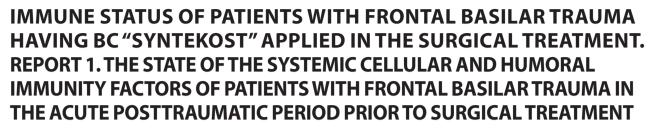
ORIGINAL ARTICLE PRACA ORYGINALNA



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ABSTRACT

Introduction: At present biocomposite materials are used in the surgical treatment of frontal bone fracture. They improve osteogenesis, reduce the number of complications. Immunologic aspects of application of these materials are studied insufficiently, therefore this report presents the results of immunoassay of patients with frontal bone fracture in the proximate posttraumatic period before implanting preparation "Syntekost".

The aim: To define the role of immune mechanisms in the realization of the biocomposite material's positive influence on the development of effective posstraumatic rehabilitation schemes. **Materials and methods:** 16 patients with frontal bone fracture (FBF) were examined on admission to the Otolaryngology Clinics of Vinnitsa Region Hospital. Additionally, 10 patients of the similar age were examined as a control group. The content of cells with markers of surface antigens-CD3,14,16,20,25, concentration of immunoglobulins of classes M,G,A,E, C₄ complement component and lactoferrin was determined in blood. Immunoenzyme methods were applied. Nonparametric Wilcoxon — Mann — Whitney test, computer programme WIN Pepi were used for statistical measurements.

Results: A decrease in the level of IgM in comparison with practically healthy donors and an increase in the concentration of lactoferrin were identified as humoral immunity factors of patients with frontal basilar trauma. The most significant deviation in the peripheral blood cellular makeup in CD-markers was an increase in cells with markers CD14 and CD16.

Conclusions: The level of cells and prodefensin-lactoferrin that maintain inborn immunity increases and the concentration of coarse defensive protein decreases in the initial period after frontal bone fracture, which must be taken into consideration during post-surgical treatment.

KEY WORDS: trauma, immunoglobulins, CD-markers, C, complement component, lactoferrin

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INTRODUCTION

It is known that both in the initial and remote periods of skull fractures there is a significant immune response to trauma, which becomes apparent through changes in immune response level at initial stages after a trauma and possible development of autoimmune response at the late stage [1,2,3]. In the recent years synthetic compositions on the basis of hydroxyapatite (BC"Syntekost") that can accelerate osteogenesis and prevent the development of liquorrhea and other posttraumatic complications have been used in the surgical treatment of frontal bone fracture [4]. The present report offers results of immunoassay of patients with frontal bone fracture in the proximate posttraumatic period before implanting preparation "Syntekost".

THE AIM

To define the role of immune mechanisms in the realization of the biocomposite material's positive influence on

the development of effective posstraumatic rehabilitation schemes.

MATERIALS AND METHODS

16 patients with frontal bone fracture (FBF) were examined on admission to the Otolaryngology Clinics of Vinnitsa Region Hospital. Additionally, 10 patients of the similar age were examined as a control group. Blood was drawn from an ulnar vein and divided into two parts: with heparin to get mononuclears and without it to get blood serum. Mononuclears were received from the heparinized blood by means of centrifugal sedimentation (120g, 10 min, cooling centrifuge NU 800R, Turkey) in the ficoll paque density gradient (1.077, Pan-Eco, RF), they were weighed in medium 199 being enriched (vitamins, aminoacids, embryonic calf serum, reagents Serva, Germany) up to density of 2 mln cells/ml. T-lymphocytes (CD3), B-lymphocytes

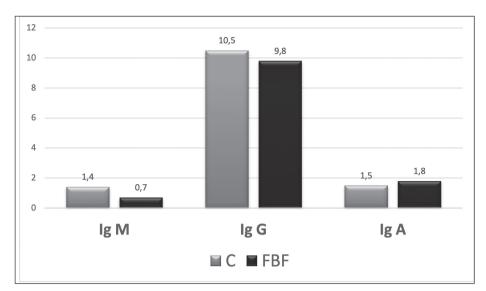


Fig. 1. The content (g/l) of immunoglobulins of M,G,A classes in the blood serum of the control group patients and of the patients with FBF. C — control groupe

FBF – fracture of frontal bone

Ig M - Immunoglobulin M

Ig G - Immunoglobulin G

Ig A - Immunoglobulin A

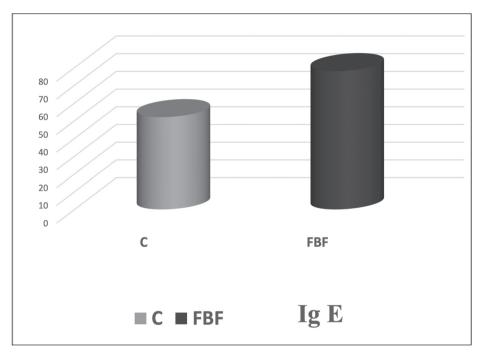


Fig. 2. The content of E class immunoglobulins (ME/mI) in the blood serum of the control group patients and the patients with FBF. C – control groupe FBF – fracture of frontal bone Ig E - Immunoglobulin E

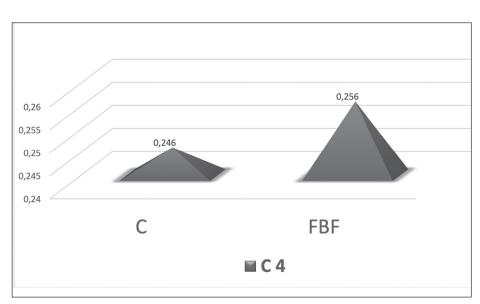


Fig. 3. The content of C4 complement component in the blood serum of both examined groups.

C – control groupe

FBF – fracture of frontal bone

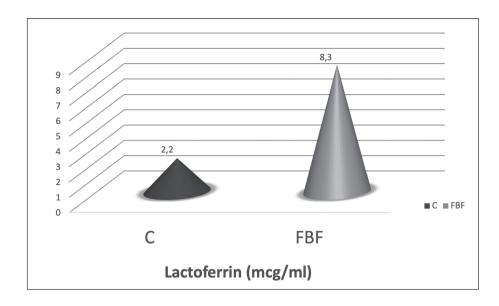


Fig. 4. Lactoferrin content in the blood of the examined groups. C — control groupe FBF — fracture of frontal bone

Table 1. The content of different types of blood cells in the patients with FBF and in the control group patients (by mucous tunic).

Groups and indices —	Relative content (%)				
	CD3	CD14	CD16	CD20	CD25
C,M (min.max.)	55,5 (40-65)	6,5 (4-8,5)	6,5(4-7,5)	17,8(12-19)	5,5(5-7)
FBF, M (minmax.)	48,2(38-55)	12,5(7-16)	13,5(6-17)	15,5(10-15)	8,5(5-12)
р	> 0,05	< 0,05	< 0,05	> 0,05	< 0,07

(CD20), monocytes (CD14), natural killer cells (CD16), activated lymphocytes (CD25) were detected applying monoclonal antibodies to cell surface antigens (CD) and using rosette method [5].

The content of immunoglobulins of M,G,A,E classes in the blood serum was analyzed using immunoenzyme method (reagents Xema-Medica Co.Ltd., RF and Lab line reader, Austria). Furthermore, the content of $\mathrm{C_4}$ complement component (Microgen, RF) and prodefensin-lactoferrin, which is an indicator of an acute phase of any inflammation (Belamy e.a., 1989). Statistical analysis was undertaken using Nonparametric Wilcoxon — Mann — Whitney test as recommended by E.V. Gubler (1990) [6] according to Biostatistics-6 programme.

RESULTS

Received data showed that the content of immunoglobulins of classes M,G,A,E in patients with FBF did not differ from the one in the control group patients, whereas the level of IgM was significantly lower than in the control group patients: 0,3 and 1,5 g/l correspondingly (figure 1,2). The content of $\rm C_4$ complement component in the stated period after FBF did not differ from the one of the control group. (figure 3)

At the same time there was a significant increase (p<0,02) in lactoferrin – from 3 mkg/ml, which is the norm, to 10 in patients with FBF (figure 4).

When studying the content of cells of different types according to clasters of differentiation, significant deviations were

identified in macrophages and natural cytotoxic cells, CD14 and CD16 correspondingly (table I), the content of which increased during the first 24 hours of FBF. The tendency towards the content increase was also noted while determining the number of activated cells that reflect to a certain extent the activation of regulatory T-cells [7]. The decrease in IgM concentration in the blood serum may relate to the usage of coarse proteins from blood as a plastic material in extreme situations [8]. The increase in cells with CD14 and CD16 markers proves that innate immunity factors take part in defense and adaptation processes as quickly responsing components of the immune system.

CONCLUSIONS

- A decrease in the IgM level and an increase in the lactoferrin concentration were noted in patients with frontal basilar trauma while determining systemic humoral immunity element.
- 2. The most significant deviation in the peripheral blood cellular makeup in CD-markers was an increase in cells with markers CD14 and CD16.

REFERENCES

- Gorbunov V. Immunology of traumatic brain injury. In: Gorbunov V, Likhterman L, eds. Uliianovsk; 2008, p. 397–399.
- Lysianyi N.I., Pedachenko E.G. The peculiarities of autoimmune reactions' development during recurring TBI. Immunology and allergology. 2006;3:53-56.
- 3. Zhuang J., Shacktoro S., Schmöker J.D., et al. The association of leukocytes with secondary brain injury. J Trauma. 1993;35:415-422.

- 4. Kishchuk V.V., Bondarchuk O.D. Using biocomposites "Syntekist" to liquidate bone defects of ENT-organs. Journal of the Ear, Nose and Throat Diseases. 2007;5:43-44.
- 5. Novikov D.K., Novikov P.D. The method of defining T and B lymphocites using a diagnostics based on monoclonal antibodies. Immunology. 2000:2:31-33.
- 6. Gubler E.V. Informatics in pathology, clinical medicine and pediatrics. In: Gubler E.V, eds. L. Medicine; 1990, p. 176.
- 7. Drannik G.N. Clinical immunology and allergology. In: Drannik G.N., eds. Kiev: Astra Print Ltd, 2006, p. 480.
- 8. Jager L. Clinical immunology and allergology. M: Medicine (translation from German). 1986;3:344-364.

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Conflict of interest:

The Authors declare no conflict of interest.

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