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HUMORAL IMMUNITY INDICATORS AS PREDICTION FACTORS FOR DYSBIOSIS DEVELOPMENT RISK

O. A. Hryzevskyi[1], V. V. Minukhin[2], S. M. Kobylnik[1], O. F. Dzygal[1], K. S. Shumilina[1]

[1]Odesa National Medical University, Odesa, Ukraine
[2]I.I. Mechnikov Institute of Microbiology and Immunology of National Academy of Medical Sciences of Ukraine, Kharkov, Ukraine

Abstract

Diseases associated with bacterial vaginosis lead to chronic inflammatory processes of the internal genitals, the development of adhesions of the pelvic organs, infertility, spontaneous abortion at different times, as well as the development of malignant neoplasms. Vaginal microflora is an indicator of a woman's health which can form changing in hormonal and immunological status during various pathological conditions. The aim of the study was to create a system for prediction of the dysbiosis development according to the levels of nonspecific humoral factor of immune defence.

The study was performed in 298 women aged 16 to 64 years, 53 of whom were diagnosed with normocenosis, and 245 have dysbiosis. Women were divided into 3 groups according to age. Regression analysis was used.

Our previous researches have shown a correlation between increased levels of anti-

inflammatory cytokines in the blood and vaginal secretions with the stage of dysbiosis. A logistic regression model was constructed during the study, which showed that the risk of developing dysbiosis in terms of normobiota increases with increasing levels of interleukin 2 in the blood, tumor necrosis factor α. Significant features of the three-factor model for predicting the risk of developing dysbiosis (IL2, IL4 and TNFα) were selected by the method of genetic algorithm. The levels of these indicators in the blood were related to the severity of dysbiosis according to the results of discriminant analysis. Thus, a linear neural network model was developed for determination of dysbiosis severity according to the levels of nonspecific humoral factors of immune defence such as the C4 component of the complement system and γ-interferon in vaginal secretions, as well as the amount of circulating immune complexes and tumor necrosis factor α in the blood. Kappa Cohen's agreement for this model on the training set was 0.87 (95% CI 0.82-0.91), and on the confirmatory set was 0.89 (95% CI 0.77-1.00). These indicators show the adequacy of the constructed model. The interface of the expert system for the dysbiosis severity prediction has been created.

Keywords: normobiota; bacterial vaginosis; linear neural network model for determination of the dysbiosis severity; humoral factor of immune defence

Introduction. Diseases of the genitourinary organs caused by pathogenic and opportunistic pathogenic microorganisms, which to some extent are associated with dysbiosis of these organs, remain an urgent problem of modern dermatovenerology, gynecology and urology [1, 2, 3]. Diseases caused by opportunistic pathogenic microorganisms are characterized by extremely high prevalence, the possibility of severe complications, especially those that affect the reproductive function, as well as resistance to treatment [4]. In addition, diagnostics of such infections is associated with certain difficulties. Diseases associated with bacterial vaginosis (BV) lead to chronic inflammatory processes of the internal genitals, the development of adhesions of the pelvic organs, infertility, spontaneous abortion at different times, as well as the development of malignant neoplasms [5, 6]. However, the cultural study of vaginal secretions with quantitative assessment of the main indicators of microbiocenosis has not been widely used due to its high cost, laboriousness, as well as lack of a single methodical approach. In most cases, diagnostics and treatment are based on the detection of the main pathogen without taking into account quantitative criteria; there is no microbiological control of the treatment efficiency, degree of disruption of the normal microflora and terms of its recovery [7, 2]. Vaginal microflora is an indicator of a

woman's health, which can form changing in hormonal and immunological status during various pathological conditions. The normal microflora of the vagina is divided into obligate (resident, indigenous), facultative and transitory [8, 9].

The aim of the work is to create a system for prediction of the dysbiosis development based on the normbiota indicator.

Materials and methods. The study was performed in 298 women aged 16 to 64 years, 53 of whom were diagnosed with normocenosis, and 245 had dysbiosis. Women were divided into 3 groups according to age. Regression analysis was used, i.e. detecting of influence of one or more independent (factor) variables on the dependent (outcome) variable.

Results

Our previous studies showed a correlation between an increase in the level of anti-inflammatory cytokines in blood and vaginal secretions and the stage of dysbiosis development [10]. In the course of this study, a logistic regression model was built, which showed that the risk of development of dysbiosis based on the normbiota indicator (NBI) statistically significantly (p=0,002) increases with an increase in the level of IL2 in blood (OR=1,22; 95% CI 1,08 -1,39) by each measurement unit (ng/ml). It was also established, that an increase of content of TNFα (OR=1.11; 95% CI 1,04-1,18) in blood for each unit of measurement (ng/ml) causes an increase (p=0,001) in the risk of development of dysbiosisbased on the NBI.

For determination of dysbiosis severity according to NB indicator, a linear neural network model was developed; it includes C4 component of the complement system and γ-interferon (γ-INF) in vaginal secretions, as well as the amount of circulating immune complexes (CIC) and tumor necrosis factor α (TNFα) in blood. Kappa Cohen's indicator for this model on the training set was 0,87 (95% CI 0,82-0,91), and on the confirmatory set was 0.89 (95% CI 0,77-1,00). These indicators show the adequacy of the constructed model. Thus, the interface of the expert system for the prediction of dysbiosis severity based on the NBI has been created.

At the first stage of the analysis, NBI (variable Y) was considered as the resulting characteristic; in case of normocenosis, Y acquired the value Y=0, in the case of dysbiosis of the 1st or 2nd degree, Y acquired the value Y=1.

Results of the examination of 298 patients was analyzed in such a way: 53 patients were diagnosed with normocenosis, and 245 – with dysbiosis.

58 indicators were used as factor characteristics during the primary analysis (Table 1).

Table 1

Input characteristics of the primary analysis of indicators of vaginal colonial resistance, immune system and hormonal regulation system

X1	Age	X20	IL10	X39	CD22
X2	MC day	X21	TNFα	X40	LPA
VS indicators:		X22	TGF-1β	X41	LPA in.
X3	IgM	X23	pH	X42	CIR
X4	IgA	Blood indicators:		X33	C3
X5	IgG	X24	FSH	X44	C4
X6	IgG$_2$	X25	LT	X45	γ-INF
X7	sIgA	X26	E$_2$	X46	IL1β
X8	Lysozyme	X27	PG	X47	IL2
X9	LPA	X28	TS	X48	IL4
X10	LPA in.	X29	CR	X49	IL6
X11	IC	X30	PRL	X50	IL8
X12	C3	X31	free T$_3$	X51	IL10
X13	C4,	X32	free T$_4$	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	IRI	X58	sIgA

Notes: MC – menstrual cycle; VS– vaginal secretion; LPA in.–Leucocyte Phagocytic Activity index; T$_3$ free– free T$_3$; T$_4$ free– free T$_4$; LC– lymphocytes; IRI – immune reactivity index; PRL – prolactin; CR – cortisol; TS– testosterone; FSH – follicle-stimulating hormone

To test the quality of prediction, all observations (using a random number generator) were divided into three sets: training (used to calculate model parameters, 248 cases), control (used to control model retraining, 20 cases), and validation set (used to test the adequacy of the model when making predictions on new data, 30 cases).

A linear neural network model was built and trained on the full set of 58 factor features. Sensitivity of the model built on the full set of factor features on the training set was

99.4% (95% CI 97,6%-100%), specificity – 100% (95% CI 97,7%-100%); on the confirmatory multiple sensitivity of the model was 100% (95% CI 88.8%-100%), specificity – 100% (95% CI 87.3%-100%). As we can see, sensitivity and specificity of the model on the training and confirmation sets did not differ with statistical significance (p=0,15 and p>0,99, respectively, when compared according to the χ^2 criterion), which indicates the adequacy of the model.

To identify the factors most associated with the risk of dysbiosisbased on the NBI, significant features were selected using the genetic algorithm method. As a result, three factors were selected: levels of IL2 (X47), IL4 (X48) and TNFα (X52) in blood.

A linear neural network model was built and trained on the selected set of factor features. On the training set, sensitivity of the linear neural network model built on three factor features was 80.5% (95% CI 74,1%-86,2%), specificity – 82,1% (95% CI 73,1%-89, 6%); on the validation set, sensitivity of the model was 81,3% (95% CI 57,1%-96,7%), specificity – 92,9% (95% CI 71,9%-100%). Sensitivity and specificity on the training and validation sets did not differ with statistical significance (p=0.80 and p=0.54, respectively, when compared by $\chi2$ criterion), which testified to the adequacy of the model.

To identify possible non-linear relationships of factor features associated with the risk of development of dysbiosis based on the NBI, a nonlinear neural network model (of multilayer perceptron type) was also built on a selected set of features. The architecture of the model is presented in Figure 1.

After optimizing the model acceptance/rejection threshold, the following was received: on the training set, sensitivity of this model was 100% (95% CI 98.8%-100%), specificity – 69.0% (95% CI 58.6%-78, 6%); on the validation set, the sensitivity of the model was 100% (95% CI 88.8%-100%), specificity – 85.7% (95% CI 60.8%-99.0%).
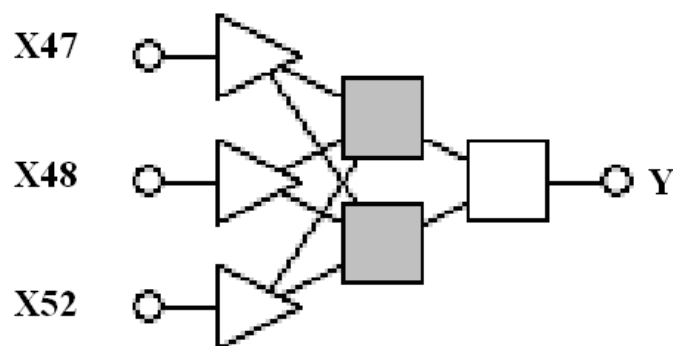

Fig. 1. Architecture of nonlinear neural network model for predicting the risk of development of dysbiosis based on the NBI (triangles indicate neurons of the input layer, gray squares – neurons of the hidden layer, white square – a neuron of the output layer)

Thus, sensitivity and specificity of the model both on training and validation sets did not differ significantly (p>0,99 and p=0,34, respectively, when compared by χ2 criterion), which testified to the adequacy of the model built.

For prognostic characteristics of the models, the method of Receiver Operating Characteristic Curve (ROC curves) was used (Fig. 2).
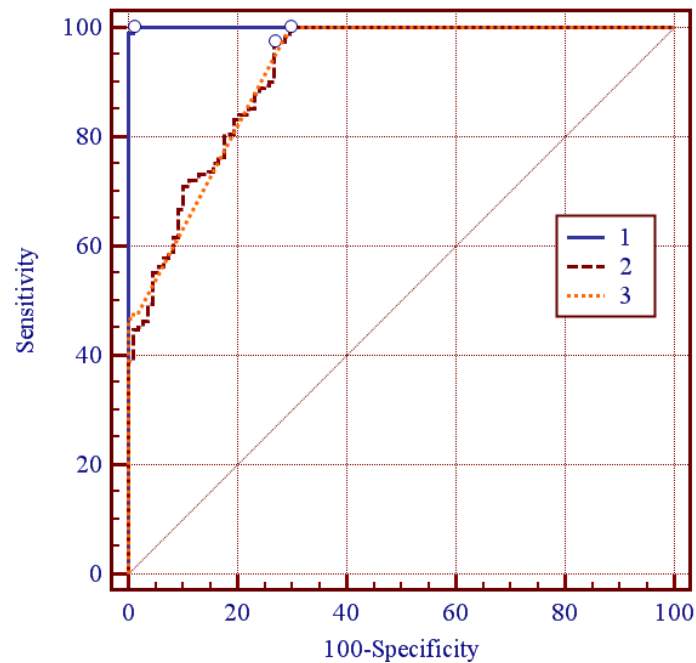

Fig. 2. ROC-curves for models for predicting the risk of development of dysbiosis based on the NBI; 1 – a model built on all 58 factor features; 2 – a linear neural network model built on three selected factor features, 3 – a non-linear neural network model built on three factor features.

In course of analysis, the area under the ROC-curve of the linear neural network model built on all 58 factor features, $AUC_1=1,00$ (95% CI 0,99-1,00), differed with statistical significance (p<0.001) from 0.5. The area under the ROC-curve of the linear neural network model built on three selected factor features, $AUC_2=0,92$ (95% CI 0,88-0,95), differed with statistical significance (p<0,001) from 0,5. The area under the ROC-curve of the nonlinear neural network model built on three selected factor features, $AUC_3=0,92$ (95% CI 0,88-0,95), differed with statistical significance (p<0,001) from 0,5.

To identify the strength and direction of influence of the three selected factor features a logistic regression model was built; the model turned out to be adequate ($\chi^2=221,4$ when p<0,001). Results of the coefficient analysis are shown in Table 2.

Table 2

Coefficients of the three-factor model for predicting the risk of development ofdysbiosis based on the normbiota indicator (logistic regression model)

Factor sign	Values of coefficients of the prediction model, b±m	Level of significance of differences from 0	OR (95% CI OR)
X47	0,20±0,07	0,002*	1,22 (1,08-1,39)
X48	−0,31±0,17	0,070	–
X52	0,10±0,03	0,001*	1,11 (1,04-1,18)

Notes: OR – odds ratio; CI – confidence interval

From the analysis of coefficients of the logistic regression model it turns out that the risk of development of dysbiosis based on the NBI increases with statistical significance (p=0,002) with an increase of IL2 level in blood (OR=1,22; 95% CI 1,08-1,39) for each unit measurement (ng/ml). An increase (p=0.001) in the risk of development of dysbiosis based on the NBI was also established with an increase TNFα level in blood (OR=1,11; 95% CI 1,04-1,18) for each unit of measurement (ng/ml).

Therefore, the risk of development of dysbiosis based on the NBI may be calculated in nonlinear neural network model for predicting the risk of development of dysbiosis based on levels (pg/ml) of IL2, IL4 and TNFα in blood. Previously, these parameters were discussed when analyzing the condition of the immune system in dysbiosis [10]. It was established that the level of all the pro-inflammatory cytokines in blood, which include IL2 and TNFα, increased along with the deepening of the degree of dysbiosis, and reached a maximum in BV. That is, IL2 and TNFα indicators in dysbiosis increased by 3.6 times in comparison with growth by 3.0 times in normocenosis (p<0,001). It is interesting that the participants of the logistic model took the last places in the ranking of increase in the level of pro-inflammatory cytokines in blood: IL1β > IL6 > IL8 > TNFα > IL2. At the same time, it was shown that activation of the "interleukin cascade" had both systemic and local nature, and the systemic one (in terms of increase in levels) turned out to be 1,5-2 times more significant.

Level of IL4 in blood in subgroups decreased according to the dysbiosis degree, which was expressed to the maximum extent in BV (by 5,5 times). In general, the level of anti-inflammatory cytokines, as opposed to pro-inflammatory ones, during the development of BV decreased sharply, and not only in blood, but also in the vaginal secretion. This may be the reason why the level of significance of differences from 0 of the coefficient of the logistic regression model for the factor X48 (IL4) turned out to be statistically insignificant (p=0,07).

According to the results of the discriminant analysis, levels of IL2, IL4 and TNFα in blood were related to the distribution into groups, that is, to the degree of severity of dysbiosis (F=10,7; F=23,6 and F=14,2; p<0,001, respectively).

Discussion

All these facts established the existence of a significant relationship between the participants of the three-factor model for predicting the risk of development of dysbiosis based on the NBI (IL2, IL4 and TNFα). But almost all these properties were to certain degree inherent in other indicators of the immune system during the development of vaginal dysbiosis. Moreover, it is obvious to expect the involvement of effector factors of vaginal colonial resistance that bind or destroy bacterial antigens, such as lysozyme, components of the complement system, sIgA, FAL, CD8, CD16, and so on, as prognostic indicators.

From our point of view, this situation is explained by the formation of a pathological hormonal and immune system, which is activated when the vaginal dysbiosis progresses and which supports its further development. That is why the indicators of the "interleukin cascade", which objectively reflect the presence of regulatory disorders, come to the fore, and BV, accordingly, can be considered a disregulatory pathology. It is the fault of hormonal and immune regulation that determines the progression of dysbiosis and its transition to BV.

Conclusions. Thus, a linear neural network model was developed and built to determine the degree of severity of dysbiosis based on the normobiota indicator, which included the levels of complement component C4 and γ-interferon in vaginal secretions, as well as the amount of circulating immune complexes (CIC) and tumor necrosis factor α (TNFα) in blood. Kappa Cohen's index for this model on the training set was 0.87 (95% CI 0,82-0,91), and on the validation set – 0,89 (95% CI 0,77-1,00). Also, the interface of the expert system for the prediction of dysbiosis severity based on the NBI has been created.

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