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Huminskyi Y.Y.,
Rykalo N.A.,
Huminska O.Y.

Department of pathophysiology of the M.I. Pyrohov Vinnytsia National Medical University, oguminska@gmail.com

REGRESSICE MODEL FOR DETERMINING A RELATIVE NUMBER OF THYMOCYTES IN THE THYMIC CORTEX OF IMMATURE RATS BASED ON THE SERUM TRANSFORMING GROWTH FACTOR-β1

Abstract. *When the serum transforming growth factor-β1 (TGF-β1) rate increases, which is typical for liver damage, there is a suppression of the immune system and the quantitative morphometric changes in the thymus are morphological reflection of these abnormalities. This correlation between the serum growth factor and a relative number of thymocytes in the thymic cortex of immature rats is embodied in polynomial regression and allows an indirect and non-invasive method to determine the morphological indices of the thymus.*
Key words: *immature rats, thymus, transforming growth–β1 factor, polynomial regression.*

Introduction. TGF–β1, transforming growth factor beta is a multifunctional cytokine involved in the regulation of proliferation, apoptosis and metabolic reactions in various target cells, it is a cytokine with systemic effect as the expression of its high-affinity receptor is widespread, the factor suppresses hematopoiesis and synthesis of pro-inflammatory cytokines, it also stimulates neoangiogenesis (J.A. Mengshol, L . Golden-Mason et al, 2010; J.P. Edwards, H. Fujii, 2013). The role of TGF-β1 in the pathology of the liver is known - it is a profibrinogen factor that accelerates the pace of fibrosing through the activation of stellate cells, increased production of extracellular matrix proteins and collagen type I, besides TGF-β1 is predictive of accidental thymic involution (M.Y. Kapitonov, 2006; A.A. Yarylin, 2003; J. P. Alekseeva, T.M. Bryzhina et al., 1991).

The issue of the role of TGF-β1 in the development of secondary immunodeficiency is not clarified in an experiment adequately, the involvement of TGF-β1 in the accidental involution of the thymus and the impact on cellular and humoral immunity has been proved (O.Y. Huminska, 2014; R.T. Robinson, J.D. Gorham 2007). However, quantitative characteristics of this relationship need further studying.

Objective: to establish a quantitative relationship based on the qualitative relationship between the serum transforming growth factor-β1 and a relative number of thymocytes of the thymic cortex of immature rats.

Materials and methods. The research involved 20 white nonlinear immature female rats aged 60 days. The animals were kept in vivarium conditions of M.I. Pyrohov VNMU with free access to water and food. All experiments were conducted in compliance with the "General ethical principles in experiments with animals" (Kyiv, 2001) and were consistent with the provisions of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes." Sampling was performed under general thiopental anesthesia by cervical dislocation, fixation of morphological material was carried out by the conventional method. The study of histological structure of the thymic tissue was performed on the left lobe, the resulting material was fixed in 10% neutral formalin. After a standard procedure, it was embedded in paraffin, histological sections 5 microns thick were made in microtome HM-360 company "Zeiss". The sections were stained with hematoxylin-eosin. Obtained specimens were examined with a microscope Olympus BX 41 at different magnifications and photographed.

In addition to a qualitative descriptive assessment of histological structure of laboratory animals thymus, we also determined the following indices using Weibull's net [G.G. Avtandilov, 1990]: the relative area of the cortex and medulla, ratio of relative areas of cortex and medulla, the relative amount of thymocytes and epitelioretykulotsytiv cortex, the relative number of thymocytes and epithelioreticulocytes of the cortex, the relative number of thymocytes and epithelioreticulocytes of the medulla for morphological verification of the presence and assess of the extent of accidental thymus involution.

The serum transforming growth factor-β1 rate was determined by ELISA on a stripped immunosorbent analyzer StatFax 303+ (Awareness Technology), using reagents of a firm «DRG» (Germany).

Results and discussion. We have proved a clear link between qualitative characteristics of a relative number of thymocytes of the cortex and those of the serum transforming growth factor-β1, these characteristics can be transformed into quantitative ones by using regression analysis.

While studying the transforming growth factor in the serum of rats, we found that the content of TGF-β1 is - 251,04 ± 4,820 pg / L, the relative number of thymocytes in the thymic cortex of immature rats is 51,20 ± 0,49.

In this case, the relationship between the number of thymocytes and the TGF-β1 rate as well as values of the population of lymphocytes in the peripheral blood in intact rats can be transformed into quantitative ones using regression analysis. In the simulation by means of step-by-step direct regression analysis of quantitative relationship between correlations, the regression model included the number of thymocytes of the cortex and TGF-β1 index with quite high levels of predictivity. Evaluation of prognostic contribution of an independent variable (TGF-β1) in a step by step procedure was determined by R^2, that is 0.9807 in this formula, i.e. in 98.07% a chance of matching the actual value (the number of thymocytes in the cortex of the thymus) calculated (for this model) at p <0.001.

Table 1

Indices of regression model of the dependence of number of thymocytes in the thymic cortex on the index of transforming growth factor-β1 in the serum of intact rats

Results of regression for the dependent variable: number of thymocytes R=0, 99; RI=0, 98; adjusted RI=0, 9807; F(2,17)= 48,15;p<0,0001; Standard error: 0,25				
variable	B	Standard error. B	t (34)	p-level
absolute term	31,29	0,64	48,71	0,000
TGF–β1 (i)	0,079	0,002	31,11	0,000

The coefficient of determination R^2, as a measure of the quality of forecasting approximates the estimated dependent variable for 98.07%. The standard error of the estimation in this case is 2.368, that in terms of the maximum and minimum value could reach approximately from 4.47% to 4.8% (i.e. theoretically, the maximum deviation can be up to 5%).

In carrying out serial analysis of residues of the dependent variable, none of the observations extends beyond two sigmas, besides, the average of the difference of the actual and calculated theoretically is "0". With this number of observations F critical is 2.17, in fact, in this formula it is 48.15, which is much more than the critical value. On this basis, we can state that the regression linear polynomial is significant at p <0.001.

The graph of residue distribution (differences of theoretical and actual normal number of thymocytes in the cortex of the thymus) clearly shows their location on the line without deviation beyond the 95% confidence interval (Fig. 1). In the final version of this formula is as follows:

N = 31,29+(0,079×TGF β-1)±0,25 (або±5%);

Where: N – a relative number of thymocytes in the thymic cortex of immature rats; 31.29 is a free coefficient;

RI=0,9807 (an adjusted connected R) determines the predictivity of the model at 98,07 % at p<0,001; the value TGF–β1 for this
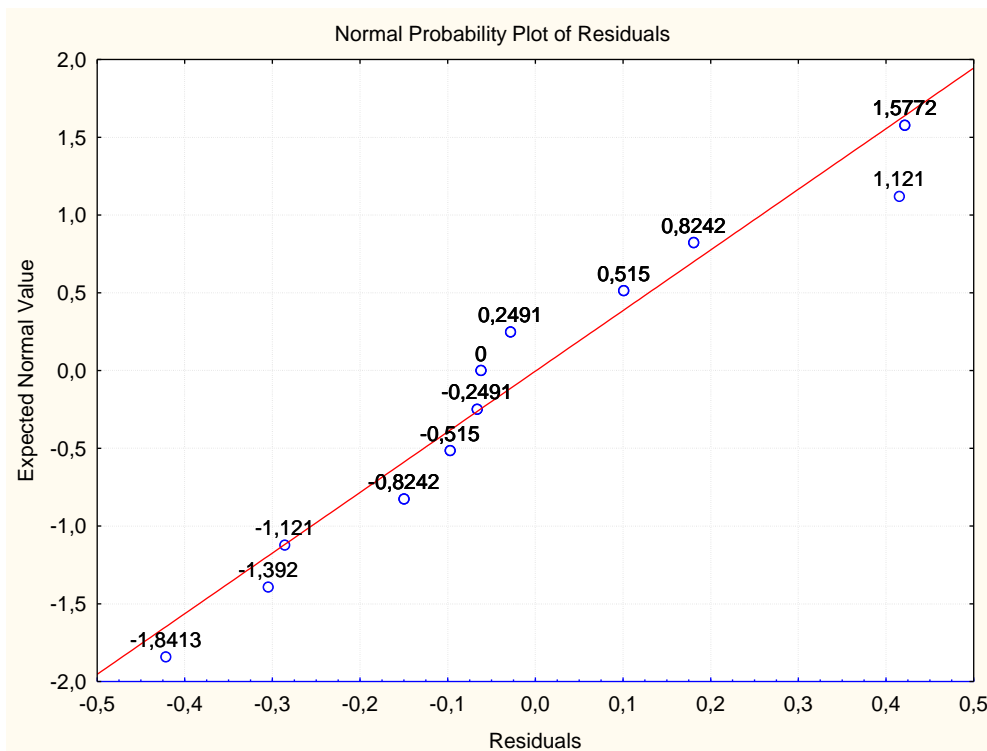
Fig. 1. Value of deviations in a modeled number of thymocytes in the cortex of the thymus in immature rats.

group of immature rats is 251,04± 4,820 pg/l; ±0,25 is a value of the standard deviation relatively the average value.

Conclusions. The above leads to the following conclusion that the number of thymocytes of thymic cortex is in a qualitative and quantitative dependence on the TGF-β1rate, which with high probability allows theoretically predict the number of thymocytes depending on the normal rate of the latter, polynomial is patented to the useful model number 100926.

Prospects of further research. The quality of the polynomial (formula) is high, which indicates its prospects of use to determine the relative number of thymocytes in the thymic cortex in terms of transforming growth factor-β1 in the serum of immature rats as a method of indirect determination of morphological index with 95% probability   this polynomial can be used in research activities.

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