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Current approaches to diagnosis of fracture consolidation disorders

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Purpose Identification of personalized diagnostic criteria of consolidation disorders in limb bone fractures. **Materials and methods** 108 patients at the age from 20 to 40 years (according to WHO classification) with limb bone fractures were included into the study. Group 1 were patients (n = 62) with uncomplicated fractures. Group 2 (n = 46) included patients with consolidation disorders. The presence of acute or chronic associated diseases was the exclusion criteria. Clinical, laboratory and instrumentation findings (210 parameters) were assessed in a multivariate predictive regression model. These parameters were studied at admission, then on days 2, 5, 10 and 90 of the study. Statistic processing was performed using STATISTICA 6.1 software package (StatSoft, Russia). **Results** The multivariate regression analysis used revealed high association of -25Pro/Pro genotype of $TGF\beta_1$ gene and -2073T/T genotype of *EGFR-2073A > T* gene in patients with consolidation disorders that ran as delayed consolidation type ($p < 0.0000001$). **Conclusion** The combination of genotypes -25Pro/Pro of $TGF\beta_1$ gene and -2073T/T of *EGFR* gene can be used as one of the markers of consolidation disorder in limb bone fractures, and that will enable to use personalized prophylactic programs to prevent such complications.

Keywords: fractures, consolidation disorder, polymorphism, genes, prediction

INTRODUCTION

Consolidation disorders in fracture repair remain a current unsolved problem [1, 2, 3].

Several factors play an important role in the course and outcome of consolidation disorders in the traumatic disease [1, 4], including the hereditary ones [5, 6, 7]. The development of consolidation disorders results not only in the impairment of patients' moral and physical conditions but also in a considerable economic burden in their management. Therefore, the search for methods of a personified prognosis is a priority for current medical studies [8].

Purpose of the study To reveal personified diagnostic criteria to detect consolidation disorders in limb fractures.

MATERIAL AND METHODS

One hundred and eight patients in the age range from 20 to 40 years (WHO age classification) with limb fractures that underwent in-patient treatment at the city hospital #1 of Chita were included into the study. The patients were divided into two groups. Group 1 (n = 62) were patients with an uncomplicated course of long bone fracture repair, and group 2 patients (n = 48) had a delayed fracture consolidation.

Ethical principles laid down by the World Medical Association Declaration of Helsinki (1964, updated in 2011) and Rules of Clinical Practice in the Russian Federation adopted by the RF Ministry of Health order dated 19.06.2003 # 266 were observed.

Groups were formed according to the classification of fractures proposed by M.E. Muller et al. (1995) (**Fig. 1**).

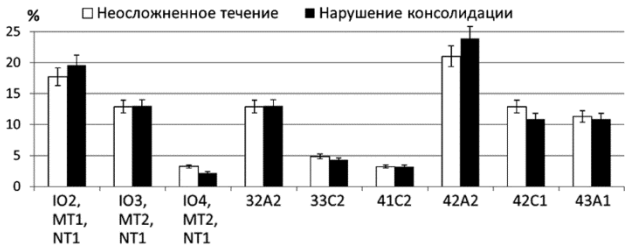

Fig. 1 Distribution of patients according to M.E. Muller's et al.'s classification (1996)

Distribution of patients according to the type of operative method is given in **Table 1**.

Operations were performed during the first 2 to 4 hours since the admission. Postoperative care was carried out according to the guidelines in force [9].

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Table 1

Distribution of patients according to the methods of operative treatment used

Method	Open fractures		Closed fractures		Total	
	Number	%	Number	%	Number	%
Uncomplicated consolidation (n=62)						
PSD*, AEF**, draining	21	33.87	–	–	21	33.87
Plating	–	–	28	45.2	28	45.16
Intramedullary nailing	–	–	13	20.97	13	20.97
Consolidation disorders (n=46)						
PSD*, AEF**, draining	16	34.78	–	–	16	34.78
Plating	–	–	21	45.65	21	45.65
Intramedullary nailing	–	–	9	19.57	9	19.57

Note: * – primary surgical debridement; ** – apparatus of external fixation

Consolidation disorders were verified with the use of clinical, laboratory and instrumentation methods of study [9].

Clinical, laboratory and instrumentation findings (210 parameters) were assessed in a multivariate predictive regression model. These parameters were studied at admission, then on days 2, 5, 10 and 90 after injury.

Anamnesis and clinical data studied corresponded to the ones recommended for diagnosis of fractures and their complications [9].

Laboratory tests were:

– index of lymphocyte-platelet adhesion (LTA);

– several cytokines (IL1α, IL1β, ΦНОα, IL-4, IL-10, TGFα, TGF1β, EGF);

– parameters of adenylyl (ATP, ADP, AMP) and antiprotease systems (α_2-macroglobulin, α_1-antitrypsin);

– values of the LPS (lipid perioxidation system) (ketodien and conjugated triens, TBA active products, malondialdehyde, conjugated diens, general antioxidant activity).

The study of the parameters above mentioned was conducted according to the standard methods [1].

Bearing of the polymorphyc genetic markers of genes 9TNFα (G-308A), gene IL-4 (C 589T), gene IL-10 (G-1082A, C-592A, C-819T), TGFβ_1 (Arg-25Pro) and EGFR (A-2073T)) was detected using the Litex-SNP kit (Moscow, Russia) [10].

Instrumentation methods were:

– Laser Doppler flowmetry (parameters of microcirculation)

– Radiography [1].

Statistical processing used the software package STATISTICA 6.1 (StatSoft, Russia). Regression analysis was used as a multivariate method of statistical analysis [11].

RESULTS

The multivariate regression analysis used (stepwise with sequence inclusion) revealed high association of -25Pro/Pro genotype of *TGFβ_1* gene with consolidation disorders in the late trauma period. The accuracy of this mathematical model increased significantly by adding the -2073T/T genotype of *EGFR* gene while other indices did not have significant influence on the prognosis (**Table 2**).

Coefficient (K) of correlation (multiple) was recorded at the level 0.985; K of determination (R^2) was 0.967, and the significance level of regression model was < 0.0000001. Genotype -25Pro/Pro of gene *TGFβ_1* appeared to be a more significant prognostic factor in fracture consolidation disorder (a 53-fold risk increase). Genotype -2073T/T of *EGFR* gene also considerably contributes to the diagnosis of bone tissue reparation disorders (a 39-fold increase of the risks) (**Table 2**).

Table 2

Prognostic significance of the indices in the multivatiate model of delayed fracture consolidation

N=108	*Beta	Statistic error Beta	B	Statistic error B	**p
Associated member			2.486932	0.123786	0.000000
-25Pro/Pro of gene *TGFβ_1*	-0.53048	0.030101	-0.296475	0.016823	0.000000
-2073T/T of gene *EGFR*	-0.39622	0.030789	-0.248905	0.019342	0.000000

Note: ∗beta – regression coefficient; **p – level of statistical significance (significant by p≤0,05).

DISCUSSION

The prognostic model obtained has a sufficiently high sensitivity and reliability ($p < 0.0000001$).

This fact is proven not only by a high degree of correspondence to empiric findings (R^2), linear dependence of the influence and response factors, or complication development (K), but also by the parameters accountable that have an impact on complications. These were the two parameters revealed – -25Pro/Pro of *TGFβ$_1$* gene and -2073T/T *of EGFR* gene (adjusted R^2 did not differ from the initial one) [11].

Currently, more than 10 polymorphic sites have been detected in *TGFβ$_1$* gene. Their role has not been established yet. However, there are data on the influence of several genotypes of polymorphic sites on the condition of bone tissue [6, 14-16] and in several disease of the gastrointestinal tract [17]. Having examined the impact of the hereditary factor on the course of bone tissue reparative processes in fractures, it was noted that the bearing of genotype -25Pro/Pro of gene *TGFβ$_1$-25Arg>Pro* results in the reduction of the coded protein expression ($TGF\beta_1$) that in its turn contributes to disorganization of bone tissue remodeling processes and, consequently, delays fracture consolidation [12].

As for gene *EGFR*, its influence has been actively studied in malignant neoformations [13], though there are some studies that deal with long bone fractures [5].

It seems possible that different combinations of bearing the genotypes of various polymorphic sites of genes might promote the synthesis of coded proteins in different directions, and, consequently, result in either a disease (complications) or have a protective effect. This fact has been confirmed by several studies [13, 14].

CONCLUSION

Consequently, the combination of genotypes – 25Pro/Pro of gene *TGFβ$_1$* and -2073T/T of gene *EGFR* may be used as one of the markers of consolidation disorders in limb fractures that would enable to use a personified prophylactic program for prevention of such complications.

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