# THE INFLUENCE OF CONCOMITANT IMMUNE PHARMACOTHERAPY ON ANTIOXIDANT DEFENSE SYSTEM IN PATIENTS WITH CERVICAL CANCER

***Kamishov S.V., Pulatov D.A., Enikeeva Z.M., Gildieva M.S.***
*Republican Specilized Scientific and Practical Medical Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan, Tashkent*

**Abstract.** The level of H2O2-induced chemiluminescence (CHL), product lipoperoxidation (LP) and the state of enzymatic antioxidant protection (AP) of the system was studied in 95 patients with cervical cancer stage II-III, who received accompanying extracorporeal immunopharmacotherapy (EIPT). The content of LP products in patients' erythrocytes and the activity of glutathione-dependent enzymes were initially high. After EIPT, the intensity of these processes was significantly reduced. Immunotherapy increased the activity of catalase and superoxide dismutase. In CC(cervical cancer) patients, the underlying disease and chemoradiotherapy can increase the content of hydroxide in the body, causing an imbalance in the LP/AP system. EIPT as a concomitant therapy can significantly reduce this imbalance.

**Аннотация.** Уровень H2O2-индуцированной хемилюминесценции (ХЛ), продукции липопероксидации (ЛП) и состояние ферментативной антиоксидантной защиты (АП) системы изучали в крови 95 больных раком шейки матки (РШМ) II-III стадией, которые получили сопроводительную экстракорпоральную иммунофармакотерапию (ЭИФТ). Содержание продуктов ЛП в эритроцитах пациентов и активность глутатионзависимых ферментов изначально были достоверно высокими. После ЭИФТ интенсивность этих процессов была достоверно снижена. Иммунотерапия увеличила активность каталазы и супероксиддисмутазы. У пациенток раком шейки матки, основное заболевание и химиолучевая может увеличить содержание гидроперекисей в организме, вызывая дисбаланс в системе ЛП/АП. ЭИФТ как сопутствующая терапия позволяет значительно уменьшить этот дисбаланс.

**Keywords:** glutathione-s-peroxidase (HSP); glutathione-s-reductase (GR); glutathione-s-transferase (GST); catalase; chemoluminescence; lipid peroxidation (LP); plasmapheresis (PP); cervical cancer (CC); extracorporeal immunopharmacotherapy (EIPT).

**Ключевые слова:** глутатион-s-пероксидазы (ГСП); глутатион-s-редуктазы (КГР); глутатион-s-трансферазы (ГСТ); каталаза; хемолюминесценция; перекисного окисления липидов (ЛП); плазмаферез (ПП); рак шейки матки (РШМ); экстракорпоральная иммунофармакотерапия (ЭИФТ).

**Introduction.** Deepening of knowledge about the immune system potential in combating cancer has promoted the development of immunotherapy (IT) of cancer. The interest for using various methods of IT in cancer treatment is growing worldwide. The results obtained during the last decade enhance the role of IT approaches in the treatment of cancer diseases [4,8,10].

The modern methods of extracorporeal immune pharmacotherapy (EIPT) are per se an effective extension of therapeutic PP. During PP, the cellular elements are returned into the patient immediately after deplasma. EIPT includes additional separation of the leukocytes which are then extra corporeally treated with medicinal product aimed at increasing or decreasing (depending on the disease) the functional activity of the cells participating in the inflammation and the immune response [7,9]. Free-radical oxidation of blood lipids is an important mechanism of maintaining the body homeostasis as it regulates the functioning of biological membranes [10]. The lipid peroxidation (LP) induction and regulation system includes the formation of activated oxygen metabolites such as superoxidanion radical, singlet oxygen, hydroxyl radical, hydrogen peroxide, etc. An adequate ratio of oxidative and antioxidative processes is a prerequisite for the normal functioning of the body [7].

The development of malignant neoplasms significantly changes the lipid composition and the intensity of LP reactions in the tumour and the body as a whole. Numerous studies have shown the activation of free-radical hematic processes in cancer patients accompanied by a decrease in the content and activity of the antioxidant protection (AP) system. Indicators of AP system activity in tumours, organs, and serum change regularly during the malignant growth and depend on the tumourcharacteristics and the body condition. The tumour tissue is able to accumulate natural antioxidants which suppress the peroxidation of lipids in the tumour. Still, the antioxidant protection in normal tissues is decreased [9,10].

The intensity of free-radical oxidation in serum lipids shall be characterized by at least three criteria: oxidation, antioxidative activity, and oxidation rate. Oxidation is defined as the rate of initiation of primary free radicals and depends mainly on unsaturation of lipids. However, this ability does not always express the rate of oxidation in multicomponent systems. Lipids with high oxidability can show a low oxidation rate in the presence of a high concentration of antioxidants [2,7,9]. Induced chemiluminescence (CL) is an integral indicator of the LP condition in the interaction of substrates, catalysts and inhibitors of free-radical oxidation. It allows an in vitro assessment of the functional state of the body as a whole and can be used as an additional test in differentiated diagnostics of cancer diseases [1,3]. To-

day, cervical cancer (CC) remains one of the most common malignant tumours in women. Certain prognostically unfavourable systemic metabolic changes, such as the state of LP processes and the AP of cells,are associated with the development of CC and, to a certain extent, contribute to the progression of tumour. Such disorders have not been sufficiently studied yet [6,10]. At the same time, an excessive formation of free radicals is a known possible pathogenetic factor of carcinogenesis [3]. The predominance of products of activated oxygen metabolites due to the increased formation or the depletion of antioxidants accompanied by the activation of destructive processes was called "oxidative stress" [9]. Thus, for many types of tumours, the activation of LP processes is an important pathogenetic factor adversely affecting the efficiency of treatment and the disease prognosis. The growth of malignancy increases the imbalance between the intensity of products of antioxidative enzymes and free-radical oxidation and the APS functional activity. Still, the impact of treatment on the LP processes in CC patients was not evidenced yet. These parameters are important for the study of the body-tumour interaction, the assessment of efficiency of treatment and the disease prognosis [4,6].

**Purpose of the study** was to study the effect of concomitant immunopharmacotherapy on various parts of the antioxidant defense system in patients with cervical cancer.

**Materials and methods.** The study included 95 patients with clinical stages II-III CC who received standard combined treatment in the oncogynecology department of the Republican Research Center on Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan during 2011-2014. All the patients had squamous cell carcinoma of the cervix histologically confirmed by the morphological analysis of surgical and biopsy material. The patients were aged 23 to 72, average – 46.2±6.74 years. All patients received complex treatment including polychemotherapy (PCT), surgery and radiotherapy (RT). In the first stage of treatment, the CC patients received intavenouse or intra-arterial polychemotherapy in the regimen of: cisplatin 50 mg/m$^2$+5-fluorouracil 1000mg/m$^2$, 4-6 courses of 4 days every 3 weeks, both in adjuvant and neoadjuvant regimen. The second stage included radical surgery or combined radiotherapy in radical regimen. The RT conducted at the third stage included remote telegamma-therapy (RTGT) and intracavitary brachytherapy. RTGT was conducted using "Theratron" or "AGAT-R" apparatus by split courses at 2 Gy fraction dose to 50 Gy summary dose , 5 QW. The brachytherapy was performed every other day, using “Gammamed” apparatus at 5 Gy fraction dose to 45÷55 Gy total dose. Group 1 (control group) of patients received no immunotherapy (Table 1).

Table 1.

| Groups of patients (CC patients, n=95) | Abs. | % |
|---|---|---|
| 1. Control group (without immunotherapy) | 40 | 42.1 |
| 2. Group receiving EIPT | 30 | 31.6 |
| 3. Group receiving EIPT + plasmapheresis | 25 | 26.3 |

In Group 2, EIPT included the exfusion of 200-250 ml of auto blood into the sterile containers "Gemakon" or "Terumo" followed by the incubation with immune modulators «Cycloferon» (meglumine acridonacetates — derived acridonacetic acid, low molecular weight inducer of interferon synthesis. Is manufactured by the firm "Polysan", Russia) in the total dosage of 750 mg (during 3 procedures) at 37°C during 60-100 minutes, with the future return of the conjugate into the circulatory system of the patient [5]. Group 3 received EIFT as an extension of plasmapheresis (PP). The buffy coat was incubated with immune modulators the same way as in Group 2, with the future return of the conjugate into the circulatory system of the patient.

**Laboratory tests.** Blood for serum was sampled from the ulnae vein of fasting patients. Blood serum was obtained by centrifugation of whole blood during 15 minutes at 1200 rpm. Induced CL was registered at the temperature of 37°C using electronic quantimeter-consisting of the radiation detector FEU-38, the power source VS-22, the high-resistance millivoltmeter LPU-01 and the logger KSP-4 with a measurement range of 10 mV. Centrifuged serum was placed in a light-tight cubicle where CL was initiated with 1 ml of 1% $H_2O_2$and recorded. The kinetic CL characteristics included: $I_i$ – initial (fast) flash intensity; $I_f$ – final CL intensity after 5 minutes of reaction, and $\Sigma I_5$– light-sum of LP reaction during 5 minutes of observation [10].

To determine the activity of antioxidant enzymes in erythrocytes, the latter were washed 3 times by 10-folds dilution of plasma with cold physiological saline with future repeated centrifugation at 2000 rpm and the temperature of 40°C. The haemoglobin hindering the determination of enzyme activity was precipitated by alcohol and chloroform mixture [1, 9].

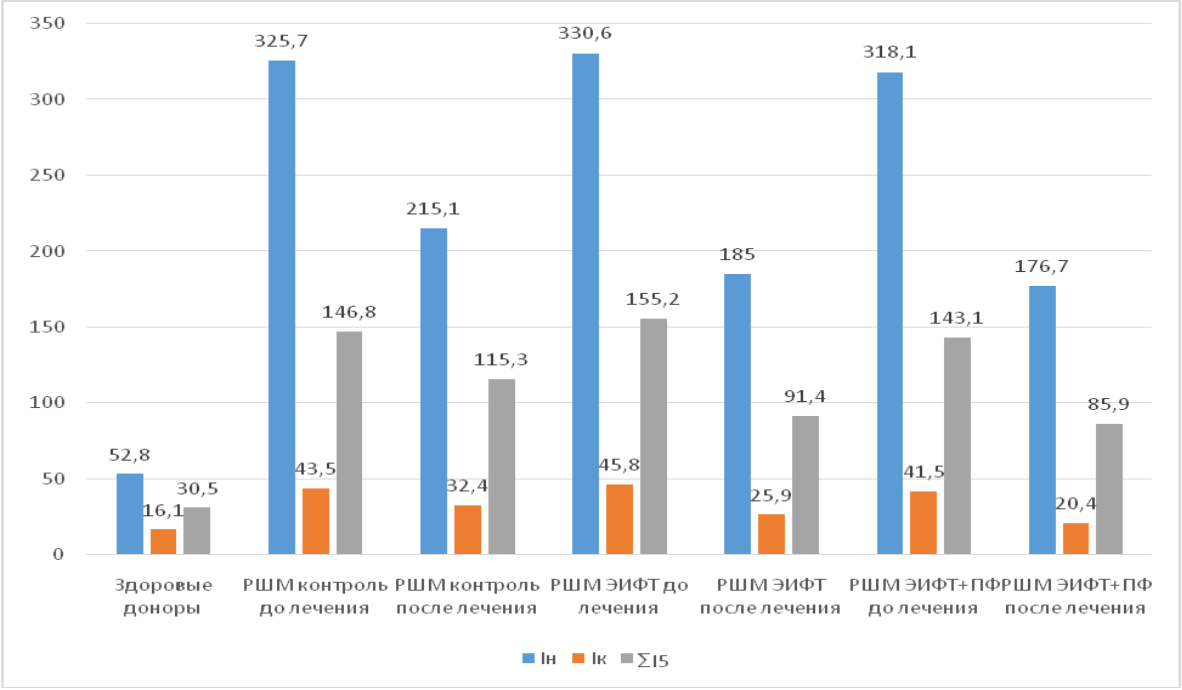
The concentration of malondialdehyde (MDA) was determined according to L.I. Andreyeva [2]. The activity of superoxide dismutase (SOD) was determined by reducing the nitroblue tetrazolium reduction rate in the presence of reduced NAAD and phenazine methosulphate [10]. The diene conjugates (DC) level was assessed by the ratio of the intensity of absorption of isopropanol extracts at 215, 220 and 232 nm [9]. The catalase activity was determined according to A.I. Karpischenko based on the ability of hydrogen peroxide to form a stable coloured complex with molybdenum salts [18]. The activity of glutathione-S-transferase (GST) was determined according to A.I. Karpischenko by the rate of formation of glutathione-S-conjugates from reduced glutathione (G-SH) and 2,4-Dinitrochlorobenzene (DNCB) [10]. The activity of glutathione-S-peroxidase (GSP) was determined by the

rate of utilization of hydrogen peroxide due to the oxidation of glutathione [6]. The activity of glutathione-S-reductase (GSR) was determined according to Harutyunyan A.,etc [4].

The studies were conducted in the Republican scientific center of immunology Ministry of health of the Republic of Uzbekistan for 2011-2014. The patients were lab examined before the immunotherapy and immediately before their discharge. The statistical analysis was made using Statistics 6.0 software. The reliability of the differences between two samples with a normal distribution of values was determined using Student's f-test.

**Results and discussion.** In the examined CC patients, the kinetics of induced CL was significantly increased several times vs. the normal level (Figure 1). The initial burst had the average amplitude of 324.8+15.4, and the final glow – 42.6+2.4, at the light sum of 148.4±9.4 cu ($p<0.05$).

Complex therapy contributed to a significant decrease in luminescence. However, the luminescence indices did not return to normal values. The accompanying EIPT resulted in a more pronounced decrease in the studied indices of induced CL. The use of EIPT+PP was even more efficient probably due to the removal of metabolic products and toxins from blood plasma of the patients. The initial burst amplitude evidences the interaction of transition metals in the serum – Cu, Fe and others– with organic and inorganic hydroperoxides.




Healthy donors | CC control before treatment | CC control after treatment | CC EIPT before treatment | CC EIPT after treatment | CC+PP EIPT before treatment | CC+PP EIPT after treatment

*l. ...ence in CC patien...*

The increase dinitial burst in CC patients could be associated with an increased level of lipid hydroperoxides in blood and the increased content of transition metals due to the disintegration of antioxidant enzymes. CC patients had initially high intensity of LP processes judging by the content of its products, DC and MDA, in red blood cells (RBC) what was in line with the kinetics of induced CL. Its reduction in the Control group after combined therapy was not very pronounced. At the same time, the oxidative processes in RBC have significantly decreased in the groups of patients receiving EIPT, especially, EIPT+PP (Table 2).

Table 2.
LP indices in RBC of CC patients ($p<0.01$-$0.05$)

| Index | Groups of patients | | | | | |
|---|---|---|---|---|---|---|
| | Control group | | EIPT | | EIPT + PP | |
| | Before treatment | After treatment | Before treatment | After treatment | Before treatment | After treatment |
| DC, mcM/L | 528.7±24.6 | 475.2±22.0 | 536.7±26.7 | 276.4±16.7 | 530.7±21.9 | 254.4±12.4 |
| MDA, mcM/L | 415.1±21.5 | 380.6±19.2 | 410.1±21.3 | 220.6±10.4 | 421.1±20.6 | 180.8±12.3 |

The conducted studies have shown aninitially increased level of activity of glutathione-dependent enzymes, GSR, GSP and GST, in CC patients against the reduced activity of SOD and catalase. Such behaviour of enzymatic member of AP system in malignant cells indicated the leading role of glutathione-dependent enzymes in inactivation of peroxides. In addition, the reduced activity of SOD and catalase could indicate a decrease in generation of $H_2O_2$, inhibitor of cell multiplication, as CC progressed. An increase in the content of GSR, GSP and GST with a simultaneous decrease in the activity of catalase and SOD enhanced the disproportion in the formation of $O_2$ and $H_2O_2$.

After EIPT, the activity of GSR, GSP and GST was decreasing probably due to a decrease in the oxidant load thanks to a reduced content of hydroperoxides. On the contrary, the activity of catalase increased 2-3 times after immunotherapy while in the control group the increase was not significant (Table 3).

Table 3 Indicators of antioxidant protection in red blood cells of CC patients ($p<0.01$-$0.05$)

| Index | Groups of patients | | | | | |
|---|---|---|---|---|---|---|
| | Control | | EIPT | | EIPT + PP | |
| | Before treatment | After treatment | Before treatment | After treatment | Before treatment | After treatment |
| SOD, cu | 0.18±0.015 | 0.24±0.018 | 0.19±0.012 | 0.32±0.020 | 0.18±0.016 | 0.37±0.022 |
| GSP, mM/GSH, min. | 0.15±0.012 | 0.12±0.009 | 0.017±0.014 | 0.09±0.007 | 0.015±0.013 | 0.07±0.004 |
| GSR, mcM/NADP*R, min. | 0.09±0.007 | 0.07±0.006 | 0.08±0.006 | 0.05±0.004 | 0.09±0.007 | 0.04±0.002 |
| GST, mcM/DNCB*GSH, min. | 0.38±0.023 | 0.33±0.014 | 0.37±0.026 | 0.22±0.012 | 0.40±0.027 | 0.18±0.015 |
| Catalase, mM/$H_2O_2$, min. | 10.51±0.97 | 18.43±0.12 | 11.26±0.84 | 24.6±0.14 | 10.72±0.16 | 31.5±0.18 |

SOD was taken as a key enzyme of cell AP as it inactivated the superoxidanion radical and worked in the cell in the cascade with catalase and GSP – the enzymes capable of decomposing hydrogen peroxide. After the immunotherapy, that indicator was much higher in comparison to the control group. Certain prognostically unfavourable systemic metabolic changes, such as the state of LP processes and the AP of cells, are associated with the development of CC and, to a certain extent, contribute to the progression of tumour. Such disorders have not been sufficiently studied yet [3]. At the same time, an excessive formation of free radicals is a known possible pathogenetic factor of carcinogenesis [9,10]. The observed dynamics of MDA and catalase in the control group indicated a disproportion in the formation of $O_2$ and $H_2O_2$ enhanced by an increased content of glutathione-dependent enzymes (GSR, GSP and GST) involved in the decomposition of hydroperoxides.

**Discussion.** When the tumour and the body interact, the system of nonspecific cellular immunity the malignant and cancer cells are not recognized as genetically homogeneous to the body. It produces a stress factor noted by many researchers and known as an "oxidative burst" which rapidly activates the membrane enzyme phospholipase $A_2$. Phospholipase $A_2$ breaks the unsaturated higher fatty acids like arachidonic acid in the membranes of body tissues. Then the arachidonic acid is turned into prostaglandin $D_2$ which in turn activates the nonspecific cellular immunity (neutrophils, macrophages, etc.) with superoxidanion radical presented in the active cell centre.

These substrates in the tumour carrier define the duration of the own antitumor protection of the body thus supporting the body's response to various tumour, infectious and other diseases. Theformation of radicals in the body is opposed by theenzymatic system of regulation of LP products represented by superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and other natural antioxidants acting as non-enzymatic inhibitors of LP products [6,8]. In the conducted study, the exceeded level of CL vs. the normal level of healthy donors indicated by a significant activation of oxidative processes. That could be due to the activity of nonspecific cellular immunity associated by activation of the reaction with a significant circulation of oxygen superoxidanion radicals. The efficiency of the conducted treatment was shown to correlate with the activity of free-radical oxidation of blood serum.The main disease and chemoradiation could increase the content of hydroperoxides in CCpatientscausing the imbalance in their LP/AP system. EIPT as accompanying therapy allowed a significant reduction of that imbalance.

**Conclusion.**

1. The indices of induced CL of CC patients'blood serum were several times higher than normal values. At that, the level of LP processes in red blood cells of CC patientswas initially high. EIPT methods effectively reduced the intensity of those processes.

2. The activity of glutathione-dependent enzymes in CC patients was initially high and tended to decrease after EIPT probably due to a decrease in the content of hydroperoxides.

3. The activity of catalase increased 2-3 times after immunotherapy while in the control group the increase was not significant. There was also a pronounced increase in SOD activity compared to the control group.

4. The observed dynamics of MDA and catalase in the control group evidenced a disproportion in the formation of $O_2$ and $H_2O_2$ enhanced by an increase in the content of glutathione-dependent enzymes (GSR, GSP and GST) involved in the decomposition of hydroperoxides.

5. EIPT as accompanying therapy allowed a significant reduction of the imbalance in the LP/AP system of patients with CC that could be caused by the main disease and chemoradiation that increased the content of hydroperoxides in the body.

**References**

1. Arslanova D.R., Antoneyeva I.I., Abakumova T.V., Sidorenko E.G., Voronova O.S. (2011) Antioxidant protection enzymes in tumor tissue with cervical cancer]. Siberian OncoJ, 1:13.

2. Barsukov V., Chesnokova N. (2008) Activation of lipid peroxidation and failure of antioxidative system in the development of structural and functional disorganization of breast tissue in the area of neoplasia. Russian J Onco, 3:31-32.

3. GoroshinskayaI., Kachesova P., Nerodo G., Kalabanova E., Shalashnaya E., Surikova E., Nemashkalova L., Neskubina I. (2011) A comparative study of oxidation of proteins and lipids in the blood plasma of patients with cervical cancer with and without metastases. Palliative medicine and rehabilitation, 1: 45-49 (in Russian).

4. Gushchin I.S. Experimental validation of extracorporeal immunopharmacotherapy. In: Gushchin I.S., Leskov V.P., Prozorovskij N.S., Pisarev V.M. Topical of issues immunopharmacotherapy.– Moscow: Medicine, 1987. pp. 71-76 (in Russian).

5. Kamishov S.V., Pulatov D.A. Supportive immunotherapy in complex treatment of patients with oncogynecologycal diseases.// The Scientific Heritage (Budapest, Hungary) 18 (18) 2017 P.1, p. 23-27.

6. Maneo A., Colombo A., Landoni F. (2005) Treatment of stage IIIB cervical carcinoma. A comparison between radiotherapy, concurrent chemo-radiotherapy and neoadjuvant chemotherapy. Minerva Ginecology, 57 (2): 141-152.

7. Manju V., Kalaivani J. Sailaja, Nalini N. (2002) Circulating lipid peroxidation and antioxidative status in cervical cancer patients: a case-control study. Clin Biochem, 35(8): 621-625.

8. Manoharan S., Klanjiappan K., Kayalvizi M. (2004) Enhanced Lipid peroxidation and impaired enzymatic antioxidative activities in the erythrocytes of the patients with cervical carcinoma. Cell Mol Bio Lett,9, (4A): 699-707.

9. Ray G., Husain S.A. (2002) Oxidants, antioxidatives and carcinogenesis Indian. J Exp Biol, 40(11): 1213-1232.

10. Rosenberg S., Restifo N., Yang J., Morgan R., Dudley M. (2008) Adoptive cell transfer a clinical path to effective cancer immunotherapy. Nature Reviews Cancer, 8:299-308.

# ГЕМОСТАЗ У НЕДОНОШЕННЫХ НОВОРОЖДЕННЫХ НА ФОНЕ ВНУТРИУТРОБНОЙ ПНЕВМОНИИ И ГЕМОРРАГИЧЕСКОМ СИНДРОМЕ.

***Катюхина Анастасия Викторовна.***
*Аспирант, Кафедра госпитальной педиатрии им. академика В.А. Таболина*
*Педиатрический факультет РНИМУ им. Н.И. Пирогова, Москва.*
*Городское бюджетное учреждение «Городская клиническая больница*
*№ 24 Департамента здравоохранения города Москвы», врач неонатолог.*

**Аннотация**. Изучить показатели гемостаза у недоношенных новорожденных с внутриутробной пневмонией. Оценить частоту встречаемости геморрагического синдрома на фоне течения внутриутробной пневмонии. В обследовании участвовало 60 недоношенных новорожденных детей, родившихся на сроке гестации с 27 по 36 неделю. Были изучены показатели гемостаза у всех 60 детей в течение 4 недель наблюдения. Проанализированы полученные результаты у детей на фоне течения пневмонии и геморрагического синдрома. Выявлена склонность к гиперкоагуляции на фоне течения внутриутробной инфекции. Установлено повышение частоты развития геморрагических проявлений как осложнений течения инфекционного процесса.

**Ключевые слова**: Недоношенный новорожденный, гемостаз, геморрагический синдром, пневмония, фибринолиз.

**Annotation**. To study the parameters of hemostasis in premature newborns with intrauterine pneumonia. To assess the incidence of hemorrhagic syndrome in the course of intrauterine pneumonia. The survey involved 60 premature newborns born at the gestational age from 27 to 36 weeks. Hemostatic parameters were studied in all 60 children during 4 weeks of follow-up. The results are analyzed in children against the background of pneumonia and hemorrhagic syndrome. A tendency to hypercoagulable against a background of intrauterine infection was revealed. An increase in the frequency of development of hemorrhagic manifestations as complications of the course of the infectious process has been established.

**Keywords**: Premature newborn, hemostasis, hemorrhagic syndrome, pneumonia, fibrinolysis.