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Model for predicting the vaginal dysbiosis' severity according to the index of conditionally pathogenic microflora

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Abstract

A model for predicting the severity of dysbiosis according to the index of opportunistic pathogenic microflora has been developed. *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. Statistical processing of data was carried out using variational and correlation analysis methods using the Application software package Statistica v.10 (StatSoft, Inc.). 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 IOPM, a selection of significant traits was performed using a genetic selection algorithm. The prediction of the severity of dysbiosis with IOPM was considered. The nine factor attributes obtained with the help of mathematical analysis allowed to predict the severity of vaginal dysbiosis with high accuracy and to calculate the IOPM

indices. Phasal nature of development of the immune system reaction during the development of vaginal dysbiosis is revealed. Possibility of practical use of the developed model is shown.

Key words: index of opportunistic pathogenic microflora; normobiota; prediction model; dysbiosis.

Symbiotic microflora plays an important role in maintaining the colonization resistance of the vaginal biotope – a complex, multicomponent and equilibrium-dynamic mechanism that ensures the stability of the population-quantitative composition of components of the normal biocenosis [7, 16, 18, 21]. Modern ideas about the composition of vaginal biota are based primarily on the results of microscopic and microbiological examination of vaginal secretions, which have known drawbacks that limit the objectivity and reliability of data on all the participants of the biocenosis [1, 2, 3, 15]. In this regard, in recent years, identification of microorganisms is based on molecular-genetic analysis of differences in the structure of their genome [4]. A full-fledged multi-factor quantitative analysis of the structure of the microbiota of the urogenital tract became possible only after development and implementation into clinical practice of the real time PCR method [19].

Due to the great capabilities of molecular-genetic analysis, the problem of determining the qualitative and quantitative characteristics being part of the concept of norm, in relation to all the participants in the vaginal biota, is not of the least importance [9, 13]. In addition, the question of influence of age and obstetric and gynecological history on the characteristics of normocenosis of the vagina and the severity of dysbiosis, as well as on the method of forming a group of clinically healthy patients (comparison group) in clinical, social and other scientific studies, still stands [17, 20].

The aim of the work was to develop a model for predicting the severity of dysbiosis according to the index of opportunistic pathogenic microflora (IOPM).

Materials and methods. Results of examination of 298 patients were analyzed: 53 of the patients were diagnosed with normocenosis, 128 had grade I dysbiosis and 117 had grade II dysbiosis [6-7, 10-14].

58 indicators were chosen as factor features (Table 1). [5-7, 10-12]

To check the quality of the prediction model, all observations (using a random number generator) were divided into three sets: training (used to calculate the model parameters, 248 cases), control (used to control model retraining, 20 cases), confirmatory (used to check adequacy of the models when predicting on the new data, 30 cases).

Table 1

Input signs of the primary analysis of indicators of colonization resistance of vagina, immune system and system of hormonal regulation

X1	Age	X20	IL10	X39	CD22
X2	MC day	X21	TNFα	X40	LPA
VS indicators:		X22	TGF-1β	X41	LPA index
X3	IgM	X23	pH	X42	CIC
X4	IgA	Blood indicators:		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	LPA	X28	TS	X48	IL4
X10	LPA index	X29	CR	X49	IL6
X11	CIC	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.–LPA index; T3 free– free T3; T4 free– free T4; LC– lymphocytes; IRI – immune reactivity index; PRL – prolactin; CR – cortisol; TS– testosterone; LH – luteotropic hormone; FSH – follicle-stimulating hormone; CIC- circulating immune complexes; PG-progesterone;

A linear neural network model was constructed and trained on a complete set of 58 factor features. Cohen's Kappa agreement rate for this model was k = 1.00 (95% CI 0.99-1.00) on the training set, and k = 0.95 (95% CI 0.86-1.00)on the confirmatory set, which indicated the adequacy of the created model.

To identify the factors most associated with risk the development of dysbiosis according to IOPM, significant traits were selected using a genetic selection algorithm. As a result, six factor traits were selected: content of sIgA (X7), lysozyme (X8), γ-INF (X14),

TGF-1β (X22) in the vaginal secretionand content of C4 (X44) and IL8 (X50) components in blood.

Results and discussion.A linear neural network model was constructed and trained on a selected set of six factor features [8]. Cohen's Kappa coefficient for this model was k = 0.99 (95% CI 0.97-0.91) on the training set, and k = 0.95 (95% CI 0.86-1.00)on the confirmatory set, which indicated the adequacy of the constructed model.

The constructed model can be expressed by a system of equations:

$$\begin{cases} V0 = 0{,}003 \cdot X7 - 0{,}063 \cdot X8 + 0{,}136 \cdot X14 - 0{,}005 \cdot X22 - 0{,}593 \cdot X44 - \\ \qquad - 0{,}021 \cdot X50 + 1{,}360 \\ V1 = -0{,}001 \cdot X7 + 0{,}067 \cdot X8 - 0{,}112 \cdot X14 - 0{,}003 \cdot X22 + 0{,}936 \cdot X44 + \\ \qquad + 0{,}012 \cdot X50 - 0{,}455 \\ V2 = -0{,}002 \cdot X7 - 0{,}004 \cdot X8 - 0{,}023 \cdot X14 + 0{,}008 \cdot X22 - 0{,}344 \cdot X44 + \\ \qquad + 0{,}009 \cdot X50 + 0{,}095 \end{cases}$$

where V0 corresponds to “normocenosis” according to IOPM, V1 corresponds to “grade I dysbiosis” according to IOPM, V2 corresponds to “grade II dysbiosis” according to IOPM; the decision is made taking into account maximum value of the indicator.

Thus, the model for predicting the severity of dysbiosis by IOPM, which was based on six factor features, shows “very good” (k > 0.81, according to the scale) agreement, which indicated the high relevance of the selected factor features for predicting the severity of dysbiosis by IOPM.

Analysis of the system of equations showed and supplemented the regularities of dysbiosis progression, which were previously revealed for Normobiota index (NBI). These factors are reflected in Fig. 1.

In normocenosis, negative signs of the coefficients (i.e. reduced IOPM) contained lysozyme and TGF-1β in vaginal secretion and C4 and IL8 components in blood. Therefore, lysozyme and activation of complement (C4) in blood could be added to the effector factors supporting normocenosis in vaginal secretion, in addition to complement (C4), as shown for NBI.

Thus, we finally can state that the factors of colonization resistance of vagina such as complement (C4) and lysozyme were responsible for maintaining normocenosis.

Level of sIgA in vaginal secretion had a positive sign of the coefficient, i.e. increased in parallel with IOPM, which indicated the presence of this immune response, but also – the ineffectiveness of this factor in terms of inhibiting the growth of opportunistic pathogenic

microflora. This condition of immune system in normocenosis can be characterized as controlled in relation to the development of vaginal dysbiosis.

Group	**Sign on the coef.**	**NBI**		**IOPM**	
		VS	**Blood**	**VS**	**Blood**
Normocenosis	+	-	CIC (X42)	sIgA (X7) γ-INF (X14)	-
	-	**C4 (X13)** γ-INF (X14)	TNFα (X52)	**lysozyme (X8)** TGF-1β (X22)	**C4 (X44)** IL8 (X50)
Grade I dysbiosis	+	C4 (X13) γ-INF (X14)	CIC (X42) TNFα (X52)	lysozyme (X8)	C4 (X44) IL8 (X50)
	-	-	-	**sIgA (X7)** γ-INF (X14) TGF-1β (X22)	-
Grade II dysbiosis	+	C4 (X13)	TNFα (X52)	TGF-1β (X22)	IL8 (X50)
	-	γ-INF (X14)	**CIC (X42)**	**sIgA (X7)** **lysozyme (X8)** γ-INF (X14)	**C4 (X44)**

Fig. 1.Role of significant factors of colonization resistance of vagina and immune system in determining the severity of dysbiosis (according to NBI and IOPM); VS– vaginal secretion; "+" – positive sign of the coefficient in a linear neural network model, "-" – negative sign; effector factors limiting the activation of opportunistic pathogenic microflora are highlighted in bold.

Among the regulatory factors, γ-INF in the vaginal secretion and TNFα in blood reduces the progression of vaginosis, as shown for NBI and, according to the effect on IOPM, we could add TGF-1β in vaginal secretion and IL8 in blood.

Factors such as Circulating Immune Complexes level in blood (according to NBI) and content of sIgA and γ-INF in vaginal secretions (according to IOPM) increased in parallel with IOPM. Therefore, these three factors can be considered markers of growth of opportunistic pathogenic microflora in normocenosis, and they can be recommended to be used as preclinical tests for the development of vaginal dysbiosis.

In grade I dysbiosis according to NBI, none of the indicators had a negative coefficient sign, i.e. all the indicators had positive signs, and, therefore, contributed to the increase of NBI. According to IOPM, we could add lysozyme content in vaginal secretion, and C4 and IL8 content in blood. That is, this number of factors reflected the activation of opportunistic pathogenic microflora, which thus acquired the properties of an uncontrolled process: both the complement system and lysozyme lost the ability to inhibit the activation of opportunistic pathogenic microflora. In our opinion, these mechanisms were the basis for the beginning of

development of immunoresistance and formation of local and systemic immunodeficiency in grade II dysbiosis. This, in turn, contributed to acquisition by pathogenic microflora of the ability to sustain itself and stimulate itself progressively.

According to IOPM, factors counteracting the growth of opportunistic pathogenic microflora were also revealed. These were levels of sIgA, γ-INF and TGF-1βin vaginal secretion. These indicators, in our opinion, reflected the development of secondary immune reactions, which unfolded later –in grade II dysbiosis – and were caused by an increase in antigenic load.

In grade II dysbiosis according to NBI, negative coefficient signs appeared for γ-INF content in vaginal secretion and CIC content in blood (see Fig. 1). According to IOPM, we could add sIgA, lysozyme and γ-INF content in vaginal secretion, and level of complement (C4) in blood.

Thus, as shown by mathematical analysis, despite the development of complex immunodeficiency in severe dysbiosis, indicators of effector systems - lysozyme, sIgA, complement (C4), CIC – acquire the ability to control the microbial flora.But, as shown in this comprehensive study, at the time of BV, pathological hormonal-immune system, which supports the combined immunodepressionon the principle of the dysregulatory mechanism, is already formed.

Condition of the immune system in grade II dysbiosis according to NBI was reflected by the activation of complement (C4) in vaginal secretions and progressive increase of TNFα in blood, and according to IOPM – also by levels of TGF-1βin vaginal secretions and IL8in blood.

For practical usage of the model for predicting the severity of dysbiosis according to IOPM, an expert system was created in the Excel spreadsheet environment (file “Prognosis IOPM.xls”).

Figure 2 shows its interface.

To work in the program it is necessary to enter indicators’ values for a particular patient into the appropriate cells of the spreadsheet. The expert system will give a forecast on the severity of dysbiosis according to IOPM.

Thus, patient P., 40 years old, had the following indicators: vaginal secretion: sIgA– 107.2 μg/ml, lysozyme – 9.7 μg/ml, γ-INF – 1.94 pg/mg, TGF-1β– 36.9 pg/ml; blood: C4 – 0.36 mg/ml and IL8 – 9.35 pg/ml. Predicted NBI – “Normocenosis”; actual IOPM value – 3.49; diagnosis –normocenosis.

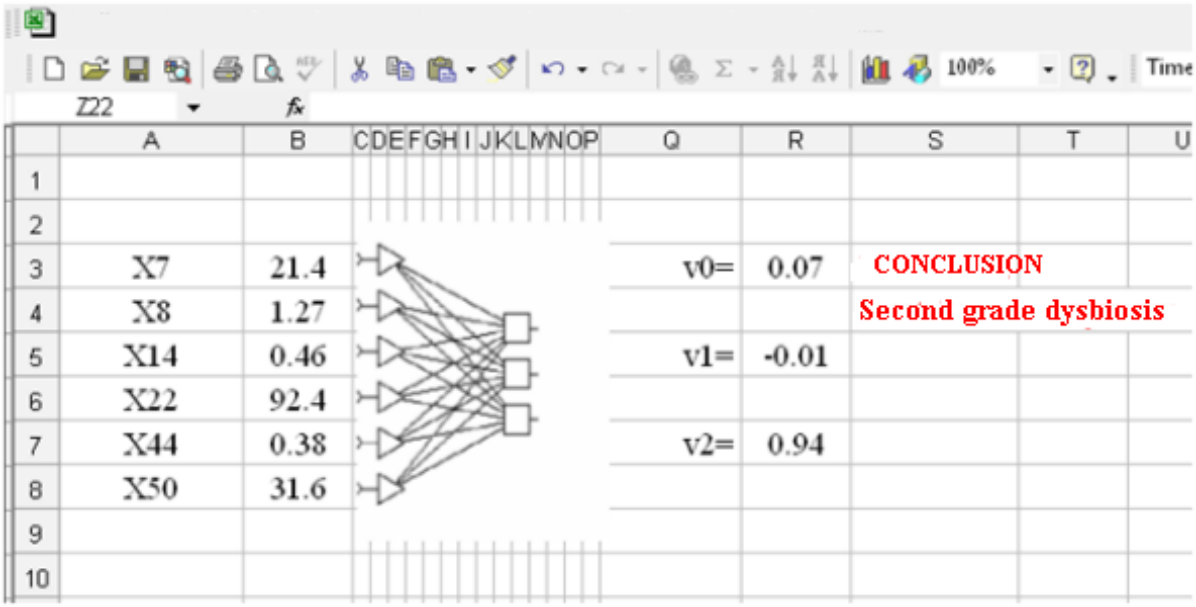

Fig. 2. Interface of the expert system for predicting the severity of dysbiosis according to IOPM

Patient P., 31 years old, had the following indicators: vaginal secretion: sIgA– 146.3 μg/ml, lysozyme – 15.4 μg/ml, γ-INF – 1.76 pg/ml, TGF-1β– 38.1 pg/ml; blood: C4 – 0.80 mg/ml and IL8 – 25.3 pg/ml. Predicted NBI – "Grade Idysbiosis", actual IOPMvalue -1.09; diagnosis –grade I dysbiosis.

Patient S., 16 years old, had the following indicators: vaginal secretion: sIgA– 21.4 μg/ml, lysozyme – 1.27 μg/ml, γ-INF – 0.46 pg/ml ;, TGF-1β – 92,4 pg/ml; blood: C4 – 0.38 mg/ml and IL8 – 31.6 pg/ml. Predicted NBI – "Grade II dysbiosis", actual IOMP value – 4.47; diagnosis – grade II dysbiosis.

Conclusions. Thus, the nine factor traits obtained using mathematical analysis allowed to predict the severity of vaginal dysbiosis with a high level of accuracy and to calculate NBI and IOPM. In addition, the phasal nature of immune system reaction to the development of vaginal dysbiosis is shown – from the state of control in normocenosis to the development of immunoresistance in grade I dysbiosis, and severe combined immunodeficiency in the presence of specific humoral response to bacterial antigens in grade II dysbiosis.

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