SUMMARY OF FINAL ANALYTICAL REPORT

Topic I: Study of molecular-genetic and immunomorphological bases of clonal diseases of the blood system in order to improve diagnosis and identify prognostic factors for optimizing differential therapy

The recruitment of patients to the interregional clinical protocol "AML21" and the maintenance of a unified electronic database of the results of laboratory examination at the onset of the disease and during course therapy continued. The optimization of complex molecular genetic analysis in AML patients has continued (the range of studies is being expanded and diagnostic methods for the main clinically significant targets are being improved). The protocols of programmed therapy based on the primary molecular and genetic risk factors and monitoring of a malignant clone in different molecular types of AML are being compared. The effect of mutations in the genes of epigenetic regulation and genes of transcription activators on hyperleukocytosis in patients with AML was evaluated. The work was carried out to identify key parameters that affect the correct assessment of the allele load in mutations in the FLT3 gene during fragment analysis. The increased sample was used to show the new features in the profile of genetic disturbances in patients with CLL and most common SAR in Russia. Data about a specter of TP53 mutations were obtained in Russian patients with B-cell lymphomas. Based on the outcomes of FISH, the structure of disturbances in the 17p13.1 locus in dim-TP53 was specified with the arrayCGH method. Pilot studies estimating the diagnostic possibilities of cfDNA trial in patients with oncohematological diseases were conducted. The prospective trial included 97 patients with newly diagnosed MM (41 males and 56 females) aged 30 to 82 years (median of 58 years) who were obtaining treatment at the National Medical Research Center for Hematology of the Ministry of Health of Russia from March 2019 to February 2022. Expression of the MAGE-C1 gene and MAGE-C1 protein within the bone marrow plasma cells of patients with MM was examined. It was shown that an increased expression of the MAGE-C1 gene and MAGE-C1 protein can be considered as an additional biomarker of an unfavorable course of MM. The outcomes of real-time PCR and IHC methods have demonstrated a high level of correlation while examining the expression of the MAGE-C1 gene and MAGE-C1 protein. The retrospective trial included 39 patients with MM (13 males, 26 females) aged 24 to 67 (median 57 years) with bone plasmacytomas detected at onset. A method for determining the risk group for patients with MM complicated by plasmacytoma, based on the use of an immunohistochemical method, has been developed. The substrate of extramedullary plasmacytoma and bone marrow in a patient with MM was studied by various molecular biological methods. There were differences in the bone marrow tumor substrate and extramedullary plasmacytoma. When comparing STR markers of tumor DNA freely circulating in plasma, CD138+ bone marrow cells, plasmacytoma, the largest number of involved loci was found in the DNA of the plasmacytoma. A mutation in the KRAS gene was detected only in the plasmacytoma DNA. A comprehensive molecular genetic study of the substrate of extramedullary plasmacytoma is necessary to understand the mechanisms of pathogenesis and, on this basis, to develop differentiated therapeutic approaches.

Results of detecting CD26+ leukemic stem cells in patients with CML with different levels of molecular response (MR). Recent studies show that there is a population of stem cells (SC)

which is highly specific to patients with chronic myeloid leukemia (CML). These are immature CD34+CD38low/neg SC cells with CD26 expression not typical for normal SC. These cells are assumed to be leukemic stem cells (LSC) in CML. The effect of residual LSCs on remission without treatment and the reproducibility of methods for their determination require study. The aim of the work was to determine the representation of residual LSCs in patients with deep MR (dMR) and their significance for maintaining remission after discontinuation of therapy. 185 samples from 125 patients with the chronic phase of CML (133 blood samples and 52 bone marrow samples) were analyzed. Patients with dMR (minimum MR4) and a control group of patients with de novo CML were included into analysis. In CML, LSC were detected with BC CytoFlex flow cytometry (FC) (4 antigens (CD45, CD34, CD38 and CD26)). Within the control group of patients with de novo CML, the median of LSC level was 50.76 per mcl (ICD 16.37-116.96) in bone marrow and 26.29 per mcl (ICD 4.67-83.93) in blood (p=0.09). CD26+ LSC was not detected in blood or bone marrow samples in any of the patients with CML both during treatment and after discontinuation of TKI therapy. Thus, the CD 26+LSTC compartment is detected in the blood and bone marrow of patients at the onset of CML and is absent in patients with dMR both prior to therapy with TKI and after discontinuation of therapy. This method is neither an alternative nor an addition to quantitative real-time PCR for the selection of patients for discontinuation of TKI therapy. According to clinical, morphological and cytogenetic data, c-MYC/BCL2 HGBL DH and HGBL TH represent a more homogeneous group of lymphomas with pathogenetic association with FL. In the majority of cases, it is represented by FL transformation. C-MYC/BCL6 HGBL DH resembles DLBCL. We suggest that an additional discordant lesion of the bone marrow and long-term (at least 6 months) case history should be treated as the signs of transformation. All of the aforesaid justifies the use of some therapeutic approaches in treatment of FL and MZL to treat patients with c-MYC/BCL2 HGBL DH, c-MYC/BCL6 HGBL DH and HGBL NOS. .

The phenomena of MSI, EMAST and LOH, in particular, occur in the presence of FL, DLBCL and high-grade B-cell lymphoma (HGBL). Lymphomas with a more aggressive morphology have a higher rate of genetic aberrations. There was no strict relationship between nosological forms and primary involvement of separate micro-satellite loci. MSI in NHL does not correspond to the definition of MSI-H, which is a type of instability typical of Lynch syndrome and similar solid tumors. It affects pathogenetic pathways of MSI-L and EMAST to the largest extent. During the second six months of 2022, morphology and megakaryocyte histoarchitecture were compared between previously isolated groups taking into account IHC drugs with CD42b antibodies. The results were put into information sheets; the statistical analyses of the results was performed. Microfilms were made. They were compared with clinical data.

Characteristics of newborns and evolution in the molecular response (MR) in pregnant women with chronic myelogenous leukemia (CML) during interruption of TKI therapy were examined. From 2000 to 2020, 147 women (146 women with a chronic phase, and 1 woman with the phase of acceleration) had 205 pregnancies during an observational study named 'Registry of pregnant women with CML'; 144 (70%) of them concluded with a delivery. In 101 (70%) of 144 cases, the pregnancy was diagnosed against the background of TKI therapy. In 96 (96%) of cases, TKI were cancelled in the early stages (4-6 weeks). 10 (6.8%) of 146 newborns (including 2 pairs of twins) had congenital developmental abnormalities (DA), which are common in the population. No severe or life-threatening DA were seen. There was no significant correlation between DA and therapy during pregnancy (p=0.81). During TKI pregnancy in the II-III trimester, a decrease in the mass and weight of newborns (less than 55.5) was detected as compared to IFN (p=0,009) and therapy-free observation (p<0.001). However, subsequent development of children was normal with the median being 4 years (ranging from 3 months to 11 years). The possibility of MR2

preservation at month 9 was 61% (95% CI 45-76%) after TKI withdrawal. In 12 (43%) cases, non-compliance and loss of hematological response were detected in the lack of primary MR2; three patients died after labor due to progression of CML. Analysis of pregnancy outcomes and kinetics of MR made it possible to develop a differentiated scheme of administration to patients with CML. Imatinib or nilotinib therapy can be used in patients with CML in the II-III trimester (after week 15). It is optimal to plan pregnancy in case of stable deep MR and TKI therapy for more than 3 years.

Preliminary survival results with no molecular recurrence were analyzed in patients with CML after complete withdrawal of TKI with previous observation and therapy with low TKI doses. The trial consisted of two consecutive phases: 1) observation with low TKI doses for ≥12 months; 2) treatment-free remission (TFR). 103 patients were included into the trial from 12.2019 to 11.2021. 29 patients had a history of at least 1 case of TKI withdrawal therapy. The first stage of TKI dose reduction was completed by 59 patients; 4 patients had lost a deep MR; no MMR was detected. 53 patients completed the second stage of reduction of doses of TKI: loss of MMR was detected in 2 patients, loss of deep MR - in 9 patients. TFR phase included 46 patients with 15 (32.6%) of them having at least one attempt of treatment withdrawal. Observational Me following treatment withdrawal was 8 months (1-21 months). At month 12, survival without MMR loss within the entire group was 60% (CI: 45-75%). Survival with no MMR loss for patients with the first attempt of therapy withdrawal and patients with repeated treatment withdrawal was 66% and 46% respectively. Therapy with low TKI doses in patients with CML and deep MR for ≥1 year is a safe treatment option in regular molecular monitoring. Survival with no loss of MMR in the TFR phase was promising in patients with the first attempt of therapy withdrawal and amounted to 66%. However, the obtained results are preliminary. Observation of patients during the trial is being continued. Detailed description of the reasons for changing therapy with multiple treatment lines (more than three) in patients with chronic myeloid leukemia (CML) has not been studied in detail. Most often, publications provide data on the reason for the transfer to the last line of treatment without detailed indication of anamnestic data characterizing the patient population. A detailed analysis of these data will help to more fully represent the population of CML patients. The analysis includes 82 patients with chronic Ph+ CML resistant to 2 and more therapy lines who were administered asciminib within the MAP (Managed Access Program) program. The reasons for TKI switch are as follows: resistance, intolerance, combined resistance and intolerance, and other reasons (no access to medical drugs, a doctor's decision, etc.) The most common reason for therapy switch in patients who were administered 2-3 generation TKI in 2 and more lines includes resistance in all previous lines (70-100%). Isolated intolerance was much less common (10-25%). Sometimes the cause was a combination of resistance and intolerance (1-25%). In patients who received more than 6 TKI resistance was the main problem and the reason for changing therapy, sometimes in combination with toxicity.

Topic 2: Study of the possibility of using hematopoietic alloantigens as targets of cellular immunotherapy of hemoblastoses

Methods of bioinformatics are used to describe the HA-1 TCR repertoire; combinations of functional chains are identified, the most affine TCR without alloreactivity are selected; their ability to effectively identify the endogenic fragmented antigen in PBMC of healthy patients and patients with hematological disturbances are shown. A method of CD8+ T-cell modification was described using HA-1-specific transgenic TCR combining knockout of endogenous TCR with CRISPR/Cas. The obtained T-cells demonstrated a cytotoxic activity in relation to PBMC of patients with different oncohematological diseases including AML, B- and T-cell ALL. The trend

can be used to develop therapy with minor histocompatibility antigens as a very promising class of antigens for the treatment of hemoblastoses.

Topic 3: Implementation of molecular-genetic and biological mechanisms in the human body in normal and in diseases of the blood system

Statistical processing of the data obtained after the blood analysis of donors continues. Median values of parameters of depletion of the T-cell link and the subpopulation composition of T-helpers were formed. The values that significantly differ in the group of donors and patients after transplantation (on +185 and +365 days after allo-HSCT) were selected. The results of the analysis of the study of co-inhibitory molecules on small subpopulations of T cells were processed. A cohort of 30 healthy donors was selected. The reference values of qualitative characteristics of T cell populations were set at different stages of maturation such as naive cells, central memory stem cells, central memory cells, 1, 2 and 3 effector memory T cells, 1, 2 and 3 pre-effector T cells, and effector cells. Expression of depletion markers (PD-1, TIGIT, CD160) was determined for these cells. Differences in subpopulation compositions were found depending on the gender and age of the donors. Hematopoietic stem cell (HSC) engraftment monitoring was done in 24 patients along with serological and molecular methods using ABO, Rhesus, MNSs (Er-PCR) and short tandem repeats (STR-PCR). 36 molecular trials were conducted at 30, 50-70, 90-100 and over 115 days following HSC transplantation. Donor chimerism was determined in all patients following successful HSCT with both molecular methods. Mixed chimerism based on all examined RBC systems were found in patients with primary disease recurrence with the Er-PCR method; mixed chimerism with a high value (over 30%) of recipient marker was detected with the STR-PCR method. Return to own hematopoiesis was recorded in one patient who had graft rejection using both molecular methods. The Er-PCR and STR-PCR methods coincided in 31 of 36 cases (86.1%).

A set of materials has been obtained for testing the operation of a cloud platform for assessing thrombotic risks and for debugging a technology that allows scaling the detail of the desired assessment at the user's request. Validation experiments on vascular network thrombosis using "microfluidity" technology were carried out. Various modes of thrombosis on the model of manufactured chips were studied. The correspondence between hypotheses for blood flow centralization issued during mathematical modelling for various mechanisms of blood coagulation activation mechanisms was qualitatively described. The method of manufacturing an experimental unit that reproduces the geometry of a real vessel fragment has been improved. The protocol of trials has been standardized. The technology improvement made it possible not only to significantly increase the productivity of the method and reduce the MRI/CT-related technological loop to test results, but also to examine the processes of platelet activation within the wide specter of specific hydrodynamic conditions. The mechanisms of saturation of tissues with oxygen were tested with the methods of numerical simulation when red blood cells were passing through capillaries. The obtained data allow to estimate the effects of transformation of hydrodynamic flows in the capillary bed if abnormal processes are developed. Targeted sequencing was used to search for mutations in the genes of 41 thrombocytopoesis proteins of patients diagnosed with immune thrombocytopenia or thrombocytopathy/unspecified thrombocytopenia. It was shown that in many cases the disease is hereditary. There was a great variety of diagnosed nosologies (von Willebrand disease, MYH9-associated diseases, amegakaryocytic thrombocytopenia, macrothrombocytopenia of various origin). The conducted trials and analysis of the most recent literature data allow to conclude about whether it is advisable to move from targeted sequencing to whole-exome sequencing at the next stages of project implementation. Standard sequencing

with the Sanger method was used to detect mutations in the ADAMTS13 gene of 5 patients with Upshaw-Schulman syndrome (USS). Two of these mutations had not been previously described (p.Asp235Gly and p.Pro487Leu). Thus, at the moment, this pathology has been genetically verified in 11 patients. Also, during the reporting period, systems of classical mutation analysis using the Sanger method for the RASGRP2 gene were put into practice.

Topic 4: Development of a unified risk-adapted strategy for transplantation of allogeneic hematopoietic stem cells, combining the stages of pre-transplantation conditioning, prevention of GVHD and post-transplantation patient-oriented exposure

It was shown that the regimens of GVHD prevention with post-transplantation cyclophosphamide (PT-CP) and T-cell depletion produce a more powerful effect on reconstitution of CMV-specific immunity, including at a later date following allo-HSCT. Thus, these patients, who went through the types of GVHD prevention, can be the target group requiring the use of cellular technologies, for instance, reinfusion of CD45RA- donor lymphocytes, and CMV-specific T cells of the donor. Determining the CMV status of the donor and recipient prior to the planned HSCT is especially important as patients with the highest risk of CMV infection are identified at this stage of diagnosis. A group of patients who require an individual approach to the prevention of CMV infections and a longer monitoring of CMV infection during the post-transplantation period was identified. It was shown that when the recipient had no 'best' genotype of killer immunoglobulin-like receptor (KIR) (the value was shown to reduce the possibility of relapse), the risk of transplant failure was reduced. Changes in the MMSC at onset and upon remission prior to allo-HSCT were detected during the study of relative expression level (REL) in the genes as compared to healthy donors. The levels of IL6, JAG1 and PPARγ were significantly increased. This may indicate that the anti-inflammatory properties of the MMSC in patients with AL during remission were weakened. According to a molecular trial, a complete donor chimera can be preserved in the bone marrow cells in the later stages. However, some populations of cells including regulatory ones can significantly differ by their genetic structure as over half of the cells can have a mixture of host hematopoiesis, which may affect the results of allo-HSCT. While studying infections of blood flow following allo-HSCT, gram-negative bacteria predominated in the etiological structure during both phases of immune reconstitution. This allowed to administer an adequate anti-microbial first-line therapy in patients following transplantation. It was shown that the use of fluoroquinolones of patients colonized with polyresistant gram-negative bacteria is unreasonable during neutropenia and that their use in patients not colonized with these bacteria is reasonable. Effectiveness and safety of using allo-HSCT in patients with NHL during the first and the last therapy lines were compared. The advantage of allo-HSCT was demonstrated in patients with mutated TP53 gene during the first line of therapy.

Topic 5: Study of molecular and genetic mechanisms of resistance and pathogenicity in nosocomial bacteria and fungi in hematology and features of the novel coronavirus infection due to COVID-19 in patients with blood system diseases, donors of blood and its components and hematopoietic stem cells

Distribution of virulent genes (iucA, rmpA and rmpA2) and capsular K1/K2/K5/K20/K54/K57 types were studied among lebsiella pneumoniae isolated from the hemoculture of patients with hematological diseases. The virulent genes were detected in 24.1% of K.pneumoniae. A high incidence of their detection among carbapenemase -producing K.pneumoniaec (55.3%) was recorded. The iucA (23.3%) and rmpA2 (19.8%) genes predominated

among the virulent genes, which could be used as markers of hypervirulent strains. The majority of hypervirulent CP-K.pneumoniae were of two genetic lines (ST395/K2 and ST23/K57). The in house method of accelerated identification of fungi isolated from the positive hemoculture was compared with a classical method of mass spectrometry (C.parapsilosis n=5, C.tropicalis n=4, C.albicans n=3, C.krusei n=1, C.guilliermondii n=1, Rhodotorula mucilaginosa n=1, Fusarium dimerum n=1). With the accelerated in house method, successful verification of fungi was 75% (12/16) of isolates for genus, and 68.8% (11/16) for species. With the accelerated in house method, identification of fungi was statistically shorter as compared to the classical method and amounted to 36 hours 20 minutes vs 55 hours 31 minutes respectively ($p = 0.028$). The suggested method of in house accelerated fungal identification isolated from the positive hemoculture made it possible to start the precision treatment of invasive mycosis 19 hours earlier. A high rate of blood flow infections was detected following allogenic transplantation of hematopoietic stem cells (allo HSCT). It accounted for 29.9% following the first all-HSCT and 35.1% following the second. Identification of carbapenems-resistant gram-negative bacteria was significantly higher following the second allo-HSCT as compared to the first one (57.1% vs 13.6%; $p=0.048$). Survival following bloodstream infections (BSI) was lower during the second all-HSCT as compared with the first one (71.4% vs 97.9%; $p<0.0001$), especially during isolation of carbapenems-resistant gram-negative bacteria (25.0% vs100.0%; $p=0.0023$). Analysis of the data obtained showed a correlation between the epidemiological situation in Russia and Moscow and the frequency of detection of antibodies to the RBD domain of the viral S-protein SARS-CoV-2. So, the rate of detecting antiviral AT among donors of blood and related components increased from 8.52% to 58.09%. In employees of the National Medical Research Center for Hematology of the Ministry of Health of Russia, the cumulative humoral immunity was around 92% by November 2021. The rate of SARS-CoV-2 RNA detection did not depend on gender of the examined patients and amounted to 1.3% and 1.2% among males and females respectively ($p=0.154$). Viral RNA was detected in 1.42% and 1.09% ($p<0.001$) of cases among employees and patients of the Center respectively. Meanwhile, there were no significant differences in the rate of RNA detection among patients with tumor and non-tumor blood system diseases (1.24 % vs 0.92 %, $p=0.147$). Sequencing of viral RNA samples obtained from employees of clinical units in which an outbreak of infection was observed showed that there was no association of a certain variant of SARS-CoV-2 with the severity of the disease, but allowed an epidemiological study to be carried out and identify a variant likely to be characterized by high contagiousness. The study of etiology of recurrent BSI in patients following all-HSCT showed a low probability of coincidence with the first episode of BSI. The rate of recurrent blood flow infections was 22.1% (in 23 of 284). They were associated with the primary ($p=0.021$) and secondary ($p=0.024$) graft incompetence and repeated allo-HSCT ($p<0.0001$). The rate of identification of carbapanem-resistant gram-negative bacteria was significantly higher during repeated blood flow infections as compared to the first ones (23.7% vs 6.0%; $p=0.003$). Only 3.9% of patients with repeated BSIs were found to have isolated a phenotypically identical strain from the hemoculture.

Topic 6: Optimization of diagnostics, treatment and monitoring of non-tumor diseases of the blood system in adults based on molecular-genetic, immunophenotypic and biological parameters

The registration of patients with Gaucher's disease continued, which included 346 adult patients (> 90% of the total population), which allowed us to obtain reliable information about the phenotypic characteristics of the disease and the dynamics of clinical and laboratory parameters in the process of pathogenetic therapy. The duration of enzyme replacement therapy in 50% of

Russian patients exceeds 12.5 years. The goals of Gaucher disease therapy were achieved in 45% of adult patients; maintenance enzyme replacement therapy was still provided. Since 2006, a novel method of Gaucher disease therapy has been used (substrate-reducing therapy (SRT). The results of a long-term (single-center) follow-up of patients who underwent eliglustat SRT confirm the data obtained during multi-center clinical trials that took place in 2006-2020 (good tolerance and high effectiveness). The National Clinical Recommendations on Gaucher disease were updated and transferred to the Expertise Center. Enrollment of patients to assess the T-cell immunity link in patients with AIHA was continued prior to and during the immunosuppressive therapy with flow cytometry. 26 patients were included into the trial. The subpopulation composition of T - lymphocytes in patients with AIHA before treatment was analyzed in comparison with the control group (56 donors). The study of the subpopulation composition of T-lymphocytes improves the understanding of the pathogenesis of AIHA and can be the basis for optimizing treatment programs and prevention of relapses of this disease in the future. Subsequent filling of the Register of patients with paroxysmal night haemoglobinuria (registry study No PNH-R01 Epidemiological and clinical characteristics of paroxysmal night haemoglobinuria at the National Medical Research Center for Hematology of the Ministry of Health of Russia) continued. Long-term clinical effectiveness of pathogenetic therapy of PNG with a Russian-made inhibitor of complement C5 component and factors associated with favorable and unfavorable prognosis of PNG and response to pathogenetic therapy was studied. Effectiveness of hemostatic therapy with various drugs (FVIII concentrates of prolonged action, monoclonal biospecific antibodies with subcutaneous mode of administration) in patients with hemophilia was assessed. Criteria for novel preparation crossover were developed. Clinical recommendations related to the management of patients with hereditary coagulopathies were improved. An algorithm of diagnostics of antithrombin III hereditary deficiency was developed; groups of the risk of thrombotic complications were identified. It was shown that screening for AT III activity should be done in the early gestational period or in the period of pre-conceptional preparation in the presence of aggravated history. The course of coronavirus infection in patients with hereditary coagulopathies continued to be studied. It was shown that thrombotic complications of SARS-CoV-2 were lacking in this cohort; 8% of patients had bleedings. The molecular and genetic defects were studied in patients with rare blood coagulation disturbances. Data about patients with FXII deficiency were collected: 33 patients underwent a genetic study, the clinical phenotype in correlation with detected mutations was analyzed. The clinical picture of 19 patients of moderate hemorrhagic syndrome was presented; thromboses were identified in 2 patients only. Quality of life in patients with hereditary coagulopathies was assessed. It was found out that in spite of active implementation of novel medications to treat patients with coagulopathies, some problems remain unsolved. For the first time in Russia, major and minor surgeries of the locomotor system were performed in patients with hemophilia A with and without FVIII inhibitor obtaining a novel non-factor therapy within the same department. The therapy allowed to reduce the intraoperative blood loss, improve the results of restoration during the postoperative period, significantly improve the quality of life and significantly reduce a number of used FVIII preparations and shunting mechanisms of action during surgeries. For the first time in Russia, major surgeries of the locomotor system were performed with novel recombinant prolonged FVIII preparations. This allowed to have the desired level of the scarce factor on a long-term basis without drop peaks resulting in early and more effective rehabilitation, and FVIII saving by 40%. Studies of using tranexamic acid during surgeries in patients with hemophilia were continued. This made it possible to reduce the intraoperative blood loss, a number of used FVIII by improving the postoperative results and decreasing the economic losses.

Immune thrombocytopenia (IT) is an autoimmune diseases with isolated thrombocytopenia. A group of patients with IT is heterogeneous due to pathogenetic mechanisms, clinical signs and therapy response. The therapy method is selected depending on the hemorrhagic syndrome intensity , PLT quantity, age and concomitant diseases, and predicted adverse effects. A wide use of thrombopoietin receptor agonists (Romiplostim and Eltrombopag) and rituximab in adults led to significant changes in the IT therapy. Based on the latest international clinical recommendations, splenectomy should be performed in 12-24 months from the onset due to possible remission. However, a clear algorithm for choosing second-line therapy has not been developed. The aim of the study is to optimize therapy in patients with immune thrombocytopenia resistant to first-line therapy. 112 patients with primary immune thrombocytopenia with resistance to first-line therapy were included into the study. The patients were divided into 2 groups: 82 of them underwent splenectomy at the he National Medical Research Center for Hematology of the Ministry of Health of Russia, 30 of them were administered thrombopoietin receptor agonists with Romiplostim and Eltrombopag being given to 17 and 13 patients respectively. Methods and effectiveness of preoperative preparation, intra- and postoperative preparation, intra- and postoperative complications and remote results of splenectomy were analyzed.

Topic 7: Effect of architecture and structural changes of new polymeric compounds on their hemocompatibility and provision of hemostatic reactions in in vivo and in vitro experiments

Experimental monocomponent and complex local coating based on sodium alginate in the form of a sponge, film, powder and their combinations were developed and studied during the reporting period. It was shown that coatings based on 2.0, 3.0 and 4.0% of sodium alginate solutions in the form of a film (88.52, 81.89 and 86.79%) and in the form of powder with active admixture 1 in the ratio 3: 1 had a high hemostatic effect. The effect of material of forms used for sublimation drying (glass or metal) on the sponge-shaped coating was studied. The changes in the coating properties were determined under the effect of micro- and nanoparticles introduced into iron oxides. The next section of our project was associated with the need to use raw materials of primarily Russian origin. A comparative study of kappa carrageenan made in Philippines and four novel lots of carrageenan samples made in Russia was performed in the lab. Sponge-shaped coatings were made from batch 1 and 2 samples, and samples 3 and 4 were examined in vitro. The thromboelastometry indicators during contact of donor blood with 1.5% solutions of Russian carrageenan (sample No. 3) and Filipino production practically did not differ, which proves the possibility of obtaining Russian-made carrageenan, which can subsequently replace imported analogues.

It was determined that the mechanism of anticoagulant activity of galactoglucomannan (GGM; mm 14.4 kDa) sulfates/galactomannan (GM; mm 276.8 kDa) is associated with an independent and antithrombin-mediated decrease in the amidolytic activity of thrombin. Intravenous administration of GGM and GM to guinea pigs at the dose of 3 mg/kg results in a significant increase of plasma clotting time while testing aPTT and PT.

Starch-based terpenophenol conjugates produced no in vitro effect on the occurrence of a fibrin clot during testing aPTT, PT and values of platelet aggregation. During incubation with conjugates, the rate of human red blood cell dialysis was < 2 %.

Intravenous administration of conjugates at the doses of 2 and 4 mg/kg did not result in a significant change in plasma clotting time of guinea pigs while testing aPTT and PT.