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INDEX OF CONDITIONALLY-PATHOGENIC MICROFLORA: FORECASTING THE RISK OF DYSBIOSIS DEVELOPMENT

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Abstract

Bacterial vaginosis is one of the most common types of infectious pathology of the reproductive organs of women mainly in reproductive age. The frequency of bacterial vaginosis over the past decade has doubled, ranging from 26% to 40-45%. Until now, the leading role in the emergence of bacterial vaginosis of obligate anaerobic bacteria has been proved, which is why it is regarded as a polymicrobial vaginal syndrome and is characterized not only by vaginal discharge, but also by damage to the cervix, the uterus, its appendages, childbirth. The etiological structure of pathogens of infectious processes in the last decade has changed significantly, which is due to the constant evolution of bacteria and the involvement of conventionally pathogenic microorganisms into pathological processes.

The aim of the study is to identify the most informative indicators that objectively reflect the condition of the pathological process and develop a system for predicting the risk of occurrence and severity of dysbiosis behind these indicators.

Materials and methods of research

Studies were conducted in 298 women aged 16 to 64 years. 53 of whom were diagnosed with normocenosis, and 245 had dysbiosis. Statistical processing of data was carried out using variational and correlation analysis methods using the Statistica v. Application software package. 10 (StatSoft, Inc.).

The results of the study and their discussion

At the first stage of the analysis, the index of conditionally pathogenic microflora was considered as a resultant trait. To identify factors that are more associated with the risk of developing dysbiosis with IPM, a selection of significant traits was performed using a genetic selection algorithm. At the second stage of the analysis, the prediction of the severity of dysbiosis with ICPM was considered.

Conclusions. The nine factor attributes obtained with the help of mathematical analysis allowed us to predict the severity of vaginal dysbiosis with high accuracy and to calculate the IPM indices. In addition, the phase of the development of the immune system reaction is shown in the development of vaginal dysbiosis - from the control state during normocoenosis to the development of immunorequency in dysbiosis of grade 1 and expressed combined immunodeficiency in the presence of specific humoral response to bacterial antigens in dysbiosis of the 2nd degree.

Key words: bacterial vaginosis, dysbiosis, conditionally pathogenic microflora

Introduction

Bacterial vaginosis is one of the most common types of infectious pathology of reproductive organs of women predominantly in reproductive age. Its share among all vulvovaginal infections of the lower section of the genital tract of women ranges from 12% to 80% [6]. The frequency of bacterial vaginosis in the last decade has doubled and is, according to various authors, from 26% to 40-45% [1, 2]. Bacterial vaginosis is the most common cause of unusual secretions from women's vagina of childbearing age, and it is found in 35% of women referred to skin and venereal dispensaries, in 15-20% of pregnant women, in 5-15% of women observed in gynecologists [4, 7].

To date, the leading role in the emergence of bacterial vaginosis of obligate anaerobic bacteria has been convincingly proven, in connection with which it is considered as a polymicrobial vaginal syndrome and is characterized not only by vaginal secretions but also by the lesion of the cervix, uterine body, and its appendages, is the cause pathology of pregnancy and childbirth [3].

The etiological structure of pathogens of infectious processes in the last decade has changed significantly, which is associated with the constant evolution of bacteria and the involvement of opportunistic microorganisms in the pathological processes. Clinicians often have difficulties in evaluating the results of the examination, determining the appropriateness of the prescribed treatment and the choice of medicinal products [5, 7, 8].

The purpose of the study is to identify the most informative indicators that objectively reflect the state of the pathological process and develop a prediction system based on these indicators of the risk of occurrence and severity of dysbiosis.

Materials and methods of research

The study was conducted in 298 women aged 16-64. In 53 of them the diagnosis of normocytosis was established, and in 245 - dysbiosis. Statistical processing of data was carried out by means of variational and correlation analyzes using a package of applications Statistica v. 10 (StatSoft, Inc.).

Results of the research and their discussion

At the first stage of the analysis as a resultant feature, the index of paranoid microflora (IPM) (variable Z) was considered, while in the case of normocenosis, the variable Z became Z = 0; in the case of a dysbiosis of the 1st or 2nd degree, the variable acquired Z = 1.

As factors in the initial analysis, 58 indicators were subjected (Table 1).

To check the prediction quality of the model, all observations (using the random number generator) were divided into three sets: educational (used to calculate the model parameters, 248 cases), control (used to control the re-evaluation of the model, 20 cases), confirmatory (used to verify adequacy models in prediction of new data, 30 cases).

In a complete set of 58 factors, a linear neural network model was constructed and trained. Sensitivity and specificity on the training and confirmation sets did not differ statistically significantly ($p = 0.20$ and $p > 0.99$, respectively, when compared with the criterion χ^2), which indicated the adequacy of the constructed model.

To identify the factors most associated with the risk of development of dysbiosis for IPM, a selection of significant features was performed using a genetic selection algorithm. As a result, four factors were selected: the content in the vaginal secretion of IL10 (X20), as well as the level of blood IL2 (X47), IL4 (X48) and IL6 (X49).

A linear neural network model was constructed and trained on a selected set of four factor characteristics. Sensitivity and specificity on training and confirmation sets did not differ statistically significantly ($p = 0.17$ and $p = 0.58$, respectively, when compared with the criterion χ^2), which indicated the adequacy of the model.

Table 1

Inbound signs of primary analysis of indicators of colonial resistance of the vagina, the immune system and the system of hormonal

X1	**Age**	X20	**IL10**	X39	**CD22**
X2	**MC day**	X21	**TNFα**	X40	**PhAL**
Parameters VS:		X22	**TGF-1β**	X41	**IPhAL**
X3	**IgM**	X23	**pH**	X42	**CIC**
X4	**IgA**	**Blood parameters:**		X33	**C3**
X5	**IgG**	X24	**FSH**	X44	**C4**
X6	**IgG_2**	X25	**LH**	X45	**γ-INF**
X7	**sIgA**	X26	**E_2**	X46	**IL1β**
X8	**Lysozyme**	X27	**PG**	X47	**IL2**
X9	**PhAL**	X28	**TS**	X48	**IL4**
X10	**IPhAL**	X29	**Kr**	X49	**IL6**
X11	**IK**	X30	**PRL**	X50	**IL8**
X12	**C3**	X31	**T_3 fr.**	X51	**IL10**
X13	**C4,**	X32	**T_4 fr.**	X52	**TNFα**
X14	**γ-INF**	X33	**Lc**	X53	**TGF-1β**
X15	**IL1β**	X34	**CD16**	X54	**IgM**
X16	**IL2**	X35	**CD3**	X55	**IgA**
X17	**IL4**	X36	**CD4**	X56	**IgG**
X18	**IL6**	X37	**CD8**	X57	**IgG_2**
X19	**IL8**	X38	**IPI**	X58	**sIgA**

Notes: **MC** – Menstrual cycle; **VS** – vaginal secret; **PhAL** - phagocytic activity of leukocytes; **IPhAL** – index PhAL; **T_3 fr**. – free T_3; **T_4 fr.** – free T_4; **Lc** – lymphocytes; **CIC** - circulating immune complexes; **FSH**– follicle stimulating hormone; **LH** - luteotropic hormone; **E_2**– estradiol; **PG** - progesterone; **TS** – testosterone; **Kr** – cortisol; **PRL** - prolactin

To identify the strength and direction of the influence of the four distinguished factor characteristics, a logistic regression model was constructed which proved to be adequate (χ^2 = 234.9 at p <0.001). The results of the analysis of the coefficients are given in Table 2.

From the analysis of the coefficients of the logistic regression model, it was concluded that the risk of dysbiosis for IPM was statistically significant (p = 0.002) decreasing with elevation of the level of vaginal secretion of IL10 (OR = 0.54; 95% PI 0.36-0.80) per unit (pg / ml). There was also a decrease (p = 0.019) of the risk of developing dysbiosis for IPM with

an increase in blood levels of IL4 (OR = 0.58; 95% PI 0.37-0.91) per unit (pg / ml).

Table 2

The coefficients of the four-factor model of risk prediction of dysbiosis for IPM (logistic regression model)

Factor sign	The value of the coefficients of the forecasting model, b±m	The level of significance is different from 0	OR (95% PI OR)
X20	-0,62±0,20	0,002*	0,54 (0,36-0,80)
X47	0,19±0,17	0,171	–
X48	-0,55±0,23	0,019*	0,58 (0,37-0,91)
X49	0,27±0,20	0,200	–

Notes: OR – odds ratio; PI – probable interval

IPM is a key indicator that allows the objective distribution of patients to groups, that is, to establish a diagnosis of normocenosis, dysbiosis of the 1st or 2nd degree. Consequently, the risk of developing IPM dysbiosis reflects the contribution of significant factor factors to the magnitude of the indicator. The construction of a nonlinear neural network model for predicting the risk of developing dysbiosis has shown that IpM can be calculated based on the content of IL10 (pg / ml) in the vaginal secretion, as well as the level of blood (IL / IL, IL4 and IL6) in blood (pg / ml). Consequently, the components of the "interleukin cascade" included prophylactic IL2 and IL6 and anti-inflammatory drugs IL4 and IL10 as significant factors. It was found that levels in the vaginal secretion and blood levels of all interleukins had similar dynamics - an increase for pro-and a decrease for proinflammatory drugs, which depended directly on the degree of severity of dysbiosis and was expressed to the maximum extent in BV.

These results substantiated the assumption of the pathogenetic role of dysregulation of hormonal and immune systems in the event of dysbiosis and BV. The level of significance of the difference from the 0 coefficients of the logistic regression model for the factor characteristics of X47 (blood level of IL2) and X49 (blood level of IL6) were statistically insignificant (respectively, p = 0.171 and 0.200). It was these indicators that had positive values of the coefficients of the forecasting model, that is, increased the value of the IPM. Coefficients of factor X20 (content in the vaginal secretion of IL10) and X48 (content in blood IL4) had negative signs, that is, their high values contributed to the reduction of IPM. With the progression of dysbiosis, these indicators were significantly reduced - respectively,

4.1 times and 5.5 times ($p < 0.001$). Inclusion of these indicators reflected the formation of immunodeficiency, both at the local and at the system level.

The second stage of the analysis considered the prediction of the severity of the dysbiosis for IPM.

To check the prediction quality of the model, all observations (using the random number generator) were divided into three sets: educational (used to calculate the model parameters, 248 cases), control (used to control the model overview, 20 cases), confirmatory (used to verify adequacy models for prediction of new data, 30 cases).

On a complete set of 58 factor characteristics, a linear neural network model was built and trained. The Cohen Kappa agreement for this model on the training set was kappa = 1.00 (95% BA 0.99-1.00), on the confirmation set kappa = 0.95 (95% BI 0.86-1.00), which testified to the adequacy of the constructed model.

To identify the factors most related to the value of IPM, a selection of significant features was carried out using the method of genetic selection algorithm. As a result, six factors were selected: the content in the vaginal secretion of sIgA (X7), lysozyme (X8), γ-INF (X14), TGF-1β (X22), and the blood component of the complement of C4 (X44) and IL8 (X50) .

On a selected set of six factor characteristics, a linear neural network model was built and trained. The Cohen Kappa agreement for this model on the training set was kappa = 0.99 (95% VI 0.97-0.91), in the confirmatory set kappa = 0.95 (95% BI 0.86-1.00), which testified to the adequacy of the constructed model.

Thus, the prediction model for the severity of the dysbiosis for IPM, which was built on six factors, gives "very good" (kappa > 0.81, according to the scale) agreement, indicating the high significance of the selected factor factors for predicting the severity of dysbiosis for IPM.

In normocenosis, negative symbols of the coefficients (i.e., lowered by IPM) contained contents in the vaginal secretion of lysozyme and TGF-1β and the blood component of the complement of C4 and IL8. Thus, among the effector factors supporting the normocenosis in the vaginal secretion, in addition to the complement (C4), as was shown for the norm of the normobioti, lysozyme could be added, and the complement (C4) in the blood was activated.

Thus, it can finally be argued that the factors of colonial resistance of the vagina, such as complement (C4) and lysozyme, corresponded to the normocenosis's support.

The level in the vaginal secretion of sIgA had a positive sign of the coefficient, that is, it increased in parallel with the growth of IPM, indicating the presence of this immune

response, but - and the lack of effectiveness of this factor in terms of inhibiting the growth of opportunistic microflora. Such a state of the immune system under normocenosis can be characterized as controlled in relation to the development of vaginal dysbiosis.

According to the IPM, there were also factors that counteract the growth of the conditionally pathogenic microflora. These were levels in the vaginal secretion of sIgA, γ-INF and TGF-1β. These indicators, in our opinion, reflected the development of secondary immune reactions, which unfolded later - with dysbiosis II degree, and were caused by an increase in antigenic loading.

Group	**Sign coeff.**	**IPM**	
		VS	**Blood**
Normocenosis	+	sIgA (X7) γ-INF (X14)	-
	-	**Lysozyme (X8)** TGF-1β (X22)	**C4 (X44)** IL8 (X50)
Dysbiosis of the 1st degree	+	Lysozyme (X8)	C4 (X44) IL8 (X50)
	-	**sIgA (X7)** γ-INF (X14) TGF-1β (X22)	-
Dysbiosis of the 2 st degree	+	TGF-1β (X22)	IL8 (X50)
	-	**sIgA (X7)** **Lysozyme (X8)** γ-INF (X14)	**C4 (X44)**

Fig 1. The role of significant factors in the colonial resistance of the vagina and the immune system in determining the severity of dysbiosis (for IPM); VS – vaginal secret; «+» – positive sign of the coefficient in the linear neural network model, «-» – negative sign; bold features effector factors limiting the activation of opportunistic microflora

Conclusions Thus, the nine factor factors obtained by means of a mathematical analysis allowed a high degree of accuracy to predict the degree of severity of vaginal dysbiosis and to calculate IPM indices. In addition, the phasicity of the development of the reaction of the immune system in the development of vaginal dysbiosis is shown from the state of control in normocenosis to the development of immunoreactivity at dysbiosis of the I degree and the expressed combined immunodeficiency in the presence of reactions of a specific humoral response to bacterial antigens at dysbiosis II degree.

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