive forms of prothrombin; RRF - residual renal function. * - probable differences in the control group on average values. ** - probable differences in patients with the lowest hemodialysis experience, preserved RRF and no AH.

ISSN1861-031X eISSN 2616-6208 Biomedical and Biosocial Anthropology
regular dialysis treatment, there is a clear imbalance in the blood coagulation system, characterized by hyperfibrinogenemia and decreased serum protein C compared to healthy patients. According to Japanese researchers, protein C deficiency and hyperfibrinogenemia were found in 28% and 37% of patients without CKD stage IV [4]. A significant imbalance in the markers of coagulation and fibrinolysis in patients on hemodialysis has been reported in a number of other studies [1, 13]. At the same time, there are other studies that show that with the progression of renal failure (CKD stage 1-4) there was a significant increase in coagulation markers - fibrinogen, coagulation factor VIII, D-dimer, and a decrease in anti-thrombin III, but they do not acquire statistical value [12].

We have shown that hemostasis disorders are more common in patients with no RRF, long history of hemodialysis, the presence of AH and have virtually no connection with sex, age of patients, and the presence and degree of anemia. Only one study has been found in the literature indicating that age >65 years was independently associated with D-dimer positive in hemodialysis patients [3].

Thus, the course of the disease, namely the absence of RRF, long experience in hemodialysis and the presence of AH may be important pathogenetic factors that initiate disorders of the hemostasis system in patients with CKD stage IV, who are on program hemodialysis, and, obviously, may be factors in the activation of thrombotic complications in this category of patients.

A comprehensive study of the main links of hemostasis in patients with CKD stage IV expands the possibilities of preventive measures to prevent thrombophilia.

Conclusions
1. In patients with CKD stage IV, who are treated with programmed hemodialysis, in 95.5% of cases there are violations of hemostasis. The most common are high levels of sF - 68.2% and hyperfibrinogenemia - 64.7%, less often - protein C deficiency - 56.8% and the presence of F1+2 - 45.5%. High levels of D-dimer are registered in 20.5% of people.
2. Disorders of the hemostasis system are closely associated with the absence of RRF, long history of hemodialysis and the presence of AH and have virtually no connection with sex, age and the presence of anemic syndrome.

References
[1] Costa, E., Rocha, S., Rocha-Pereira, P., Castro, E., Reis, F., Teixeira, F., ... & Santos-Silva, A. (2008). Cross-talk between inflammation, coagulation/fibrinolysis and vascular access in hemodialysis patients. J. Vasc. Access., 9(4), 248-253. https://doi.org/10.1177/112972980800900405
[2] Dohlback, B., & Villoutreix, B. O. (2005). Regulation of blood coagulation by the protein C anticoagulant pathway: Novel Insights Into Structure-Function Relationships and Molecular Recognition. Arterioscler. Thromb. Vasc. Biol., 25(7), 1311-1320. doi: 10.1161/01.ATV.0000168421.13467.82
[3] Gubensek, J., Lolic, M., Ponikvar, R., & Buturovic-Ponikvar, J. (2016). D-dimer levels in maintenance hemodialysis patients: High prevalence of positive values also in the group without predisposing diseases. Hemodial Int., 20(2), 198-203. doi: 10.1111/hdi.12371
[4] Ichinose, M., Sasagawa, N., Chiba, T., Toyama, K., Kayamori, Y., & Kang, D. (2019). Protein C and protein S deficiencies may be related to survival among hemodialysis patients. BMC Nephrol., 20(1), 191. doi: 10.1186/s12882-019-1344-8
[5] Komisarenko, S. V. (2017). Research of molecular mechanisms of thrombosis and creation of hemostatic agents. Visn. Nac. Acad. Nauk Ukr., 3, 38-44. ISSN 1027-3239
[6] Lugovskoy, E. V., Gritsenko, N. G., Lugovskaya, N. E., Kolesnikova, I. N., & Komisarenko, S. V. (2006). Soluble fibrin. Molecular structure and quantification. Laboratory diagnostics, 3(37), 11-17.
[7] Lugovskoy, E. V., Kolesnikova, I. N., Lugovskaya, N. E., Gritsenko, P. G., Litvinova, L. M., Gogolinskaia, G. K., ... & Komisarenko, S. V. (2006). Soluble fibrin and D-dimer at normal pregnancy and pregnancy with risk miscarriage. Ukr. Biochem. Zhurn., 78(4), 120-129. Retrieved from https://pubmed.ncbi.nlm.nih.gov/17236629/
[8] Lugovskoy, E. V., Komisarenko, S. V., Platonova, T. M., Rubenko, A. M., Fischchenko, V. O., & Kolesnikova, I. M. (2013). Determination of soluble fibrin and D-dimer content for prediction of thrombotic complications by hip arthroplasty. Laboratory diagnostics, 2(64), 3-8. Retrieved from http://nbuv.gov.ua/UJRN/labdia_2013_2_2
[9] Meamar, R., Shafeii, M., Abedini, A., Ghazvini, M. R., Roomizadeh, P., Taheri, S. & Gheissari, A. (2016). Association of E-selectin with hematological, hormonal levels and plasma proteins in children with end stage renal disease. Adv. Biomed. Res., 29(5), 118. doi: 10.4103/2277-9175.186992
[10] Melnik, A. A. (2016). The hemostatic system and its regulation in case of impaired renal function. News of medicine and pharmacy in Ukraine, 9(583), 24-31. doi: 10.22141/2307-1257.3.17.2016.76541
[11] Milburn, J. A., Ford, I., Mitch, N., Fluck, N., & Britenden, J. (2013). Thrombin-Anti-Thrombin Levels and Patency of Arterio-Venous Fistula in Patients Undergoing Haemodialysis Compared to Healthy Volunteers: A Prospective Analysis. PLoS One, 8(7), e67799. doi: 10.1371
[12] Muslimovic, A., Rasic, S., Tulumovic, D., Hasanspahic, S., & Rebic, D. (2015). Inflammatory Markers and Procoagulants in Chronic Renal Disease Stages 1-4. Med. Arch., 69(5), 307-310. doi: 10.5455/medarh.2015.69.307-310
[13] Nunnis, G. R., Moore, E. E., Chapman, M. P., Moore, H. B., Stettler, G. R., Peltz, E., ... & Sauaia, A. (2017). The hypercoagulability paradox of chronic kidney disease: The role of fibrinogen. Am. J. Surg., 214(6), 1215-1218. doi: 10.1016/j.amjsurg.2017.08.039
[14] Platonova, T. N., Zaichko, N. V., Chernyshechna, T. M., Gorfitskaya, O. V., & Grischuk, V. I. (2010). Assessment of the informative value and prognostic value of traditional screening and additional laboratory tests for the diagnosis of thrombophilia. Laboratory diagnostics, 4(54), 3-10.
[15] Popova, J. A., Yadrinshinskaya, V. N., Krylova, M. I., Sleptsova, S. S., & Borisova, N. V. (2016). Comparison of clinical and laboratory parameters in patients with end-stage renal failure in the outcome of chronic glomerulonephritis and patients with...
Особенности нарушений гемостаза у больных ХБП VД стадии и их связь с течением заболевания

Цель - изучить особенности нарушений гемостаза у больных ХБП VД стадии, находящихся на программном гемодиализе (52 мужчины и 36 женщин), доследить особенности поражения гемостаза залежно від клінічних характеристик пацієнтів. У 88 хворих ХХН VД стадії, які перебувають на програмному гемодіалізі, та оцінити їх зв’язок з особливостями перебігу захворювання. У 88 хворих ХХН VД стадії, які перебувають на програмному гемодіалізі (52 чоловіки та 36 жінок), досліджено особливості порушень гемостаза залежно від клінічних характеристик пацієнтів. Визначалося показники пораненої фібриногенемії, активних форм протромбина, активных форм протеина С, а також функціонально неактивні форми протромбіну.

Ключевые слова: ХХН VД стадії, гемодіаліз, гемостаз, розчинний фібрин, Д-димер, фібриноген, протеїн С, функціонально не активні форми протромбіну.