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ESTIMATION OF PROBIOTIC AND VITAMINE D EFFICIENCY IN PROPHYLACTICS OF INFECTIOUS DISEASES IN PREMATURE INFANTS

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Abstract

The objective: to study the efficacy of probiotic and vitamin D use in prophylaxis of infectious diseases in premature infants. Materials and methods. The reseach has been conducted on the base of the Odessa National Medical University – neonatal unit of the maternity hospital N 7 (Odessa). The I group consisted of 20 premature infants (PI) who got BioGiy drops. The II group conssted of 19 PI, who got BioGiy drops and vitamin D3. Control group- 23 PI who did not get the preparations mentioned above. At birth and at the age of 3-4 months complex immunological investigation was carried out. Every month the morbidity of PI was observed. Results. PI are more likely to suffer from infectious diseases, functional disorders of the gastrointestinal tract and hypovitaminosis D. The results obtained indicate a high effectiveness of probiotic with vitamin D3 use for the prevention of infectious diseases in PI. The greatest effect was observed in the prevention of skin infections and respiratory diseases of the upper respiratory tract as a reduction of respiratory episodes frequency, acceleration of recovery and lighter course of the disease. Also, the combination of probiotic and vitamin D has proven itself as a means of preventing hypovitaminosis D in PI.

Key words: immunity, premature infant, probiotic, vitamin D, infectious disease.

Introduction. Despite the progress of modern medicine and introduction of highly effective perinatal technologies, about 13 million premature infants (PI) are born in the world annually. They account for 60-70% of early neonatal and 65-75% of infant mortality. Perinatal mortality in PI is 33 times more frequent than in term infants [1, 2, 3]. Children born prematurely have higher incidence rates of cerebral palsy, attention deficit disorder, respiratory pathology, they often have learning problems compared to the timely born children.

The most dangerous complication in preterm pregnancy is postpartum infectious inflammatory processes in the mother, Infection of the fetus and newborn [4]. In connection with the immature defense mechanisms the potential risk of fetal infection is much higher than in mother. The incidence of infectious disease is higher the less gestational age of the fetus, which is determined by the relative immaturity of the mechanisms of antibacterial protection of the fetus and the undeveloped bacteriostatic properties of the amniotic fluid in preterm pregnancy [5].

Infectious diseases are an important cause of morbidity and mortality in PI, that many of them require intensive care and invasive procedures. A congenital immune system provides the first line of defense against infectious agents invading. The main cells that provide an adaptive immune response are neutrophils, monocytes, macrophages and dendritic cells. These cells develop and mature during the period of intrauterine life at different times.

The function of all components of innate immunity is weak in newborns compared with later stages of life. [6] The state of the immune system determines the survival of a newborn child, the peculiarity of the course of the neonatal period of life, the effectiveness of therapeutic and rehabilitation measures, as well as the degree of disability, as it is one of the most sensitive to any pathogenic factors and regulating the functioning of the homeostasis system in the development of pathological processes [7, 8].

Probiotics are living microorganisms and substances of microbial origin that have positive effects on the physiological, biochemical and immune responses of the host organism through the stabilization and optimization of the function of its normal microflora.

During the last 10 years, probiotics have been widely used in children in the treatment of infectious, allergic, autoimmune and gastroenterological diseases. Probiotics are widely used in pediatrics for the prevention of acute intestinal and respiratory infections [9]. Stimulation of the immune response by probiotics is manifested by an increase in phagocytic activity and an increase in IgA, T-killers, interferon concentrations, increased mucin production, which leads to a decrease in mucosal permeability [10, 11].

Primary microbial colonization of a newborn child begins in the process of birth and depends on many factors, with the determining role of the morther's microflora and the environment. It is known that the process of intestinal microflora formation can be disturbed by various factors: the mother's and child antibacterial therapy, prematurity, Caesarean section, artificial or parenteral nutrition, etc.

In the extremely PI there are additional unfavorable factors: immaturity of the intestine, insufficient exposure of the maternal microflora, delay of enteral nutrition, high risk of contamination with hospital microflora. The administration of probiotics to the risk group newborns in the early neonatal period can have a beneficial effect on the formation of the intestinal microflora. Currently, the effectiveness of various probiotics in preventing the development of neonatal diseases: necrotizing enterocolitis (NEC), sepsis, various forms of nosocomial infections has been proven [12].

Recently, more and more reports about the various clinical effects of vitamin D [13] have appeared. Experimental data indicate that vitamin D is involved in the antiviral response, especially with respect to enveloped viruses. In all likelihood, the virucidal activity of vitamin D is mediated by its ability to induce the expression of antimicrobial peptides – HBD-2 and catelicidin (LL-37) [14]. Based on the findings of the "case-control" study conducted in Turkey, it was concluded that in newborns, infants and children with subclinical form of vitamin D deficiency the risk of lower respiratory tract acute infections [15] increases.

There are studies that prove that vitamin D deficiency in young children is associated with an increased risk of developing urinary tract infections [16].

The objective: to study the effectiveness of vitamin Dc probiotic use in prevention of infectious diseases in PI.

Material and methods. The study was conducted in the neonatal department of the maternity hospital No 7 (Odessa, Ukraine) - the basis of the Odessa National medical university. 62 PI (gestational age 34-36 weeks) without perinatal pathological conditions were involved in the study. Their relatives got oral information about all the procedures of the study and gave informed consent for participation in it. They patients were randomized into 3 groups.

The first group consisted of 20 children who received BioGia drops - 5 drops (100 million of viable bacteria *Lactobacillus reuteri* DSM 17938) inside during the first 20 days of life.

The second group consisted of 19 newborns who received BioGia drops with vitamin D3 - 5 drops (100 million viable bacteria *Lactobacillus reuteri* DSM 17938 and vitamin D3

400 IU) inside during the first 20 days of life. In the first and second group after the instruction, the mother gave drops in the maternity hospital and at home independently.

The third (control) group was represented by 23 patients who did not receive the above mentioned drugs.

A complex immunological study was conducted in the first 3 days of the child's life by flow cytofluorimetry. To monitor the morbidity, telephone calls were made monthly, and at 3-4 months of age, a control visit and blood sampling for study in dynamics (with the mother's consent). In the first group, immunological study was conducted in dynamics in 14 patients, in the second group – 12, and in the third group - in 18 children. Also for comparison, a similar analysis of parameters of a complex immunological study was carried out in 15 healthy full-term children.

Statistical analysis of the data obtained was carried out using STATISTICA 10.0, MedCalc14.8.1, and MicrosoftExcel 2010 packages with the AtestStat 12.5 add-in of the SISA (Simple Interactive Statistical Analysis) ionline - calculator. Proportion (percent) are presented with 95% confidence intervals (CI). In all statistical analysis procedures, when testing null hypotheses, the critical level of significance *p* was taken to be 0.05. To evaluate the effectiveness of different therapy methods, an analysis of the ROC curves - area under the curve (AUC), odds ratio (OR), absolute (ARD) and relative risk (RRD) decrease, the number of patients who need to be treated for a certain time to achieve a positive result in one patient (CPPR) with the definition of 95% confidence intervals.

The results obtained and their discussion. According to the age parameters, the number of pregnancies and childbirths, there were no statistically significant differences between the groups. According to the gender, no statistically significant differences were observed among the children in the groups under study. The effectiveness of the drugs used was assessed by studying the morbidity in the groups and normalizing the immunogram indices.

The highest morbidity took place in the third (control) group, the incidence rates in the first and second groups did not differ significantly from each other (except for data on hypovitaminosis D, Table 1).

According to the results of the comparative analysis of the incidence, presented in Fig. 1, the most pronounced effect on the prevention of skin diseases (OR = 0.21, 95% CI 0.01-2.26), acute respiratory diseases (OR = 0.14, 95 % CI 0.01-1.46), decrease in the number of intestinal colic (OR = 0.21, 95% CI 0.05-0.97). At the same time, in the first group the chances of hypovitaminosis D increase (OR = 2.16, 95% CI 0.35-3.11) more than twice.

Table 1

Morbidity in the groups under study for 3-4 months of life

	Group 1, n=20		Group 2, n=19		Control, n=23	
	N	%, 95% CI	N	%, 95% CI	N	%, 95%CI
Skin infections	1	5.00 (-4.55-14.55)	1	5.26 (-4.78-14.79)	6	26.08 (8.07-43.92)
Intestinal infections	2	10.00 (-3.14-23.14)	2	10.52 (-3.06-25.06)	5	21.73 (5.07-38.92)
Acute respiratory diseases	1	5.00 (-4.55-14.55)	1	5.26 (-4.78-14.79)	7	30.43 (11.27-48.72)
Otitis media	0	0.00	0	0.00	1	4.34 (-4.00-12.00)
Skin allergic reactions	3	15.00 (-0.64-30.64)	2	10.52 (-3.06-25.06)	6	26.08 (8.07-43.92)
Intestinal colic	4	20.00 (2.46-37.53)	3	15.78 (-048-32.48)	14	60.86 (41.06-80.93)
Hypovitaminosis D	5	25.00 (6.02-43.97)	1	5.26 (-4.78-14.79)	6	26.08 (8.07-43.92)

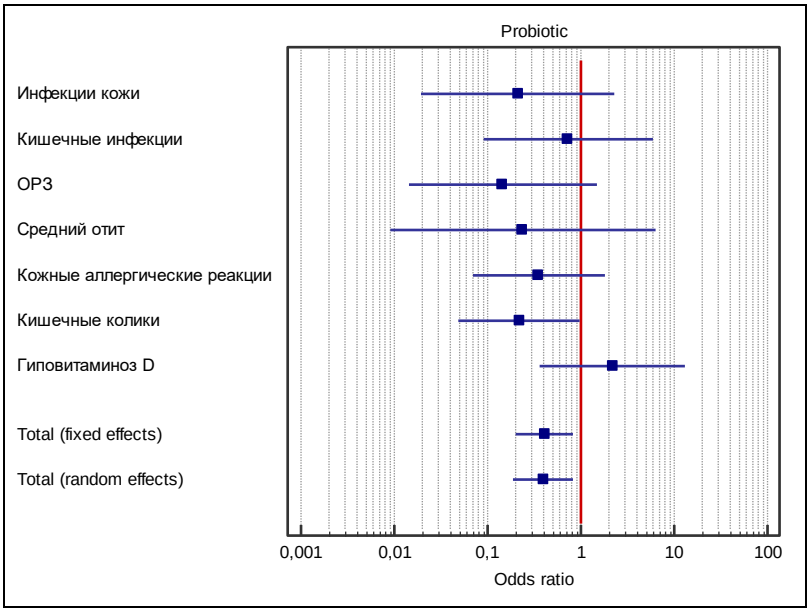

Fig. 1. Comparative analysis of morbidity in the first group of preterm infants who got probiotic in relation to the group of full-term newborns (odds ratio).

In the second group of PI who got probiotic with vitamin D, the incidence reduction rates, except for hypovitaminosis D (OR = 0.36, 95% CI 0.02-4.41), did not statistically significantly differ from those of the first group (Fig. 2). The chances of respiratory infections decreased 6.5 times (OR = 0.15, 95% CI 0.03-1.44), skin infections 4.5 times (OR = 0.22, 95% CI 0.02 -2.39).

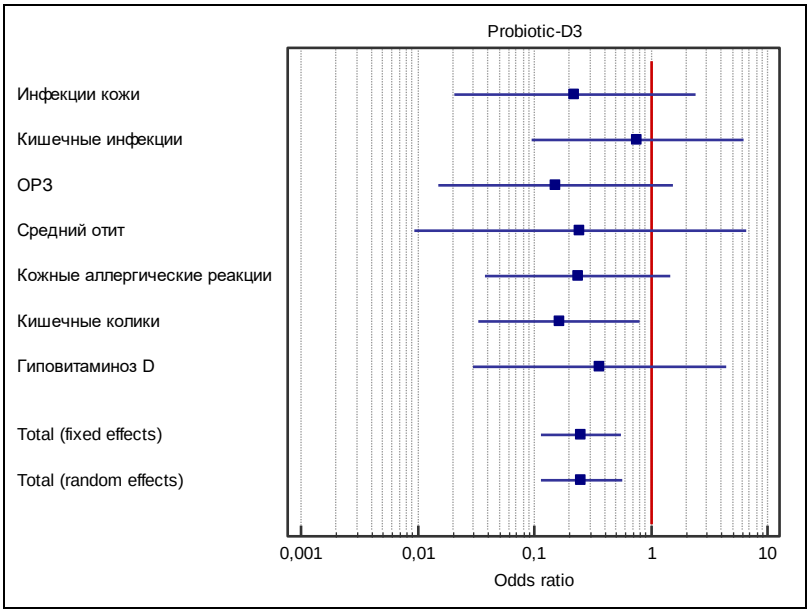

Fig. 2. Comparative analysis of morbidity in the first group of preterm infants who got probiotic with vitamin D, in relation to the group of full-term children (odds ratio)

In the third group, consisting of PI who did not get probiotic and vitamin D, increase in the level of infectious diseases in 1,2 - 1,8 times, hypovitaminosis D in 2,29 times (that corresponds to the literature data [17]) and intestinal colic in 1.36 times (Fig. 3) took place.

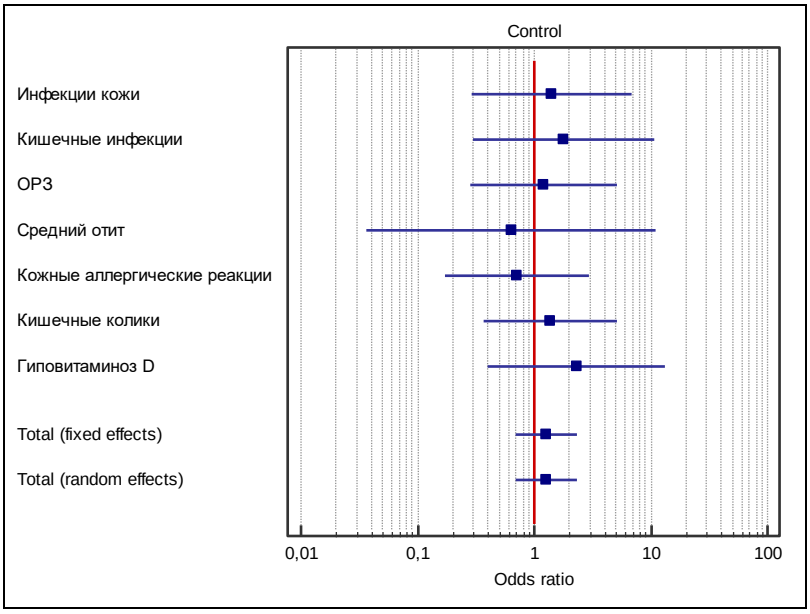

Fig. 3. Comparative analysis of morbidity in the control group of premature infants relative to the group of full-term newborns (odds ratio).

According to the results of PI immunity links studies, the greatest effectiveness of

probiotic in combination with vitamin D has been revealed. This combination was most effective in the degree of normalization of the level of B-lymphocytes (Table 2 and Fig. 4, 5), Ig A (Fig. 6, 7), phagocytic index, complement components and macrophages number.

Table 2

Estimation of probiotic and vitamin D effectiveness in the groups of premature infants by the results of B-lymphocytes level normalization

	First group, n=14	Second group, n=12	Third group, n=18
OR(95% CI)	0.04 (0.005-0.36)	0.01 (0.003-0.17)	0.26 (0.04-1.31)
ARD (95% CI)	0.63(0.37-0.83)	0.81 (0.41-0.94)	0.27(-0.05-0.57)
RRD(95% CI)	80(44-94)	88 (61-94)	60(-21-88)
CPPR(95% CI)	2(1-4)	1(1-2)	4(2-∞)
AUC(95% CI)	0.92(0.73-0.99)	0.99(0.85-1.00)	0.67(0.45-0.85)
Significance level *p*	0.001	0.000	0.12

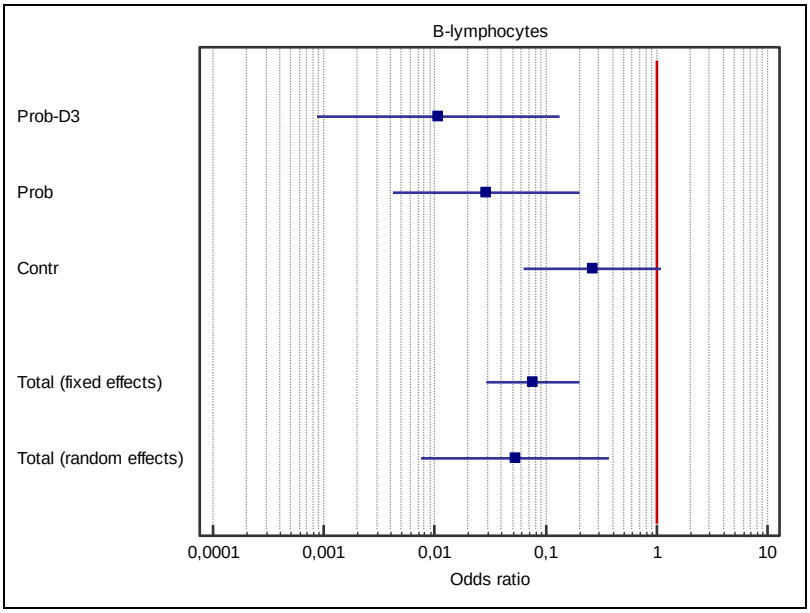

Fig. 4. Estimation of probiotic and vitamin D effectiveness in the groups of premature infants on the normalization of B-lymphocyte level (odds ratio)

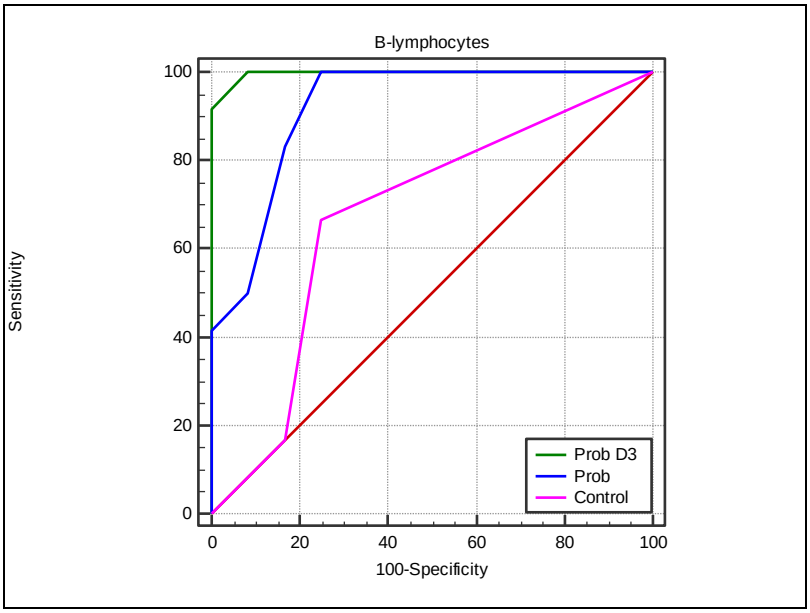

Fig. 5. Estimation of the effectiveness of the use of probiotic and vitamin D in the study groups of premature infants on the normalization of B-lymphocyte level (ROC-analysis in graphical expression)

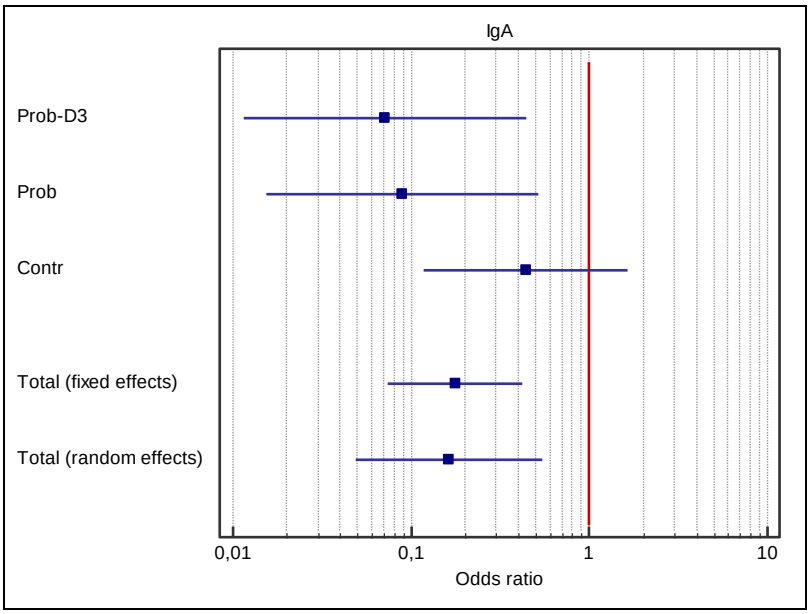

Fig. 6. Estimation of the effectiveness of the use of probiotic and vitamin D in the study groups of preterm infants for the normalization of IgA (odds ratio)

In the presented Figures 6 and 7, the maximum effectiveness of normalization of the IgA level is noted with the complex application of the probiotic and vitamin D (PPC = 0.96,

95% CI 0.79-0.99, OR = 0.07, 95% CI 0.007-0, 55), followed by a probiotic without vitamin D (AUC = 0.94, 95% CI 0.76-0.99, OR = 0.09, 95% CI 0.01-0.63). The lowest efficiency is presented in the control group (AUC = 0.66, 95% CI 0.44-0.84, OR = 0.44, 95% CI 0.09 - 1.97).

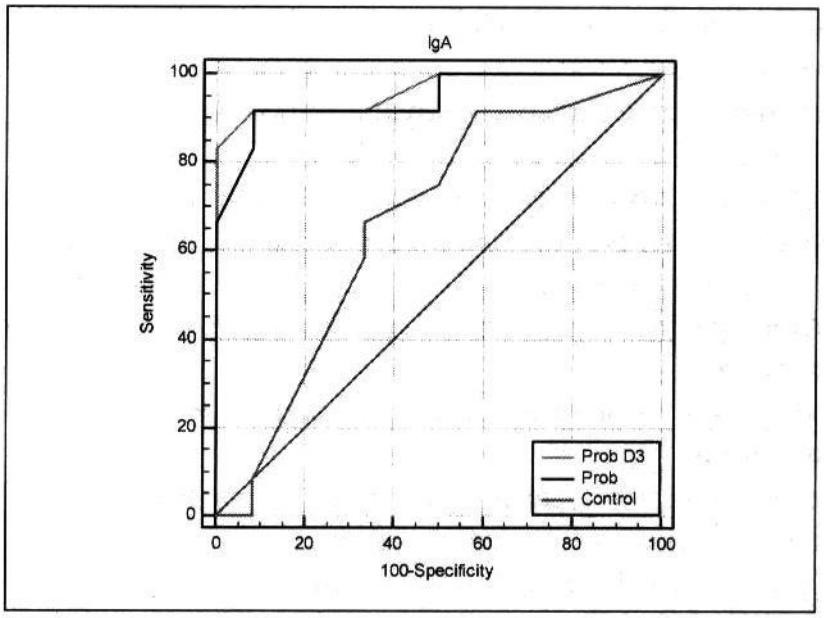

Fig. 7. Estimation of the effectiveness of the use of probiotic and vitamin D in the study groups of preterm infants for the normalization of IgA level (ROC analysis in graphical expression)

To the level of T-lymphocytes, neither the administration of a probiotic nor the combination of probiotic and vitamin D had any significant effect (Table 3, Fig. 8).

Table 3

Effectiveness of probiotic and vitamin D use in the groups of premature infants according to the results of T-lymphocytes levels normalization

	First group, n=14	Second group, n=12	Control group, n=18
OR (95% CI)	0.27 (0.04 -1.57)	0.22(0.02-1.60)	0.35(0.07-1.56)
ARD(95% CI)	0.29 (-0.1-0.54)	0.30(-01-0.53)	0.24(-0.1-0.52)
RRD(95% CI)	36(-19-58)	36 (-18-54)	33(-20-59)
PPR(95% CI)	3(2-∞)	3(2-∞)	4(2-∞)
AUC(95% CI)	0.69(0.47-0.86)	0.72(0.50-0.88)	0.65(0.433-0.83)
Significance level *p*	0.18	0.17	0.21

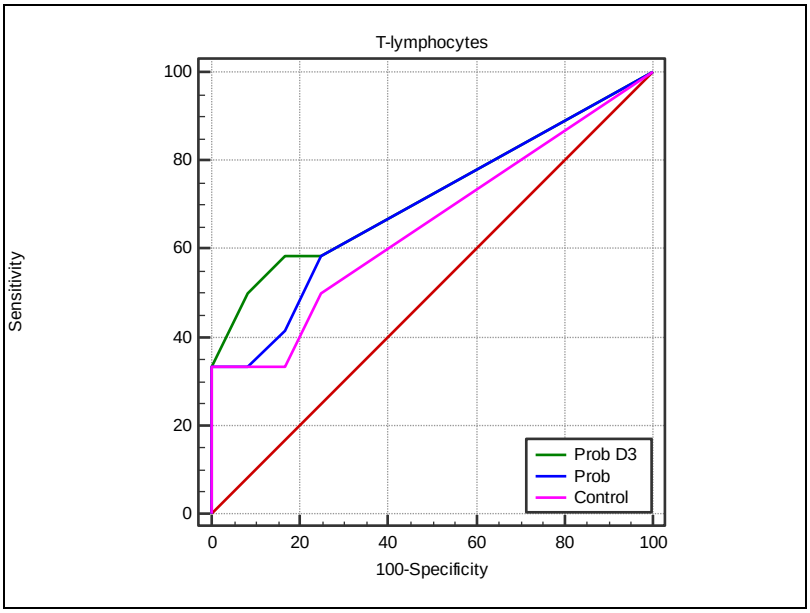

Fig. 8. Estimation of the effectiveness of probiotic and vitamin D use in the groups of premature infants for the normalization of B-lymphocyte level (ROC-analysis in graphical expression)

The discriminant analysis of parameters of a complex immunological investigation in PI groups (Fig. 9) revealed the absence of statistically significant differences in the indices of the first and second groups (p = 0.99). According to the results of the ROC analysis of similar indicators, the prevalence of positive results in the group of children who got probiotic with vitamin D.

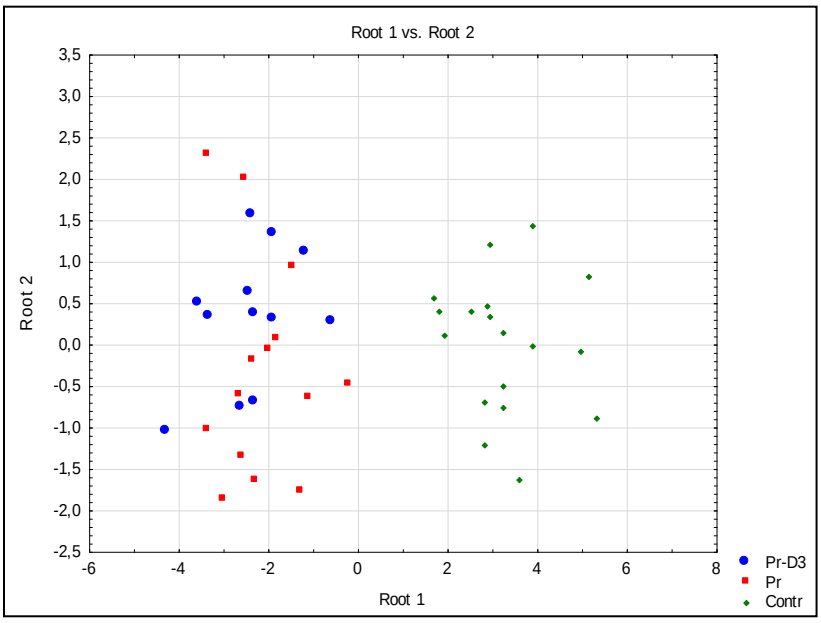

Fig. 9. The result of a discriminant analysis of the parameters of a complex immunological investigation in the groups of children under study in graphical expression.

Conclusions. Preterm infants are more likely to suffer from infectious diseases, functional disorders of the gastrointestinal tract and hypovitaminosis D. The results of the study indicate a high effectiveness of probiotic with vitamin D3 use for the prevention of infectious diseases in premature infants. The greatest effect was observed in the prevention of skin infections (OR = 0.22, 95% CI 0.02-2.39) and respiratory diseases of the upper respiratory tract (OR = 0.15, 95% CI 0.03-1.44) as a reduction of respiratory episodes frequency, acceleration of recovery and lighter course of the disease. Also, the combination of probiotic and vitamin D has proven itself as a means of preventing hypovitaminosis D in premature infants.

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