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ORIGINAL RESEARCH

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The anatomical variations of the posterior circumflex humeral artery

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Abstract

Background: Arteries have a very variable origin, diameter, path, correlation, branching and terminal pattern. An honorable mention is necessary for the individual anatomical variations of the axillary artery branches, because this is a spot of frequent vascular lesions that are the result of blunt weapon traumas, proximal humerus traumas and glenohumeral dislocations.
Material and methods: The morphologic variability of the posterior circumflex humeral artery was studied on a male adult cadaver, on 10 angiographies and 9 ultrasonographies of the upper limb arteries which also constituted the study poll. The origin variations were identified in 3 cases.
Results: In correlation with gender, and the body part that is taken into consideration, the posterior circumflex humeral artery morphological variations are found more often in the male gender, predominantly on the right upper limb.
Conclusion: The posterior circumflex humeral artery has a wide range of anatomical variations. More frequently the anatomical variations are found on the right upper limbs of male gender. The obtained data will become useful in the approaches of the axillary artery during arteriographies and also in choosing the right surgical intervention tactic on this topographical region.
Key words: axilla, posterior circumflex humeral artery, arterial variation.

Introduction

The axillary artery through its branches supplies the anatomical formations in the deltoid region, the lateral thoracic wall and the superior portion of the arm.

In the classical description of the axillary artery ramification, the specialized literature relates about a pattern of branching that implies 6 main branches and 5-6 accessory branches [1, 2].

The main branches have a very high variation not only in origin but also in path and number. The most variable of them being the lateral thoracic artery, posterior circumflex humeral artery and subscapular artery [3].

The posterior circumflex humeral artery has its origin in the infrapectoral portion of the axillary artery, 1-2 cm superiorly to the superior margin of the latissimus dorsi muscle insertion. During its path, to the posterior surface of the quadrilateral space, it passes infero-laterally between the subscapular muscle and the latissimus dorsi muscle, being variable in its position towards the axillary nerve, that in most cases is superior to the artery, in less cases inferiorly or between its branches in cases when the artery splits at the quadrilateral space level [4].

In the subdeltoidian space, the branches of the posterior circumflex humeral artery are distributing collaterally supplying the glenohumeral articulation, the deltoid muscle, the teres major and minor muscles, the long head of the arm triceps muscle [5].

In the surgical neck traumas, the posterior circumflex humeral artery can be traumatized along with the axillary nerve, leading to major complications of the gleno-humeral dislocations, which are present in 15.8 – 48% of cases [6].

According to the data found in the specialized literature, surgical neck traumas represent 6% of the registered fractures, where in 64% of cases the axillary nerve is traumatized [7].

Along with that, the anatomical variations of the posterior circumflex humeral artery became more important in the last years, because of the gradual growth of the vascular surgical interventions, radiological interventions and not less importantly – reconstructive surgical interventions. Many errors in medical practice are caused by the misrecognition of the anatomical variation of these arteries [8, 9].

The study goal consists in the recognition and description of the anatomical variations of the posterior circumflex humeral arteries depending on the gender and the part of the body that is studied.

Material and methods

The study of the posterior circumflex humeral artery was performed on a male adult cadaver fixed in 10% formalin

and also on 10 angiographies (6 selective angiographies and 4 angio-CT) and 9 ultrasonography images (Vascular Dopplerographies) of the upper limb arteries that were obtained from the database of the Republican Center of Medical Diagnostics and the Timofei Mosneaga Republican Clinical Hospital.

The upper limb arteries of the cadaver were studied using the anatomical dissection method; we were able to establish the origin, number, path and correlation with the adjacent anatomical structures.

The imagistic poll that was implied in the study included 10 males and 8 females (one patient of female gender had a bilateral ultrasonography of the upper limb arteries), the age poll was between 49-65 years.

The arteries of the right axilla were studied at 11 patients (6 males and 5 females), and the left axilla – at 8 patients (4 males and 4 females).

The CT Angiographies were made on a Lightspeed VCT with 64 slices; the tomographic sections were made with a 5.0 mm thickness, the reconstructions – 1.5 mm in "Angio-RunOff" regime, MPP, MIP Thin and VRT. The imagistic study with this method offered us a very accurate topography of the posterior circumflex humeral artery, and the 3D reconstruction highlighted its origin.

The landmark for the recognition of the posterior circumflex humeral artery was the subscapular artery and the humerus, which helped us to identify the level and type of branching, and its anatomical variations.

Results and discussion

During the upper limb dissection we identified anatomical variations on the infrapectoral portion of the right axillary artery, while on the left upper limb, we found a classical pattern of the axillary artery branches. The posterior and anterior circumflex humeral arteries have their origin from a common arterial trunk, which emerges from the lateral hemicircumference of the axillar artery. The common trunk length constituted 0.4 cm, the external diameter – 0.64 cm. The posterior circumflex humeral artery had a 0.45 cm diameter and crossed the axillary nerve antero-superiorly, the anterior circumflex humeral artery – a smaller diameter of 0.15 cm and a transverse path that was following the surgical neck of the humerus.

The subscapular artery with an external diameter of 0.52 cm before its usual bifurcation (into the circumflex scapular artery and the thoracodorsal artery), launched 2 muscular branches that had a relatively big diameter (0.31 cm and 0.24 cm) and an unusual path.

The first muscular branch had its origin 0.5 cm inferiorly from the subscapular artery origin, following up to the superior scapular angle where it realised an anastomosis with the suprascapular artery, launching on its path 2 branches that penetrated the subscapular muscle.

The second muscular branch had its origin 2 cm inferiorly from the subscapular artery origin, it crossed with an oblique trajectory the subscapular muscle from the anterior side, and on its middle portion it penetrated the muscle (fig. 1).

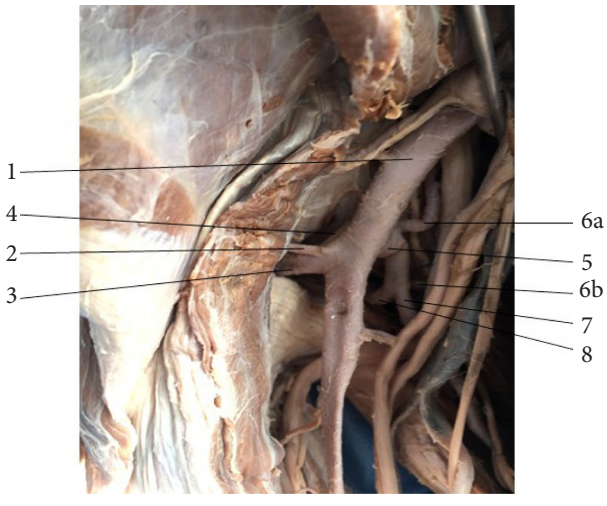

Fig. 1. Arteries of the axilla. 1 – axillary artery, 2 – anterior circumflex humeral artery, 3 – posterior circumflex humeral artery, 4 – axillary nerve, 5 – subscapular artery, 6a, 6b – muscular branches, 7 – thoracodorsal artery, 8 – circumflex scapular artery (Dissected by Zinovia Zorina).

The studied ecographic images of the upper limb offered us classical data that are relevant to the morphology and topography of the posterior circumflex humeral artery and no anatomical variations were identified, while the study of the angiographies offered us possibilities in their recognition. A variation model of the posterior circumflex humeral artery was identified at 3 upper limbs: 2 from the right side (one of male gender, the second of female gender) and 1 from the left side, of male gender.

The apparition of different anatomical variations of the posterior circumflex humeral artery can be caused by genetic factors or by the disturbance in the development of the primitive arterial axis of the upper limb, that takes place during the embryonic stage, also we can mention local factors like the fetus position, early limb movements and unusual muscular development [10].

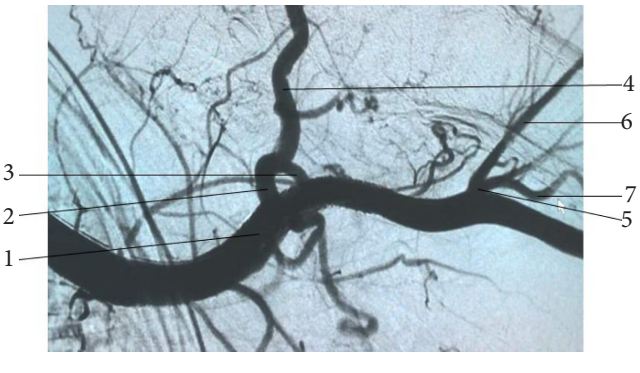

Fig. 2. Selective angiography. 1 – axillar artery, 2 – common trunk I, 3 – subscapular artery, 4 – posterior circumflex humeral artery, 5 – common trunk II, 6 – anterior circumflex humeral artery, 7 – deep brachial artery.

In the first case of a left upper limb of male gender, the origin of the posterior circumflex humeral artery and sub-

scapular artery was from a common trunk, that was emerging from the subpectoral portion of the axillary artery, and inferiorly, a common trunk from the brachial artery that was splitting into the anterior circumflex humeral artery and the profound brachial artery was identified (fig. 2).

The common arterial trunks appear because of the vasculogenesis disturbances, the consequences that are following, most commonly are related to unusual paths of the primary vascular plexus and fusion of the blood vessels that usually are solitary [11].

According to the literature data, the posterior circumflex humeral artery very often forms a common trunk with the subscapular artery, in 15.7 – 26% of cases [12].

In the second case, at the right upper limb of male gender was identified a common trunk that splits into the anterior and posterior circumflex humeral. This trunk has its origin from the third portion of the axillary artery, from its lateral hemicircumferince, next to the subscapular artery. The anterior circumflex humeral artery had a smaller caliber and a sinuous ascendant path (fig. 3).

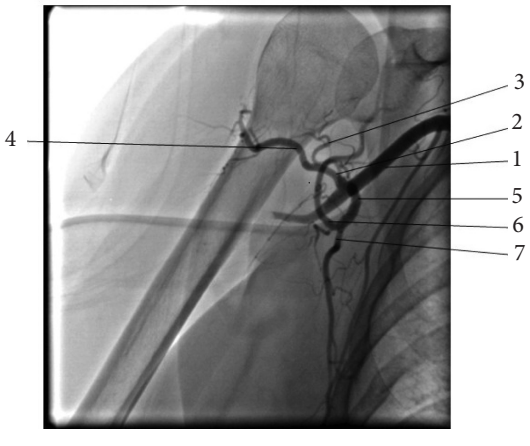

Fig. 3. Selective angiography. 1 – axillary artery, 2 – common trunk, 3 – anterior circumflex humeral artery, 4 – posterior circumflex humeral artery, 5 – subscapular artery, 6 – circumflex scapular artery, 7 – thoracodorsal artery.

Saeed M. [13] reported about the existence of a common trunk that splits into the posterior and anterior circumflex humeral arteries and the subscapular arteries in 3.8% of cases, and Astik R. [14] mentioned about a higher incidence of this variant for the female gender.

Mahendra K. [15] describes a common trunk, identified at the left upper limb of a male cadaver of adult age during a routine dissection that emerges from the subpectoral portion of the axillary artery and branches into the lateral thoracic artery, the thoracoacromial artery and subscapular artery. The subscapular artery is also branching into circumflex scapular artery, thoracodorsal artery and the posterior and anterior circumflex humeral arteries. In the third case, on a right upper limb of female gender was identified a common trunk that emerges from the postscalenic portion of the subclavian artery that subsequently splits into the posterior circumflex humeral artery and the profound brachial artery. The path of the posterior circumflex humeral artery before the quadrilateral space was straight, after that it was sinuous (fig. 4).

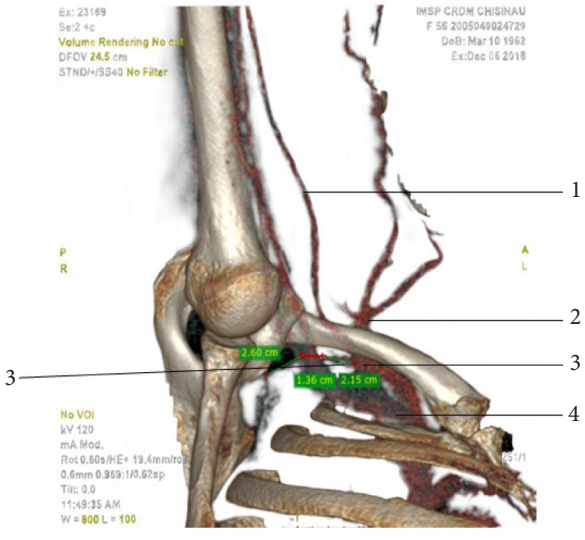

Fig. 4. Angiography with compurterized tomography. 1 – profound brachial artery, 2 – thyrocervical trunk, 3 – common trunk, 4 – subclavian artery, 5 – posterior circumflex humeral artery.

In specialized literature the presence of such a trunk that had a diameter of 0.5 cm, was reported by Rajani Singh into a study of case [16].

By Kovalevich K. [12] reports, the posterior circumflex humeral artery is a branch of the axillary artery only in 40 – 62.8%, in other cases different origin variations are present.

The 3D reconstruction of the arteries offered us the possibility to perform a morphometry of the common trunk and its branches from an anterior and posterior aspect, measuring the length, diameters and their angle of emergence (tab. 1).

Table 1

Morphometric parameters of the variational arteries in the axillar region

N/o	Morphometric parameters	ScvA	CT	PCHA	PBA
1.	Length, cm	5.01	3.75	4.48	14.42
2.	Internal diameter, cm (anterior aspect)	1.26	0.52	0.38	0.41
3.	Internal diameter, cm (posterior aspect)	1.36	0.48	0.33	0.49
4.	Angle of emergence, º		90	85	55

Note: ScvA – subclavian artery; CT – common trunk; PCHA – posterior circumflex humeral artery; PBA – profound brachial artery.

The number of variations of the posterior circumflex humeral artery was identified at 2 right upper limbs of male gender. On one of these limbs, 2 posterior circumflex humeral arteries were identified; the first artery had its classical origin, from the subpectoral portion of the axillar artery, the second – from the profound brachial (fig. 5).

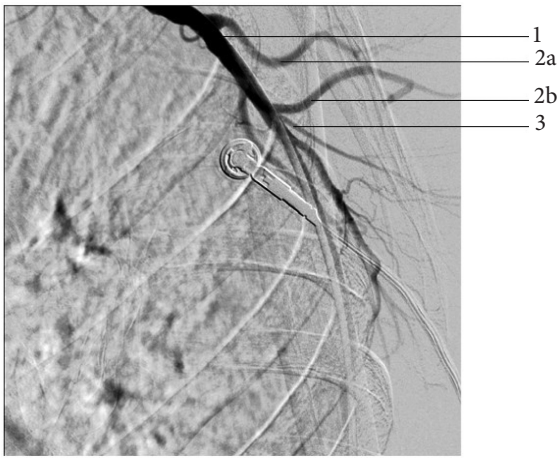

Fig. 5. Selective angiography. 1 – axillar artery, 2a, 2b – posterior circumflex humeral arteries, 3 – deep brachial artery.

Daimi S. [17] reports about a double posterior circumflex humeral artery in 1.28% of cases, and Gadzhieva F. [18] about its origin from the subscapular – in 23% of cases.

The exposed data from the specialized literature reports about the laterality character of the anatomical variations of the axillary artery and its branches, it relates to the fact that they are found 2 times more often on the right upper limb opposing the left upper limb and 2.5 times more often only on one upper limb rather than on both [19, 20, 21].

Conclusions

1. The posterior circumflex humeral artery has a wide range of anatomical variations.

2. More frequently the anatomical variations are found on the right upper limbs of male gender.

3. The obtained data will become useful in the approaches to the axillary artery during arteriographies and also in choosing the right surgical intervention tactic on this topographical region.

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Effect of induction of general anesthesia with propofol and fentanyl on hemodynamic response

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Abstract

Background: Induction of general anesthesia with propofol and fentanyl is frequently associated with changes in arterial blood pressure and heart rate. At present, there are no clinical studies investigating the relation between baseline cardiac autonomic tonus and cardiovascular instability after induction of general anesthesia with propofol and fentanyl.

Material and methods: A randomized prospective study was performed with approval of Ethic Committee. Written informed consent was obtained from all patients. We enrolled in the study 47 ASA physical status I–II patients scheduled for elective surgical procedures. Heart rate variability by Holter ECG, arterial blood pressure (systolic, diastolic, mean), and heart rate were measured at baseline, after premedication, as well as after induction of general anesthesia with propofol 2.5mg/kg and fentanyl 1.0 mkg/kg.

Results: our research revealed that increased baseline cardiac parasympathetic tonus was a risk factor for development of sinus bradycardia (OR = 21.0 (95%CI 3.9-112.8, p<0.0002) and sinus bradycardia associated with arterial hypotension (OR = 19.2 (95%CI 4.1-88.6, p<0.0001).

Conclusions: Induction of general anesthesia with propofol and fentanyl was associated frequently with arterial hypotension and sinus bradycardia. Increased cardiac parasympathetic tonus at rest represents a risk factor for development of arterial hypotension and sinus bradycardia after administration of propofol and fentanyl for induction of general anesthesia.

Key words: arterial hypotension, sinus bradycardia, cardiac autonomic tonus.

Introduction

Propofol is a frequently used hypnotic for sedation as well as for induction of general anesthesia. But, when this drug is injected rapidly it can lead to hemodynamic instability, mainly to arterial hypotension and changes in heart rhythm. Both, sinus tachycardia and sinus bradycardia were reported after administration of propofol for sedation or for induction of general anesthesia [1-3]. Many mechanisms have been involved for explanation of propofol induced arterial hypotension, mainly direct depression of myocardium, reduced peripheral vascular resistance caused by direct vasodilatory effect of the drug, reduction of preload and afterload. The studies anyway, showed controversial results, and any of these factors could be imputed for hemodynamic instability after administration of propofol for sedation or for induction of general anesthesia [2-7]. Administration of propofol for moderate or deep sedation is frequently associated with a significant decrease in mean blood pressure. This hypotensive effect of the drug can be caused by reduction of sympathetic cardiac tonus or disturbances in baroreceptor-mediated cardiac activity [4-6]. Similar to other intravenous anesthetic agents like benzodiazepines and barbiturates, propofol exerts its hypnotic actions by activation of the central inhibitory neurotransmitter – gamma-aminobutyric acid (GABA) [7].

Most often propofol administration is combined with opioid (fentanyl or sufentanyl). This combination has a beneficial effect as can reduce the requirement in myorelaxants in the course of general anesthesia. But, on the other hand the combination between propofol and opioid can enhance the risk for arterial hypotension and bradycardia in patients [8,9]

Heart rate variability (HRV) is a noninvasive electrocardiographic marker which reflects the sympathetic and parasympathetic influences on sinus node of the heart. In other words, HRV analysis shows the baseline autonomic function of the heart. Measurements of HRV are noninvasive, and highly reproducible. They may be performed on the basis of 24 hour Holter recordings or on shorter periods ranging from 0.5 to 5 minutes particularly in the field of dynamic electrocardiography. Most studies in anesthesia and intensive care which used the HRV for analysis of changes in sympathetic-parasympathetic balance of the heart performed the 5 minutes analysis of HRV [10,11].

The purpose of this clinical research was to find a relationship between autonomic heart tonus at rest and the risk for development of arterial hypotension and changes in heart rate after induction of general anesthesia with propofol and fentanyl.

Material and methods

We performed a prospective randomized study to evaluate the relationship between baseline cardiac autonomic tonus of the heart and the risk for development of cardiovascular instability after induction of general anesthesia with fentanyl and propofol. The study protocol was approved by the Ethic Committee of the State University of Medicine and Pharmacy "Nicolae Testemițanu", Chișinău (No.20, 2.02.2016).

Between March 2017 and September 2017, ASA physical status I-II patients aged less than 60 years (to exclude age-related changes of HRV) scheduled for elective surgeries with normal sinus rhythm on ECG were enrolled in the study. We obtained an informed consent from all research participants. Patients with diseases that could affect autonomic cardiac regulation (endocrine, neurological, cardiovascular diseases) were excluded from the study.

In the operating room, the patients were monitored (Holter ECG (Holter TLC 5000, USA)), non-invasive blood pressure, pulse oximetry and capnography). Baseline heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and respiratory rate were recorded. During induction of general anesthesia, oxygen was delivered to maintain SpO_2 above 95%. All patients received 10 ml/kg of crystalloid before induction of anaesthesia.

HRV parameters, HR, SBP, DBP and respiratory rate were recorded at baseline (T1), 5 minutes after premedication with Fentanyl 1.0 mkg/kg (T2) and 5 minutes after induction of general anesthesia with propofol 2.5 mg/kg and fentanyl 1.0 mkg/kg (T3). If after receiving propofol and fentanyl, patients developed bradypnea or apnea, the mask ventilation was initiated at a rate of 14-16 breaths/min and tidal volume of 7-8 ml/kg, an important requirement for correct registration and analysis of HRV.

HRV parameters were analyzed by Holter computerized system. HRV was interpreted according to the recommendations of the *Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology* [12]. In this clinical study HRV was used for assessment of autonomic heart tonus at rest.

Sinus tachycardia was considered in any patient who had a heart rate more than 100 beats/min, and sinus bradycardia – a heart rate less than 60 beats/min.

We considered systolic arterial hypertension when SBP was more than 140 mmHg or an increase in SBP of more than 20% from baseline values, systolic arterial hypotension – when SBP was less than 90 mmHg or a decrease in SBP more than 20% below baseline, and diastolic hypotension – when DBP was less than 60 mmHg or a decrease in DBP more than 20% below baseline.

Statistical analysis of the results was performed using GraphPad Prism 6 (GraphPad Software, San Diego, California, SUA). Values with parametric distribution were analyzed by t-pair and repeated measures ANOVA tests. Values with non-parametric distribution were analyzed by Wilcoxon and Friedman tests. The Fisher's exact test was used to compare categorical variables. Results are expressed as 95% confidence interval of odd ratio (parametric data) and median with interquartile range (IQR, non-parametric data). A p value of less than 0.05 was considered statistically significant.

Results and discussion

The study group consisted of 47 patients (26 females and 21 males), aged 37.5±11.9 years. The mean body mass index was 24.6±3.4 kg/m^2 (it ranged between 16.1 and 30.0 kg/m^2).

Holter heart rate variability analysis revealed that in baseline 38.3% of patients were with enhanced sympathetic tonus of the heart and 38.3% – with enhanced parasympathetic tonus of the heart. Another 23.4% of patients presented with heart eutonia (fig. 1).

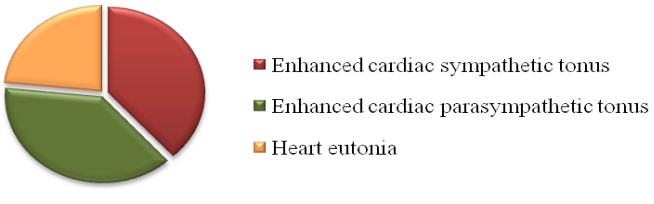

Fig. 1. Structure of the study group in function of vegetative tonus of the heart at rest.

There were no significant changes in SBP, DBP, MAP and HR after premedication with fentanyl (1.0 mkg/kg), but after induction of general anesthesia with propofol 2.5 mkg/kg and fentanyl 1.0 mkg/kg, SBP decreased by

Table 1

Changes in blood pressure and heart rate after premedication and induction of general anesthesia

Parameters	T1	T2	T3	p
SBP	138.9 (134.0-143.7)	133.9 (129.6-138.1)	99.7 (95.9-103.5)	0.001
DBP	85.4* (81.5-89.2)	79.0* (75.5-82.5)	51.1* (48.6-53.5)	0.001
MAP	103.1* (98.9-107.3)	97.7 * (94.1-101.3)	67.9*` (65.5-70.4)	0.001
HR	73.1 (69.9-76.4)	72.8 (69.4-76.1)	61.4 (59.1-63.6)	0.001

*Blood pressure and HR values are represented as mean and 95%CI or as median and interquartile range for data with nonparametric distribution.

25.5% (from 133.9 mmHg at T2 to 99.7 mmHg at T3; p=0.001), DBP – decreased by 35.5% (from 79.0 mmHg at T2 to 51.1 mmHg at T3; p<0.001), MAP – decreased by 30.5% (from 97.7 mmHg at T2 to 67.9 mmHg at T3; p<0.001), and HR – decreased by 15.7% (from 72.8 beats/min at T2 to 61.4 beats/min at T3; p<0.001), (tab. 1).

After induction of general anesthesia with propofol and fentanyl most patients developed systolic-diastolic or diastolic hypotension (41 patients – 87.2%) and sinus bradycardia (24 patients – 51.1%) (fig. 2, fig. 4). More frequently there was attested diastolic hypotension (35 patients – 74.5%), only in 6 patients (12.8%) there was found systolic-diastolic hypotension. Minimal SBP was 69.0 mmHg, minimal DBP was 37.0 mmHg and minimal registered MAP was 49.0 mmHg. More frequently systolic-diastolic hypotension or diastolic hypotension was registered at 3-5 minutes (4.6±0.3 min) after administration of propofol and fentanyl. Arterial hypotension was corrected with fluids, and none of the patients required vasopressor support. In the study group only in 3 patients (6.4%) was found arterial hypertension. It is worth mentioning the fact that in all these patients arterial hypertension was present only the first 1-3 minutes (1.1±0.6 min) after administration of propofol and fentanyl. Maximal SBP was 169.0 mmHg, maximal DBP was 109.0 mmHg, and maximal registered MAP was 134.0 mmHg. All these 3 patients who developed arterial hypertension after induction of general anesthesia presented enhanced sympathetic tonus of the heart at rest.

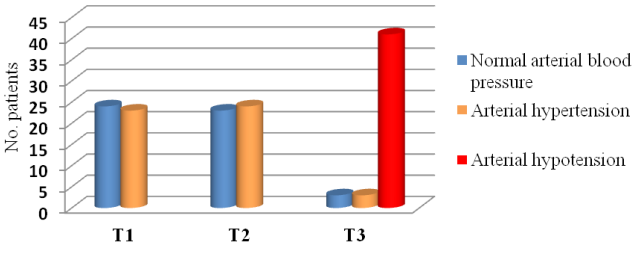

Fig. 2. The number of patients with normal BP, arterial hypertension and arterial hypotension at rest, after premedication and induction of general anesthesia.

It is important to remark the fact that out of 41 patients who developed arterial hypotension after induction of general anesthesia, in 18 patients (43.9%) was attested enhanced parasympathetic tonus of the heart at rest. Holter ECG analysis revealed that all 18 patients with enhanced cardiac parasympathetic tonus in baseline developed arterial hypotension after administration of propofol and fentanyl. Other 15 patients (36.6%) who developed arterial hypotension were with enhanced sympathetic tonus of the heart at rest, and 8 patients (19.5%) – with heart eutonia at rest.

Fisher's exact test of the relation between presence of enhanced parasympathetic tonus of the heart at rest and the risk for development of arterial hypotension after administration of propofol and fentanyl for induction of general anesthesia revealed: Odds ratio – 10.2 (95%CI 0.54-19.8)

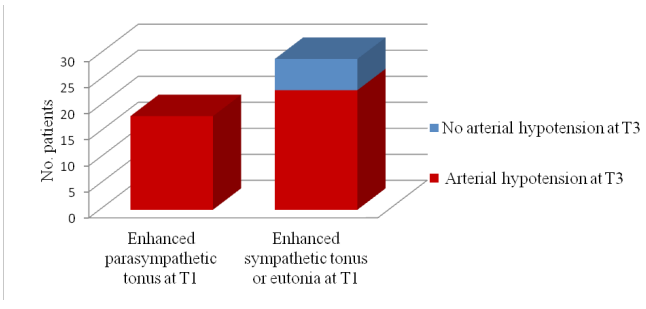

Fig. 3. Relation between autonomic cardiac tonus at rest and development of arterial hypotension after induction of general anesthesia with propofol and fentanyl.

(p=0.06), with sensitivity – 1.0 (95%CI 0.8-1.0), specificity – 0.2 (95%CI 0.08-0.39). Even if, OR and sensitivity are high, specificity is reduced, such enhanced parasympathetic tonus of the heart in baseline doesn't represent a risk factor for development of arterial hypotension after administration of propofol and fentanyl (fig. 3).

After administration of propofol and fentanyl for induction of general anesthesia there were found changes of HR on ECG. Most patients (51.1%) developed sinus bradycardia (fig. 4). Minimal HR registered by Holter ECG was 43/min. Minimal HR was registered at 3-5 minutes (4.1±0.6 min) after injection of propofol and fentanyl. Sinus tachycardia was registered in 3 patients (6.4%) from the study group. All these 3 patients presented enhanced sympathetic tonus of the heart at rest. Maximal HR registered by ECG Holter was 116/min, and was attested most frequently at

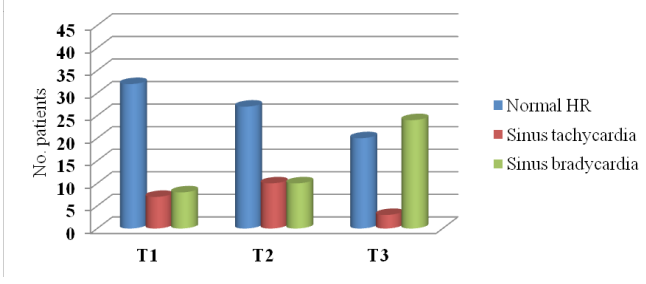

Fig. 4. The number of patients with normal HR, sinus tachycardia and sinus bradycardia at rest, after premedication and induction of general anesthesia.

It is worth mentioning that most patients (66.6%) who developed sinus bradycardia after administration of propofol and fentanyl had increased parasympathetic heart tonus at rest. Sixteen of the 18 patients with enhanced baseline parasympathetic tonus of the heart developed sinus bradycardia after administration of fentanyl and propofol. On the other hand, only 4 of the 18 patients with enhanced basal sympathetic cardiac tonus, and 4 of the 11 patients with heart eutonia at rest developed sinus bradycardia after induction of general anesthesia.

It can therefore be concluded that enhanced baseline parasympathetic tonus of the heart is a risk factor for development of sinus bradycardia after administration of propofol and fentanyl:

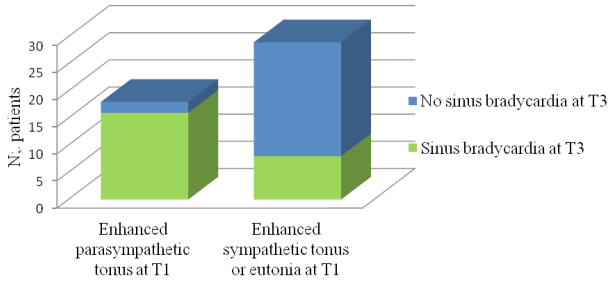

Fig. 5. Relation between autonomic cardiac tonus at rest and development of sinus bradycardia after induction of general anesthesia.

Odds Ratio – 21.0 (95%CI 3.9-112.8; p<0.0002); sensibility 0.89 (95%CI 0.65-0.99) and specificity 0.72 (95%CI 0.53-0.87) (fig. 5).

In 21 patients from the study group (44.7%) after injection of propofol and fentanyl was attested arterial hypotension associated with sinus bradycardia. It is important to mention the fact that Holter ECG analysis revelead that out of these 21 patients in 15 (71.4%) was attested enhanced parasimpathetic cardiac tonus at rest. On the other hand, only 2 of the 18 patients with enhanced baseline sympathetic cardiac tonus, and 4 of the 11 patients with heart eutonia at rest developed sinus bradycardia associated with arterial hypotension after induction of general anesthesia.

Fisher's exact test of the relation between cardiac autonomic tonus at rest and the risc for development of arterial hypotension associated with sinus bradycardia revealed: Odds Ratio – 19.2 (95%CI 4.1–88.6; p<0.0001); sensitivity – 0.83 (95%CI 0.58-0.96) and specificity – 0.79 (95%CI 0.60-0.92) (fig. 6). It can therefore be concluded that enhanced parasympathetic tonus of the heart at rest is a risk factor for development of sinus bradycardia associated with arterial hypotension after administration of propofol and fentanyl for induction of general anesthesia.

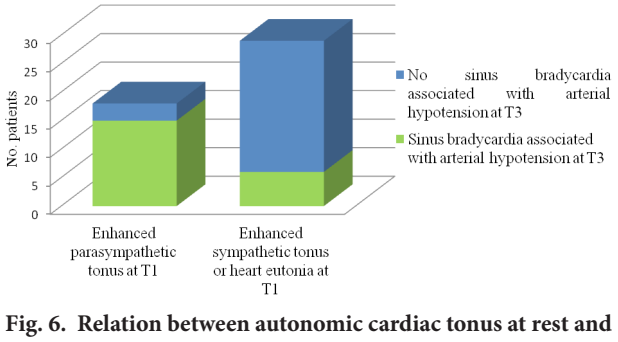

Fig. 6. Relation between autonomic cardiac tonus at rest and development of arterial hypotension associated with sinus bradycardia after induction of general anesthesia

Discussion

Propofol is an intravenous hypnotic agent, which commonly is used for anesthesia induction due to rapid onset, short duration of action, anti-nausea and vomiting effect and feeling comfortable after surgery. The most prominent effect of propofol is a decrease in arterial blood pressure during induction of anesthesia and is associated with a decrease in cardiac output, stroke volume, and systemic vascular resistance. Furthermore, propofol induces severe vasodilation while the effects of myocardial depression are not exactly clear. Vasodilation occurs in both venous and arterial circulation, which leads to reduced preload and afterload [1, 5, 7, 13, 14].

Although there are many studies that evaluated the effect of propofol and fentanyl on cardiac autonomic nervous system using heart rate variability [2-6], the relation between cardiac autonomic tonus at rest and the risk for cardiovascular instability after induction of anesthesia with propofol and fentanyl have not been investigated before.

In the literature there are published several studies which compare the hemodynamic effects of propofol with other hypnotic drugs, most often from the group of barbiturates or benzodiazepines.

In a study by Frolich M.A. et al. which involved 60 healthy volunteers ASA I physical status, subjects received 4 dose level ranges of propofol to provide anxyolitic level to moderate sedative effect. Predicted propofol concentrations in this study were 0.1, 0.2, 0.4 and 0.8 mkg/kg. A significant dose dependent blood pressure reduction was in the propofol group compared to midazolam group. Significant decreases in blood pressure were at propofol concentration 0.4 and 0.8 mkg/kg [13].

In another prospective, double-blinded, randomized clinical study by Kilic E. et al., proved that combination of alfentanil (10.0 mkg/kg) with propofol 0.7 mg/kg for sedation in upper gastrointestinal system endoscopy in morbidly obese patient is more frequently combined with bradycardia and arterial hypotension than combination of propofol with ketamine [8]. Chidambaran V. et al. conducted a study to evaluate total intravenous anesthesia with propofol and fentanyl in obese children and adolescents (aged 9-18 years) during laparoscopic surgery. Propofol was administered at a standardized infusion rate of 1000 μg/kg/min combined with fentanyl 50-100 μg. In all patients were attested hemodynamic changes and drop in SBP, DBP and MAP was more than 20% from the baseline value [9].

In a recent study, aiming to compare the effect of the ketamine-propofol mixture (ketofol) and propofol on the insertion conditions of laryngeal mask airway and hemodynamic stability in pediatrics patient (age ranging from 2 to 15 years) physical status ASA I and II, and undergoing elective surgical procedures, Aberra B. et al., proved a significant decrease in blood pressure and heart rate in the group of patients who received propofol (3.5 mg/kg) compared with the group of patients who received a mixture of propofol and ketamine [14]. Soleimani A. et al. proved that induction of general anesthesia in patients with left ventricular dysfunction with diazepam is safer than induction with propofol in term of hemodynamics. In this study in the group of patients who received propofol (1.5 mg/kg) after premedication with fentanyl (2.0 μg/kg) the decrease in SBP, DBP and MAP was significantly grater than in the group of

patients who received midazolam or etomidate for induction of general anesthesia [15]. Another group of authors [16], compared the hemodynamic effects of propofol with the hemodynamic effects of etomidate when used for induction of general anesthesia in patients undergoing coronary artery bypass grafting /mitral valve and aortic valve replacement surgery. In this study all patients received fentanyl 2.0 mkg/kg 3 minutes prior to induction. The dose of propofol for induction of general anesthesia was 2.0 mg/kg. There was significant decrease in SBP, DBP and MAP between the groups after induction, after intubation and 5 min postintubation. There was significant decrease in cardiac output and cardiac index in propofol group when compared to baseline values after induction, after intubation and 5 minutes after intubation, but not in etomidate group.

There are several studies which compare the hemodynamic effects of propofol used for sedation in patients undergoing gastrointestinal endoscopy [17-19]. In a prospective, randomized, double-blind study by Usman S. et al. [17] was compared the hemodynamic effects of propofol (1.0 mg/kg propofol followed by repeated doses of 10 to 20 mg propofol intravenously) with that of midazolam associated with meperidine (0.4 mg/kg meperidine intravenously followed three minutes later by 0.05 mg/kg midazolam intravenously) in 100 patients scheduled for diagnostic upper gastrointestinal endoscopy. The authors observed significantly more adverse cardiopulmonary events with propofol compared to meperidine/midazolam (20% vs. 4%, p = 0.025). Hypotension incidence was significantly higher in the propofol group compared to the meperidine/midazolam group (12% vs. 0%, p=0.027). In this study, the authors found that midazolam/meperidine is superior to propofol with respect to the occurrence of adverse cardiopulmonary events, particularly hypotension. Tsai H. C. et al. performed a meta-analysis of randomized clinical trials aiming to compare the efficacy and safety of propofol and midazolam for sedation in cirrhotic patients undergoing endoscopy. Five studies between 2003 and 2012, including 433 patients, were included. In four of the selected randomized clinical trials arterial hypotension was more frequently in the patients who receive propofol than in patients who receive midazolam. In three of the selected studies was evaluated the incidence of bradycardia based on a heart rate (HR) < 55 beats per minute. The incidence of bradycardia was 6% (9/150) in the propofol group and 2.86% (4/140) in the midazolam group [18]. In another recent meta-analysis conducted to compare the efficacy and safety of midazolam and propofol in gastrointestinal endoscopy five randomized controlled trials involving 552 patients were included [19]. The conclusion of this meta-analysis was that propofol sedation for gastrointestinal endoscopy results in higher endoscopist satisfaction scores, but may increase the incidence of hypotension and bradycardia.

Another study examined the safety and effectiveness of the procedural sedation analgesia technique carried out in the emergency department. The research was done to compare the effectiveness and efficacy of moderate sedation of fentanyl (0.1 mkg/kg) combined with propofol (1.0 mg/kg) or midazolam (1.0 mg/kg). None of the patients in either group developed any adverse events during and after the procedures. No significant drops in blood pressure and heart rate were observed during and after the procedures. Even though a few parameters, such as MAP, SBP and DBP, dropped intra-procedure, these values normalized post-procedure, and the changes were statistically insignificant within and between the groups [20].

Most studies which analyze the hemodynamic effects of propofol alone or propofol associated with opioid (as in this study) proved that a drop in SBP, DBP, MAP and HR can develop. These hemodynamic changes can be present even after administration of propofol and fentanyl in doses lower than in this study. However, there is not a single clinical research which studied the relation between baseline cardiac autonomic tonus of the heart and the risk for development of arterial hypotension and changes of heart rate after administration of propofol and fentanyl for induction of general anesthesia. This study revealed that enhanced baseline cardiac parasympathetic tonus represents a risk factor for development of sinus bradycardia and arterial hypotension associated with sinus bradycardia after administration of propofol and fentanyl for induction of general anesthesia.

Conclusions

1. Induction of general anesthesia with propofol and fentanyl is frequently associated with arterial hypotension and sinus bradycardia.

2. Enhanced parasympathetic tonus of the heart at rest is a risk factor for development of sinus bradycardia and sinus bradycardia associated with arterial hypotension after injection of propofol and fentanyl.

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Antibiotic susceptibility and some persistence factors of Gram-negative bacilli isolated from trophic ulcers

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Abstract

Background: Infections that are difficult to treat might lead to high morbidity and mortality rates. In some infections, however, despite a proper antibiotic therapy, microorganisms might persist, under certain circumstances, and produce recurrent or chronic infections. It is a well-known fact that the persistence of microorganisms might influence their viability within the macro-organism, whereas the suppression of the microbial persistence via drug preparations might greatly reduce therapeutic duration. This study is aimed at assessing the antibiotic sensitivity and some factors, contributing to persistence of Gram-negative bacilli strains isolated from trophic ulcers.

Material and methods: Data were collected and examined from 128 samples of patients with trophic ulcers. The bacteriological examinations, factors determining the persistence and the antibiotic susceptibility of the isolated strains were carried out in accordance with the current method.

Results: 211 microbial strains were isolated. The identified microorganisms revealed a high taxonomic diversity, whereas Gram-negative bacilli made up 50.2%. Isolates showed multiple resistances to antimicrobial drugs in 76.4% of cases, 43.4% strains showed hemolytic, 88.7% – anti-lysozyme and 93.4% – anti-complementary activities, whereas 70.8% strains produced a detectable biofilm. The strains isolated from mixed infections exhibited a higher percentage of pathogenicity factors compared to those isolated from monoinfections.

Conclusions: Gram-negative bacteria showed great resistance to the antimicrobial drug tests and multiple persistence factors. The results of the study proved that trophic ulcers are difficult to treat, thus being a major problem, which requires coherent monitoring and control.

Key words: trophic ulcer, Gram-negative bacilli, antibiotic resistance, persistence factors.

Introduction

Infections may cause serious complications in patients with trophic ulcers that commonly lead to inappropriate treatment, long-term hospital stay, high morbidity and mortality rates within medical units [1].

Infected trophic ulcers may result from the interaction between the macroorganism and the microorganism inoculated at this level. This interaction is influenced by the level of its contamination and the immune status of the host organism [2]. The denuded tissue is contaminated with microorganisms from the skin microbiota or spread by foreign bodies [3]. The risk of developing infections is directly proportional to the dose of microorganisms, as well as deficiencies in both general and local body's immune defenses [4]. The critical concentration of the disease-causing pathogens at which the bacterial colonization might shift to infection is also related to the accumulation of pathogenicity factors within the tissue, produced by microorganisms (enzymes, toxins, etc.) [5].

Recently, a qualitative transformation has been recorded in some species of microorganisms involved in the infectious disease pathology, which tends to increase the incidence of mixed infections, due to a simultaneous exposure of certain etiological agents. Each of these species revealed a complex of pathogenicity factors, such as adherent, hemolytic, anti-lysozyme, anti-complementary, and anti-interferon activity, etc. [6].

Long-term persistence of microorganisms in trophic ulcers is due to multiple factors that inactivate the antimicrobial activity of the immune system. Therefore, it is advisable to study the persistence properties of microorganisms in purulent infections, since these are responsible for the elimination rate at the site of inflammation, as well as for the disease prognosis. The microbial persistence determines the length of time that pathogens can persist within the macroorganism, whereas its suppression via drug preparations may potentially weaken the infectious microorganisms [7, 8].

Studies, which have been reported across different countries, revealed a range of species isolated from trophic ulcers, as well as antibiotic susceptibility cases and an increased number of patients associated with multiple resistance, thus, suggesting that administration of empirical antimicrobial therapy might increase the chances of a treatment failure [9, 10].

In other instances, some species of microorganisms may produce biofilms, which show a far greater resistance to both treatment and immune effector actions. The biofilm represents a microbial complex, wherein the cells adhere firmly to each other or to various surfaces, surrounded by the cells' exopolysaccharide matrices and exhibiting a modified, as well as a different rate of gene transcription that of planktonic cells [11]. Microbial biofilms are responsible for chronic, persistent, difficult to treat infections [12].

Treatment of trophic ulcer is a challenging task for clinicians and remains a current and relevant issue [13].

As regarding to the aforementioned, this study was aimed at identifying the spectrum of microorganisms isolated from trophic ulcers, studying the antibiotic susceptibility of Gram-negative bacilli and determining the hemolytic, anti-lysozyme, and anti-complementary properties, as well as the biofilm-forming capacity.

Material and methods

The study was carried on 128 samples of trophic ulcers. The microbial strains involved in the process were isolated in pure cultures, under laboratory conditions, and subsequently identified by classical microbiological methods and Vitek2 Compact system (BioMerieux), based on the morpho-tinctorial, biological and biochemical properties.

Antibiotic susceptibility test of Gram-negative bacilli was carried out and interpreted according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) recommendations, using phenotypic methods (Kirby Bauer disc diffusion test, synergy test) [14]. The assessed antibiotic discs included ciprofloxacin (5mg), levofloxacin (5 mg), amikacin (30 mg), gentamicin (10 mg), aztreonam (30 mg) cefepime (30 mg), ceftazidime (30 mg), amoxicillin-clavulanic acid (30mg), imipenem (10mg), meropenem (10 mg), piperacillin (30 mg) and ampicillin (10 mg).

Strains that showed resistance to three or more antibiotic groups were considered poly- resistant ones [15].

The anti-lysozyme activity was determined according to the method described by Gordina E. et al. [16]. The tested strain was cultured on an agar slant for 18-24 hours at 37°C, then subcultured in peptone water and grown for 6 hours at 37°C. The culture was adjusted to 0.15 optical density in peptone water, which corresponds to $1x10^8$CFU/ml. Simultaneously, the lysozyme suspension was prepared in peptone water with a concentration of 12.5μg/ml. The use of a higher concentration of lysozyme inhibits the growth of microorganisms, whereas lower concentrations do not allow indicating this phenomenon. Then, 100μl of lysozyme broth at a concentration of 12.5μg/ml and 25μl of microbial suspension were dispensed to the wells of the plate used for enzyme immunoassay. 100μl of peptone water and 25μl of microbial suspension were added to the control wells (n=2). The culture was thermostated for 4 hours and the optical density was measured over 2 and 4 hours. The results were read by the ELISA reader and the optical density was measured at 600 nm wavelength (A600). The distribution of the strains according to the level of expression was performed according to the following criteria: low expression levels ($K<0.49$); medium expression levels (within the limits of $0.5\leq K\geq 2.49$) and high expression levels ($K>2.5$), where K stands for the coefficient of anti-lysozyme activity of the assessed strain.

Anti-complementary activity was determined by the method described by Bukharin O. et.al. [17]. A microbial suspension (the optical density of which corresponded to the McFarland turbidity standard 1.0) was inoculated with the inoculation loop on a 1.5% agar plate surface. The inoculated plates were thermostated at 37°C for 18-24 hours, in order to reveal the biological properties of the microorganisms. Afterwards, the cultured plates were exposed to chloroform vapors for 10 minutes and then covered with a second layer of 1.5 ml of agar and 1 μl of complement on a flat surface (trated in the hemolytic system until 50 HU/ml, 25 HU/ml and 12.5 HU/ml activity), so that the final complement concentration corresponds to 20; 10 and 5 UH/ml, respectively. Plates were incubated in the inverted position at 37°C for one hour in order to perform the anti-complementary activity of bacteria and vital products. Then, the plates were covered with a third 0.7% agar layer, containing 0.1 ml of bacterial suspension from indicator culture of *Escherichia coli* ГИСК 212 (optical density of microbial suspension corresponded to McFarland turbidity standard 0.5), exhibiting an increased sensitivity to the bactericidal action of complement system. Plates were incubated at 37°C for 18-24 hours to allow inactivation of complement by bacteria. Anti-complementary action was assessed based on the growth areas of indicated culture around bacteria, where the inactivation of the complement occurred.

Blood agar culture medium was used to study the hemolytic activity of the isolates [5]. The quantitative determination of biofilm-forming capacity of isolates from trophic ulcers was determined by a microtiter test [18]. Thus, 150μl of peptone water and 15μl of bacterial suspension were dispensed to a 96-well plate according to the McFarland turbidity standard 0.5 (1.5×10^8CFU/ml, respectively), previously harvested from 18- to 24-hour cultures on 5% blood agar. Duplicate laboratory tests were performed. The plates were then covered and incubated aerobically for 24-48 hours at 37°C. In order to assess the bacterial attachment to the inert substrate, the wells were rinsed five times in sterile saline solution and fixed with cold methanol for 5 minutes. Methanol was then removed; the dry plates were stained for 30 minutes with 0.1% crystal violet solution. The slide was then washed with tap water to remove the excess stain and the stained biofilm was resuspended with a 33% glacial acetic acid solution. Thus, the obtained suspensions were used to determine the optical density (OD), based on the absorbance spectrophotometer readings of stained suspensions at 490 nm (A490).

The cut-off optical density (ODc) is defined as the average OD of negative control + 3x standard deviation (SD) of negative control. The strains were tested for biofilm production and classified based on the adsorption of the Crystal Violet dye. The isolates were classified into four categories: non-adherent, the optical density lower than 0.056; poor adherent ($0.056<OD\leq 0.112$), moderately adherent ($0.112<OD\leq 0.222$) and strongly adherent, the optical density greater than 0.222.

Escherichia coli (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603) and *Acinetobacter baumannii* (ATCC 11778) reference strains were used for quality control. Statistical data analysis was carried out via EpiInfo 2000.

Ethical issues

The studied strains were obtained from routine analysis of clinical specimens. Sample collection did not involve direct contact with the patient, thus no consent was required. Permission to conduct the study was obtained from the Head of the Microbiology Laboratory. The study was conducted and approved by the ethics committee No 65/12.04.2017 of Nicolae Testemitsanu State University of Medicine and Pharmacy of the Republic of Moldova.

Results

Bacteriological examination was carried out on 128 samples collected from patients with trophic ulcers. A single species of microorganisms was isolated in 35.9% of cases, two and more species in 53.1% and no microorganisms were isolated in 10.9% of cases. A total of 211 microbial strains were isolated and identified. The most common strains isolated from trophic ulcers were the *Staphylococcus* (predominantly *S. aureus*), then enterobacteria (*Proteus* spp., *Klebsiella* spp., *Escherichia* spp.), non-fermenting bacilli *Pseudomonas* spp., *Acinetobacter* spp. and yeast-like fungi of the genus *Candida.*

Among the infections caused by a single microbial species, the most often involved was *Staphylococcus aureus* (41.3%), as well as other isolated species like *Proteus* (15.2%), *Staphylococcus haemolyticus* (10.9%), *Pseudomonas aeruginosa* (8.7%), *Acinetobacter baumannii* (8.7%), *Klebsiella pneumoniae* (8.7%) and *Escherichia coli* (6.5%). Mixed infections were caused by associations of strains like *S. aureus* and *P.aeruginosa* (23.1%), followed by associations of *S.aureus* and *A.baumannii* (20.5%). Association between two species was registered in 57.4% of mixed infections and three species associations were found in 42.6%.

In this study, 106 (50.2%) strains of Gram-negative bacilli were isolated, of which 61.3% were glucose-fermenting and 38.7% – glucose-non-fermenting.

The antibiotic susceptibility tests of Gram-negative bacilli strains, isolated from trophic ulcers, showed a high level of resistance to these drug preparations. *Enterobacteriaceae* strains exhibited a marked resistance to penicillins (100%), cephalosporins (87.7%), fluorquinolones (84.6%) and aminoglycosides (70.8%). Carbapenems proved to be the most effective antibacterial drugs (83.1% strains were sensitive to meropenem) against infections caused by enterobacteria (fig. 1).

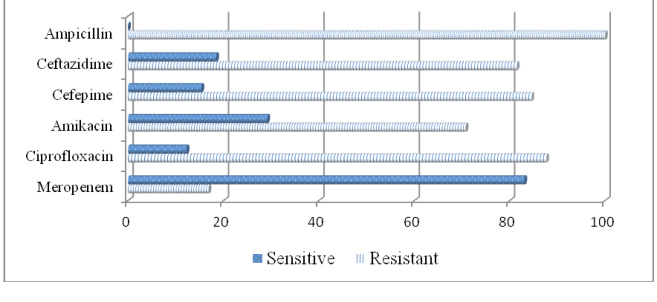

Fig. 1. Antibiotic susceptibility of *Enterobacteriaceae* strains (%).

Moreover, 21 (32.3%) extended-spectrum beta-lactamase producing strains (ESBL) have been identified during this study, which were sensitive to meropenem (76.2%), followed by amickacin (28.6%) and ciprofloxacin (19.0%).

Antibiotic susceptibility assessment of *P. aeruginosa* strains revealed a large number of multiple antibiotic resistant strains and only three strains (13.0%) were resistant to a single drug preparation. Of 23 strains, 20 (87.0%) were multidrug-resistant. Aminoglycoside was the most active agent tested against *P. aeruginosa* (47.8%). A high resistance level was observed in the following drug groups: penicillins (100%), cephalosporins (86.9%), monobactam (82.6%), carbapenems (78.3%) and fluorquinolones (73.9%) (fig. 2).

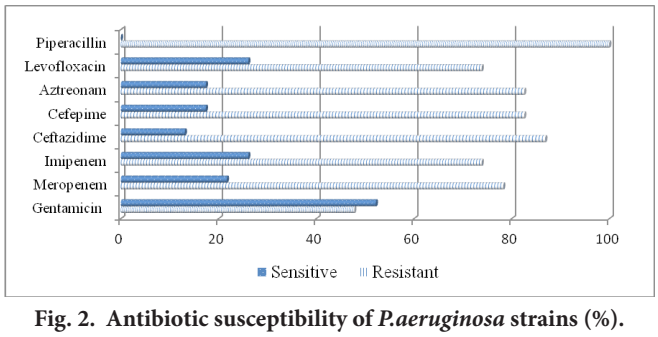

Fig. 2. Antibiotic susceptibility of *P.aeruginosa* strains (%).

Acinetobacter baumannii strains showed high resistance to most antibiotic groups. Carbapenems exhibited a higher level of sensitivity, including imipenem (72.2%) and meropenem (66.7%). Over 50% of the strains were resistant to aminoglycosides, fluorquinolones, third- and fourth-generation cephalosporins. Multiple antibiotic resistance was detected in 77.8% of strains and only 4 strains (22.2%) were sensitive to all antibiotics that were chosen for testing (fig. 3).

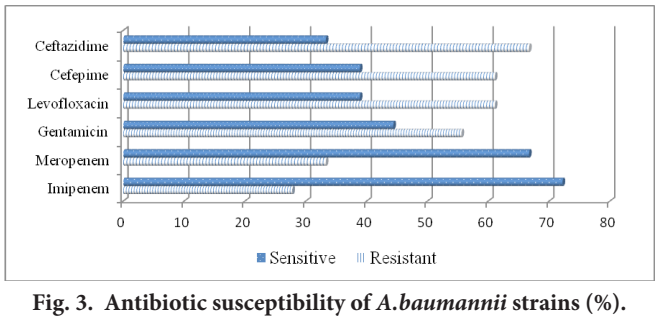

Fig. 3. Antibiotic susceptibility of *A.baumannii* strains (%).

The next stage of the study determined the level of expression of some persistence factors of Gram-negative bacilli isolated from trophic ulcers (tab. 1).

Hemolysin, which is an exotoxin, appeared to be one of the persistence factors leading to chronic infectious process [5]. Hemolytic activity was recorded in 46 (44.3%) strains of Gram-negative bacilli isolated from trophic ulcers.

Lysozyme was also determined as being a universal resistance factor of the macro-organism. It is a peptidoglycan-degrading enzyme, which commonly works in Gram-positive bacteria; however, Gram-negative bacteria might be also affected by increasing the permeability of the outer membrane and lipopolysaccharides [6]. Therefore, micro-

Table 1

Hemolytic, anti-lysozyme and anti-complementary activity of Gram-negative bacilli isolated from trophic ulcers

Species	Hemolytic activity		Anti-lysozyme activity		Anti-complementary activity		Total strain number
	Abs.	%	Abs.	%	Abs.	%	
P. mirabilis	12	42.8	25	89.3	26	92.9	28
P. vulgaris	0	0	2	100	2	100	2
K. pneumoniae	12	54.5	20	90.9	21	95.5	22
E. coli	4	30.7	7	53.8	12	92.3	13
P. aeruginosa	8	34.8	22	95.7	21	91.3	23
A. baumannii	10	55.5	8	100	17	94.4	18
Total	45	43.4	94	88.7	99	93.4	106

Table 2

***In vitro* biofilm-forming ability of Gram-negative bacillus strains isolated from trophic ulcers**

Biofilm-forming ability	*Proteus* spp.		*Klebsiella pneumoniae*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Acinetobacter baumannii*		Total	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Non-producing	4	13.3	3	13.6	4	30.8	6	26.1	5	27.2	31	29.2
Producing	26	86.7	19	86.4	9	69.2	17	73.9	13	72.2	75	70.8
– strong	6	23.1	13	68.4	3	33.3	7	41.2	4	30.8	33	39.3
– moderate	18	69.2	5	26.3	5	55.6	9	52.9	2	15.4	39	46.4
– weak	2	7.7	1	5.3	1	1.1	1	5.9	7	53.8	12	14.3

organisms tend to protect themselves against this enzyme in order to survive longer within the host organism. Anti-lysozyme activity was recorded in 94 (88.6%) out of 106 strains, whereas 12 (11.3%) were inactive. 24 (25.5%) strains showed a high level of expression of anti-lysozyme activity, 32 (34.1%) – a medium level and 38 (40.4%) strains – a low level of expression.

Another important factor responsible for microbial persistence within the infection site is the ability of bacterial cells to inactivate the complement system of the macro-organism [6]. Of the 106 Gram-negative bacilli strains involved within the study, 99 strains (93.4%) exhibited anti-complementary activity, of which 81 (81.8%) strains inactivated the complement at a concentration greater than 15 CH50/ml, 16 (16.2%) strains – at a concentration ranging between 5 to 15 CH50/ml and 2 (2.0%) strains – 5 CH50/ml. Only 7 strains (6.6%) did not inactivate the complement.

The data study of the anti-complementary activities in monocultures compared to isolated cultures in associations showed that the latter strains are often related to medium and high anti-complementary activity ($P < 0.05$).

Studies on the persistence factors of the isolated microorganisms showed that the level of expression is higher in isolates of mixed infections (1.0-1.5 times) compared to those in monoinfections ($P < 0.05$).

Of the 106 strains of Gram-negative bacilli isolated from trophic ulcers, 75 (70.8%) strains produced detectable biofilms (OD>0.112). As regarding the biofilm status, 33 (39.3%) isolates produced strong biofilms (OD>0.220), 39 isolates (46.4%) – moderate biofilms (OD 0.112-0.220) and 12 isolates (14.3%) – weak biofilms (tab. 2).

All Gram-negative bacilli strains isolated from trophic ulcers exhibited a high ability of biofilm formation (>70%).

The antibiotic resistance of biofilm-forming compared to non-biofilm-forming strains showed that biofilm-forming strains had a higher resistance to all groups of drugs tested.

Conclusions

1. The study of the spectrum of microorganisms isolated from the major trophic ulcers has shown the important roles of the genus *Staphylococcus*, followed by Gram-negative bacilli, yeast-like fungi of the genus *Candida* and *streptococci*.

2. Gram-negative bacilli strains isolated from trophic ulcers showed a marked resistance to the antimicrobial drugs tested.

3. The study of the persistence factors of gram-negative bacilli showed that the isolated strains have a range of abilities to inactivate the natural resistance mechanisms of the macroorganisms.

4. Understanding the bacterial persistence factors might allow selecting effective targeted therapies for controlling the microbial growth in trophic ulcers.

5. The study results show that treatment of trophic ulcers is both a challenging task and a major issue requiring current management strategies.

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REVIEW ARTICLE

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Antiviral therapy in chronic hepatitis C virus infection

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Abstract

Background: Hepatitis C is a disease with significant global impact. According to the World Health Organization there are 71 million people chronically infected with the hepatitis C virus. About 399.000 people die each year, mostly from cirrhosis and hepatocarcinoma. GT 1 and 3 are the most common causes of infection. Chronic HCV infection is accompanied by extrahepatic manifestations reported in up to 75% of patients, rapid development of hepatic fibrosis and accelerated time to cirrhosis and increased risk for liver failure, HCC and liver-related mortality. HCV therapy is one of the interventions necessary to reduce global burden of disease. Because of their high virological efficacy, ease of use, safety and tolerability, IFN-free, ribavirin-free, DAA-based regimens must be used in HCV-infected patients without cirrhosis or with compensated cirrhosis, including: treatment-naive patients: never been treated for their HCV infection, treatment-experienced patients: previously treated with PEG-IFNa + RBV. From pangenotypic drugs or drug combinations for treatment HCV in Europe are recommended: sofosbuvir/velpatasvir, sofosbuvir/velpatasvir/voxilaprevir and glecaprevir/pibrentasvir. Genotype-specific drugs sofosbuvir/ledipasvir, ombitasvir/paritaprevir/ritonavir, or grazoprevir /elbasvir are recommended for (GT 1, 4, 5 and 6).
Conclusions: The new direct-acting antiviral treatment regimens can be given to most patients with chronic hepatitis C virus infection, including those with liver cirrhosis, they have shown high efficacy, achieving sustained virologic response in over 90% of patients. DAA are well tolerated and have minimal side effects that do not require treatment discontinuation.

Key words: viral hepatitis C, direct-acting antiviral combination therapies.

Introduction

Prior to the identification of HCV, only a small number of Non-A Non-B hepatitis patients have been successfully treated by long-term administration of interferon-alpha. However, the molecular features of hepatitis C virus made it possible to develop specific treatment and laboratory tests for the diagnosis and monitoring of HCV infection [1].

According to WHO data, around 70 million people with HCV infection were estimated by the end of 2017 viz. approximately 2-2.5% of the world's total population [2]. In the republic of Moldova, the incidence rate makes up 4.5% of people aged 30-49 and more prevalent in males than in females [3]. According to the National Public Health Agency data, the analysis of the dynamics of viral hepatitis morbidity over the last 10 years shows a continuous increase in cases of chronic hepatitis and liver cirrhosis in the Republic of Moldova. The prevalence of chronic HCV tends to increase from 189.4 in 2008 to 441.5 cases per 100.000 persons in 2017. This tendency of HCV morbidity growth is due to the lack of specific HCV preventive measures, as well as high level of viral hepatitis detection. It also should be noted that liver cirrhosis mortality, including hepatitis C virus has decreased from 88.3 to 100 thousand individuals in 2009 to 62.7 per 100 thousand population in 2017 as a result of the National Program for Combating Hepatitis B, C and Delta viruses [4, 5]. Chronic hepatitis C virus infection is one of the most common causes of chronic liver disease and liver transplantation all over Europe and the US. It is difficult to determine the number of new HCV infections since the most acute cases have not been clinically identified. Less than 25% of acute HCV cases are detected based on clinical manifestations [6].

Antiviral therapy: historical background, objectives and adverse reactions to treatment

Prior to of HCV identification, interferon-alpha was considered as a potential therapy in non-A, non-B viral hepatitis, which contributed to both normalization of transaminases and improvement of liver histology in some patients [1]. Since the identification of HCV by Choo in 1989, it has become possible to quantitatively determine the level of serum HCV RNA and evaluate the effectiveness of long-term viz. obtaining a sustained virologic response (SVR).

The first studies showed that Interferon-alpha-2b 3MU administered 3 times / week for 6 months has achieved SVR in 8% of cases, and increased to only 12% when therapy continued up to 12 months [7, 8].

Ribavirin alone was used in the treatment of chronic

HCV for the first time in 1991. Ten patients were administered Ribavirin 1000-1200 mg / day for 12 weeks. There was a considerable decrease in transaminases during treatment, however these returned to initial level after drug withdrawal. Hepatitis C virus has not been completely removed in any of the patients [9]. An increase in the effectiveness of antiviral treatment was observed in combined therapy viz. Ribavirin and IFN-alpha. Two major randomized trials were conducted in order to compare the efficacy of combined IFN-alpha-2b + Ribavirin and Interferon-alpha-2b alone administered to naive patients. The first results established a sustained virological response in 47-50% of cases following a combined therapy, whereas only 13% patients with Interferon alone and 0% in patients with Ribavirin monotherapy [10, 11]. The synthesis of pegylated interferon (PEG-IFN) that contains pegylated proteins and shows a much longer half-life, has improved the pharmacokinetics of IFN, thus reducing the dosing intervals. Two types of IFN-PEG are currently available: PEG-IFNα-2b (PEG-Intron, Merck) and PEG-IFNα-2α (PEGASYS, Roche). A large multicenter study in the US has not established any significant difference between the two PEG-IFN and RBV regarding SVR [12].

The combined therapy of PEG-IFN α-2a (180 μg / kg / week) and RBV at a dose of 1000 mg was found to be effective if the body weight <75 kg or 1200 mg if the body weight ≥75 kg in patients with GT1 HCV [13]. In case the hemoglobin level drops below 10 g / dl, the dose of ribavirin should be reduced by 200 mg and discontinued if the hemoglobin level is below 8.5 g / dl [14, 15]. Several studies were conducted on chronic HVC patients following a PEG-IFN + RBV treatment during the period of 2011-2013 years. SVR was reported in 42-52% of GT1 patients within 48 weeks and 76-84 % of GT2 and GT3 individuals within 24 weeks [16, 17]. Treatment regimens with PEG-IFN-α and Ribavirin are still valid for countries with limited access to DAA.

A good treatment adherence is an important factor in achieving optimal outcomes in the antiviral treatment of chronic hepatitis C virus infection. Adherence to interferon and ribavirin treatment was particularly difficult, since these have been the only option available over the past two decades. Almost all patients treated with interferon and ribavirin exhibited adverse reactions that significantly influenced the treatment adherence. The most common side effects were reported in patients treated with pegylated interferon and ribavirin showing symptoms of general intoxication and asthenia – 66%, headache – 50%, nausea – 43%, insomnia – 39%, pyrexia – 35%, anemia(10 g / dl) – 34%, myalgia – 27%, neutropenia (<1000 cells / μl) – 26%, depression – 26%, irritability – 25% and rash – 22%. According to Seyam et al. [18], 6-10% of patients who administered interferon therapy for 48 weeks lost weight. Weight regained quickly after therapy discontinuation.

The most frequent psychiatric adverse events induced by IFN α include fatigue – 40-80%, sleep disturbances – 20-45%, irritability – 20-45%, cognitive disorders affecting concentration and memory – 20-30 %, depressive episodes – 20-70%, delirium, psychosis, mania – 1-3%, suicidal thoughts – 3-10%, and suicide attempts – 0-0.02% of individuals [19, 20]. Interferon therapy is accompanied by a 30-50% decrease in the absolute number of WBCs within the first 4-8 weeks from the treatment onset and a rapid increase after its withdrawal. Anemia (<10 g/dl) was reported in up to 20% of patients [13]. The dose of ribavirin should have been reduced in severe cases of anemia. Thrombocytopenia occurs within the first 2 months after the onset of IFN therapy, reducing the platelet count by 30-40% compared to values before the treatment. As a rule, the platelet count returns to its normal values after 4 weeks from the antiviral therapy withdrawal [21]. Combination of Boceprevir and Telaprevir protease inhibitors in the treatment of chronic HCV increased the SVR rate up to 75% in HBV naïve patients [22, 23] and up to 29-88% in patients pretreated with antiviral drugs [24, 25]. However, both protease inhibitors required combination with PEG-IFN and Ribavirin, since the monotherapy may develop a rapid resistance and severe side effects such as anemia.

A French cohort study was conducted on patients with compensated liver cirrhosis who underwent telaprevir or boceprevir regimen. Severe adverse reactions (anemia in up to 50% of patients), including sepsis, hepatic decompensation and even death were reported [26, 27]. Renal failure was registered in patients following a triple therapy with telaprevir and bocepravir. Impaired renal function was reported in patients with pre-existing risk factors for renal disorders associated with a more marked decrease in hemoglobin level that was reversible in most cases after the treatment withdrawal [28, 29]. Due to a wide range of side effects of interferon, telaprevir and boceprevir-based triple therapy, their production was discontinued after approval of interferon-free treatment with DAA.

Sofosbuvir, the first polymerase inhibitor, has substantially improved the therapeutic efficacy in patients with chronic HCV. Treatment with PEG-IFN / RBV and SOF for a period of 12 weeks showed 89% SVR rate. When administered in combination with interferon and ribavirin, it exhibited minor adverse effects and a limited drug interaction [30, 31].

However, due to its renal excretion, Sofosbuvir should cautiously be administered in patients with advanced kidney disease and glomerular filtration rate <30 ml / min, as well as in end-stage renal diseases, unless an alternative treatment is available.

Interferon-free antiviral treatment: indications, contraindications, new treatment regimens with direct-acting antivirals

As new therapeutic opportunities have emerged, each patient with confirmed chronic hepatitis C should receive antiviral treatment. Patients who undergo a HCV infection treatment may experience a higher quality of life and a lower risk of developing liver cirrhosis, hepatocellular carcinoma and mortality associated with hepatic and extrahepatic

pathology [32, 33]. According to EASL 2018 recommendations, patients with chronic HCV should be preferably administered direct-acting antivirals with IFN-free and RBV-free regimens. The treatment should be initiated as soon as possible in patients with advanced fibrosis and an increased risk of liver complications. Moreover, a priority for immediate treatment of patients with hepatitis C virus is the severity of extrahepatic manifestations. Another reason for an early initiation of treatment in all individuals diseased with HCV infection is its further prevention and transmission to people at high risk (intravenous drug users, men who have sex with men, women in childbearing age, hemodialysis patients, inmates in prisons) [15].

Antiviral treatment is not recommended in patients with short-life expectancy due to non-HVC comorbidities [15].

The predictive factors for the selected treatment regimen should be considered prior to initiating the antiviral therapy in order to increase the SVR rate. Even though, HCV genotype, liver fibrosis and steatosis, initial viral load, insulin resistance, age, gender, BMI, ethnicity, and HIV co-infection are the SVR predictive factors suggesting the initiation of PEG-IFN / RBV therapy, then most of these factors are much less important for DAA therapy. The HCV genotypes 1a and 1b, antiviral resistance and in most countries the treatment cost are the other important parameters for IFN-free therapy. However, the severity of the disease at time of treatment initiation is still important to assess [34, 12]. Although there is a number of available antiviral regimens, not all of them show a pangenotypic effect, thus genotyping is mandatory when initiating an antiviral treatment in order to select the optimal regimen and treatment duration of chronic HCV [35].

If the HCV RNA level has been and remains the most important predictive factor of SVR in PEG-IFN + RBV treatment, at present, when new DAA regimens have been used, the HCV load does not appear to have a significant predictive value. Concomitantly, according to 2018 EASL recommendations, RNA-HCV concentration (<600.000-800.000 IU/ml) is a condition for reducing treatment duration in naive non-cirrhotic patients who initiate IFN-free treatment with sofosbuvir and ledipasvir [36, 37].

According to 2018 EASL guidelines, the viral load assessment is recommended only prior to treatment and at 12 or 24 weeks after the antiviral therapy ceases. Instead of HCV RNA, the HCV core antigen may be performed if HCV RNA tests are not available [15]. At the same time, quantitative HCV-RNA testing is recommended by the AASLD/IDSA guidance on week 4 of DAA therapy to monitor the patient compliance [48].

Interleukin IL28B plays an important role as a predictive factor for SVR in PEG-INF / RBV / IP treatment. IL28B has a much greater significance than the HCV load. IL28B-related data explain the SVR difference in PEG-INF / RBV treatment among different ethnic groups, such as reduced SVR in African and American patients, and a high SVR in Asian patients. Female gender, initial viremia of <6 log10 IU/ml and body mass <30 kg/m^2 are additional factors that may influence the SVR in SOF / LDV therapy. Patients with HCV GT1b respond better to some approved DAA therapies. The stages of liver damage and previous experience with PEG-IFN + RBV are important predictors in treatment response [38].

The emergence of DAA therapies has given rise to another major problem viz. developing drug-resistance. Patients receiving first-generation HCV-protease inhibitors boceprevir and telaprevir developed resistance within a few days. If resistance develops, it is not known yet how long it will persist and what are the significant consequences for future therapies. Some studies suggest that most IP-resistant variants revert to wild type within 1-2 years after the completion of treatment [39].

The combination of different DAA classes might solve out the problem of resistance. SOF has a very high resistance barrier [40]. Combined SOF and NS5A inhibitor (SOF / DCV or SOF / LDV or SOF / VEL) exhibit a SVR > 90%. However, according to the studies, NS5A-associated resistance may become a problem within the clinical practice.

Several studies on initial resistance prior to treatment initiation with NS5A inhibitors have been performed. The resistance made up approximately 16% in SOF / LDV group [39] and 20% in the GZR / EBR-treated patients [41]. The resistance levels have no impact on SVR in HCV GT1b patients, though it may be significant in GT1a and GT3 cases, particularly if other negative predictors (previous non-responder or advanced cirrhosis) are present. For these reasons, there is no point in assessing the resistance level prior to treatment initiation via first-line preparations; this should be done when selecting the optimal therapy unless the combined DAA therapy has failed.

Interferon-free direct-acting antiviral therapy has become available in many countries since 2014 and has substituted the standard interferon therapy. DAAs have shown a much better efficacy, a substantially improved tolerance and shorter treatment duration compared to interferon therapy [42, 15].

The DAA groups are as following NS3 / 4A protease inhibitors, NS5B polymerase inhibitors and NS5A replication complex inhibitors. The combination of at least two of these three major drug classes results in ≥95% of SVR in just 8-12 weeks of treatment [42]. Treatment options are different across the world, as not all countries have access to new treatment regimens, whereas the generic preparations [43] are available in few countries.

Since 2016, in the Republic of Moldova, the National Treatment Program of Viral Hepatitis has developed a program on antiviral treatment with DAAs of chronic HVC that has become available now. Two regimens were approved: sofosbuvir + ledipasvir and sofosbuvir + daclatasvir.

Sofosbuvir (SOF) is an all-genotype NS5B polymerase inhibitor with high resistance barrier that is combined with other antivirals. Sofosbuvir alone is not allowed. The combination of sofosbuvir with NS5A-daclatasvir (DCV) or ledipasvir (LDV) inhibitors has reached SVR in over 90% of cases [38, 44].

The SOF + LDV treatment regimen is available in a single tablet containing SOF (400 mg) and LDV (90 mg). This once-daily taken preparation is an advantage since it improves the treatment adherence. According to the EASL 2018 guide, SOF / LDV is recommended for patients infected with genotype 1, 4, 5 and 6 and is not recommended for patients with genotype 2 and 3. It should be used with caution in patients with chronic kidney disease where GFR <30 ml / min, unless other recommended therapeutic regimens are available and can be used without restrictions in patients with decompensated cirrhosis.

In patients GT1 chronic HCV-infected patients without cirrhosis, the treatment with SOF/LDV for 12 weeks reached a SVR of 96%, while treatment for 24 weeks – in 99% cases.

Most studies conducted in naive patients with HVC GT 2 and 3 [45] and treated with SOF/LDV experienced reduced SVR rates (64-68%), and those who received SOF/LDV regimen + RBV result in SVR of 97 – 100%. Based on these results, the SOF/LDV treatment regimen is not recommended in patients with HVC GT 2 and 3 because there are more optimal treatment options.

Based on study analysis of patients with GT4, 5 and 6 there is little data on IFN-free regimens. A study conducted on 41 naïve patients with GT5 and 25 with GT6 for 12 weeks with SOF / LDV, SVR resulted in 95-96% [45, 46].

HCV genotyping is required in order to select the best Direct-Acting Antiviral regimen. However, there are patients in whom genotyping was not possible to determine or mixed genotypes were recorded. In this case, AAD pangenotypic regimens are available. Combination of sofosbuvir and daclatasvir (DCV) is an example of such a regimen. Daclatasvir is an inhibitor of the NS5A replication complex and is given once daily at a standard dose of 60 mg [47].

The results of several studies confirmed that treatment with SOF / DCV and RBV-free for 12 weeks is recommended for naïve patients without cirrhosis. Treatment of cirrhotic patients should last up to 24 weeks. The treatment might be reduced up to 12 weeks in patients with cirrhosis, previously untreated and showing positive prognostic factors [38, 44].

A study was conducted on 41 patients with non-cirrhotic HVC GT1 who underwent PEG-IFN + RBV + IP and demonstrated a 100% SVR in both SOF + DCV therapy for 24 weeks and in SOF + DCV + RBV regimen for 24 weeks [44]. Since 2018, Daclatasvir has not longer been used in a series of countries, such as Germany, because it has to be combined with sofosbuvir, and this treatment regimen is more costly than any other pangenotypic AAD recommended in the 2018 EASL guide. However, the combination of sofosbuvir and daclatasvir is the basic treatment in countries where the generic drugs are used.

Among the pangenotypic combinations approved in 2017, the association of Glecaprevir – NS3 / 4A protease inhibitor and Pibrentasvir that is the second-generation selective inhibitor of the NS5A replication complex (GLE / PIB) is also possible. An integrated analysis of all Phase 2 and 3 studies in cirrhotic and non-cirrhotic patients showed very good results within a 12-week GLE / PID treatment, thus SVR was established in 99.8% of patients with GT1, 99% in patients with GT2, 95% in GT3, 99-100% in GT4-GT6 patients. There were no statistically significant differences in naive and pre-treated patients. The SVR rate was quite high within the group of patients treated for 8 weeks and ranged from 99% in GT1 and GT2 up to 92% in GT6 [48]. Based on these study results, an 8-week treatment with GLE / GDP is recommended for naive, non-cirrhotic patients and 12 weeks for naive patients with cirrhosis.

A 16-week GLE / PIB treatment [15, 49] is recommended in cirrhotic and non-cirrhotic patients treated with AADs. Another pangenotypic regimen includes sofosbuvir (SOF) and velpatasvir (VEL) that are available in a fixed dose of 400 / 100mg SOF / VEL in one tablet. According to the results of the Phase 3 trials, the standard treatment for 12 weeks in all chronic HCV patients, GT1, 2,4,5,6 and non-cirrhotic CT3 patients has recorded SVR in 97-100% and there was no obvious difference between experienced and naive patients [50, 51, 52]. A complete analysis of patients with advanced fibrosis and cirrhosis demonstrated SVR in 98% and 99%, respectively. Therefore, this therapeutic regimen may be recommended for all HCV patients, regardless of the stage of fibrosis, including those experienced, however not recommended for patients with chronic kidney disease (GFR <30 ml / min) [52, 53].

Nevertheless, the new DAA treatment regimens show very good results in the treatment of chronic HCV, there were subjects who did not achieve SVR. The combination of sofosbuvir, velpatasvir and voxilaprevir (VOX), approved in 2017, should be used as an alternative regimen for the treatment of patients who failed to respond to NS5A inhibitor therapy. This treatment has a pangenotypic effect and can be used as a first-line treatment regimen [54]. Two studies of Phase 3 investigated SOF / VEL / VOX treatment in both naïve and DAA patients.SVR recorded 96-100% of patients infected with all HCV genotypes, with or without cirrhosis [55]. Antiviral treatment exhibits some adverse effects, as well. Once the new DAA interferon-/ribavirin-free regimens have been introduced, the rate of adverse effects has decreased substantially, thus most patients complain of minor manifestations such as fatigue, headache, insomnia and nausea [56]. These side effects do not require discontinuation of treatment.

Patients treated with ribavirin, in addition to anemia, may experience pruritus, dry skin, coughing, and dyspnoea [57]. These side effects have already been described in combined ribavirin and interferon regimen but their incidence and severity are lower when ribavirin is combined with direct-acting antivirals. However, there have been a number of severe complications as bradycardia and cardiac arrest, including some lethal outcomes in patients taking antiarrhythmic drugs, particularly amiodarone, in combination with DAA including sofosbuvir [58].

Conclusions

1. Hepatitis C virus (HCV)-related morbidity is a major current issue both worldwide and in the Republic of Moldova. This disease predominantly affects persons aged 30-49 years, and tends to develop into hepatic cirrhosis and hepatocellular carcinoma.

2. Lack of specific HCV prophylaxis requires the identification of cases of HCV infection and the application of effective treatment regimens.

3. Over the last two decades, the only available treatment option for chronic HCV was PEG-IFN and RBV for 48 weeks, which recorded a 42-52% SVR in patients with GT1 and for 24 weeks – 76-84% in those with GT2 and GT3. However, the treatment was accompanied by a series of side effects in over 50% of patients, which reduced adherence to therapy.

4. The new direct-acting antiviral treatment regimens can be administered in most patients with HCV infection, including those with liver cirrhosis. These proved to be highly effective, resulting in a sustained virologic response in over 90% of patients.

5. DAAs are well tolerated and have minimal side effects that do not require treatment discontinuation.

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Specific conventional epileptic crisis

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Abstract

Background: The brain controls how the body moves by sending small electrical signals along the nerves to the muscles. Seizures occur when abnormal signs in the brain change the way the body works. Seizures vary from person to person. Some people only have light handshakes and do not lose consciousness. Other people may become unconscious and experience violent whole body trembling. The article focuses on the cases of the seizure, order and behavior during and after the epileptic crisis, the child's febrile convulsions and recommendations.
Conclusions: Our knowledge about the causes of epileptic seizures increases the chance of preventing them. Correct actions in the crisis, follow-up of the crisis particularity minimize the risk of traumatization and ensures correct therapy of the pathology that causes the crisis. Correct treatment and cognition of contraindications reduce syncope repetition. Correct informing people about the crises particularities allows understanding the seriousness of the consequences of a crisis.

Key words: epileptic crisis, causes of seizures, help during the epileptic seizure.

Introduction

The brain controls how the body moves by sending small electrical signals along the nerves to the muscles. Seizures occur when abnormal signs in the brain change the way the body works [1].

Seizures vary from person to person. Some people only have light handshakes and do not lose consciousness. Other people may become unconscious and experience violent whole body trembling [2].

Body shakes, both, mild and violent, do not always occur in seizures. Some patients who have crises lose briefly contact with the environment and look at a fixed point. Although the person is awake, he does not normally respond to what's happening around him. After the crisis, the patient does not remember the episode. Not every tremor of the body is caused by seizures. Many medical conditions can cause a type of body trembling that usually affects your hands and head (tremors) [3-5].

A small number of patients will have only one life-threatening seizure. A single crisis usually takes less than 3 minutes and is not followed by a second crisis. Any normal healthy person may have a single crisis under certain conditions. A heavy head blow may trigger such a seizure. A crisis does not always mean that there is a serious health problem [6-8].

The patient who has a first seizure should consult a psychiatrist. It is important to remove from diagnosis severe conditions that can cause seizures. Febrile seizures are the most common causes of single seizures, especially in childhood [9-11].

Causes of seizures

Epilepsy is a central nervous system disorder that can cause seizures. It may appear at any age. A seizure may be a symptom of another condition such as:

- A cerebral tumor or a structural defect of the brain, such as a cerebral aneurysm;
- An extremely low blood sugar level in a diabetic patient;
- An infection such as meningitis or encephalitis;
- Cerebral surgery or a craniocerebral trauma;
- Fast-growing fever (febrile seizure);
- Giving up alcohol;
- Parasitic infections such as toxoplasmosis;
- Prescribed drugs or illegal drugs;
- Problems that have been present since birth (congenital problems) [12-14].

Eclampsia is a pregnancy-related seizure that is usually caused by high blood pressure. It is a dangerous situation for the mother and the fetus during the convulsion, the intake of oxygen in the fetus decreases very drastically. Eclampsia occurs most often after the 20th week of pregnancy [15-17].

Non-epileptic convulsions, also called pseudocrises, are a condition that can cause seizure-like activity [18].

Non-epileptic seizures are characterized by the loss or alteration of physical functions without the presence of a central nervous system problem. This loss or change results in periods of physical activity and inactivity that resemble epileptic seizures [19, 20].

These types of crises usually relate to mental health problems. Physical symptoms can be caused by emotional

conflicts or stress. Symptoms usually occur suddenly in times of extreme emotional stress. Regardless of the seizure causes, various measures can be taken to protect the patient during convulsions and to get specialized help after the episode [21-23].

During the crisis:

- Guide the person to stretch on the floor;
- Human bites may also occur;
- If the patient has a convulsion and is on the floor, it is good to put something soft under his head;
- If the patient sheds it is better to turn him on one side;
- It is advisable not to try to keep the patient on the floor or move him;
- It is good for the person who helps the patient to be careful about the way the crisis manifests itself so that he can describe it to the medical staff;
- Keeping calm;
- Moving furniture and objects that can injure the patient during a crisis;
- Placement of an object in the patient's mouth may result in additional injuries, such as tooth pricking and mandible fracture;
- Protecting the person from the impact;
- Turning the patient to one side, mouth down, unless the person opposes mobilization;
- Trying to keep the person from falling [24, 25].

After the crisis:

- The patient should not drive, swim, climb, until he or she consults a physician;
- Checking if the person has struck during the seizure;
- If the patient can not be turned back during the crisis, this can be done after the seizure, when the patient is more relaxed;
- If the patient has breathing problems, fingers can be used to release the saliva from the airways;
- Most patients are sleepy or confused after a convulsion;
- Providing a safe area where the patient can relax;
- The patient must be accompanied until he becomes fully aware of the environment;
- The patient should not drink or eat until he is fully awake and conscious;
- Widening too tight garments from the neck or the middle [26-28].

The help given to a person during an epileptic seizure:

An epileptic seizure or seizure crisis can be scary. A convulsive crisis temporarily interferes with muscle control, movement, speech, vision or consciousness. It can cause violent shaking of a person's whole body for seconds or minutes, accompanied by loss of consciousness [29, 30].

Convulsions can be mild or severe and affect patients differently. Even if people feel helpless when they are near a sick person who has seizures and is difficult to look at, there are many things that can help [31, 32].

Help granted during seizures:

- Do not insert anything, including fingers, into the patient's mouth;
- Do not try to keep the patient lying down;
- Inserting a material into the patient's mouth with epilepsy can be harmful;
- Move away furniture or other objects that could harm the person during the crisis;
- Protect from falls if possible;
- Turn the patient to one side, with his mouth down, only if he can be moved;
- Try to protect the person from injuries [33-35].

After a convulsive crisis:

- Examine whether the patient has suffered injuries;
- If the patient can not turn to one side during convulsions help him to do this after the crisis is over when the patient is more relaxed;
- If the patient has dyspnea, clean the mouth;
- Choose a safe area where the patient can sit;
- Do not offer anything to eat or drink;
- A person who has just experienced a seizure is often confused for a short time after the crisis;
- To oversee the patient until he / she is aware and familiar with the surroundings.

Things to follow during a seizure

Try to remember and remind the doctor:

- The way the patient's body moves;
- Seizure duration;
- The patient's behavior before the crisis;
- The patient's behavior mode immediately after convulsions;
- If the person has suffered an injury during the seizure;
- Call for medical help [36-39].

Seizures do not always require urgent medical attention. However, call for emergency services immediately if:

- The person experiencing a seizure has a breathing stop (apnea) for more than 30 seconds;
- The seizure has duration longer than 5 minutes (the patient may experience a life-threatening condition due to prolonged convulsions called epileptic status);
- More than one convulsive crisis occurs within one hour;
- The person who has a seizure does not normally react one hour after seizures or has any of the following symptoms:
- The patient is not completely awake;
- Confusion;
- Nausea or vomiting;
- Dizziness;
- Inability to walk or sit;
- Fever;
- Seizures occur after the patient complains of sudden headache;
- Seizures occur after signs of vascular accident as a disorder;
- Speech dyslexia;
- Understanding of speech presents some difficulty;
- Visual disturbances;

- Inability to move different body segments to one side (apraxies);
- Seizures occur after a lesion in the cephalic extremity;
- A pregnant woman who has seizures;
- It may be a sign of preeclampsia (pregnancy toxemia, manifested by increased blood pressure);
- A person with diabetes has seizures: low blood sugar level (hypoglycaemia) or high blood sugar level (hyperglycemia) can cause seizures in a diabetic patient;
- Seizures have occurred after drug use [40-45].

The child's febrile convulsions

Convulsion is a short period of time when the child may become unconscious and has muscle spasms in various areas of the body.

The febrile convulsions are caused by high fever. Fever can be due to an infection (a respiratory viral infection). If the seizures occur, a psychiatrist should be consulted to determine their causes. Febrile convulsions are not signs of epilepsy.

However, patients with epilepsy are most susceptible to febrile seizures [46-48].

What happens during convulsions?

Febrile convulsions duration is less than 5 minutes, but may extend for up to 15 minutes and may be accompanied by the following reactions:

- After the seizures, the child may be indisposed or tired.
- Can lose control of the urinary and anal sphincter.
- May occur changes in the eyes position.
- During convulsions, the baby's hands and feet are spasmodic, convulsive (muscle spasms, contractions).
- They may lose consciousness.

At what age febrile seizures can appear

They usually appear between the ages of 6 months to 5 years. The highest incidence is at the age of 12 to 18 months. The febrile convulsions disappear after the age of 5 to 6 years.

Children of parents with a history of febrile seizures have a higher risk of developing them.

What can we do when febrile convulsions occur?

Fearful convulsions usually scare parents, but they are harmless to the child. There are two important things to do:

- We need to make sure that the baby breathes.
- We need to be careful so that the child is not injured during convulsions [49-52].

Recommendations

Parents should remain calm and ensure that the child's breathing is normal. Bending the lips is a sign that he does not breathe. If the child stops breathing, an ambulance service must be called quickly. Ideally, parents should know the cardio-respiration technique and while waiting for the ambulance try to restore the child's breath. If the child breathes, he is better to be placed on the floor or on the ground, so as not to fall during convulsions [45, 53].

The child should be set to the side to avoid choking (he can suffocate by blocking the airways from the base of the tongue). All objects that may cause injury during the convulsion (chairs, tables) must be removed farther. He must not be immobilized or interfere in any way with the movements of his body. It is forbidden to place any object or substance (drugs, water) in the child's mouth, to avoid breathing difficulties. After the seizures, the baby will be very tired and will fall asleep. This is normal. You do not have to wake up the child or give him/her something to drink or eat while he/she is sleeping. Medical staff will recommend decreasing the baby's temperature [35, 54-56].

Conclusions

1. Knowledge of the causes of epileptic seizures increases the chance of preventing them.
2. Correct actions in the crisis, follow-up of the particularity of a crisis, minimize the risk of traumatization and correct therapy of the pathology that causes the crisis.
3. Correct treatment and cognition of contraindications reduce syncope repetition.
4. Correct informing people about the crises particularities allows understanding the seriousness of the consequences of a crisis.

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The importance of blood cultures in the effective management of bloodstream infections

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Abstract

Background: Bloodstream infection (BSI) is a major public health concern due to its severity-related consequences. These infections pose a human health risk, as they can result in human morbidity and mortality over a short period of time. Blood culture remains the gold standard and major tool for the diagnosis of BSI. Blood culture sampling is commonly indicated before administering antimicrobial therapy, whereas the daily therapeutic adjustment to the antibiogram is an effective intervention in management of BSIs. Compliance with the microbiological criteria-based protocols for pathogen identification and antimicrobial susceptibility testing allow treatment correction within 48-72 hours. Interpretation of positive blood cultures may sometimes present a dilemma for clinicians and microbiologists and, therefore, the test findings should be evaluated in the context of the clinical picture.
Conclusions: Over the last decades, we have witnessed an outbreak in the number of BSI studies. The implementation of a standardized algorithm on criteria of a complete blood count sampling, processing and interpretation of the results will help increase the yield rate of BSI pathogens and ultimately improve care management of the patients with BSI. The education and training of medical staff, engaged in BSI patient care is vital in developing good practice, preventing blood culture contamination and obtaining fast and accurate outcomes.
Key words: bloodstream infections, blood cultures, antimicrobials.

Introduction

Bloodstream infection (BSI) has become a subject of medical concern worldwide due to its severity-related consequences. BSI represents a growing public health concern, with an estimated burden of 1.200.000 episodes across Europe and 157.000 deaths annually [1].

The reviewed studies highlight the most common microbial agents involved in the aetiology of BSI, namely: *Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae*, with approximately 35%, 25% and 10% per 100.000 population, respectively. These studies revealed that BSI incidence varies significantly across different geographic regions, and this is largely due to timing of BC specimen collection, demographic population differences and distribution of risk factors within these regions [2].

These infections show high morbidity and mortality rates across the globe and are among the top seven causes of death in North America and Europe [3].

However, accurate data on the incidence of BSI-associated morbidity and mortality in some countries are limited, due to misdiagnosis and insufficient patient follow-up [4-8].

The risk of BSI is due to the immunocompromised human organism that might favor the invasion of various pathogens, which commonly show resistance to a number of antimicrobial groups [9, 10].

BSI diagnosis is based on the detection of bacterial and fungal pathogen isolates from the blood cultures. Over the recent decades, great progress has been made in the development of rapid diagnostic tests based on innovative technologies; BC sampling remains the gold standard for diagnosis of BSIs. This method is one of the most important techniques if any BSI-related suspicions arise. Blood, being normally sterile, has a considerable diagnostic significance in isolating and identifying bacteria or fungi from the blood cultures. It allows establishing the diagnosis for BSI by isolating and rapidly identifying the pathogen (within 2-7 days). Moreover, it guides the physician in choosing the antimicrobial therapy based on the results of Antimicrobial Susceptibility Testing (AST) of pathogen isolates to antimicrobial drugs, thereby contributing to reducing the phenomenon of antibiotic resistance [11].

Most studies have reported variations, from country to country, on recommendations for good blood culture sampling practices [12-14].

At present, there are international guidelines on the collection, processing, interpretation of blood cultures, where-

as this paper will present the rigors stipulated therein [15-18].

Despite the fact that, there are guidelines, methodologies, instructions on BC sampling, however inadequate practices continue to be registered, which exhibit an approximately 3% rate of blood culture contamination [19].

Blood culture contamination produces false-positive results, leading to irrational use of antimicrobial drugs, increased length of hospitalization and higher costs [20].

Furthermore, the authors of the studies highlight the importance of accurate diagnosis for BSI, which requires both laboratory findings on isolation of the microbial agent from blood cultures and presence of compatible clinical signs of the patient. The same studies show that the pathogen, detected as an etiological agent for BSI, should be, preferably, isolated from several blood samples. Moreover, studies recommend identifying the site of primary infection and isolating the same microbial agent from this outbreak [21].

According to some studies, in most cases, only 5 to 13% of blood cultures turn out to be positive with isolation of the microbial agents, and of those, 20–56% represent the contaminating microbial flora, despite the scientific advance in the use of skin antiseptics, which have been successful since 25 years ago, and thus, reducing the risk of contamination by 2.1-6% [22, 23].

Effective management in reducing the overall incidence of BSI is a major reason for indicating BC sampling prior to the administration of antimicrobial therapy, as well as for daily reassessment and appropriate adjustment of this therapy based on definitive pathogen identification and antimicrobial susceptibility testing to antimicrobial preparations. Since BSI patient survival is inversely proportional to time of initiation of appropriate antimicrobial therapy, it is vital to obtain the BC results as soon as possible. Therefore, early diagnosis and appropriate treatment are crucial in the efficient management of BSI. Delays in the initiation of antimicrobial treatment may lower dramatically the survival rate among BSI patients. Patients treated within the first hour of diagnosis may have a survival rate of almost 80%, subsequently, the chances of survival decrease by 7.6% for every hour after. Inappropriate initial antimicrobial treatment is likely to be fatal in these patients [5].

Key terms and definitions

Antiseptic. A substance that inhibits the growth and development of microorganisms [24].

Bloodstream infection. A bacteria or fungi-associated infection [24].

Bacteremia. The presence of bacteria in the blood. It may be transient, intermittent or continuous [24].

Transient bacteremia / fungemia. Transient presence of bacteria or fungi in the blood for a period of several minutes [24].

Intermittent bacteremia. Intermittent BSI is a "recurrent transient infection" that is associated with undrained and intra-abdominal abscesses [24].

Continuous bacteremia. Continuous bacteremia suggests a severe infection that inhibited the host's defense mechanisms. It is a characteristic of BSI, such as infective endocarditis or suppurative thrombophlebitis [24].

Pseudobacteriemia. Pseudobacteremia occurs when blood culture isolates come from outside the patient's bloodstream [24].

SIRS (Systemic Inflammatory Response Syndrome). Systemic inflammatory response syndrome features the early body response to damages of infectious or non-infectious origin [25-27].

Sepsis. Life-threatening organic dysfunction caused by impaired host response to infection [28, 29].

Septic shock. It is defined as a subgroup of sepsis, associated in particular with profound circulatory, cellular metabolism abnormalities, with a higher risk of death than sepsis [30].

Neonatal sepsis. Neonatal sepsis is defined as clinically diagnosed SIRS, caused by infection occurring within the first four weeks after birth. The incidence of neonatal sepsis increases with early-onset birth weight decrease and can be divided into two types: early-onset neonatal sepsis (occurs in the first 72 hours of life); late-onset neonatal sepsis (occurs after the first 72 hours of life) [31].

Bloodstream infections in immunocompetent patients include:

Community-acquired infection. Bacteremia and fungemia may often occur in previously healthy individuals, commonly associated with focal infections such as pneumococcal pneumonia. Moreover, bacteria can penetrate into the bloodstream from the patient's own commensal flora or from an undetectable infected site, leading to metastatic infections [31].

Hospital-acquired infection. The increased number of invasive procedures, such as catheterization, immunosuppressive therapy, antibiotic therapy and life support measures, has resulted in higher overall incidence of hospital-acquired bacteremia, candidemia and other fungal infections. These procedures may allow microorganisms to invade into the blood or weaken the host defense system [31].

Health care-associated infections (HCAIs). HCAIs are infections that occur as a result of medical assistance and treatment procedures within outpatient care units, medical offices, healthcare clinics or hospitals [31].

Anaerobic bacteremia. Studies have shown that anaerobic microorganisms make up 1-17% of positive blood cultures. Anaerobic microorganisms are a common cause of bacteremia and, therefore, routine testing should be carried out [31].

Pediatric BSI. The etiology of pediatric bacteremia has changed over the last few years. *Haemophilus influenzae type b* (Hib) infections decreased dramatically after the introduction of national immunization programs, whereas the systemic HCAIs have increased [31].

Catheter-related bloodstream infections. Intravenous catheter (IVC)-related bacteremia or fungemia are difficult to confirm. There is often no evidence of infection at the

catheter insertion site, whereas the involved microorganisms are part of the normal skin flora and common contaminants of BCs [31].

Infective endocarditis (IE). IE is defined as an infection of the endocardium, particularly involving the heart valves, characterized by functional impairment, and termed as an infection of the heart valves and / or other endocardium areas [31].

BSI in immunocompromised patients. Immunocompromised patients are individuals with acquired or drug-induced autoimmune disorders. Defects in phagocytes, complement, antibody response and cell-mediated immunity are often associated with specific disorders or conditions, such as malignancy, HIV infection, organ transplantation, immunosuppressive therapies, and steroid administration. Patients with neutropenia are at highest risk of infection [31].

Recommendations for blood culture sampling

Most of the reviewed studies highlight the importance of doctor's indication for BC sampling in patients exhibiting the following signs: septicemia, septic shock; severe localized infections (meningitis, pneumonia, intra-abdominal abscess); fever or fever history and suspected or detected neutropenia; fever and immunocompromised status; individuals undergoing invasive procedures (catheter, dialysis or surgery); fever and recent trips abroad; suspected bacterial endocarditis; syndromes, suggestive of BSIs with specific germs (enteric fever, brucellosis, leptospirosis), under certain conditions (like pregnancy, cardiac diseases, diabetes, renal failure, hepatic failure, leukocytosis, granulocytopenia) [32, 33].

However, the academic society has not come to a common denominator on the best clinical predictors for BSIs [34, 35].

Key elements and general principles for blood culture sampling

The research underlines the importance of standardization of the blood sampling procedure for microbiological investigations. Therefore, the study outcomes on the implementation of a standardized procedure of BC sampling performed under aseptic conditions , showed a reduction in the contamination rate up to 1.6% ($p < 0.001$), with a previously recorded rate of 3.9% [36]. According to another study by Self et al. (2013) on the development and implementation of a standardized set of sterile tools required for blood sampling , as well as the user's checklist, the contamination rate decreased from 4.3% to 1.7% ($p < 0.001$) [9, 37].

The studies emphasize the importance of timing blood sampling and recommend to be collected soon after the clinical signs appear and before the initiation of antimicrobial therapy. In case if, due to certain circumstances, the patient is already undergoing antimicrobial therapy, blood sampling should be carried out immediately prior to administering the next dose and by inoculating the blood into bottles containing specialized antimicrobial neutralization media. It is not recommended to collect blood through the same needle / lumen through which an antimicrobial drug has been given within the last hour. Information on previously administered antimicrobial preparations should be indicated on the laboratory application form [32, 10].

First, blood samples must be collected from the peripheral vein. Sets withdrawn from either the central or the peripheral vessels must be taken successively or at intervals of 12 hours apart. To obtain reliable BC data, the peripheral blood samples are taken first before other types of investigations [38, 17].

Some authors of the recent studies do not recommend blood sampling through the peripheral intravenous cannula. Moreover, there is also an obvious risk of contamination due to the difficulty of skin disinfection when taking blood from a femoral vein. Therefore, the authors suggest avoiding these sites and, and if no other options for sampling are available, this procedure will be carried out by documenting into the patient's sample accompanying form or clinical record [39, 40].

The study analysis reveals the number and amount of sampling, required to obtain reliable and accurate results. To optimize the yield of bacteria and fungi in the blood, an adequate amount of blood is required. A sufficient volume of blood sampling allows a better detection of small amounts of bacterial or fungal pathogens. It is essential in case if an endovascular infection (such as endocarditis) is suspected. The blood volume obtained for each BC set is the most important variable when isolating microorganisms from patients with BSIs [38].

The blood amount collected from both sampling sites must be sufficient to ensure BC accuracy, e.g. if only 10 ml of blood is obtained from the peripheral vein, additional 10 ml is taken from the central vein. When collecting blood from both central and peripheral veins, the sampling site is clearly indicated on the culture bottle and reference sheet [41].

Researchers demonstrated that blood culture bottles are designed to accommodate the optimal blood- to-broth ratio (1:5 to 1:10) and to allow maximum bacterial and fungal isolation. Commercial continuous monitoring blood culture systems may use a lower blood / broth ratio (<1:5) due to added sodium polyanethol sulfonate or sodium citrate , non-toxic anticoagulants that promote bacterial proliferation by neutralizing the bactericidal activity of human serum and inhibiting the action of some antibiotics [42].

Most studies have reported that adult bacteremia and fungemia in adults commonly develop with a reduced amount of circulating microorganisms, ranging from 1-30 colony-forming units per mL (CFU / mL) of blood. The concentration is over 100 CFU / mL in newborns, babies and older children, therefore the volume of blood sampling differs in both adults and children. For an adult, the recommended blood volume to be obtained per culture is 20 to 30 ml [32, 42, 43].

The standard indicates that BC set should include two bottles (for aerobic and anaerobic microorganisms), and about 10 ml of blood should be collected per each in adults. This volume is required to optimize pathogen isolation

when the amount of bacteria or fungi is less than 1 UFC / mL of blood. Two or three bottle sets (two bottles per set) are recommended to be used for each septic episode, viz. in adults, 40-60 mL of blood is collected for 4-6 bottles, 10 mL per each bottle [44].

Few studies have been carried out on the optimum blood volume taken from infants and children, however, the available data indicate that the yield of pathogens also increases in direct proportion to the volume of blood cultured and inoculated. Also, the recommended blood volume to be collected should depend on the patient's body weight, and only one aerobic bottle is used, if no anaerobic infection is suspected. In this context, in children under 2 years, specifically designed bottles to maintain the blood-to-broth ratio (1:5 to 1:10) are used, with smaller blood volumes [32, 45].

The researchers have shown that, as bacteria and fungi are not always present in the bloodstream; the sensitivity of a single BC set might be compromised.

The study results on the cumulative sensitivity of blood cultures, using continuous-monitoring blood culture systems for 24 hours demonstrated that four blood cultures might be needed to achieve a detection rate of > 99% in BSIs. Thus, the authors observed that the cumulative yield of pathogens from three blood culture sets (2 bottles per set) with a 20 mL blood volume in each set (10 mL per bottle) was 73.1% for the first set, 89.7% – the first two sets and 98.3% – the first three sets [46].

Most studies emphasize that, it is not generally recommended for adult patients to collect a single bottle or a single blood culture sample set since this practice will result in insufficient BC volume and failure to detect the causative agent in a considerable number of bacteremias [43].

Furthermore, international guidelines recommend collecting 2, or preferably 3 blood culture sample sets for each septic episode in order to differentiate BC contamination during sampling of true bacteremia. In case of BC contamination, the microbial flora will be present in only one bottle or a set of blood culture bottles, unlike true BSI, in which multiple bottles or sets will be positive. In case of 2-3 blood culture sets sampling, followed by a 24-48 hour incubation period, the results are negative and the patient is still septic, 2-3 additional BCs should be taken [15, 22, 42].

A number of studies have described the variety and usefulness of inoculation of blood culture media. Various BSI-causing microorganisms (aerobic and anaerobic pathogens, fungi, fastidious microorganisms, etc.) require specific growth factors and incubation conditions. In cases when a patient is administered antimicrobial therapy, specialized media with antibiotic neutralization factors should be used. Some studies have shown that media, containing antibiotic inhibitors, increase the degree of isolation and identification of pathogens in a shorter time compared to standard media [47-50].

An essential element in blood culture sampling is the sequence of bottles inoculation. Therefore, when using vacuum blood-sampling system, the blood is first transferred into the aerobic bottle to prevent transfer of air from the sampling device to the anaerobic bottle. When using a needle or syringe, the anaerobic bottle is first inoculated to avoid air ingress. If the taken blood volume is less than the recommended one, then 10 ml of blood is first inoculated into the aerobic bottle, as most bacterial cases are caused by aerobic and facultatively anaerobic bacteria. In addition, pathogenic fungi and strictly aerobic bacteria (e.g. Pseudomonas spp.) are almost exclusively isolated from aerobic bottles. Any remaining blood amount is recommended to be inoculated into the anaerobic bottle [11].

The study analysis reported that the time interval between taking two blood samples is not considered a critical factor, since the yield of diagnosis remains the same [51].

At the same time, international guidelines recommend that the first two / three blood culture sets (two bottles / set) should be obtained over a short period of time (e.g. within one hour) or as a single sample taken at one time. Blood sampling at long intervals, such as 1-2 hour intervals, is recommended when monitoring continuous bacteremia/ fungemia in patients with suspected infective endocarditis or other endovascular infections [32, 43].

In severe infections or to increase detection sensitivity (e.g. pyelonephritis), two or three additional blood culture sets are collected, in case if the first 2-3 hemocultures are negative after 24-48 hours of incubation. Moreover, the time interval depends on the suspected BSI-causing agents: the sensitivity is relatively good for such microorganisms as *Escherichia coli* or *Staphylococcus aureus*, and lower for *Pseudomonas aeruginosa, streptococci or fungi* [52].

Methods and techniques for the processing and interpretation of blood culture results

Most microbiology laboratories use incubation, automated continuous monitoring, shaking, and automated blood culture systems. Many manufacturers provide such devices with almost similar performance characteristics [53-56].

Automated systems place the BC bottles for a predetermined incubation period, giving a visual or warning signal if increase is detected. Each automated blood culture system produces its own media, which should be carefully assessed and selected by the user. The blood culture bottles typically contain culture medium approved mixtures, anticoagulants and, in many cases, resins or coal mixtures to neutralize antimicrobial and other toxic compounds. Commonly, the mixture of different substances is complementary to each other and is chosen to improve the range of bacterial life, including the fastidious microorganisms. Blood culture media formulas allow the detection of aerobes (including fungi), anaerobes and mycobacteria [51, 57, 22].

Other studies, comparing the performance of media with and without the addition of antimicrobial neutralizing agents (resins and / or coal compounds), have repeatedly demonstrated that these substances are obviously superior in the recovery of microorganisms, particularly the fastidious ones and levuriform mycetes [58-60].

Studies in the field examined the requirements for blood

culture media and highlight the most essential rule to be followed. Thus, media must be sufficiently sensitive to yield a wide range of clinically relevant microorganisms, even the most fastidious ones (e.g. .*Neisseria spp., Haemophilus spp.*) or that require lower amount of CO2 (e.g., *Brucella spp., Acinetobacter spp*.) [57, 61].

Blood cultures are usually incubated for 5 days via automated systems. Multiple studies have proven that this is an appropriate incubation period for detection of most pathogens, including the bacterial strains like *Haemophilus, Actinobacillus, Cardiobacterium, Eikinella and Kingella* (HACEK) group, whereas the incubation period over 5 days increases the number of contaminant isolates [62, 63].

However, studies emphasize that a longer incubation period is required in case of suspected fungemia or bacteremia caused by *Legionella, Brucella, Bartonella or Nocardia spp*. *Mycobacterial* and blood culture should be incubated for 4 weeks [64, 65].

Bloodstream infections of suspected fungal etiology do not require special culture media since most fungi grow on conventional aerobic media within 2 to 3 days. *Candida glabrata* and *Cryptococcus neoformans* are exceptions to this rule that usually require 3 to 5 days of incubation. *Fusarium* and *Paecilomyces* can be isolated in the conventional blood culture broth, while other filamentous fungi are not detected [61, 66].

Furthermore, there are no sufficient data on dimorphic fungi growth, such as *Histoplasma* and *Blastomyces,* which grow in blood culture broth requiring more than 2-week incubation period. Therefore, some studies recommend that slow growth of fungi and fastidious bacteria should be carried out via more specialized systems such as the lysis centrifugation system [67].

BSI-diseased patients, under certain circumstances, are often administered antimicrobials prior to sampling, thus suppressing the bacterial and fungal growth. Therefore, manufacturers of blood culture systems might complement their own media formulations with antibiotic binding resins or absorbent carbon. The analysis of these compounds characteristics has shown significant outcomes in the absorption of antibacterial and antifungal preparations, thus increasing bacterial and fungal yield rates and reducing the detection time of positive cultures [58, 68-70].

The identification of BSI causative agents can be performed by both manual technique and automated systems. This technique is initiated by taking a Gram stain, which is absolutely necessary for the management of these samples. A positive Gram stain with gram positive or negative microbial flora identification is immediately reported to the clinician in order to provide prompt antimicrobial therapy and measures of controlling these infections. Subcultures are performed later on, to allow identification and susceptibility testing to be carried out normally within the next 24–48 hours. Laboratories should have a standardized protocol to guide the lab staff activity on blood sampling in order to optimize the use of resources for complete organism identification and organism-specific susceptibility testing of clinically important organisms that are probably the contaminating flora [71, 72].

Interpretation of positive blood culture results is often simple, but sometimes presents a dilemma for both clinicians and microbiologists. Therefore, laboratory data must be evaluated in the context of clinical symptoms to achieve an accurate interpretation. A true positive blood culture result is obtained when most or all of the blood culture sets, withdrawn by independent venipuncture, are positive for the same microorganism. Thus, the probability that the isolated microorganism represents the BSI-causing agent is very high, regardless of the organism's identity [52].

The identification of microorganisms isolated from positive blood cultures is also significant, namely: *Staphylococcus aureus, Streptococcus pneumoniae, Enterobacteriaceae, Pseudomonas aeruginosa* and *Candida albicans* are almost always predictive of true BSI. However, *Corynebacterium spp.* and *Propionibacterium spp*. often represent the contaminating flora. Isolation of viridans group streptococci, coagulase-negative staphylococci (CoNS) and enterococci is more difficult to interpret, as some studies have reported that they cause BSIs in 38%, 15% and 78%, respectively. Coagulase-negative staphylococci are particularly the most common blood culture contaminants. Moreover, a number of studies have reported that these microorganisms have an obvious clinical importance in BSI etiology among patients with implanted medical devices and localized catheters. Therefore, the accurate result interpretation can be achieved, when a few sets of blood cultures are positive with the same CoNS species. Under these circumstances, the probability of these microorganisms to represent the true cause of bacteremia is much higher [73].

Rapid methods for identification and susceptibility testing of blood culture isolates responsible for BSI are crucial and may guide clinicians in decision-making on therapeutic interventions. Blood cultures incubated in modern systems commonly signal a positive result in a mean time of 12-36 hours, whereas the positive time from sampling to detection is longer for some fastidious microorganisms, anaerobes and fungi [55, 56].

According to the microbilogical protocols on blood culture sampling, even from the initial steps, the gram stained smear provides immediate useful information to the clinician, who might determine both the importance of the positive result and / or the initial antimicrobial therapy. Compliance with standard microbiological protocols, based on the biochemical identification of microorganisms and antimicrobial susceptibility testing, allow the adjustment of antimicrobial therapy after 48-72 hours. However, microorganisms that are difficult to be identified biochemically or slowly grow "in vitro", may require a longer period. Therefore, the prolonged result delivery (3-5 days from the blood culture collection to final identification and testing of antimicrobial susceptibility) is one of the main challenges encountered within the microbiology laboratory. In this context, the researchers highlight the continuous interest to reduce this time by developing rapid methods. For example,

the coagulase test, traditionally used to distinguish CoNS isolates from coagulase-positive isolates, can be conducted directly on positive blood culture broths, containing Gram-positive cocci on Gram staining [74].

This approach allows a rapid distinction between CoNS and coagulase-positive staphylococci (for example, *S. aureus*) and may influence clinicians' ability to interpret the clinical significance of a positive blood culture result and initiate an appropriate antimicrobial therapy. Clearly, this is not a complete solution as it does not definitively detect the micro-organism and does not provide data on susceptibility. Similarly, it is possible to couple direct coagulase testing with the use of chromogenic media, which allows identification of methicillin-resistant S. aureus isolates within 18-24 hours [75].

Some studies come with stronger evidence and approach to improve the laboratory diagnosis of BSIs by using new and rapid methods. Molecular methods, including nucleic acid amplification assays (NAATs) and DNA sequencing approaches have emerged as highly useful tools for identifying microorganisms and, in some cases, predicting antimicrobial susceptibility for selected antibiotics [76-79] .

Furthermore, a series of studies have proven that novel phenotypic approaches reduce the time for identification and antimicrobial susceptibility testing for selected microorganisms [80].

Researchers also describe another rapid method, such as Mass Spectrometry, which is widely used within clinical microbiology laboratories as a routine method for rapid identification of microorganisms directly from positive blood culture broth [81, 82].

This method provides much more specific results within a limited time, regarding the microorganism identification and the present resistance mechanisms, compared to traditional methodology. Clinicians should get familiar with the criteria for interpreting these results, as well as the initial antimicrobial treatment schemes and subsequent adjustment of therapy on the basis of final outcomes [83, 67].

Therefore, these methods can only be used as additional assessment to the already existing standard protocols on BSI patient management. Many infections show similar clinical picture, whereas the laboratory diagnosis of infectious diseases is limited to testing, as only the most common pathogens are associated with clinical syndrome. Thus, it results in a number of undiagnosed infections, requiring additional sampling, patient dissatisfaction and a compromised health care. The syndrome-related approach to diagnosing infectious diseases might change this situation. This might become a symptom-based diagnostic method that might assess multiple common pathogens using a single rapid test (multiplex PCR). This method allows physicians to quickly choose the right test and improve treatment management for a number of infections. Hospital-related mortality rate among septic patients ranges from 10% to 50%. Inappropriate antimicrobial therapy for septic shock occurs in approximately 20% of patients and is associated with a fivefold reduction in patient survival rate. Rapid diagnosis and targeted treatment might prevent up to 80% of sepsis-related deaths [84, 85].

Rapid multiplex PCR systems used in positive blood culture sampling detect over 20 types of microorganisms, including antibiotic-resistant genes (carbapenemases, MRSA, VRE), which provide fast and reliable results within several hours for rapid clinical decision-making. Ease of sample collection, rapidity and ability to cover a wide range of pathogens, including the antibiotic- resistant genes, provide excellent laboratory opportunities for delivering data for further positive care assistance among patients with BSIs [86, 87].

Unlike the traditional blood culture method, the multiplex PCR system rapidly reduces the time required for identification of microorganisms in positive BC bottles, viz. from 26.5 hours to less than 3 hours. The mean time required to identify the potential antibiotic resistance mechanisms was 2.2 hours, whereas the results of bacterial antibiotic sensitivity testing (phenotypic) were available within 33.3 hours on average. The multiplex PCR system test accurately identified the presence or absence of antibiotic resistance mechanisms in all 70 bacteria detected within the study (35 samples with *S. aureus*, 6 – *Enterococcus spp*., 29 – *Enterobacteriaceae* and *P. aeruginosa*). These study results allowed clinicians to adjust an empirical combined treatment in 22 out of 112 patients [88].

Most studies emphasize the importance of implementation of rapid diagnostic solutions along with manual method of BC sampling, in order to identify and differentiate the underlying pathogens of BSI etiology. The implementation of these rapid diagnostic methods (multiplex PCR) is crucial in urgent therapeutic management and patient follow-up, which helps reduce the intervention time and targeted treatment, as well as additional sampling and length of hospitalization [88].

Overall considerations on blood culture contamination

Inappropriate practices result in blood culture contamination with skin commensals on the venipuncture site. Blood culture contamination during the sampling process may lead to false positive results, which may have a negative impact on the patient condition. A false positive result is defined as an increase of bacteria in the blood culture bottle that are not normally present in patient's blood , which might have been introduced during blood sampling. Contamination may originate from a number of sources: the patient's skin, the equipment used for sampling, the hands of the person taking the blood sample or the environment [61, 81].

Taking uncontaminated blood specimens is essential in providing a blood culture result that has a clinical value. Certain microorganisms such as coagulase-negative staphylococci and streptococci from the group of *Viridans, Bacillus spp, Propionibacterium spp., Difteroides, Micrococcus spp* may rarely cause severe bacterial infections or BSIs. These microorganisms are common skin contaminants and, al-

though they are able to cause severe infections, under appropriate conditions, their detection in a single, blood culture set can be reasonably identified as a possible contaminant with no clinical significance. However, coagulase-negative staphylococci are considered the primary cause of catheter-associated infections and may exhibit clinical significance in up to 20% of cases [19].

The most difficult challenge for physicians is to interpret whether the microorganism isolated from a blood culture is a BSI-causing pathogen or a contaminant. If it is a contaminant, the antibiotic therapy in patients is unnecessary or inappropriate, resulting in life-threatening condition and additional costs for the health care assistance. The microbial isolate should be distinguished as a real etiological factor from the contaminant one, based on venipuncture blood sampling or via an intra-vascular device and multiple isolation of the same species of microorganisms. Therefore, it is highly important to include information regarding the site of blood culture sampling in the application form before sending the sample to the laboratory [32].

Prevention of blood culture contamination

The most effective way to reduce contamination rate is to strictly comply with the rules of hand hygiene, as well as follow the best practices in blood sampling, skin processing, venipuncture and blood transfer in BC bottles. However, although the best blood sampling protocols have been carried out, it is impossible to reduce the contamination rate below 2% [37, 61, 89].

The American Society for Microbiology (ASM) and CLSI recommend that the contamination rates should not exceed 3% of the total of collected sets [32].

The study outcomes on the analysis of contamination sources showed a good understanding of blood culture sampling time but described a variety of methods and equipment used. Subsequently, the study authors suggested measures on rationalization and standardization of blood sampling equipment and techniques, as well as personnel training regarding their compliance. This project operated for 12 months within an Emergency Department and successfully reduced local contamination rates up to 2.0% [90].

Blood culture contamination of skin microbiota is an important issue regarding the patient outcome and management, and might lead to inappropriate use of antibiotics, additional laboratory and radiological tests, antimicrobial side effects and increased length of hospitalization [91].

Personnel training in sampling, processing and interpretation of blood cultures

Education and professional training of medical staff (doctors, nurses, phlebotomists or technicians), engaged in caring of patients with BSIs is paramount in developing best clinical practices and preventing blood culture contamination [22, 92-94].

Over the last decades, a number of studies have focused on the study of causes and measures to prevent blood culture contamination. Thus, according to some authors, training of the staff on the proper sampling techniques, monitoring of blood culture contamination rates and getting familiar with these data might reduce the contamination rate [95, 22].

Other studies have reported that continuous training and their efficiency assessment have been associated with lower rates of blood culture contamination (from 2.59% to 2.23%). Moreover, the decrease in the contamination rates from 5.7% to 1.95% due to personnel training was registered in other studies, as well [96].

Studies that described the use of combined interactive learning methods (video materials, simulations for developing practical skills), and determined their impact on blood culture contamination rates were performed. The study revealed that contamination rates remained the same, except for the experienced staff (from 4.1% to 2.7%), hence theoretical education is considered inefficient without coherent practices [95, 96].

A group of researchers demonstrated a decrease of 44% in the contamination rate (from 1.82% to 1.01%) as a result of personal training and counseling. Considerable results (from 11.8% to 7.4%) were recorded when using the same mechanisms (individual training and counseling) but with more frequent sessions, e.g. twice a month [22, 96].

Another approach in staff training on good practices, in terms of preventing blood culture contamination, was conducted by another group of researchers, who introduced theoretical education along with proper using of blood culture sampling containers, hence reporting a decrease of 42% in the contamination rate [95].

Thus, the reviewed studies presented evidence-based data on the importance of staff training in sampling, processing and interpretation of blood cultures, using various interactive methods, as well as counseling sessions in terms blood culture sampling.

In order to increase the yield of BSI-causing pathogens, as well as to obtain fast and reliable outcomes, prevent blood culture contamination and improve patient management of BSIs, it is paramount to implement a standardized algorithm for the sampling, processing and interpretation of blood cultures.

Conclusions

1. Bloodstream infections represent a serious medical issue for public health care worldwide, as well as a challenge in their diagnosis and management.

2. Data collection on BSI etiology and microorganism resistance at a regional level provides a rational basis for optimizing further potential preventive strategies such as immunization, environmental hygiene and chronic disease management.

3. Implementation and compliance to a standardized algorithm, blood culture identification by the clinician, and proper sampling procedure will help increase the pathogen isolation rate of BSIs.

4. The epidemiological surveillance of these infections is a continuous and permanent multidisciplinary activity of healthcare professionals.

5. Standardization of blood culture sampling methods, blood volume optimization, using of a checklist, using effective antiseptics and staff training, lead to the development of good practices for collecting blood culture samples and thus reducing their contamination at the lowest possible rate, not more than 2%.

6. Education of medical staff, trained in the sampling, processing and interpretation of blood cultures is vital for improving patient care and management in case of BSIs.

7. The study calls attention to insufficient data on patient evolution with BSI due to misdiagnosis, resulting in irrational microbial administration and uncontrolled spread of MDR microorganisms that generally occur within the hospital and community.

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The orthodontic miniscrew implants in tooth migration

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Abstract

Background: The bone loss, tooth loss and gingival inflammation are the most common harmful factors that might cause pathologic tooth migration. Taking into consideration that miniscrews implants are used in orthodontic treatment of both pathologic and physiologic tooth migration, we were interested to mark out the advantages and disadvantages of miniscrew usage.

Conclusions: Development of implants industry and particularly miniscrew anchorage has been rapidly increased in the recent years, but nevertheless there still are some risk factors of miniscrews use and success of treatment depends a lot on techniques of miniscrew insertion and stability elements. The anchorage control during tooth movement is one of the main factors for ensuring successful orthodontic treatment and the most important keys for achievement of success in clinical orthodontics. The advantages of miniscrews, in comparison with other methods of orthodontic treatment might be resumed to: lower costs, easy placement, no need for patient cooperation, high quality and efficiency of treatment. Among the most undesirable side effects of miniscrews use can be marked out the screw fracture, screw-root proximity, damages of soft and hard tissues, displacement under orthodontic loading, etc.

Key words: miniscrew, pathologic tooth migration, anchorage devices, tooth movement.

Introduction

The etiology of tooth migration includes a wide range of harmful factors such as: periodontal and gingival inflammation diseases, dystrophy of the attaching and supporting structures of the teeth, bone loss and loss of the approximating, or opposing teeth, lingual interposition, parafunctions, age and oral habits [1], as well it might be caused by movement of teeth during eruption, or out of their normal position in the dental arch.

According to Martinez-Canut et al. [2], among the most common etiological factors that may cause pathologic tooth migration, were bone loss, tooth loss and gingival inflammation that resulted in PTM prevalence in 55.8% of patients.

Two types of tooth migration are distinguished:

- ***Physiologic tooth movement*** due to tooth eruption, migration or drifting, changes of tooth position during mastication.

Usually the posterior teeth migrate to the mesial direction and anterior teeth migrate to the distal direction.

- ***Pathologic tooth movement*** occurs in periodontium and gingival diseases, bones loss, tooth loss and loss of periodontal ligament that may result in loss of supporting structures of the tooth.

As a common complication of moderate to severe periodontitis may occur teeth migration and those patients usually, apply for periodontal therapy.

Pursuant to Khorshidi et al. [3] the "pathologic migration was not observed in patients with mild chronic periodontitis". The same author pointed out that in patients with moderate chronic periodontitis, PTM was marked out in 5.2% of cases, but in patients with severe chronic periodontitis the rate of patients with PTM was 51% and in cases of aggressive localized, or generalized periodontitis the PTM was marked out in 50% of patients.

According to Brunsvold [4] "prevalence of PTM among periodontal patients has been reported to range from 30.03 to 55.8%", with an average of 42.9%.

For example Towfighi et al. [1] in their paper concluded that PTM was one of the most common compliant of patients that addressed do dental clinics.

The rate of pathologic tooth migration according to different authors, periods of time and countries is given in the diagram (fig. 1).

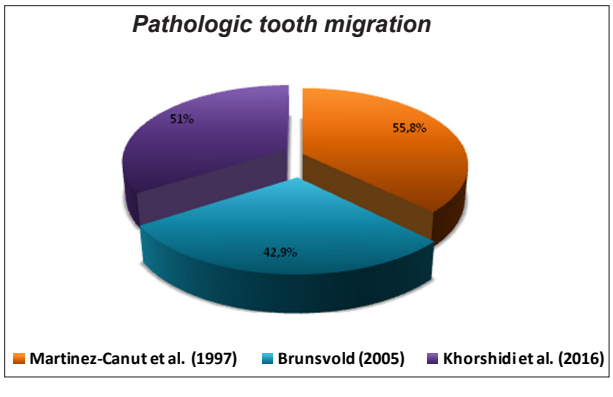

Fig. 1. The rate of pathologic tooth migration according to [2, 3, 4].

Results and discussion

First publications on osseointegration appeared in 1940 when Bothe, Beaton and Davenport did their researches on

titanium implant in an animal. As a result it was discovered that "titanium has a property to fuse in bone", and they proposed to use it as prosthetic material [5].

Later Leventhal [6] in published papers pointed out that "no reaction to the metal was found" and "in no animal there was any infection, no induration, or discoloration about the site of the screw in the soft tissue".

The history of usage of temporary anchorage devices started its development after significant discovery done by Professor Per-Ingvar Branemark when in the 1950s he proved that bone can integrate with titanium components. After his discovery, Professor Branemark introduced the term «osseointegration», which principles served as foundation for clinical applications of implants.

Per-Ingvar Branemark is considered the «*father of modern implantology*» and his discovery was really significant for medicine and dentistry. Due to Branemark's discovery an increasing interest in osseointegration of implants in bone was mentioned, and got started the era of using implants for orthodontic anchorage.

Jokstad et al. [7] emphasized in his paper that «the uniqueness of Professor Per-Ingvar Branemark research» was «the discovery of osseointegration phenomenon».

Nowadays, due to the development of implants technology, this method of treatment is widely used. In dentistry, the temporary anchorage devices are used with the aim to produce an orthodontic tooth movement that occurs as a result of a force being placed on a tooth application forces, producing change of a tooth position.

In case of tooth migration, or malocclusion the orthodontic miniscrew implants may serve as an optimal variant of treatment. The "anchorage control during tooth movement is one of the main factors for ensuring successful orthodontic treatment" [8].

Temporary anchorage devices (TADs), or miniscrew implants are small titanium alloy, or stainless steel surgical bone screws, used in orthopedics in order to achieve quicker tooth movement with more efficiency and comfort. TADs are temporarily fixed to bone for the purpose "of enhancing orthodontic anchorage either by supporting the teeth of the reactive unit or by obviating the need for the reactive unit altogether" [9] as well as for controlling the tooth movement during orthodontic treatment, and they are removed when the treatment is completed. TADs are used in addition to braces, or as an alternative to headgear.

Labanauskaite et al. [10] proposed to classify the implants for orthodontic anchorage into three groups.

I. According to shape and size:

1. Conical (cylindrical) implants
   a) Miniscrew implants
   b) Palatal implants
   c) Prosthodontic implants
2. Mini plate implants
3. Disc implants (onplants)

II. According to implant bone contact:

1. Osteointegrated
2. Non-osteointegrated

III. According to application:

1. Used only for orthodontic purposes (orthodontic implants)
2. Used for prosthodontic and orthodontic purposes (prosthodontic implants).

The TADs, according to Singh et al. [9] can be "located transosteally, subperiosteally, or endosteally; and they can be fixed to bone either mechanically (cortically stabilized) or biochemically (osseointegrated)". An important fact mentioned by Singh et al. [9] was that "incorporation of dental implants and TADs into orthodontic treatment made possible *infinite anchorage*, which has been defined in terms of implants as showing no movement (zero anchorage loss) as a consequence of reaction forces».

Creation of space in case of tooth migration by using orthodontics miniscrew implants, known as temporary anchorage devices may be used in patients with PTM.

Due to high biocompatibility of nickel-free wires the «development of new b-titanium alloys and other titanium alloys has rapidly increased» [11].

TADs provide stable anchorage for tooth movements, and excellent treatment results were obtained by using miniscrews for orthodontic anchorage in different types of malocclusion, when it is necessary to perform the midline coordination and changes in the occlusal plane, as well as for intrusion, extrusion, distalization and protraction.

For a successful treatment, first of all there should be done an exhaustive orthodontic diagnosis to select the miniscrews implants of a correct length and diameter, and to take into consideration the distribution of orthodontic forces.

However, there is no common, or single opinion about the length and diameter of miniscrew used in orthodontic therapy. For example, Cheng et al. [12] emphasized that in their study «the length of miniscrews had no effect on implant survival», because «the short screws used for the fixation of miniplate implants did not jeopardize their performance» and «longer implants did not necessarily result in greater bone support».

For modern man the physical appearance, sometimes plays a decisive role in carrier and even life quality, that is why people of 21st century are very much concerned about their physiognomy. The malocclusion and other problems of the dental apparatus may cause discomfort in psychically liable people.

The temporary anchorage devices successfully are used in orthodontics therapy, and according to Song et al. [13] "in comparison with the traditional orthodontics, miniscrew implant anchorage can increase the success rate and efficacy of oral orthodontics, with shorter complete closing time of extraction space, lower incidence rate of the postoperative adverse reactions and promising stability and safety".

The miniscrews "have relatively lower costs and are simple to insert and remove; therefore, they can be easily placed by an orthodontist with minimal tissue invasion", and "obtain their stability mainly from mechanical retention in the bone, so they can be loaded immediately after placement" [8].

The temporary anchorage devices are used in orthodontics for the following purposes:

A. Closure of extraction space by using miniscrew implants;
B. Retraction of incisors and canines;
C. For symmetric incisor intrusion, deep bite-intrusion;
D. Molar intrusion, open bite;
E. Molar mezialization;
F. Molar distalization;
G. Dental midline corrections;
H. Extrusion of impacted canines;
I. Canted of occlusion, or occlusal cant

The occlusion pathology is too various, but nevertheless for a better understanding of miniscrew implants usage in orthodontics, below is given a more detailed description of TADs clinical appliance.

A. Closure of extraction space by using miniscrew implants is done with the aim to close a space that was created after extraction of a tooth and in order to pull the adjusted tooth. Due to such orthodontic treatment a patient does not need a prosthodontics implant.

According to Lee et al. [14] «the combination of two midpalatal miniscrews and a modified transpalatal arch serve as a skeletal anchorage» and the «miniscrew anchorage system allows the maxillary anterior teeth to be retracted effectively without undesirable side-effects such as anchorage loss. There is no need to wear Class II elastics to retract the maxillary incisors and to maintain the molar relationship».

B. Retraction of incisors and canines means to move the teeth back. Sometimes the canines and incisors have a tendency to stand out. To retract the incisors and canines and to correct the position of those teeth the miniscrew implants might be applied.

According to Koteswara et al. [15] the «conventional methods of canine retraction are generally grouped into frictional and frictionless mechanics».

Huffman et al. [16] pointed out that the highest mean rate of movement in canine retraction «was 1.37 mm per month», and thus for complete canines retraction about 4-6 months are needed, but Koteswara et al. [15], applying the distraction of the periodontal ligament, achieved a rate of maxillary canines retraction about 2.53 mm per week.

C. For symmetric incisor intrusion, deep bite it is necessary to move a tooth into the supporting structures. In case of a deep bite the upper front teeth almost completely overlap the lower front teeth and for correction of such a malocclusion type, there should be applied a force on the upper anterior teeth to move them deeper into the alveolar bone, and towards the mandibular anterior teeth a force for protrusion movement must be applied.

According to Upadhyay et al. [17] «correction of deep bite by extrusion of posterior teeth is difficult to accomplish in non-growing individuals, also the results might not be stable» and «conventional appliances frequently use posterior teeth for facilitating anterior teeth intrusion».

Due to their simple design and small size, the patient does not feel discomfort and extrusion of adjacent teeth as side effect is minimized. The «mini-implants can solve some problems associated with conventional intrusion devices» [17].

The possibility to be inserted in the oral cavity, even on the alveolar bone between the dental roots, as well as relatively simple implantation technique, that ensures controlling of the direction and amount of force characterize the mini-implants as the most reliable for orthodontic treatment [17]. However, an extreme caution is necessary while placing the implants on some specific dangerous sites in order to avoid inflicting injury on delicate anatomic structures such as vessels, nerves or dental roots [18].

According to Upadhyay et al. [17] a combination «of factors can provide excellent results in the treatment of malocclusions, by increasing anchorage, moving and controlling the teeth and dentoalveolar process in all three planes of space» sagittal, vertical, and transverse one.

D. Two types of molar intrusion, open bite are distinguished:

- *Anterior open bite* – when the front teeth fail to touch antagonists there is no overlap between upper incisors and lower incisors teeth.
- *Posterior open bite* – when posterior teeth such as molars or premolars fail to touch their antagonists teeth.

According to Park et al. [19] «anterior open bite is considered to be one of the most difficult problems to treat in orthodontics». Among various methods of treatment the «miniscrews have many advantages over other various temporary anchorage devices», due to the fact that «miniscrews are relatively simple and easy to insert, less traumatic, stable for the optimal force, and make it possible to apply a force immediately after insertion». Park et al. [19] agree with many authors on the point of «fewer limitations of the implantation site and lower costs».

Cambiano et al. [20] opinion was that «molar intrusion might be effectively achieved by using miniscrews as anchorage in patients with an anterior open-bite» as alternative to surgical treatment.

E. Molar distalization is moving a tooth along the occlusal plane away from the midline. In that case the molar is moving its position from distal inclination to vertical position due to temporary anchorage device by applying force on the tooth.

The miniscrew can be used as stationary anchorage for maxillary molar distalization, especially for «class II malocclusions, without extractions», that «usually requires distalization of maxillary molars» and «with the use of dental implants, mini-plates, and mini-screw implants as anchorage, the distal movement of anterior teeth or posterior teeth (or both) without anchorage loss has become possible» [21].

According to Celebi [21], the miniscrew treatment is an advantage over implants because treatment with miniscrew does not require that long period of healing and osseointegration.

Another advantage of the miniscrews in comparison

with other methods of orthodontic treatment, according to Singh et al. [22] is that a «miniscrew implant can be immediately loaded and used for group movement of teeth».

F. Molar mezialization is the movement of a tooth along the occlusal plane towards the midline. In those cases, a molar is moving its position from mesial inclination to a vertical one, due to temporary anchorage device with the function to apply a force on the tooth.

Wilmes et al. [23] considers that «anchorage control is crucial in treatment of patients», «when protraction of the molars is required without retraction of the anterior teeth and premolars».

Due to cost efficiency and more convenient use in comparison with endosseous implants the «titanium mini-implants are commonly used as a source of absolute anchorage during various types of tooth movement» and the «direct-anchorage mechanics» can be used for «successful closure of a maxillary first permanent molar space with the use of an implant supported appliance (Mesialslider)» [23].

G. Dental midline corrections is a midline deviation, and one of the most difficult problems that orthodontists encounter. This problem might be seen in all types of malocclusions, but more commonly, it is found in Class II cases. For correction of the midline deviation, it is necessary to make a differential diagnosis in order to determine the etiology and to evaluate the effects on the occlusion. General causes of midline deviation are:

- Asymmetry of the upper and/or lower arch;
- Lateral mandibular deviation that might be related with posterior cross-bite or not;
- Tipping and/or drifting on the upper and/or lower incisors;
- Any combination of the named above factors.

Unilateral or bilateral placement of miniscrews on one or both arches might help to correct a severely deviated midline without the use of intermaxillary elastics that requires the patient's cooperation, and in this respect the usage of miniscrew is a great advantage.

H. Extrusion of impacted canines, or moving of a tooth out of the supporting structures. In case of impacted canines, there can be used miniscrew implants to push out the impacted canine to the dental arch.

According to Kocsis et al. [24] results the «mini-screw anchorage should be taken into consideration when extrusion of an impacted canine is planned».

Philip et al. [25] consider that «application of optimal traction forces will lead to a stress distribution all around the periodontal ligament» that as a consequence will result in «marginal apposition of bone at the alveolar crest».

I. Canted of occlusion or occlusal cant might be with, or without facial asymmetry and it appears, due to asymmetric development of the mandible, unilateral extruded molars, or asymmetric dentoalveolar development of the facial skeleton, and/or dentoalveolar development. Occlusal plane canting in the vertical plane is one of the parameters affecting smile esthetics. The canted occlusal plane originates from facial asymmetry and/or vertical position asymmetry of the right and/or left quadrants of the dental arches without facial asymmetry.

According to Hashimoto et al. [26] «conventionally, the combination of mandibular and maxillary osteotomy is used to correct both mandibular deviation and maxillary canted occlusal plane». As an alternative treatment instead of maxillary osteotomy in correction of mandibular deviation and canted occlusal plane, can be applied treatment with miniscrew anchorage.

By a combined treatment of miniscrew anchorage for correction of the maxillary canted occlusal plane by intrusion of the maxillary molars, and osteotomy for correction of the mandibular deviation can be achieved a good therapeutic result.

Preparation of the placement site is a meticulous and very important process for success of miniscrew implants application.

According to Martinez-Canut et al. [2] «no single factor by itself is clearly associated with PTM; the factor mainly related to PTM is bone loss, followed by tooth loss and gingival inflammation, as bone loss increases, the association of additional factors with PTM, such as tooth loss and gingival inflammation, increases».

The stability in using miniscrew implants depends on the quality and quantity of the bone and on thickness, type and health of the soft tissue. Wilmes et al. [27] pointed out that «insertion torques of orthodontic mini-implants and therefore primary stability varied greatly, depending on bone quality», and «compacta thickness, implant design and implant site preparation have a strong impact on the primary stability of mini-implants for orthodontic anchorage».

According to Motoyoshi et al. [28] the cortical bone with a thickness of less than 0.5 mm is not suitable for miniscrew placement. For successful treatment «the prepared site should have a cortical bone thickness of at least 1.0 mm, and the placement torque should be controlled up to 10 Ncm».

In order to attain a good stability effect, «it is better to place the miniscrews in the attached gingiva (keratinized) gingiva, which is more resistant to inflammation and less likely to develop soft-tissue hypertrophy» [8]. However, «if the miniscrew has to be placed in non-keratinized mucosa, a 3 mm vertical stab incision should be used to prevent the soft tissue from surrounding the miniscrew, as this small incision requires no sutures» [8].

According to Cheng et al. [12] «the absence of keratinized mucosa around mini implants significantly increased the risk of infection and failure», also they mentioned the «bacterial role in the failure of orthodontic mini-implants, since peri-implant infection was associated with a high rate of implant failure (71%)».

Branemark et al. [29], in their experiment on dogs, pointed out that for a «long term stability of intra-osseous titanium implants to restore masticatory function» and for maintenance of a «good anchorage of the implant» are required to be respected following important conditions: «1) Non-traumatic surgical preparation of soft and hard tissues and a mechanically and chemically clean implant. 2) Prima-

ry closure of the mucoperiosteal flap, to isolate the implant site from the oral cavity until a biological barrier has been reestablished. 3) Oral hygiene to prevent gingival inflammation».

The mid palatal suture region is the most favorable placement site for miniscrews in terms of both bone and soft-tissue characteristics. The high density of cortical bone and thin keratinized soft tissue in the palatal region ensures the biomechanical stability of the miniscrew at a higher success rate in comparison with the para palatal suture region that is considered the most suitable area for miniscrew placement in adolescents.

On the mandible, the most adequate bone thickness and safety region for miniscrew insertion is either between the second premolar and first molar, or between the first and second molars. Taking into consideration that the thinnest bone was found between the first premolar and the canine, the miniscrew implantation should be done 11 mm below the alveolar crest, as well the miniscrews might be placed in the alveolar mucosa and attached gingiva.

For success of treatment with miniscrews implants, the placement site plays an important role. The miniscrews can be placed in the inter-radicular space between tooth roots, either buccally or lingually; in the hard palate, below the anterior nasal spine; and in the infra zygomatic crest, maxillary tuberosity, edentulous areas, chin and retro molar areas.

In cases when there is not enough space for implant, then «additional space can be created by intentional separation of the dental roots during the initial stages of orthodontic treatment» [17].

For example, Cheng et al. [12] for retraction of the protruded anterior teeth; protraction of retruded posterior teeth; molar intrusion and molar uprighting had used several types of extradental anchors such as:

- Conventional osseointegrated implant,
- Onplant,
- Mini-implants with "a cumulative success rate of 89%" [12].

As advantages of the of the miniscrew treatment Cheng et al. [12] pointed out the "low cost, simple surgical placement, and high versatility". Similar opinions have Upadhyay et al. [17] who marked out that the "miniscrews are now accepted as a simple and effective tool in daily orthodontic practice and orthodontists commonly use them in a variety of clinical situations".

Considering all mentioned above, the success rate in miniscrew implants does not depend only on bone quality, but it depends as well on the soft-tissue thickness, oral hygiene, root proximity and other anatomo-physiological peculiarities such as dangerous areas, the maxillary tuberosity in case of the third molar eruption, etc.

Anchorage preparation is an important factor in the success of orthodontic treatment. Reducing the need for patient cooperation, there is an increase in the quality and efficiency of treatment, ease placement and lower costs are the main advantages of mini-screws in comparison with conventional and other skeletal anchorage preparation methods.

Analyzing the «long-term stability of micro-screws under different loading protocols» on animal experiments, Zhang et al. [30] specified that, the «orthodontic microscrews tend to suffer a failure rate of about 10% to 30%, which is much higher than conventional implants», and as a risk factor «for reducing the long-term stability of microscrews» they pointed out the counterclockwise loading.

According to Arantes et al. [31] «the larger number of threads and their greater angle of inclination resulted in less resistance to deformation and induced a higher level of tension in the mini-implant and cortical bone when subjected to forces, especially when inserted at an angle of 45º to the cortical bone».

Goyal et al. [32] consider that «the popularity of titanium has been attributed to its chemical purity and its ability to form an adherent, passivating oxide film which forms at the rate of 100 A per minute» that has a significant role in development of manufacturing implants industry «with micro and submicro (nano) topography».

According to Kuroda et al. [33] the «miniscrews can provide stationary anchorages for various tooth movements and even make it possible to move the tooth in directions which have been impossible with traditional orthodontic mechanics».

Development of implants industry and particularly «miniscrew anchorage has greatly expanded the limit of clinical orthodontics» [33] but nevertheless, there still are some risk factors in miniscrews use and success of treatments depends a lot on techniques of miniscrew insertion and stability elements.

Liou et al. [34] sustain that «miniscrews are a stable anchorage but do not remain absolutely stationary throughout orthodontic loading», in some patients was mentioned their movement. In order «to prevent miniscrews hitting any vital organs because of displacement, it is recommended that they be placed in a non–tooth-bearing area that has no foramen, major nerves, or blood vessel pathways, or in a tooth-bearing area allowing 2 mm of safety clearance between the miniscrew and dental root» [34].

Regarding the risk of miniscrews treatment Mohammed et al. [35] in published paper emphasized that «miniscrews inserted in interradicular locations between the first molars and second premolars suffer from a failure rate of 9.2% for those inserted in the maxilla and 13.5% for those inserted in the mandible».

Thus, the primary stability is a major element of success in miniscrews using. The stability might be affected by many factors among which are:

- Thickness and quality of bone;
- Design of the miniscrew, including its diameter and length, tapering, thread length and pitch;
- Placement conditions (pre-drilling, penetration depth and number of involved cortical plates, insertion angle, etc.).

Possible risks of miniscrew treatment:

- The screw may touch a tooth root during placement.
- The screw becomes loose.

- Sometimes a plastic device is used as a guide to help place the screw in exactly the right position.

Nowadays, due to their advantages the miniscrew anchorage devices are widely used in orthodontic treatment, but for patient benefit an orthodontist should be aware of risk factors, as well as to take into consideration the disadvantages of miniscrew treatment.

Conclusions

1. Development of implants industry and particularly miniscrew anchorage has been rapidly increased in the recent years, but nevertheless, there still are some risk factors of miniscrews use and success of treatment depends a lot on techniques of miniscrew insertion and stability elements.

2. The "anchorage control during tooth movement is one of the main factors for ensuring successful orthodontic treatment" [8] and "the most important keys for achievement of success in clinical orthodontics" [33].

3. The advantages of miniscrews, in comparison with other methods of orthodontic treatment might be resumed to: lower costs, easy placement, no need for patient cooperation, high quality and efficiency of treatment.

4. Among the most undesirable side effects of miniscrews use can be marked out the screw fracture, screw-root proximity, damages of soft and hard tissues, displacement under orthodontic loading, etc.

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Treatment of dental subtotal coronal lesion through atraumatic surgical extrusion

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Abstract

Background: Clinical short crowns or teeth with insufficient height of the supragingival part are a provocation for restorative dentistry. Restoring severely damaged teeth requires a comprehensive approach, a well-planned pretreatment. These teeth with subtotal coronary dental lesions can be treated conventionally by surgical resection of the gum and support bone or by the atraumatic surgical extrusion of the tooth as an alternative to avoid complications that may occur following removal of tissues. Now, the world's population lives longer. The hope of life according to WHO in 2016 was 72.0 years old. Respectively, the requirements of the people are higher in order to maintain oral health and effective treatment for the preservation of their own tissues. The atraumatic surgical extrusion of the tooth is a biological solution for the preservation of the tissues, but due to the recent implementation is not fully evaluated its effectiveness.

Conclusions: The Benex system can offer certain advantages to both the patient and the clinician, including the predictability of maintaining the stability and integrity of the dental alveole after the extrusion, due to the applied vertical force. Furthermore, the axial force for several minutes minimizes the oblique and lateral force that decreases compressive manipulations in the periodontal ligament and risk of resorption defects. However, the given technique can be resulted in unpredictable results, because it is an innovative technique and there is a small number of scientific studies on the subject.

Key words: dental extrusion, atraumatic surgical extrusion, Benex system, crown lengthenig.

Introduction

Through different marketing methods population is conscious in the ability of the dentist to restore the aesthetics, less the functional part. However, the restoration of tissues with insufficient supragingival height should be enforced in compliance with biological principles. The direction must be dictated in dependence on the concrete clinical situation: apically or coronary. The compromise situation should combine concomitantly: the component of the 1:1-crown: root ratio after treatment and the component of the distance of at least 3 mm of supracrestal dental tissue between the bone and the edge of the future restoration according to the concept of "biological width" [1, 2]. This concept is supreme in understanding the apico-occlusion relationship between the edge of the restoration and the crest of the alveolar bone [1, 3, 4].

The "biological width" – is a combined dimension between the junction epithelium and connective tissue of the attachment, which represents the distance between the deepest point of the gingival attachment to the crest of the alveolar bone. Gargiulo A.W. et al. established the mathematical value of the components of this dimension such as: junction epithelium – 0.97 mm (0.71-1.35) and connective tissue of attachment to the alveolar ridge – 1.07 mm (1.06-1.08) [5]. This dimension has about 2.04 mm. There are two aspects of the crown lengthening (CL): functional and aesthetic one. In both cases surgical intervention comes to reposition the biological width apically by discovering the dental structure [6]. Therefore, in order to have a lasting restoration it is necessary to respect the space of 3 mm between the bone and the edge of the prosthetic construction, which will allow the reformation of the biological width and the sulcular depth [3, 4, 7-9].

Several studies showed that the biological width after CL intervention is restored between 6 months to 3 years. That's why it needs to be considered the periodontal status of the patient and the habits of the oral cavity hygiene. Additionally a correct diagnosis and interdisciplinary tactics will improve the achievement of predictable conservative results in the frontal aesthetic area [10].

The common causes of the short clinical crown are: deep dental caries extended to the alveolar bone, erosion, dental malformations, tooth fracture, atrition, excessive decrease of the tooth, so the tooth losing retentiveness for a subsequent restorative treatment. The fundamental purpose of the CL is to provide a retentive coronary dimension, clinically appropriate for the stable dento-gingival complex opposite the placement of the restoration edge, and an optimal aesthetic result [10].

However, there are clinical situations, which require a decision to be taken to restore or draw short clinical crowns such as: the root caries not located in cervical part of the root, the place of the perforation incompatible with a subsequent treatment. If the fracture extends to the root the clinician must appreciate the forecasts, accessibility, periodontal biotype, gum thickness and aesthetic appearance before proceeding to the procedure directly. If the fracture compromises the furcation then the radicular resection or extraction is indicated. If the fracture has a favorable localization in the coronary third of the root then the apical flap

operation is indicated with bone resection for exposing the fracture and recreating biological widths or dental extrusion. If the tooth that requires CL has periodontal bags must be appreciated the height of the outstanding support bone, the strategic value, and forecasts. Initial therapy is directed towards decreasing inflammation and stimulating a better homeostasis. Muco-gingival bone surgery can be performed for the elimination of periodontal bags and the concomitant CL. It is essential to apply specific criteria to decide the treatment of election for each individual case. However, the method of atraumatic surgical extrusion (ASE) has the advantages of minimizing the loss of dental tissue and increasing its longevity with minimal cost and in an operative time, without compromising the tooth involved [11].

Advantages and disadvantages of different methods of CL and dental extrusion

There are different methods of treatment for dental subtotal coronal lesion.

To the apical direction. 1. Gingivectomy; 2. Electrosurgery or laser surgery (a method that reduces excess tissue with good bleeding control, but contact with the bone should be avoided here because there is a danger of necrosis); 3. Technique by apical positioned flap with or without bone resection for exposure of a minimum of 3 mm of root (if the bone level is normal).

To the coronary direction: 1. Orthodontic extrusion; 2. ASE with the periotomes or Benex system (BS).

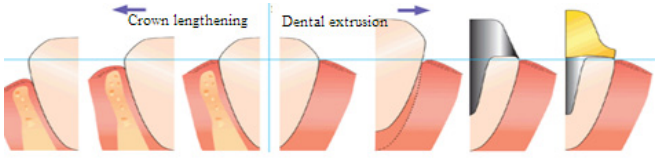

Fig. 1. Methods of treatment for dental subtotal coronal lesion [5].

Each technique has advantages and disadvantages. The method of gingivectomy requires a sufficient amount of keratinized tissue. A great disadvantage of gingivectomy is that in the frontal area it is contraindicated for aesthetic considerations, as well as the technique with the apical positioned flap. However, according to the American Academy of Periodontics 2003 CL – with/or without apically positioned flap is the most common and frequent surgical intervention [5]. A thick biotype of gum manifested by a dense area of keratinized tissue, which ensures a denser flap, makes the intervention more predictable and with a higher success rate compared to the fine biotype [10]. According to the study, apically positioned flap with osteoectomy is more effective than gingivectomy. The procedure for bone resection used in periodontological therapy has been shown to be more effective in stabilizing periodontal destruction [11]. The risks and complications that may occur in the frontal area following the gingivectomy procedure or apically positioned flap are [1, 12-14]:

1. Loss of interproximal papilla.
2. Overweighing.
3. Suprarecesia – what will require correction for root cover again.
4. Repeated increase of gum with the need for retreatment.

This is why it is necessary to include in the treatment protocol the warning of the patient with the risks and postoperative complications that can occur both immediately and tardive.

For CL in the front maxillary area it is important to study the position of the lip line and the exposure of teeth and gums, the relationship between the smile line and the position of the incisal line of the upper frontal teeth by aesthetic point of view. It is useful to examine the symmetry of the right and left side in relation to the median line. In CL through bone resection the existing gum morphology should be analyzed because the gum tends to return to its original position [4, 15].

The increase of the clinical crown to the coronary direction can be performed by orthodontic extrusion, atraumatic surgical extrusion (ASE) with the help of periotomes or Benex system(BS). Orthodontic extrusion can be achieved by traction using fixed, movable or temporary anchoring devices. This method is limited by accepting the patient, the duration of treatment, the risk of returning the tooth to its original position and it is a costly method with unfavorable aesthetics [16, 17]. Repeated rupture of fibers, the retention phase required after extrusion and the tendency to turn back are the main disadvantages for orthodontic extrusion [18]. In cases of deep subgingival cavity, subgingival fracture and when bone surgical resection is contraindicated, it is proposed the ASE with the periotomes or BS with predictable aesthetic and functional results. These techniques are an alternative approach compared to orthodontic extrusion or resection of tissues. The alternative approach through the extrusion results in a lower coronary height of the final restoration compared to the resective therapy, respectively, and the crown-root rate will be more favorable following extrusion than by the technique of resection of tissue [18, 19].

Surgical extrusion by such approach avoids undesirable consequences such as loss of interdental papilla, appearance of aesthetic or functional deformations, repeated return and rupture of periodontal fibers, etc. [1]. For the first time the tooth's luxation was made by Khanberg. He introduced the fine and gentle luxation of the tooth to the desired position. The Khanberg technique is the same like ASE – to bring the root in the desired position without osteoectomy or bone augmentation, the only difference is the utility of the modern atraumatic instruments and systems. This technique reduces the risk of dehydration of the periodontal ligament. The advantages of ASE are: 1. Reducing the time of the entire treatment compared to orthodontic extrusion; 2. Alternative conservative approach to bone architecture compared to bone resection [1, 20].

The ASE with the use of periotomes can also cause the crestale defects, fracture and deformation of the dento-alveolar complex. However, in comparison with periostal

elevators, periotomes allow minimal bone trauma, as they are placed in the periodontal trench and the Sharpey fibers are separated due to a fine blade. The periotomes provides a non-flap extrusion, decreasing discomfort and post-operative pain. The disadvantage of this technique is that it requires the possession of the polished and experienced manual practices for the success of the intervention [1].

The ASE of the tooth through the BS (Helmut Zepf Medizintechnik, GmbH, Hager & Meisinger GmbH) is considered analogically with the extrusive luxation after dental trauma. The incidence of root resorption in such a situation is 15%. According to other studies, the non-progressive resorption of the root meets at a frequency of 30% [18]. Tooth loss can occur in 5% cases, poor tooth mobility in 4.6%, marginal bone loss in 3.7% and progressive root resorption in 3.3% [21]. During extrusion, the fibers in gingival and periodontal tissues are elongated by stretching and new bone is formed in the direction of movement [22].

The indications of dental extrusion are [1, 11, 23, 24]:

• Rehabilitation of compromised teeth by extensive subgingival caries.

• Fracture of the root or endodontic perforation situated in cervical part of it.

• Severe parafunctional habits with massive coronary destructions.

Contraindications of dental extrusion are [16, 18]:

• Insufficient length of the root.

• Insufficient periodontal attachment.

• Fracture of the root or endodontic perforation not situated in cervical part of it.

• High-risk roots fracture (slim roots).

• Teeth with modest endodontic prognosis.

• Teeth with multiple roots and divergent roots.

Although the BS was originally designed for the atraumatic extraction of the tooth, the clinicians expanded its horizons. This system consists of a screwdriver (which is threaded in the root after geometric widening and the creation of the pilot hole) – the connecting part between the tooth and the wheel pulley. In this way, the root being fixed with this device is towed dosed in millimeters, with balanced manipulations being quite easily routed.

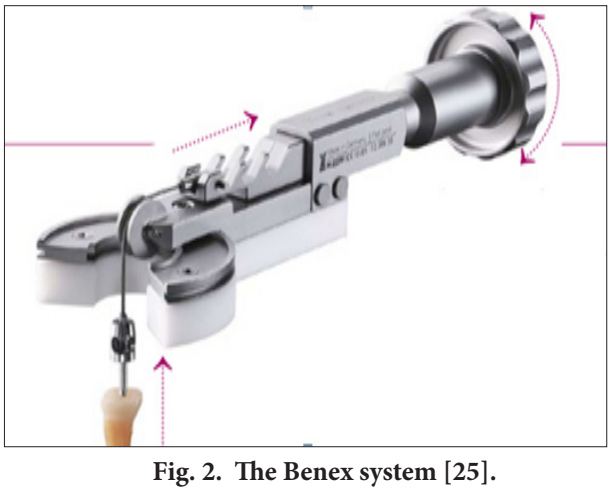

Fig. 2. The Benex system [25].

The creation of the pilot hole and the sealing of the screwdriver must be carried out quite mildly so as not to fracture the walls of the root. The traction itself lasts a few minutes [18].

ASE minimizes the deterioration of the root surface, the disruption of the root ligament and the deformation of the bone apophyseal that makes the changes that can occur more predictable.

Fig. 3. The traction forces used in dental extrusion [25].

The vertical axial traction force used produces the minimal loss of cementoblasts on the radicular surface compared to the traction using pliers. The rupture leads to the formation of an apical clot at the apex of the root, which subsequently reshape and turn into oscillating bone [18, 26]. It is not fully explained the period of rapid remodeling of the periodontal support ligaments after the extrusion, but the bibliographical analysis confirms the use of ASE in the subtotal dental coronal lesion [16, 18].

CL and dental extrusion are intended for the extension of supragingival dental tissue for restorative or aesthetic purposes. The necessity is dictated by the dental factor or the patient's wish. The clinician will have to choose the treatment of election taking into account the aesthetic, functional and biological aspects of each patient [27].

The anatomical aspects that must be taken into account when deciding CL or dental extrusion are: the anatomy of the root (length and shape), the position of the furcation, the conicity of the root, the smile line, the height of the interdental bone, the anatomy of the hard and soft tissues, insertion of muscles, the width of the gingival tissue attachment. CL depends essentially on the edge of the gum attachment and the thickness of the alveolar ridge. The option of the apical or coronary extension in the CL or dental extrusion will be decided according to the following algorithm [5]:

1. The importance of tooth in the dental arch.

2. The level of the subgingival tooth decay and the degree of apical fracture extension.

3. If the crown/root ratio may be unfavourable after the treatment performed.

4. The length and the morphology of the root.

5. Theoretically the height of the residual bone after CL.

6. The degree of abscess tissue of the remaining support.

7. Possibility of discovering the furcation also with the unwanted discovery of the root that can complicate the final result for CL.

8. Increase of tooth mobility due to diminished support tissue and its effect on occlusion.

9. Possible posttreatment aesthetics and phonetics defects.

10. Possibility of maintaining the control of the plaque after the application of the final restauration.

Conclusions

The Benex system can offer certain advantages to both the patient and the clinician, including the predictability of maintaining the stability and integrity of the dental alveole after the extrusion, due to the applied vertical force. Furthermore, the axial force for several minutes minimizes the oblique and lateral force that decreases compressive manipulations to the periodontal ligament and risk of resorption defects. However, the given technique can be resulted in unpredictable results, because it is an innovative technique and there is a small number of scientific studies on the subject.

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Janus Kinase-Signal Transducer and Activator of Transcription in target therapy of cancer

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Abstract

Background: Janus kinase-signal transducer and activator of transcription (JAK-STAT) is a family of intracellular, nonreceptor tyrosine kinases that transduce cytokine-mediated signals. In the beginning of 20th century, it was named "just another kinase" but by some reasons ultimately it was published as "Janus kinase". The name Janus was taken from the two-faced Roman god of beginnings, endings and duality, because the Janus kinases (JAKs) possess two near-identical phosphate-transferring domains, one domain exhibits the kinase activity, while the other negatively regulates the kinase activity of the first one. The article describes JAK-STAT in many aspects such as general definition, mechanism of action, biochemical qualities and the relation to cancer. Eventually the article will explain the role of JAK-STAT pathway in carcinogenesis and summarize the article through future direction in clinical medicine and research.

Conclusions: Understanding JAK-STAT pathway can help physicians, medical students and teachers apply this into clinical practice. By discovering more about the JAK-STAT pathway, many cancer diseases could be halted or treated due to their connection to cancer therapy.

Key words: Janus Kinase-Signal Transducer and Activator of Transcription, cytoplasm receptors, mutation, cancer.

Introduction

Janus kinases (JAKs) are a family of cytoplasmic receptors associated with protein tyrosine kinases required for cytokine signaling and signal transducer activators of transcription (STAT) are transcription factors which regulate some genes required for cellular proliferation, differentiation and survival. Aberrant activation of intracellular signaling of JAK-STAT pathways resulting from mutations is associated with many types of cancers. Targeting intracellular signaling pathways has been a productive strategy for drug development, with several drugs acting on signaling pathways already in use and more continually being developed. The STATs form dimers that translocate to the nucleus when phosphorylated on highly conserved tyrosine residues (termed pSTAT) by JAKs or other tyrosine kinases. The STAT dimers bind specific promoter sequences and modulate transcription of genes controlling cellular processes including proliferation, differentiation and apoptosis. In addition to the established role of the JAK-STAT signaling pathway, cytokine traditionally signals the noncoding ribonucleic acid (RNA) [1]. Understanding the crosstalk of non-coding RNA with JAK-STAT signaling in cancer is of critical importance and may result in better patient stratification not only in terms of prognosis but also in the context of therapy.

The JAK-STAT pathway is important in cytokine-mediated immune responses. Research in the JAK-STAT field has elucidated its roles in various cellular processes such as proliferation, apoptosis and migration, and has found frequent dysregulation of the JAK-STAT pathway in diverse types of cancer. A similar interaction occurs in human cells, where unphosphorylated STAT5A interacts with heterochromatin protein 1α (HP) and acts as a tumor suppressor. Nuclear JAK2 however, functions as a histone tyrosine kinase, displacing HP1α from chromatin. These data have important implications for human cancer: They suggest new drug therapies, which could target the not canonical functions of JAK and STAT [2, 3].

The (JAK-STAT) pathway plays a major role in transferring of signals from cell-membrane receptors to the nucleus. The JAKs are now recognized as an integral component of the cytokine receptor subunits, and enzyme activation, as the initiating step in a signaling cascade required for embryonic development, tissue growth, haemopoietic development and differentiation, innate and adaptive immunity and the inflammatory response. There is a reciprocal interaction between external actions and internal reactions that enables a cell to live. Each receptor like a sentinel senses stimulus and starts to transfer corps of signals to the 3d castle of the nucleus in order to provoke vital responses. The result of this process may be proliferation, differentiation (polarization), activation/inhibition and survival/apoptosis [4]. The role of JAK-STAT signalling in the pathogenesis, prognosis and treatment of solid tumours is divided into many aspects. The JAK-STAT pathway regulates embryonic development and is involved in the control of processes such as stem cell maintenance, haematopoiesis and the inflammatory response. The pathway transduces signals from cytokines, interleukins and growth factors that act through a number of transmembrane receptor families. Type I receptors include the erythropoietin receptor and the granulocyte colony-stimulating factor receptor. The granulocyte-

macrophage colony-stimulating factor receptor is a type IIa receptor and the type IIb subfamily includes the receptors for interleukin-6 and leukaemia inhibitory factor. The intracellular tails of these receptors are constitutively associated with inactive kinases named Janus kinases. While cellular overexpression studies suggested JAKs could signal promiscuously downstream of many cytokine receptors, it is evident from genetic deletion studies that cytokine receptors have clear preferences for the JAK family members which they utilize as signaling effectors. In light of this fact, here we have focused our attention on the genetic deletion studies that have illuminated which JAKs couple with which cytokine receptors. The first insights into the specificity of JAKs within each signaling pathway arose from early cell-based genetic screens to identify components of the IFNα/β and IFNγ signaling pathways [5, 6, 7].

The JAK-STAT pathway has been known for many years as a key pathway for the vitality functions of many cells in our body both in the blood system and even in the respiratory system or reproductive system. The role of JAK-STAT has been increasingly growing over the past year while more researches were published. In this article are considered many important studies that were conducted in the recent years. We will try to explain the mechanism of this apparatus, and what happens if the apparatus fails and leads to dysregulation of some cells. It is exclusively important to explain what medicines are found today on the pharmaceutical markets, their significance in different types of cancer and their function on the JAK-STAT pathway [8].

The role of the JAK-STAT pathway in carcinogenesis

The JAK-STAT pathway plays a major role in transferring of signals from cell-membrane receptors to the nucleus. Cancer involves abnormal and uncontrollable cell growth in a part of the body. Therefore, since JAK-STAT signaling can allow the transcription of genes involved in cell division, one potential effect of excessive JAK-STAT signaling is cancer formation. High levels of STAT activation have been associated with cancer; in particular, high amounts of STAT3 and STAT5 activation are mostly linked to more dangerous tumors [9, 10]. For example, too much STAT3 activity has been associated with increasing the likelihood of melanoma (skin cancer) returning after treatment and abnormally high levels of STAT5 activity have been linked to a greater probability of patient's death from prostate cancer [11, 12]. Altered JAK-STAT signaling can also be involved in developing breast cancer. JAK-STAT signaling in mammary glands (located within breasts) can promote cell division and reduce cell apoptosis during pregnancy and puberty, and therefore if excessively activated, cancer can form. High STAT3 activity plays a major role in this process, as it can allow the transcription of genes such as B-cell lymphoma 2 and c-Myc Oncogene, which are involved in cell division.

Mutations in JAK2 can lead to leukemia and lymphoma [13]. Specifically, mutations in exons 12, 13, 14 and 15 of the JAK2 gene are supposed to be a risk factor in developing lymphoma or leukemia. Additionally, mutated STAT3 and STAT5 can increase JAK-STAT signaling in natural killer and T cells, which promotes very high proliferation of these cells, and increases the likelihood for developing leukemia [14, 15, 16]. Also, a JAK-STAT signaling pathway mediated by erythropoietin, which usually allows the development of red blood cells, may be altered in patients with leukemia. Early evidence that JAK-STAT signaling is activated in solid tumors was derived from cancer cell lines [16, 17]. There is now substantial data demonstrating tyrosine phosphorylation and nuclear localization of STATs, indicative of STAT activation, in tumor tissue derived from many patients across a range of tumor types. A relationship between JAK-STAT activation and prognosis has been observed in many of these tumor types. In general, activation of STAT3 or STAT5 is associated with a worse prognosis, although in breast cancer and in some studies of colorectal cancer and head and neck squamous cell carcinoma it appears to be associated with more favorable outcomes. In breast cancer, this relationship is consistent with the role of pSTAT5 in normal physiology – constitutive phosphorylation of STAT5 is a feature of normal breast epithelial cells, where it is thought to promote differentiation [18, 19, 20].

For other tumor types, differences in the strategies are used to quantify STAT phosphorylation, which vary across all the studies described below, may account for the apparently conflicting associations between STAT phosphorylation and outcome. Interestingly, there is some evidence that in Myeloproliferative Neoplasms STAT3 may oppose malignant proliferation, suggesting this may also occur in certain situations in solid tumors. Activation of STAT1, in contrast, is generally associated with better outcomes across all tumor types (tab. 1) [21-28].

In conclusion, the table 1 shows different types of cancer in human body which relates to the STAT activation. By understanding the source of the problem and using immunochemistry, we can pinpoint the mechanism that elicits those cancers. As the clinical medicine will use those methods for cancer detection, it will be easier to prevent, treat and halt many malignant diseases.

In some research papers [29, 30, 31], we found that STAT5A/B is an important immunohistochemical marker for prostate cancer as in other research studies [21, 22, 23] were found identical findings for the assesment of the progression of prostate cancer by simple immunoshistochemistry. While understanding this phenomena, we can assess this marker in specific people and direct it for patient's management such as prostatectomy. In those researches was also found association with this STAT activation and the risk for developing non-small lung cancer.

In addition, the presence of pSTAT3 in immunohistochemistry examination was associated with the decrease in overall survival in patients with prostate cancer [32]. After getting to know this mechanism of cancer some researches work on therapy. In 2013 a research about JAK-STAT blockage did not succeed in proving that Siltuximab (JAK-

Table 1

Types of cancers associated with the JAK-STAT pathway

Cancer type	STAT activation, tissue sample	Clinical implications of STAT activation
Non-small cell lung cancer	STAT3 and pSTAT3 detection with immunohistochemistry	Positivity for STAT3 or pSTAT3 associated with reduced overall survival
Prostate	Nuclear STAT5A/B, immunohistochemistry on tissue microarrays from prostatectomy	Presence of nuclear STAT5 associated with early recurrence. Presence of nuclear STAT5 associated with prostate cancer-specific death
Breast	Immunohistochemistry for pSTAT3 on tissue microarrays Immunohistochemistry and immunofluorescence for nuclear pSTAT5 on tissue microarrays	Presence of pSTAT3 associated with improved overall survival in patients receiving adjuvant chemotherapy (10-year survival 79% for pSTAT3 positive, vs 61.5% for pSTAT3 negative). Absence of activated STAT5 associated with decreased cancer-specific survival
Rectal/colorectal	Immunohistochemistry for nuclear pSTAT3	Presence of activated STAT3 associated with better overall survival. Presence of activated STAT3 associated with worse overall survival
Oral squamous cell carcinoma	Immunohistochemistry for nuclear pSTAT3. Automated quantitative analysis immunohistochemistry for nuclear STAT3	Nuclear pSTAT3 associated with shorter median disease-free survival (13 months vs 64 months). High nuclear STAT3 associated with improved overall survival (Mean 119 months vs 57.3 months)
Cervical squamous cell carcinoma	Immunohistochemistry for nuclear pSTAT3	Nuclear pSTAT3 associated with reduced overall survival (5-year survival 79.2 months vs 95.3 months)
Malignant melanoma	Immunohistochemistry for pSTAT1 and pSTAT3	In patients with lymph node metastases, higher rates of recurrence with high pSTAT3. Lower rates of recurrence with high pSTAT1 staining in lymph node and brain metastases
Renal cell carcinoma	Immunohistochemistry for nuclear pSTAT3	Nuclear pSTAT3 associated with shortened cancer-specific survival
Glioblastoma	Immunohistochemistry for pSTAT3 on tissue microarrays	High or very high number of cells positive for pSTAT3 associated with reduced overall survival

STAT inhibitor) can halt prostate disease. Another research in 2012, found out that interleukin-6 antibody, was able to halt several types of cancer such as multiple myeloma, non-small cell lung cancer, colorectal cancer, renal cell carcinoma and prostate cancer [33].

Patients with oral squamous cell carcinoma pSTAT3 positive with special immunohistochemistry detection which is called automated quantitative analysis, had shorter disease-free survival in comparison with other patients (13 months vs 64 months) [34, 35]. But high nuclear STAT3 was surprisingly associated with improved overall survival (mean 119 months vs 57.3 months) in patients with oral squamous cell carcinoma.

Cervical squamous cell carcinoma has similar perspective with oral squamous cell carcinoma, while using immunohistochemistry for nuclear pSTAT3 associated with reduced overall survival (5-year survival 79.2 months vs 95.3 months) [34, 35].

The role of JAK-STAT signaling in the pathogenesis, prognosis and treatment of solid tumours that was described above on several types of cancer is supposed to be fascinating phenomena in biochemistry and oncology.

Finally, the prospects for treating solid tumours are analyzed using strategies targeting JAK-STAT signalling, including what can be learned from haematological malignancies and the extent to which results in solid tumours might be expected to differ [36, 37, 38].

Conclusions

1. According to recent studies JAK-STAT pathway has a significant role in the control of immunity, cell proliferation and apoptosis.

2. Many studies showed that defects which activate JAK-STAT pathway can lead to different types of cancer. Finding defected components of this pathway can help to understand the mechanism of tumor genesis.

3. Finding defected components of this pathway can help us diagnose various cancer diseases.

4. Also, we can use these defected proteins as a target to inhibit the progression of the disease and produce new drugs.

5. After many researches, we still must continue to learn more about different components of the JAK-STAT apparatus.

6. Cancer progression might be in the future less accelerated after discovering more about JAK-STAT.

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Optimizing diagnose for visual disturbances after head trauma in school children

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Abstract

Background: Despite the unprecedented evolution of medical science in recent decades, trauma, especially traumatic brain injury, is one of the most common and widespread injuries worldwide. WHO reports annually over 1 million deaths worldwide, which estimates a doubling of the rate of trauma during childhood, particularly those aged 7 to 18 years. Far from being addressed, the associated complications remain, such as disorders of the visual analyzer, other organs and systems. For this reason, the lesions of the visual analyzer are often a critical condition and a determining factor for the health of the future adult and for these reasons have an increased interest in the field of ophthalmology therapy. The diagnostic behavior is different, if we refer to children compared to the adult population, which requires a personalized, but also an objective, approach in order to assess the visual deficiencies.
Conclusions: The present paper considers the clinical aspects, corroborated with those specially selected in the certain diagnosis and treatment. The study reveals objective data, specially selected for the evaluation of a set of instrumental and laboratory investigations relevant to the age of 7-18 years. Determining an algorithm for diagnosing visual disorders that occur as a result of cranio-cerebral trauma in children will allow a relevant and objective examination to assess the treatment and behavioral tactics.

Key words: traumatic brain injury, visual disorders in children, ophthalmoscopy.

Introduction

The severity of traumatic brain injury (TBI) differs greatly depending on age. The child is not a mere reduced model of the adult. In children, brain trauma occurs on an immature brain. Anatomical differences: the larger head in relation to body mass, the absence of development of the cervical muscles and so on, facilitates the transmission of kinetic force to the brain. This aspect affects the child's response to trauma, as well as the prognosis. TBI causes a cascade of metabolic and inflammatory reactions with local and systemic consequences that lead to global cerebral ischemia with the development of cerebral edema [1]. Due to a vicious pathophysiological circle, as well as the fact that much of the brain structures are involved in the process of vision, they remain a problem far from being solved, testifies Singman EL [2]. Contusions or so-called cranio-cerebral traumas, as a rule, can induce both acute and chronic seizures [1, 3, 4]. This can be explained by the fact that a large part of the brain structures is involved in the process of vision [2]. The visual deficiency can be explained in several ways [5-7]. The mechanism of the visual changes is related to that of the trauma, which in turn can be produced by direct or indirect forces on the brain, affecting the white substance, according to the data of Cockerham GC et al. [8]. The white matter lesion occurs at the cellular and subcellular levels. Therefore, hemorrhage or organic lesions cannot be diagnosed even by the method of computed tomography or nuclear magnetic resonance, Suchoff IB et al. noted [9]. Brad P. Barnett et al. mentions that the age of the child plays an important role in the association of long-term sequelae, because the brain in its evolution is considered to be much more vulnerable, due to the sensitive blood-brain barriers, the elastic properties and the degree of myelination of the nervous tissue [10, 11].

Due to some peculiarities of the regulation of cerebral blood flow, the pediatric age group is subject to the development of intracranial hypertension (ICH), the cause of the development of which is the congestion (expansion, swelling) of the brain. Here the water content inside the nerve cell is increased as opposed to cerebral edema, where the water content is increased in the interstitial space. The incidence of brain congestion in pediatric patients is twice as high as in the adult population, resulting in a mortality of 46-53%. Diffuse cerebral edema occurs in over 35% of children aged 0-4 years and in over 40% of patients aged 4-16 years [1].

In children, the overall cerebral blood flow has a higher value (>100ml/100g/min) compared to that of the adult (30-40ml/100g/min), as well as the cerebral blood flow under physiological conditions in the child, which gives the brain better protection [1].

The treatment of post-traumatic visual impairments involves the use of visual rehabilitation therapies aimed at improving ocular convergence, accommodation and motility. Press L.J. et al. reports that 90% of patients mentioned improvement in visual symptomatology after applying visual therapy [12]. Similarly, there was an improvement, both clinical and statistical, of the accommodation, vision and visual attention [13, 14].

Most adults achieve a full neurological recovery in 1-2

weeks [15-17]. They may have prolonged symptoms and a long recovery period [18-22]. Approximately 50%-90% of adults who have had TBI have symptoms of vestibular impairment or ocular-motor dysfunction [23-26], but limited studies confirm the prevalence of vestibular-ocular disorders among children and teenagers [27, 28].

Notions of morphopathology

Based on the data presented by J. Johansson, the integrity of the vestibular, oculomotor and somatosensory systems is necessary to enable human beings to navigate and operate in a complex visuo-spatial environment [29]. This system is composed of specialized neural connections, which interact at different levels of the craniospinal axis to ensure the possibility of maintaining balance, of coordinating the movements of the eyeballs. The given system consists of sensory organs (retina, semicircular canals) and otolithic organs, as well as mechanoreceptors, having the ability to process primary information, which is then projected at the level of the spinal cord, CNS (basal, cerebellar, thalamus nuclei, cerebral cortex, basal ganglia) [30, 31]. The components of the given process include the vestibulo-spinal reflex (VSR), which determines the position of the head, neck and trunk during dynamic movements. Subsystem injury may result in injury to subsequent mechanisms. By this, it is sometimes possible to explain the exact topography of the lesion. The presence of vertigo, instability of balance, disturbance of vision, fog in front of the eyes are conclusive signs of VSR damage at different levels, and the morphopathological aspect cannot be fully elucidated. In the case of TBI, an important role in the installation of vertigo and dizziness is post-traumatic paroxysmal positional vertigo (PPPV), labyrinth contusion, perilimbal fistula, endolymphal hydrops, otolithic disorders and vestibular disorders in 46% of cases, according to data presented by J. Johansson [29]. Such accusations as, disturbed view, diplopia, reading difficulty are the result of dysfunction of accommodation, vision, insufficient convergence, visual field disorder and nerve paralysis, attests Brad P. Barnett and RE Ventura [10, 11]. Without a well-argued neuro-anatomical attitude regarding post-contusion disorder, visual rehabilitation could be compromised.

Wolf JA et al. have shown that axonal injury at the time of TBI occurs rarely upon impact [32]. More often, however, axonal stretching results in an irregular flow of ion transport, increased intra-axonal calcium ion concentration, and activation of the calcium proteolysis system (calpain proteolytic system). This promotes cytoskeletal proteolysis associated with irreversible axonal changes, in particular disassembly of the endoplasmic reticulum, notes Saatman KE et al. [32, 33].

Increased intra-cellular calcium concentration could lead to increased release of glutamate that activates N-methyl-D-aspartate receptors, and promotes depolarization of neurons, reports Barkhoudarian G et al. [34]. Damaged cells try to return to normal homeostasis by activating transport mechanisms. Increased cellular metabolism and glucose transport require numerous membrane pumps. Overloading of the given system leads to a depletion of energy sources, increased Ca influx into the mitochondria, impaired oxidative-basic metabolism, increased lactose glycolysis, and a final local acidosis as well as edema.

Evolutionary axonal inflammation becomes excessively severe and leads to a process called secondary axotomy. In patients with TBI, spectral magnetic, neurophysiological and electrophysiological data show that their recovery period can be 30-40 days, and in some patients it may take years, denotes Johnson EV [35]. Gardner RC emphasizes that, the age of patients plays an important role, as the developing brain is more vulnerable to traumatic action [36].

Visual dysfunctions after TBI can influence all the elements of the vision: visual acuity, accommodation, visual field, photosensitivity, color perception, contrast sensitivity, pupillary functions, sacadic movements, visual memory. Alteration symptoms may be present as a result of injury to the associated, efferent, or common region pathways, Singman EL and Brad P. Barnett mention [2, 10, 37].

Afferent injury after TBI can occur by a disturbance of visual acuity, contrast sensitivity and color perception. As a rule, these manifestations are bilateral. In the case of post-traumatic optic neuropathy or direct lesion of the orbit the symptoms may be unilateral. Direct trauma can be easily diagnosed with standard ophthalmological equipment. In patients with retro-bulb trauma, signs of proposis, ptosis, and decreased color perception will be suspected, according to Singman, E. L. and Brad P. Barnett [2, 10].

Visual dysfunctions may be one of the causes that would lead to poor patient integration after TBI in daily activity. The Rivermead Post Concussion Symptoms questionnaire is composed of a series of specific questions, which relate to the appreciation of visual function. The patient is asked to determine the severity of the visual symptomatology: how blurry the vision is or how clear the diplopia is. Follow-up studies in patients with average TBI and outpatients showed predominantly visual symptoms after three months, blurred vision 6.0-16.2% and diplopia 2.0-6.2% according to studies by Laborey M et al. [30]. Studies that included patients in the stage of subacute middle TBI show accommodation spasm 24.2-62.0%, convergence deficit 23.3-56.3%, and oculomotor deficit 6.0-51.3%, as Alvarez et al. mention [38]. Case-control studies that included patients with subacute mid-stage TBI and practically healthy patients revealed a higher prevalence of binocular and motor dysfunctions, Capo-Aponte et al. [39]. One of the progressive studies of the patient after the mean TBI determined the presence of an accommodative spasm 23.0% and a convergence deficit in 25.0%, Magone M. T. et al. [40]. Likewise, prospective researches assessing the dysfunction of the accommodation, as well as those regarding the assessment of the insufficiency of convergence, have determined that these are more pronounced in the patients who have suffered an average TBI, according to the data of J. Johansson [29].

Similarly, Magone M. T. et al. found situations in which the patients addressed the problem of difficulty in reading,

both during the subacute period of the trauma and over one year after the trauma [40].

Kapoor N. et al. determine a specific form of stimulation techniques for patients after TBI, called oculomotor visual rehabilitation (OVR). The latter uses combined techniques of motor training as well as the attention training to improve visual defects. Optometrists trained in the instucting patients to optimize divergence/convergence, fixation, saccadic movements choose to perform a certain spectrum of techniques that involve both motor and perceptual training. This complex of exercises helps patients determine not only the visual deficiency that is characteristic to them, but also the motor movements that could diminish them. Both manual machines to train these functions, as well as specialized computer programs can be used. Patients with concomitant strabismus and complex diplopic changes are preferred to be treated under the guidance of both an ophthalmologist and a neurologist/neurosurgeon. Disorders such as convergence/divergence and the accommodative reserve are sensitive to orthopedic treatment. However, when using such techniques, in a patient who has had a TBI, we must take into account the neurological symptoms that may include depression, fatigue, and concentration deficiency [41].

Afferent optical pathways deficits

Decreased visual acuity

The dysfunction of the afferent optic pathways could cause a decrease in visual acuity, color perception, light sensitivity and contrast sensitivity. Decreased post-traumatic vision may be omitted by the patient in case the condition is monocular. As a rule, the decrease in monocular visual acuity may be due to trauma to the orbit and the ocular, notes Cockerham GC et al. [8].

Data on visual acuity in patients after TBI are not clear and fully studied. One study shows visual acuity in patients after TBI between 6/30 and light perception at 13% and 1.6% respectively [42].

In his study, Richard A. Armstrong mentions that patients, who suffer from TBI, may experience a decrease in visual acuity that may persist for a long time [42].

Kenneth J. Ciuffreda et al. mention that, after a TBI, we could determine a growing myopia or hyperopia in the patient [43]. This seems paradoxal at first glance. Increased myopia can be explained by altered functioning of sympathetic NS, which occurs following a TBI. The disturbance of the function of the humoral systems of control of the curvature of the crystalline leads to an insufficient relaxation, in the case of distance sight, so that myopia becomes more accentuated.

On the other hand, an increase in the hypermetropic indicators could be explained by the altered functioning of parasympathetic NS, which may also occur following TBI. Thus, the ability to increase the accommodation to compensate for a residual hyperopia is compromised. That is why, a hyperopia that is not manifested becomes evident. This can also be characterized by the patient's sensation of transient fog in front of the eyes, which demonstrates a function of precarious parasympathetic NS.

These patients will not be directed to a progressive optical correction, but will choose a separate correction both at a distance and at close range. This fact can be explained by the hypersensitivity shown by patients to changes in the optical correction, a hypersensitivity that comes, in fact, from an alteration of the sensitivity caused by TBI [43].

The accommodative spasm, which indicates a pseudomyopia, as a rule seems to be associated with myosis or excessive convergence. Likewise, this spasm can appear not to be associated with anything being due to the psychogenic response [44].

The visual field

Suchoff IB mentions that visual field deficits are observed quite often after TBI. Usually, their presence signals a severe TBI, but they can also be detected in the case of an average TBI with the involvement of an optic chiasm or as a result of post-traumatic neuropathy [9].

Kenneth J. Ciuffreda et al. attest that defects in the field of vision usually refer to certain sectors, which are missing or seem to be sensory suppressed following the action of the trauma on the primary visual pathways. These areas can take shape from hemianopsies to small regions with reduced sensitivity. The symptoms in this case may be different, starting with accentuated visual difficulties up to minor visual effects. Visual field deficits were determined in 35% of the population with visual changes after TBI [43]. Some patients may benefit from using recessed prisms, such as Fresnel or Peli prisms, according to Ross NC et al. [45]. The training itself should include stimulation by luminous targets of both the deficient sector and the visual field. There are certain programs that improve the visual field by stimulating cortical function, by training the patient to better understand the visual field deficits or even to align their eyeballs to these deficits, according to data presented by Plow EB, Obretenova SN et al. [46].

In his study William V. Padula et al. determine an appearance of the visual deficiencies somewhat correlated with the anatomical area in which the tissue injury occurred afterwards in the TBI [47]. Thus, lesions that have included the visual tract damage above the optic chiasm can most often cause hemianopsy-like visual defects, but they are not always congruent. Depending on the affected area, the person may or may not be aware of the loss of visual abilities. The temporal lobe injury most often causes the appearance of an upper quadrant with oblique margins. Vision-spatial negation can be manifested either as a form of a total subjective loss of vision perception on the affected side or an objective lack of vision, notes Padula W. in his study on neurosensory rehabilitation questions of patients [47].

Richard Armstrong mentions that a number of studies reveal problems in the visual field in patients after TBI [42]. Patients with bitemporal hemianopsies reported by Padula JH et al., attributed to optic chiasm injury, were also attested [47].

Color perception, contrast sensitivity

R. Armstrong suggests that there are few studies that would talk about color perception in patients after TBI. However, data are found in the literature that would say that a case-control study of 11 patients after TBI and 11 control patients would suggest a poor perception of a primary color [42].

Lemke et al. determined that 21% of patients with TBI showed a low contrast sensitivity, which resulted in a low quality of life [48].

Efferent optical pathways deficiency

Accommodation

The accommodative dysfunctions (the accommodative step, the insufficiency of the accommodation and the inability to accommodate) can lead to an intermittent or constant blurred vision, depending on the severity of the injury. These come with the neurological changes of the trauma, but can affect other ocular manifestations.

Kenneth J. Ciuffreda et al. mentions that the accommodation disorder in patients who have had a TBI is manifested largely by a moderate impairment [43]. This seems controversial. A study in presbyopia and pre-presbyopia patients, who underwent TBI, elucidated by Thiagarajan P. et al. shows that the accommodation deficit will be determined in 24.4% and 41.1% respectively [26]. On the other hand, a case-control study of 50 patients after TBI and 50 control patients performed by Olver JH et al. determine equal numbers of the accommodation deficit [49].

Nystagmus

Nystagmus represents an oscillation of the eyeballs. This can be moderate or determined in case of major and wide oscillations. Usually, however, patients present with a slow, small amplitude nystagmus, which is accentuated if we cover an eye. Similarly, nystagmus may appear as a consequence of ophthalmoplegia. It can occur following the injury of the optical chiasm which stimulates certain pathological regions of the pituitary gland [30].

According to data presented by Geiger G. et al., which included 65 patients with central nystagmus following a TBI, favored by an extension of the spinal cord [23]. On the other hand, the patients who acted with a force that led to flexion-extension of the same sector presented associated vestibular and sensory disorders [42].

Scherer MR et al., in their research determined a pathological nystagmus with the feeling of dizziness in 50% of the patients exposed to TBI, and in the non-symptomatic patients the incidence was 33% [17].

Extra-ocular motility

The eye's motility system is highly sensitive to TBI, so the appearance of heterophoria is a common occurrence in patients after TBI. Patients who develop heterophoria most often will demonstrate diplopia. Skull muscles paralysis seems to lead to a deficiency of ocular motility commonly encountered [50]. The cranial nerves are very sensitive to TBI because their pathway is along the base of the skull. Unfortunately, the assessment of the motility of the eyeballs is difficult to establish in the first hours after the trauma because the patient is mostly often in a coma. Many signs, such as, for example, the third pair of cranial nerves are felt within a few months. Lagoftam may occur in patients after TBI due to a paresis of the facial nerve. In the case of fractures of the skull base the most frequent paresis of the nerve is that of the ipsilateral facial nerve of the motor neuron [51].

Deficiencies of oculomotor muscles can lead to binocular and accommodative dysfunctions that occur as a result of injury to the cranial nerves: CN III (oculomotor nerve), CN IV (trochlear nerve) and CN VI (abducens nerve), says Suchoff IB in his studies. But most of the time, the damage of one of the nerves is not obvious and can only be assumed. It is stipulated in the literature that computerized tomography, nuclear magnetic resonance do not cause changes [47].

As a consequence, the strabismus appears after a paresis of a cranial nerve or the injury of extraocular muscles, especially in case of damage of the integrity of the orbit. Esophoria or exotropia are also consequences of TBI. Binocular vision disorder often occurs after TBI, with the installation of latent force or fusion disorder [52]. One of the main accusations the patient has is diplopia. And the latter can occur even when the patient is ready to be discharged.

The World Health Organization estimates that strabismus accounts for 2-3% of the child population [36]; occurs more frequently at the age of 2-3 years. In 36% of cases, strabismus is complicated by amblyopia [53, 35]. In the Republic of Moldova, according to the statistical data and the annual activity reports, in the ocular nosological structure in the children taken under supervision, the strabismus holds the third place and constitutes 15-20% [54].

In general, it is proposed to evaluate the patient who has suffered TBI using the following aspects of ocular motility: fixation, amplitude of the saccades and the ability to track an object.

Stereognosia

The brain association systems together with the motor fusion are based on a mesencephalic network, where the oculomotor areas are located, as well as the areas corresponding to the view from the frontal lobes of the brain [55, 56].

A study shows that 10 patients, who have had a TBI, show an altered stereognosis at close and distant sight. Patients were investigated using stero-tests at distances of 3 m and 40 cm respectively. Thus, the data compared with those of the control patients did not show obvious differences in both the monocular and the binocular examination. The authors of the study Ciuffreda KJ et al. conclude that the disorder of stereognosis in post-TBI patients is not a problem of binocular perception, but a consequence of the disorder at a higher level associated with diffuse cortical injury [43, 57].

Convergence represents the movement of the medial eyeballs. This mechanism starts when patients look at an object closer than 5-6 m. A convergence deficit is one of the most common symptoms associated with TBI [50]. Thiagarajan et al. denotes the fact that 56.3% of patients who

have had a TBI attest some vision disorders [26]. Similarly Ciuffreda et al. points out that 42.5% of patients suffer from signs of insufficient convergence [43]. It is proposed to examine the patient using the cover test, to determine the proximity point of convergence, to evaluate the heterophoria according to the tactic Von Graefe, the amplitude of positive and negative fusion.

Pupillary reaction

Patients with TBI present a slow reaction of the pupillary response, as well as signs of anisocoria. The causes that could lead to the formation of a fixed and dilated pupil could be: transtentorial herniation (the sign of the Hutchin pupil), resembling the third pair of facial nerve, traumatic mydriasis or orbit fracture. In patients with TBI, they may have a narrow pupil, which may indicate the presence of Horner syndrome, traumatic myosis, pontine hemorrhage, and the Hutchinson pupil [52].

Kenneth J. Ciuffreda et al. notes that the assessment of pupillary reflex is important in the acute stage of TBI and could indicate the appropriate treatment options. According to a study in Portland, Oregon from 2012-2013 on a number of patients who had a TBI, 5 patients were diagnosed with pupillary reflex problems.

Similarly, a case-control study of 17 patients who had a TBI and 15 control patients revealed a difference in certain parameters: constriction velocity, maximum and average speed, maximum diameter and amplitude of constriction [43].

Papillary edema is a common neuro-ophthalmological complication. This occurs within the first 48 hours as a sign of intracerebral or extracerebral hemorrhage and is an absolute indication for surgery. If it appears after one week, it indicates a cerebral edema [52].

According to the literature data we find the notion of post-traumatic optic neuropathy (PTON) which has an incidence of about 0.7% -2.5% cases after a closed or open TBI. Its clinical picture is presented by signs such as decreased visual acuity, lack of color perception and color sensitivity, pupillary defect and a lack of changes at the back of the eye [58].

On the other hand, we find the same neuropathy, classified according to the anatomical sector involved: anterior PTON characterized by inflammation of the optic nerve papilla and posterior PTON with an unchanged image of the optic nerve papilla in the acute stage [58].

A traumatic injury of the optic nerve may occur as a result of a direct action of a bone fragment on it or it may be caused by an indirect mechanism of edema or ischemia. If we refer to the first variant, then we can say that a CT scan would help us determine the causative bone fragment. In case of ischemia or edema, an MRI examination would be more appropriate. In many cases the administration of high doses of corticosteroids would have a beneficial effect in case of worsening of tissue perfusion due to the indirect mechanism [30].

According to statistical data, the clinical picture of delayed PTON is more frequently encountered than that present in the acute stage of trauma. According to the International Optic Nerve Trauma Study (IONTS), the latter was determined in 13 cases of total patients investigated (approx. 10%), according to Levin La et al., delayed PTON appears due to a full spectrum of mechanisms [20]. Crowe et al. have described a clinical case of a patient who was accused of losing visual acuity on the 9th day after frontal TBI, as a result of secondary bleeding and inflammation of the optic nerve and optic chiasm [25]. Eidlitz-Markus et al. also described a clinical case of a 16-year-old patient who had symptoms of PTON delayed after 2 months of TBI with a blunt object [59]. The authors were not completely sure of the mechanism that triggered PTON, but the pale appearance of the optic nerve demonstrated an underlying ischemic mechanism. Likewise, Kay B. Kang et al. described a case of PTON delayed by the manifestation of a pale and edema optic papilla, with a triggering ischemic mechanism due to compression of the posterior ciliary artery, following inflammation of the medial right muscle [58].

From the point of view of an anatomical division we could say that the anterior PTON is more often encountered than the posterior one. Following a retrospective study by Goldenberg-Cohen, papillary edema was determined in 6 patients (15%) investigated [60]. Similarly, Brodsky et al. illustrated in the study three patients with previous PTON who had visual acuity of 1.0 0.1 respectively and light perception [61]. All three of these patients were previously hit by a blunt object in one eye. The patients were young people, more than that, two of them presented the so-called optical cup-less disk. Then the authors came up with the hypothesis that, in the case of an optic cup-less disk, the peripapillary inflammation of the sclera after a trauma, could induce the formation of a sclera channel, which in turn causes an interruption of the axonal transmission. This induces the formation of axonal conglomerates and, respectively, the inflammation of the optic disk [58].

Examination of the visually evoked potentials

The visually evoked potentials (VEP) refer to the electrophysiological signal arising from the neural activity correlated to the region corresponding to the visual cortex in response to a certain visual stimulus fixed in time [62, 63]. The primary activity recorded refers to the activation of the photoreceptor cones in the central area at 150 degree of the visual field [62, 64]. This corresponds to ~ 50% of the primary visual cortex [64, 65]. The registration of VEP activity was established as a method determined to assess objective and quantitative data in order to describe the integrity of the primary visual paths [66-69]. When applying a high luminescence stimulus and low temporal frequency, parvocellular pathways dominate in the processing of visual information [70], and on the other hand when using a low luminescence stimulus and high temporal frequency, the magnocellular pathways dominate. As a reference, in recent studies it has been shown that patients with TBI in the anamnesis have an increased coherent movement threshold, suggesting deterioration of the magnocellular pathways [71]. Thus, we could conclude that subjects with TBI in the anamnesis exhibit a

disturbance of the magnocellular pathways [72-74], which in turn would be responsible for processing the visual information with low luminescence, they hypothesize that the VEP data will be delayed in time and have decreased amplitude compared to control subjects [75].

Conclusions

1. A detailed clinical examination as well as a spectrum of neuro-ophthalmic investigations play a vital role in identifying the location and type of TBI lesion.

2. The mechanism for establishing visual disorders following a TBI is multifactorial. These can occur both as a result of a primary axonal injury induced by the force applied, as well as a secondary axonotomy induced by a primary ischemic process.

3. Taking into account the plasticity of the nervous tissue of the children the visual disorders can manifest themselves after the acute stage of TBI in the form of a post-contusion visual symptom.

4. The alternation of the refractometric data cannot be considered as the basis of an immediate optical correction, considering that these can be manifestations of transient pseudo-myopia or excessive accommodation.

5. The visual field defects could be an indication in the assessment of the anatomical area that was subjected to the lesion, highlighting over time the ability of the nervous tissue to restore its basic functions.

6. The long-term presence of disorders of fusion, convergence, tracking ability, stereognosis may be more pronounced in the pediatric population, as they may delay the restoration of the child's educational process.

7. Ophthalmoscopic changes in children are largely dependent on the degree of TBI, but these have a more pronounced tendency to manifest after the acute period of the disease in the form of delayed post-traumatic optic neuropathy.

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Features of signal transmission and aqueous media in tumorogenesis

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Abstract

Background: The latest studies highlight the importance of a holistic bioelectric field in the development of tumor diseases. According to some researchers, carcinogens prevent formation of a single morphogenetic field and lead to the creation of separate bioelectric fields. It has been experimentally proved that the development of a tumor in a certain part of the body depends on the bioelectric state of the distant regions. Water is one of the important links between the morphogenetic field and molecular substrates. Due to the presence of hydrogen bonds in the aqueous media, specific structures can be formed that can receive, store and transmit information. Intracellular structured water can serve as a "transformer" of various types of energy for use in the life processes of cellular structures. It was found that normal biological tissues could be distinguished from hyperplastic and malignant ones by means of magnetic resonance image scan based on recording diverse reactions of water protons.

Conclusions: the importance of a holistic bioelectric field in the development of tumor diseases is probably paramount. The development of a tumor in a certain part of the body depends on the bioelectric state of the distant regions. It is possible that carcinogens prevent formation of a single morphogenetic field and lead to the creation of separate bioelectric fields. A more in-depth study of the bioelectric and water constituents in patients with oncopathology will probably open up new facets of oncogenesis.

Key words: carcinogenesis, bioelectric patterns, aqueous media.

Introduction

At the present stage, cancer incidence and mortality remain one of the most modern pressing challenges. According to estimates from the International Agency for Research on Cancer (IARC), cancer will be the leading cause of death in the coming decades in all regions of the world. More than 22.2 million cancer cases reported each year in the world are expected to be diagnosed by 2030 [1]. The American Cancer Society has published an annual report, which shows that 1.8 million new cancer cases and 606880 deaths from cancer are expected to occur in the USA in 2019 [2].

According to January 2019 statistics, the lowest level of five-year survival in Europe after a cancer diagnosis is seen in the Russian Federation (40%), while in France, for example, it is more than 60%, and in the USA – more than 80%. The reason is that in Russia oncological diseases are diagnosed, as a rule, at stage III or IV, while in Europe and the USA – at stage I or II [3].

Despite huge financial resources invested in cancer research, the leading U.S. centers have noted a steady decline in the incidence of certain low-risk cancers and an absolute increase in intermediate and high-risk cancers [4].

The importance of intercellular communication in oncogenesis

Despite substantial efforts aimed to identify cancer "triggers", molecular cell substrate studies have been significantly more modest. Instead of a small amount of biochemical and genetic indicators of specific blastoma cells, molecular analysis of human cancers have revealed a much wider variety of such determinants [5]. The latest studies identify a number of RNA molecules that do not encode proteins as tumor markers. According to Lavrov SA et al. [6], who investigated the aspects of non-protein-coding RNAs influence on chromatin structure, the actual scale of these processes at this stage turns out not to be evaluable, but, undoubtedly, enormous. Similar conclusions can be found in the works of many researchers [7].

Modern studies confirm the historical thesis that voltage gradients can predict morphology, providing information on the structure, growth and formation of the organism as a whole, and play an important role in the process of oncogenesis [8, 9].

The results of experiments [10] serve as an example of significance of the bioelectric state of cells in tumorigenesis.

Scientists induced tumor-like structures (ITLSs) in Xenopus model by overexpression of various oncogenes, such as Xrel3, Gli1, p53Trp248 and KrasG12D, associated with the development of melanoma, leukemia, lung cancer and rhabdomyosarcoma. Experimental findings suggest that the depolarized transmembrane potential is a marker of ITLSs regardless of its genetic origin.

According to the authors [11-13] violation of the expression of some ion channels is promising in terms of the diagnosis of blastoma processes. However, the same effect was achieved by researchers [14] using any method of Vmem depolarization (by modulating chlorine, sodium, potassium, or hydrogen channels).

The researchers [15] also point out the paramount importance of the integral formation field as contrasted with molecular mechanisms at the cellular level for the integral development of an individual. The scientists' research was focused on independent methods for implementing morphogenesis. For example, renal tubules in a triton, having a constant size, can be constructed from cells of various sizes, depending on ploidy. Reaching the same macroscopic state can be realized by various underlying molecular mechanisms. Thus, the renal tubules can be formed both by bending of the cytoskeleton – twisting one very large cell around it, or by numerous small cells.

The above discrepancies can explain to some extent the absence of a frequent direct dependence between the outcome of the cancer process and the level of tumor markers.

In the context of the importance of the problem concerning intercellular communication for tumor genesis and regenerative pattern, we think it necessary to consider the early experiments of Seilern-Aspang F and Kratochwill L [16]. The author described experiments with planaria in which a carcinogen led to the formation of many head teratomas with irregular nerves and ectopic eyes, and concluded that "the cell-isolating action of the carcinogen prevents formation of a single morphogenetic field and leads to the establishment of several separated fields of reduced dimensions".

Consequently, it is possible that in their mainstay tumors have their own bioelectric autonomous field. The latter leads to a loss of integration with the host's body layout. This phenomenon is indirectly confirmed by the fact that, in contrast to normal somatic tissues, which are reconstructed during transplantation to foreign places [17], the histopathological structure of metastases reflects the structure of the tissue of origin rather than their destination [18].

The importance of the aqueous media in oncogenesis

According to the researcher [19] bioelectric control of cell differentiation and growth can be achieved using different types of water.

Spiral-shaped chain structures can be formed during the interaction between the water molecules and the structural components of the cell, which make it possible to implement the proton conduction mechanism through this universal conductor. Water molecules can interact with each other in such formations according to the principle of charge complementarity, that is, through long-range Coulomb interaction [20].

Just in a few instances the intracellular water can be considered as solely a medium [21], nevertheless it is a metabolic reactant, product, and catalyst [22]. Intracellular water is responsible for the conformations and functions of all biomolecules due to direct interaction with their hydration shells [23-25]. According to Zenin SV [26], violation of the Arrhenius dependence in determining the thermodynamic parameters for complexation of aromatic cycles indicates the presence of a structured state of the aqueous medium that controls the behavior of interacting hydrophobic molecules.

Features of the water structure are reflected in the intracellular salt composition. A 1000-fold preference for K^+ over Na^+ has been found in a halophilic organism without any energy expenditure but with a highly reduced intracellular water mobility [27].

Measurable differences in the water structure of healthy and pathologically altered tissues and cell structures are possible to detect by magnetic resonance imaging (MRI). MRI measures the response of water protons and therefore represents differences in the aquatic environment.

Hazlewood et al. [28] found that precancerous stages could be identified by an MRI scan for hyperplastic and malignant stages. Further studies have confirmed the prospects of this method [29-31]. It would be possible to determine this diverse reaction of water protons at the level of organs, tissues and cells [32, 33] and cell structures [34, 35].

Let us consider hereinafter some structural aspects of water providing to it these properties.

Water is a substance whose main structural unit is an H_2O molecule, which consists of one oxygen atom and two hydrogen atoms.

The water molecule has a structure of a kind of isosceles triangle: an oxygen atom is located at the top of this triangle, and an atom of hydrogen at its bottom corners. The O-H bond length is 0.96 nm and the H-O-H bonds make an angle of 104.27°. These parameters relate to the hypothetical equilibrium state of the water molecule without its vibrations and rotations. Because of the large difference in electronegativity between hydrogen and oxygen atoms, electron clouds are strongly biased towards oxygen. For this reason, and also because hydrogen ion has no inner electron shells and is small, it can penetrate into the electron shell of a negatively polarized atom of a neighboring molecule. Due to this fact, each oxygen atom is attracted to the hydrogen atoms of other molecules, and vice versa. Each water molecule can participate in up to four hydrogen bonds: two hydrogen atoms – each in one, and an oxygen atom – in two; the molecules in an ice crystal are in such state. The properties of water mainly depend on the magnitude of the hydrogen bonds [36]. Authors of the article [37] note that it is impossible to give an exact definition of H-bonds and even to indicate which interactions, covalent or electrostatic, play the main role in its formation.

During the formation of hydrogen bonds, water mol-

ecules can act as electron acceptors and donors at the same time. As a result, water dimers are formed [38]. According to Tretyakov MI [39] water dimers are found in the Earth's atmosphere and influence on chemical reactions, homogeneous condensation processes and the radiation balance of the planet. Using density functional method researchers [40] performed calculations for the water dimer at 20.480 points. Attempts have also been made to determine as accurately as possible the potential for the water dimer [41]. As a result, it turned out to be so complex that it cannot be used in computer modeling. The set of parameters occupies two pages (available on http://fandango.ch.cam.ac.uk).

The real physical properties of water differ substantially from the properties of other hydrides (compounds in which hydrogen is combined with another element). In other words, unlike other substances, the properties of water cannot be calculated on the basis of its position in the periodic table of the chemical elements by Mendeleev. Theoretically, based on its position in the table, water – that is, oxygen hydride – should go into the solid phase, turn into ice at -100° C.

According to the researchers Cameron IL et al. 2013 [42], multiple unfrozen water fractions in biological materials (plant and animal tissues) were most often recorded at temperature ranges of -6.5, -15.0, -30.4, -74.0 and -96° C. Interestingly, a distinct unfrozen water fraction of 0.20 to 0.28 g/g was observed below a temperature of -74° C (vitrification state) in all samples surveyed. According to Pagnotta SE and Bruni F [43], some seeds in this state remain dormant but are germinal by rehydration above this level. At the same level of hydration, cysts of shrimp embryos remain non-metabolically viable [44].

Water and aqueous solutions have anomalous properties compared to other liquids. Water is characterized by: 1. Polymorphism of crystalline structures; 2. Maximum density at 3.98° C; 3. Decrease in molar volume when ice melts; 4. High values of melting points, boiling points; 5. When many hydrocarbons are dissolved, negative values of transfer entropy from a nonpolar solvent to water can be noted; 6. Extremely high values of surface tension; 7. Decrease of viscosity with increasing pressure; 8. High values of molar heat capacity; 9. High dielectric constant; 10. Minimum isothermal compressibility at 46.5° C [5, 47].

Due to the hydrogen bond, water molecule has a quality that manifests itself only in the presence of other molecules: the ability to form hydrogen bonds between oxygen atoms of two molecules that are close to each other. Each of the water molecules attached to this molecule is itself capable of joining further molecules. This process can be called "polymerization". Using a rapid decompression technique integrated with in situ X-ray diffraction, low-density aqueous media indicate the presence of a fully developed tetrahedral network. At the same time, there is a distinct difference in terms of the tetrahedral order parameter depending on the physical state of water [48].

The authors [49, 50] found that the resulting network structure is characterized by many small condensations, in which the molecules have four bonds and the local molecular density around them is less than the global density. According to Geiger et al. [51] liquid water can be considered as a typical polymer.

The features of the physical properties of water and the numerous short-lived hydrogen bonds between neighboring hydrogen and oxygen atoms in water molecules create favorable opportunities for the formation of special structures – associates (clusters) that perceive, store and transmit a wide variety of information [52-54].

During the process of structuring, we can conditionally distinguish several levels. At the first level, classical water molecules combine with each other, forming the minimum structural units – quanta water, each of which consisting of 57 H_2O molecules.

The second level of organization of the aqueous medium is associated with the union of quanta water into more complex water structures – associates.

The third level of structuring the aquatic environment is characterized by the combination of associates into more complex structures – supermolecules (clusters). This formation is a stable, long-living structural element of the aqueous environment.

The cluster resembles an elongated rhombus-shaped crystal. Information on the interaction between these water molecules is encoded in the structure of clusters. The pattern of hydroxyl groups on the surface of rhombohedrons ensures the memory of water. In other words, dipolar water molecules making up the crystalline facet come out of it, being charged either positively or negatively. It turns out a binary code, as in a computer. In contact with a water cluster of introduced substances or other disturbing factors, they seem to imprint their electromagnetic pattern on its facet. Subsequently, this "labeled" cluster, in contact with another, "clean" one, does the same thing – it transfers its electromagnetic pattern to it, but only in a "negative" one. The value of clusters formation in water is explained by their ability to temporarily "remember", "store", and "radiate" huge amounts of information in the form of electromagnetic waves [20, 26, 55, 56].

The above water features are supported by the theoretical conclusions of the author Bushuev IuG [57]. In his opinion, the structural heterogeneity of liquid water justifies the existence of a set of phenomenological models. The researcher believes that in the system of H-bonds in water, it is possible to define an unlimited number of structures, each of which is characterized by its structural elements and connections (relationships) between them. Currently available technical tools allow us to investigate the behavior of systems consisting of hundreds and maximum of thousands of particles.

Water as an energy substrate

The spatial structure and some physical properties of water molecules depend on the spin of hydrogen atoms. Spin (literally – rotation) is an intrinsic form of angular momentum carried by elementary particles, which has a quantum nature and is not related to the movement of the par-

ticle as a whole. Para-water is a water molecule in which the spins of both atoms are aligned in the same direction. If the electrons move in the opposite direction, we refer to ortho-water. One of the main differences between the para- and ortho-spin isomers of water is that the former has a state in which the molecule does not rotate (the rotational quantum number is zero), while ortho-spin isomer does not have such a state – it is always in motion. This difference leads to the fact that different spin isomers of water have different degrees of adsorption on the surface. It is important to note that the energy of the rotational ground state of ortho-H_2O (j=1) is 23.79 cm-1 higher than the energy of the rotational ground state of para-H_2O (j=0), i.e. energy will be released during the ortho-H_2O – para-H_2O phase transition [58, 59].

The researchers (Willitsch S et al.) [60] report the relationship between the nuclear spin, rotational symmetry and its consequences for chemical reactivity. According to the author, N_2H molecules and para-water "react 25% faster than ortho-water; this effect can be explained in terms of how the spin of hydrogen atoms nucleus influences the rotation of the entire molecule".

The existence of different spin isomers of water can explain a number of experimentally observed features of energy exchange in cell biophysics. According to the authors [61-63], the increase of metabolic activity in biological structures is associated with this phenomenon.

Energy for the vital activity of most organisms is produced by aerobic respiration. According to the definition of Antoine de Lavoisier, respiration "is nothing but a slow combustion of carbon and hydrogen, which is entirely similar to that which occurs in a lighted candle, and that, from this point of view, animals that respire are true combustible bodies that burn and consume themselves. The final products of the combustion of carbon and hydrogen are carbon dioxide and water" [64].

Aerobic respiration is currently reduced to mitochondrial respiration. Here oxygen acts as a final electron acceptor that donates its energy to the electron transport chain for the synthesis of ATP molecules. As energy portions released during this process are equivalent to quanta of middle-far IR-photons (≤0.5 eV), mitochondrial respiration is analogous not to burning, but to smoldering combustion.

Genuine combustion is a stepwise reduction of oxygen to water with four electrons ("one electron reduction"). By these means, quanta of energy equivalent to energy of visible and even UV-photons (> 1 eV) are generated [65].

Almost half a century ago, Albert St. Gyordi suggested that cellular metabolism could be supported by the energy released during the direct reduction of oxygen to water. The scientist noted that cyanide, which blocks the activation of oxygen, causes rapid death, although the ATP supply in the tissues remains quite large for a long time. The described phenomenon indicates the need for continuous activation of oxygen to maintain vital activity. The author asks: "Does this not mean that there are two independent energy generation systems, both using O_2 as an electron acceptor?" [66American immunologists pointed out the possibility of water burning in living matter in 2000. Researchers noted that antibodies (immunoglobulins) and some other proteins (including beta-galactosidase, beta-lactalbumin, and ovalbumin) catalyze oxidation of water with singlet (excited) oxygen to form hydrogen peroxide, which is equivalent to water burning [67]. It was found that water is the electron donor, specially arranged by these proteins [68]. This means that water can form such structures in which it acquires the properties of a reductant. Pollack defined water adjacent to hydrophilic surfaces as the "Exclusion Zone Water (EZ-water)" [69].

According to Pollack et al., the electrogenic properties of EZ-water and its potential with respect to the bulk water are largely determined by the properties of the surface that forms EZ-water. The larger the surface area and the density of its fixed negative charge, the greater the electron-donor capacitance of EZ-water of this surface is [70]. Nucleic acids have the maximum charge density among biopolymers, and DNA has an uttermost surface area. Therefore, DNA must have a maximum density of structural energy and the ability to organize and manage the environment. EZ-water has special properties – EZ-water has negative electrical potential (it reaches 150 mV) with respect to the bulk water adjacent to it. A direct current flows through a conductor connecting an electrode placed in EZ-water and an electrode in volumetric water, the strength of which increases at illumination. At illumination of EZ-water by infrared radiation with λ = 270 nm, it emits fluorescence [71]. If charged microspheres ranging in diameter from 0.5 to 2 μm are suspended in the water bordering to hydrophilic surfaces, they are quickly displaced from the boundary water regardless of the nature of the gel and the charge sign of the microspheres. The width of the microsphere-free water layer reaches hundreds of microns [72].

Accordingly, in an aqueous system in which EZ-water and bulk water are adjacent, a small activation energy is sufficient for the transfer of electrons to oxygen and the entire chain of one-electron oxygen reduction to be realized.

The structural temperature of EZ-water is lower than bulk water [73] and EZ-water constantly extracts thermal energy from the environment thus maintaining its electron-donor capacity. Such a water system is not a generator, but a transformer of heat energy into electronic excitation energy.

The process can be initiated by an external pulse, but only if both the oxygen concentration and the electron reserve (electron-donating capacity of EZ-water) exceed certain threshold values [74]. When the electron-donating capacity of EZ-water falls below threshold levels, the combustion wave is dampened until a sufficient layer of EZ water is restored by recruiting water from the volume into it. The reaction becomes rhythmic and can provide a cyclic course of coupled reactions [75].

In the light of the above data on the energy-informational role of water, let us try to analyze some features of tumorigenesis.

Studying the growth dynamics of Ehrlich ascites tumor, the author Zamay T. [76], found that the growth curve of

tumor cells did not correlate with their energy supply. The research results showed that, starting from the 7th and up to the 12th day, the energy state of ascites cells markedly worsened. On the 12th day, the state of the studied structures was characterized by the poorest energy supply (the ratio of inorganic phosphate and nucleotide triphosphates was maximum at this time). However, during this period, the maximum acceleration of tumor growth was observed. It was also surprising that blastoma cells showed very high viability in the terminal phase of the development of the cell population, despite the fact that the number of cells in the state of apoptosis was 25% from the 11th to the 14th day. Moreover, by the 16th day, when the mass death of tumor-bearing mice was already observed, it increased even to 78%.

The researcher explains the described phenomenon by the genomic instability of the tumor and the accelerated mutational process that contributes to the emergence of cells that are characterized by the highest viability in worsening conditions. Taking into consideration the given literature, the phenomenon observed by the authors may have another explanation. To clarify our assumptions, let us consider the mechanism of apoptosis in detail.

Apoptosis is a process of programmed cell death by forming apoptotic bodies without violating the integrity of biomembranes. The phenomenon is not accompanied by the development of the inflammatory process and happens without macroscopic signs, structural or functional defects of the tissue [77, 78]. The importance of this phenomenon for the formation of both organs and the body as a whole is enormous. Apoptosis provides the proper ratio of the number of different cell types, the removal of genetically damaged cells, and the selection in cell populations. In an adult, programmed cell death balances mitotic division, provides tissue renewal by maintaining optimal cell numbers [79]. As an example, we can cite an increase in the number of endothelial cells and the dimensions of blood vessels in mice with targeted inactivation of the Braf gene that controls apoptosis of endothelial cells [80]. There is a massive neuronal cell death, reaching 25-75% of the entire neuronal population, starting from the early stage of development of the nervous system, and throughout life activity. According to Paparone S et al. [81], the delicate balance between apoptosis and cell survival regulates the development of the nervous system and homeostasis.

It was noted that apoptotic cells near the amputation site provided an increased source of Wnt3, which regulates division of neighboring stem cells. Thus, pattern information was spread by dying cells to proliferating ones [82, 83].

According to more relevant data, in blastoma cells of the tumor tissue, it is noted overexpression of mitochondrial oligomers K-Ras, BAD, p27, Bax and Bak that increase glycolysis to provide energy demand during the formation of apoptotic bodies – blebbishield [84]. The latter probably indicates the active (inducing) participation of the tumor in the process, most likely as a highly organized structure, providing itself for the next stage in the development with a substrate in the form of apoptotic cells and not a conglomerate of solitary tumor cells.

The short last stage of development indicates a low likelihood of the emergence of a *de novo* tumor cell strain. According to the author Zamay T [76] the failure of the tumor proliferation curve was observed on the 12th day and then, during the course of the day, cell population switched back to active growth. In this case, we support our assumptions by referring to the author's opinion Moiseenko VM, Galante E et al. [85, 86]. The scientists have noted that the duration of breast cancer growth from both 1 cell and 103 cells (in the case of polyclonal origin) until the clinical manifestation cannot even theoretically last several months (as previously thought), because in this case, tumor doubling time should be less than 1 day. Meanwhile, the maximum growth rate of primary breast cancer in humans is 3-8 days.

Coming back to the main topic of our research – the peculiarities of the aqueous structure of the tumor, we recall that the properties of EZ-water and its potential are largely determined by the properties of the surface that forms EZ-water.

Considering the observation of the author Zamay T [76], we can assume that the maximum acceleration of tumor growth on the 12th day under the conditions of the worst energy supply is associated with a peculiar quantitative-qualitative transition. It is possibly the critical summation of pattern information from apoptotic cells and its transmission to proliferating cells through aqueous structures during this period. In a similar vein, it is important to note the observations of Kozhokaru A et al. [87]. The authors argue that excited water molecules that have received additional energy are able to transfer it to the same structures and molecules of other living organisms (detectors), which have similar frequencies of electromagnetic waves.

Perhaps the phenomenon observed by the author Zamay T [76], was also accompanied by a transition to another type of energy metabolism not directly related to ordinary molecular substrates. This possibility is evidenced by the fact that it is impossible to detect about 30% of cancers by FDG-PET research, suggesting that these tumors use alternative (non-glucose) metabolic pathways to produce energy [88].

As previously noted, EZ-water is able to extract thermal energy from the environment by transforming low-density energy (heat) into high-density energy, into electronic excitation energy. For example, at illumination of EZ-water by infrared radiation with λ=3100 the fourfold increase in its thickness is observed. Infrared light is literally free energy and is found everywhere – it is not only inside, but also outside [71].

Another possible source of energy for tumor proliferation not directly related to conventional molecular substrates may be weak and superweak magnetic fields – relic radiation (RR), geomagnetic field. According to Kozhokaru AF et al. [87], the energy of the polarized component of the relic radiation (RR), which is a superweak cosmic photon radiation, can be absorbed by excited atomic electrons by NMR. As previously stated, EZ-water may be used as the

acceptor. In experiments on mice with transplanted tumors Novikov VV [89], showed that the action of weak combined, constant (30-49 μT) and variable (frequencies 3.5-5.0 Hz, amplitude 50-120 nT) magnetic fields modulate the development of ascites and solid forms of Mouse Ehrlich ascites carcinoma (EAC).

If the above statements are true, the maximum acceleration of tumor growth as a conglomerate of tumor cells arising *de novo* for 1 day under the conditions of the worst energy supply is unlikely. This phenomenon is probably inherent in a highly organized biological structure with the ability to structure water very specifically. The latter quality probably allows the tumor to choose energy sources depending on the phases of development or other factors.

Conclusions

Summarizing the above materials, it can be concluded that the importance of a holistic bioelectric field in the development of tumor diseases is probably paramount. As it was shown, the development of a tumor in a certain part of the body depends on the bioelectric state of the distant regions. It is possible that carcinogens prevent formation of a single morphogenetic field and lead to the creation of separate bioelectric fields. The cited materials indicate the binding role of the aqueous medium in relation to the field and molecular components of biological objects. Despite the impressive amount of research on water structures, this substance, in spite of its apparent simplicity, remains little studied.

However, the evidence that exists at the present stage indicates that water, perhaps, acts as a kind of mirror of objective reality, capable of not only reflecting the world in all its endless images, but also conveying the inner essence of processes.

An *in vivo* study of the various properties of water in blastoma cells and tissues, and extrapolation of the obtained data, will probably allow opening up new aspects of the etiopathogenesis of oncological diseases.

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The key players of tumor microenvironment and their role in breast cancer

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Abstract

Background: Cancer studies were focused mainly on tumor cells. But not that much time passed since researchers began to focus not only on neoplastic cells, but also on significant alterations in the surrounding stroma or tumor microenvironment. These alterations are now recognized as a critical element for breast cancer development and progression, as well as potential therapeutic targets. Different elements of the breast cancer microenvironment (such as immune cells, soluble factors and modified extracellular matrix) act together to stop effective antitumor immunity and stimulate breast cancer progression and metastasis. Stromal cells in the breast cancer microenvironment are characterized by molecular alterations and aberrant signaling pathways, some of which are prognostic factors for clinical outcome.

Conclusions: Tissue microenvironment has profound effects on the progression of cancer cells by its paracrine signaling. Molecular characterization of various cell types from the normal breast tissue, ductal carcinoma *in situ* and invasive breast tumor revealed significant changes in gene profile in all cell types during breast tumor progression. Microenvironment changes influence tumor progression as well as the efficacy of various cancer therapies. There is compelling evidence that the elements of tumor microenvironment respond to different stimuli and release distinct mediators, some antitumorigenic, while others protumorigenic activity. Each of the known players of breast stroma involved in tumorigenesis and cancer progression can be influenced and directed towards an "anticancer" state.

Key words: breast cancer, tumor microenvironment, myoepithelial cell, fibroblast, macrophage, mast cell.

Introduction

Cancer studies were focused mainly on tumor cells. It was 1889 when Paget proposed the "seed and soil" theory which suggests that neoplastic cells (seed) may only initiate tumor formation when in the context of a hospitable and supportive microenvironment (soil) [1]. But not that much time passed since researchers began to focus not only on neoplastic cells, but also on significant alterations in the surrounding stroma or tumor microenvironment. These alterations are now recognized as a critical element for breast cancer development and progression, as well as potential therapeutic targets. Different elements of the breast cancer microenvironment, such as immune cells, soluble factors and modified extracellular matrix, act together to stop effective antitumor immunity and stimulate breast cancer progression and metastasis. Stromal cells in the breast cancer microenvironment are characterized by molecular alterations and aberrant signaling pathways, some of which are prognostic factors for clinical outcome. Several new therapies targeting stromal components are in development or undergoing clinical trials [2]. In this paper, there will be reviewed the key players of breast stroma and their role in tumorigenesis and cancer progression. However, a key question remains: which comes first, the dysfunction of epithelial cells or the dysfunction of their microenvironment? [3].

Myoepithelial cells

The human breast represents a branching ductal system composed of two epithelial cell types: an inner layer of polarized epithelial cells and an outer layer of myoepithelial cells, separated from the stroma by a laminin-rich basement membrane (BM) [4]. BM is also composed of heparan sulfate proteoglycans, glycosaminoglycans and entactin. Myoepithelial cells are attached to luminal cells by desmosomes and to the BM by hemidesmosomes [5]. The myoepithelial cells are a fascinating type of cell, because they belong to two completely different types of tissues, namely the epithelium and the mesenchyme, these having even a distinct embryonic origin. This two-sided nature is expressed not only by their position (on the one hand they are connected in typical manner with the secreting epithelium, whereas on the other hand they interact with the stroma and the basal membrane in the same way as smooth muscle cells), but also by their possession of potentialities of both tissues [6].

The branching ductal system ends with a terminal ductal-lobular unit (TDLU), this being the basic functional and histopathological unit of the breast. The myoepithelial cells lining the ducts are spindle-shaped cells oriented parallel to the long axis of ducts as a continuous layer. The myoepithelial cells in TDLUs are discontinuous, stellate-shaped, and form a basket-like network around acini, allowing some luminal epithelial cells to directly contact the BM. Both epi-

thelial and myoepithelial cells originate from the same precursor. This precursor cell niche is believed to hold the key to the definitive origin of both luminal epithelial and myoepithelial cells, as well as providing a possible cell population for the origin of breast cancer [4].

Myoepithelial cells contain a large amount of microfilaments and smooth muscle-specific proteins such as alpha-actin and myosin that are responsible for the contractile function mediated by oxytocin during lactation. Each myoepithelial cell has long cytoplasmic processes that wrap around a secretory unit and hence, contraction of the myoepithelial cell can eject secretory product from the secretory unit into its duct. Thus, contraction is the most obvious and important function. Normal myoepithelial cells are critical for correct polarity of luminal epithelial cells, most likely via production of laminin-1 [5]. Adriance *et al.* showed that human breast luminal cells, when grown in three-dimensional type I collagen as opposed to laminin-rich gels, form structures with altered integrins that have reversed polarity and lack central lumina; however, if these same cells are cocultured with myoepithelial cells in collagen I gels they exhibit correct apicobasal polarity [5]. On the other hand, Gudjonsson *et al.* showed that myoepithelial cells present in invasive breast carcinoma have many similar features with normal myoepithelial cells but they show either complete absence or reduced expression of laminin-1. This one is strongly expressed around normal breast epithelial structures and thus tumor myoepithelial cells are unable to induce the polarization of luminal epithelial cells [4, 5].

Because breast cancer arises mainly in the luminal epithelial compartment of the TDLU, until recently little attention has been paid to the surrounding myoepithelial cells [4]. However, progression to carcinoma involves alteration of the entire organized structure of the breast; depending on tumor grade, the changes can include the loss of polarity, collapse of the glandular structure, disappearance of normal myoepithelial cells, and disruption of the BM at the epithelial–stromal border [7]. Myoepithelial cells form a natural border which is a semi-continuous protective sheet separating the human breast epithelium and the surrounding stroma. They suppress stromal invasion of tumor cells not only physically, but also by the secretion of various anti-angiogenic and anti-invasive factors. Among these, maspin is one of the most important tumor suppressors that are secreted by myoepithelial cells. It is a member of the serpin family of serine proteases which inhibits tumorigenesis, tumor cell migration and metastasic spread thus it functions as a tumor suppressor. Maspin is secreted in large quantities by the normal cells whereas tumor cells do not secrete it [5].

Myoepithelial cells regulate the flow of fluid and control the entry and exit of nutrients, electrolytes and other growth factors. They also process signals of endocrine or paracrine nature and perhaps act as an intermediary in such signaling processes by passing information both inwards and outwards in a paracrine fashion. The disruption of this cell layer results in the release of the growth factors, angiogenic factors, reactive oxygen species that cause an alteration in the microenvironment and the loss of myoepithelial cells. BM is the distinctive key feature of invasive carcinoma, because most tumor epithelial cells have to first pass through the myoepithelial cell layer and then the BM in order to physically contact the stroma [5, 8]. It is also postulated and generally accepted that primary breast carcinomas show a dramatic increase in the ratio of luminal-to-myoepithelial cells, and that many invasive breast carcinomas essentially lack myoepithelial cells entirely [4]. It may be possible that the myoepithelial cells are degraded by the overproduction of the degradative enzymes or they are selectively eliminated by apoptosis [5, 8]. Myoepithelial cells rarely transform; however, when they do transform, they generally give rise to tumors of low malignancy [4].

We need to understand what prevents myoepithelial cells to exhibit the tumor suppressive properties. It is also possible that the tumor suppressive ability of myoepithelial cells depends on their complete differentiation and that changes in their expression pattern can lead to reversal of their function, i.e., that undifferentiated myoepithelial cells may actually promote tumor progression instead of suppressing it. These observations could open up the possibility of a future differentiation therapy where cancer cells are forced to differentiate along the myoepithelial pathway, thus manufacturing cells of lower malignancy or those that could suppress the aggressive behavior of their more malignant counterparts [4].

Confirmation of the myoepithelial cell layer on routine histology can be done with the help of alpha-smooth muscle actin (α-SMA) immunostaining; however, these cells can also be identified by S-100, calponin, h-caldesmon, smooth muscle heavy chain (SMMHC) antibodies and CD10 [5]. Because of epithelial origin, they also express cytokeratins (CK) characteristic for the basal layer of stratified epithelia, such as CK 5, CK 14, and CK 17 [4].

Fibroblasts

Tumors are known as wounds that do not heal. This implies that cells that are involved in angiogenesis and the response to injury, such as endothelial cells and fibroblasts, have a prominent role in the progression, growth and spread of cancers [8]. Fibroblasts are cells that form the basic cellular component of connective tissue and contribute to its structural integrity. They play important roles in wound healing, regulation of epithelial differentiation and inflammation. In healthy organs, fibroblasts have a low proliferation index and minimum metabolic capacity. By contrast, during wound healing and in cancers, fibroblasts become activated, start to proliferate, secrete higher amounts of extracellular matrix (ECM) components, and acquire contractile features. Fibroblasts from tumors are known as reactive fibroblasts, peri-tumoral fibroblasts, myofibroblasts, tumor-associated or cancer-associated fibroblasts (CAFs) [3]. Characteristic feature of CAFs is expression of α-SMA and its expression is higher in fibroblasts derived from cancer tissues than in those derived from normal tissues [9].

Fibroblasts are associated with cancer cells at all stages of cancer progression, and their structural and functional

contributions to this process are beginning to emerge. Their production of growth factors, chemokines and ECM facilitates the angiogenic recruitment of endothelial cells and pericytes. Cancer cells tumoriginity was dramatically increased when inoculated with fibroblasts. Fibroblasts are therefore considered a key determinant in the malignant progression of cancer and represent an important target for cancer therapies [3, 10]. Normal fibroblasts maintain the extracellular environment through the production and remodeling of the ECM. CAFs have distinct characteristics and substantial data to support a role for CAFs in promoting tumor progression through morphological and phenotypic changes in various breast cancer subtype cells by production of TGF-β [8, 9]. In human breast tumors, the abundance of stromal CAFs is associated with aggressive adenocarcinomas and predicts human disease recurrence. In addition, CAFs have been shown to contribute to drug resistance and to reduce anti-tumor immunity [11].

The origin of CAFs has been actively investigated and multiple hypotheses have been proposed. One possibility is that they are derived from native interstitial fibroblasts whose phenotype has been modified by persistent aberrant signaling from neighboring tumor epithelial cells. Alternatively, they can be differentiated from bone marrow-derived mesenchymal stem cells that are recruited to the tumor site via endocrine stimulation by tumor-derived factors [8].

CAFs often express α-SMA. These cells are also positive for vimentin and desmin, but do not express CKs, CD31 and smooth muscle myosin [3].

Leucocytes

Immune cells are one of the most dynamic cell populations present within tumors and healing wounds and during the remodeling of breast tissue in pregnancy and involution. The smoldering inflammation was proposed as the seventh hallmark of cancer [1, 8]. High numbers of infiltrating leukocytes are present in ductal carcinoma *in situ* (DCIS) with focal myoepithelial cell layer disruptions, suggesting that they might play a role in invasive progression [8].

Among leucocytes, tumor associated macrophages (TAMs) represent the vast majority, sometimes more than 50%. Their importance should not be underestimated because they are able to control the immune response, cellular mobility and to stimulate/inhibit angiogenesis and lymphangiogenesis [12]. During chemically induced neoplastic transformation macrophages induce DNA damage through the release of reactive oxygen and nitrogen intermediates. Such macrophages have the potential to promote the survival of transformed cells and establish a state of chronic inflammation via secretion of the proinflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β [1]. Moreover, macrophages can modulate the drug resistance and stimulate tumor regrowth by various substances secreted into the microenvironment. For example, irradiation causes tumor necrosis, vascular damage, and hypoxia, which together or separately can induce the upregulation of several myeloid cells/monocytes chemoattractants, like vascular endothelial growth factor (VEGF) in the tumor microenvironment. *De novo* recruitment of myeloid cells drives tumor regrowth via their effects on the tumor blood vessels and, possibly, the cancer cells [12, 13].

Macrophages are highly heterogenic members of the mononuclear phagocyte system. They are distributed throughout every organ of the body and can ingest microbes and present antigens to T lymphocytes, therefore constituting a first line of defense against invading pathogens [1, 12]. In inflamed and remodeling tissues, elevated macrophage turnover is indefinitely supplied largely from hematopoietic progenitor cells (HPCs), which proliferate and differentiate into promonocytes in the bone marrow before they exit into the circulation as monocytes. This proliferation program is orchestrated through colony stimulating factor 1 (CSF1), a key growth factor regulating macrophage proliferation and survival, produced by the local tissue stroma. Monocytes then undergo final differentiation into macrophages as they strain in the target tissues. Once resident in tissues, macrophages acquire a distinct, tissue-specific phenotype in response to signals present within individual microenvironments. Depending on the microenvironmental signal type, macrophages can be polarized into "classical" (or M1) and "alternative" (or M2) phenotypes [1, 12, 14, 15].

During M1 activation, IFN-γ and other molecules are involved to bring a Th1 response, thus type I inflammation, intracellular pathogen killing and antitumor immunity. M2 activation is known to accelerate tissue repair and tissue growth. These suggest that the increase of M1 macrophages in cancer is associated with less tumor aggressiveness, while M2 macrophages stimulate tumor growth and lead to poor prognosis [14, 16].

Various mouse studies have shown that monocytes are recruited into tumors in large numbers by chemokines secreted by both malignant and stromal cells. Upon monocyte differentiation into TAMs, these cells support the proliferation, survival, and motility of the cancer cells as well as angiogenesis; suppress antitumor immunity; support progression of cancer cells at the primary tumor site and extravasation/growth at distant metastatic sites. Previously, activated macrophages were believed to exhibit antitumor activity by directly attacking tumor cells in the tumor microenvironment. However, many recent studies have indicated the protumoral functions of TAMs, and thus, TAMs are believed to be predominantly polarized in the tumor microenvironment toward an M2-like phenotype and that this underlies their ability to promote the growth and vascularization of tumors. This is supported by expression of CD163 and CD204, a characteristic feature of M2 macrophages [12, 16]. Another typical markers of M2 macrophages are MRC1, TGM2, CD23, CCl22; M1 express CD64 and CXCL10 markers [14].

TAMs are responsible for immune alterations in breast cancer. The first way is inhibition of antitumor T-cell responses by secreting anti-inflammatory cytokines, like IL-10. Other mechanisms are the recruitment of immunosuppressive leucocytes and the inhibition of tumoricidal function by decreasing of MHC class II expression.

The main function of MHC class II molecules is to present processed antigens, which are derived primarily from exogenous sources, to CD4+ T-lymphocytes. MHC class II molecules thereby are critical for the initiation of the antigen-specific immune response [14, 17]. They are doing this to limit tissue damage due to deleterious inflammation. The continually activated macrophages undergo apoptosis or functionally 'stand-down', adopting an anti-inflammatory phenotype defined by the ability to suppress persistent immunity and facilitate wound healing [1].

Anatomically, macrophages are present at high numbers at the margins of mammary tumors with decreasing frequency throughout the stroma moving in within the tumor. Within the tumor mass, macrophages, either individually or in clusters, are commonly found in association with blood vessels and orchestrate the migration of tumor cells [1].

Macrophages have emerged as an important key player in breast cancer progression and represent an attractive target for breast cancer therapy. Current interventions have focused on three strategies: blocking macrophage precursor recruitment, depletion of TAMs and their progenitors, and reprograming macrophage function within tumors [1].

Mast cells

Mast cells are granulated immune cells characterized by their cargo of inflammatory mediators, comprised of a wide array of preformed bioactive molecules stored in cytoplasmic granules, which are released upon encountering the appropriate stimuli and have beneficial roles in immunological responses against pathogens, including intestinal helminths, bacteria, and viruses. Mast cell-derived mediators also participate in tissue physiological processes, such as wound healing and tissue repair, and in some pathological conditions, such as immediate allergic reactions [18]. Human mast cells derive from $CD34^+$, $CD117^+$ pluripotent hematopoietic stem cells, which arise in the bone marrow. Mast cell progenitors enter the circulation and subsequently complete their maturation in tissues [19].

At least two major populations of mature mast cells have been described in humans based on their protease content. Mast cells containing only tryptase are termed MC_T, while those containing tryptase, chymase, carboxypeptidase A, and cathepsin G are named MC_{TC}. These mast cell subsets differ in their tissue localization; for instance, the MC_{TC} is the predominant type found in normal skin and small bowel submucosa, whereas the MC_T is almost the exclusive type found in small bowel mucosa and in bronchial/bronchiolar areas [18].

Back in 1992, Judah Folkman suggested that TAMs and mast cells play an important role in angio- and lymphangiogenesis [20]. Researchers have demonstrated that mast cells produce several proangiogenic (VEGF-A, VEGF-B, and FGF-2) and lymphangiogenic factors (VEGF-C and -D). In addition, it was shown that VEGFs are chemotactic for mast cells, indicating that mast cells are a target, in addition to be a source, for VEGF. Human mast cells produce different matrix metalloproteinases (e.g., MMP-9) and proteases (tryptase and chymase), which regulate the digestion of ECM favoring the migration of cancer cells [19].

The role of mast cells in cancer is dual and uncertain. Some scholars highlight the anticancer function of mast cells. Human mast cells contain different proinflammatory mediators, but are unique in their ability to pre-store and release potentially beneficial anticancer mediators. For example, human mast cells have pre-stored and released TNF-α within their granules. Furthermore, human mast cells release granulocyte-macrophage colony-stimulating factor (GM-CSF). Both TNF-α and GM-CSF have been used as anti-cancer agents. In this way, antitumor agents from mast cells could be used as a potential "Trojan Horse" of cancer cellular immunotherapy [21].

Xie et al. suggests that mast cells can induce prostate cancer chemotherapy and radiotherapy resistance by modulation of p38/p53/p21 [19, 22]. Mast cells have a protumor action in human bladder cancer through stimulating estrogen receptor β (ERβ). In a murine model of bladder cancer, authors showed that a selective ERβ antagonist inhibited mast cell-promoted tumor growth [19, 23].

Some groups have concluded that the prognosis is worse with a higher density of mast cells in the breast cancer tissue [24]. Xiang et al. have observed more numerous peritumoral MCs in G3 breast cancers, increased tryptase being associated with higher tumor grade and more lymph node metastasis compared to lower grades. They have also noted that tryptase promotes the invasion and migration of breast cancer cells along with the activation of matrix metalloproteinase-2, and have concluded that tryptase promotes breast cancer migration and invasion [25]. Raica et al. revealed strong positive correlations between populations of MCs and lymphatic vessels in some molecular subtypes of breast cancer, thus supporting the idea of MCs involvement in metastasis by lymphangiogenesis [26]. Ribatti et al. have pointed out that angiogenesis increased in parallel with the number of tryptase-positive MCs particularly inside lymph nodes associated with micrometastases compared to non-metastatic lymph nodes [27]. It has also been demonstrated that during breast cancer progression MCs may contribute to stromal remodeling and differentiation of myofibroblasts, through tryptase released in the stromal microenvironment [28]. All these mean that targeting MCs could be involved in the inhibition of angiogenesis, lymphangiogenesis and many other negative effects of MCs' activation. Our research showed that mast cells dynamics is strongly influenced by hormone receptors and HER2 status. Mast cells from intratumoral stroma increased in aggressive tumor types and is a worse prognostic factor [29].

Tissue microenvironment has profound effects on the progression of cancer cells by its paracrine signaling. Molecular characterization of various cell types from the normal breast tissue, DCIS and invasive breast tumor revealed significant changes in gene profile in all cell types during breast tumor progression. Microenvironment changes influence tumor progression as well as the efficacy of various cancer therapies [5]. There is compelling evidence that the

elements of tumor microenvironment respond to different stimuli and release distinct mediators, some antitumorigenic, while others protumorigenic activity [19].

Conclusions

In further researches it is necessary to unravel the factors determining the failure of breast stroma elements to exert anticancer functions. Even if a lot of things are known about breast cancer, the mortality is still high. Our findings suggest that cancer therapy should be an individual one, approved after complex diagnosis of the patient. Each of the known players of breast stroma involved in tumorigenesis and cancer progression can be influenced and directed towards an "anticancer" state. This could be the therapy of future.

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BOOK REVIEW

The monograph "Biological unity of the nature and man: an esthetics-anthropological research with emphasis on phylogenesis and ontogenesis features of oro-maxillo-facial system"

Tipografia Centrala, Chisinau, 2019, 496 p.

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The offered monograph is aimed at the comprehensive complete analysis of theoretical and practical problems of the man and medicine, particularly stomatology, seeking to cover unity of historical, philosophical and natural-science approaches relevant for modern anthropology and the world culture of the XXI century in general.

The author published 79 scientific works (articles and theses) reflecting his monograph subject: 13 in national and 66 in the international specialized editions; 1 methodical mo-dule; 1 national and 2 international collective monographs. The chosen bibliography is divided according to heads and includes 814 sources. Author's methodical module "Modern concept of development and shaping of a maxillofacial system of the man" (2012) and monograph "Esthetic, spiritual and moral heritage of N. K. Roerich: waiting for demand" (2013) are introduced in pedagogical process in higher educational institutions and practical activities of treatment and prevention institutions in Russia, Belarus and Ukraine.

It is important to emphasize that the author of monograph's foreword is A. I. Subetto, the President of Noosphere public academy of Sciences, academician, member of Presidium of Petrovsky academy of Sciences and Arts, Doctor of Philosophy, Doctor of Economics, Candidate of Technical Sciences, professor, the Honored man of science of the Russian Federation, the Grand doctor of philosophy and Full professor (in the Oxford educational network), St. Petersburg, Russia. It is noted that magnificent alloy of unity of the person and the universe, matter and form, beauty, good and the truth is presented in the monograph. It gives to the reader the chance not only to physically feel this unity, but also to show how such integrated knowledge begins to serve any particular private affair, creation of any technology, for example, technology of dental care in the present and the future. "We live during the Era of the Great Evolutionary Change", (quoted on page 9).

Part I is devoted to one of fundamental problems in science, evolution of life and differentiation of forms on Earth. It includes four chapters: 1) Nature, Man, Universe; 2) Life and crystallization; 3) Evolution of the person and biotechnologies; 4) Nano-revolution and evolution of teeth. Scientific facts, knowledge accumulated in millennia by mankind in their common cultural context are revealed in detail from various cross-disciplinary points of view (history, philosophy, biology, physics, biomechanics, etc.). Indissoluble thread of continuity between philosophers of various eras and medical science is underlined, which uniting purpose is knowledge of Man, Nature and Universe. The author comes to the conclusion that more profound study of the general evolutionary principles of development and functioning of plants and teeth, is capable to bring us closer to answers to many questions of an embryo-morphogenesis of tissues of an oro-maxillo-facial system. This conclusion is especially important because studying of mechanisms of differentiation and laws of variability of the teeth structure is one of fundamental problems of anthropological odontology.

It should be noted that through all many-sided research revealed by the author on macro and microlevels in various objects of the organic and inorganic nature can be found regularity of fractal geometry. It is fairly noted that "fractals open simplicity in the difficult material world of objects surrounding us." (quoted on page 27). Now fractal geometry is developing in different high-precision technologies including in methods of the destroyed teeth restorations.

Results of works of great scientists I. Goethe, T. Shvann on studying analogies in the nature and a human body allowed the author to carry out the comparative analysis of established facts between the structure of tooth tissues and plants. His own observations gave the idea of probable, from the evolutionary point of view, similarity between a morpho-functional role of a cambium of plants and enamel-dentine junction of teeth as according to I. Goethe "any being is an analog of everything existing" (page 39-40). In this context attracts attention thesis of V. I. Vernadsky, recognized by world science of the XX century (quoted on page 31): "empirical generalizations on the basis of the exact and indisputable facts are the strong and firm ground for the description of an overall picture of a set of the (coming true) processes taking place around us or occurred far back in the past, even if sometimes these facts do not stand the logical analysis, owing to their incomplete studying and incomprehensibility by contemporary scientific thought".

Part II is devoted to laws of the universe in the composition, development and biomechanics of an organism and oro-maxillo-facial system of the man and also includes four chapters: 5) Gold proportions, Fibonacci's numbers and law of a phyllotaxis; 6) Spiral symmetry as universe matrix; 7) Vibrations and a sound at the heart of the universe. Wave biomechanics; 8) Bionics – cross-disciplinary science about knowledge of structural unit of a living organism.

The author presented the extensive analysis of the cross-disciplinary scientific facts and results of own researches in studying of the general regularities of spiral biosymmetry, a gold proportion / golden ratio and a numerical number of Fibonacci both in the nature, and in the organization and shaping of structural elements of a oro-maxillo-facial system. The work reveals the key role of spiral biosymmetry in evolutionary formation of tissues and organs of a human body. The double spiral of DNA can be considered as one of classical examples of total perfection and compactness in the form of the information carrier of the genetic code created in natural laboratory. From these positions is proposed the phyllotaxis theory of the mechanism of a teeth eruption. At the same time, relying on the principles of dichotomic growth as the most ancient type of phyllotaxis growth in the nature, the author offers the original scheme of an embryonic development of the head and a maxillofacial system.

Understanding of the general regularities in development and differentiation of tissues in the nature helps to look from the other point of view at features of architectonics of the occlusal surface of molars. As a result the author marks out three types of expressiveness of natural furrows (fissures) on permanent molars, that play extremely important role in normal functioning of teeth rows, muscles and temporo-mandibular joints: 1) molars with full disappearance of fissures of the II order in the field of the central hole; 2) molars with simplified architectonics of occlusal surface, with deep and wide fissures; 3) molars with complicated architectonics of occlusal surface with superficial and narrow fissures.

The theoretical biomechanical model of solid tooth tissues on the basis of quantum mechanics is originally presented. Biomechanics studies mechanical properties of biological tissues, organs and an organism in general and also the mechanical phenomena occurring in them. It is emphasized that the unity of the nature and universality of its laws are not shown as brightly anywhere, as in the oscillatory and wave phenomena. With all evidence it becomes clear that interstitial reaction during the function of chewing is followed by microfluctuations which extend from the centers of occlusal contacts. As we know, fluctuation is the movement to the opposite sides around some average position. And the author has proved it convincingly in detail, leaning on the known scientific facts.

The author notes that bionics as a science has begun its formation in the second half of the XX century and great interest to it is caused by considerable practical orientation of this science studying the principles of construction and functioning of biological systems, first of all, for the purpose of creation of new technologies – materials, tools, devices, mechanisms, etc. Bionics studies the most various characteristics of living organisms, including characteristics of material, energetic and information systems. On clinical examples are demonstrated the developed biomimetic "spiral" principle of reinforcing of walls of a tooth and modeling of a pinlay (intra-root incrustation) is shown with the help of a microhybrid composite.

It is important to note that readers can open absolutely new sides of the Unity of Nature and Man which consists not only in community of molecular and genetic bases of evolutionary development on Earth, but also in architectonics of tissues on micro- and macrolevels. This fact, according to the author, can become an additional incentive for the development of bionics and more widespread introduction of its achievements in the general medicine and stomatology.

The work considers the prospects of the modern scientific directions on the basis of biomimetic nanotechnologies, robotics, genetic engineering and also their positive and probable negative influence on a human body and civilization at present and in future. The author comes to the conclusion that "prevention is the most universal medical technology which was and remains the protection of health of each person and its level is the indicator of health of the population in any country of the world" regardless the colossal rate of science, material and technological base development (quoted on page 431).

Thus, the presented monograph covers a wide range of questions and problems which are not lit enough or are practically missing in special literature and therefore it is a unique cross-disciplinary scientific work in modern stomatology and conforms to all international requirements. The monograph is recommended for a wide range of specialists, students and graduate students of medical schools, dental faculties, and doctors.

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