Научно-практический журнал для работников здравоохранения

Включён в Перечень ведущих рецензируемых периодических изданий ВАК Минобрнауки РФ

Архивъ внутренней медицины

THE RUSSIAN ARCHIVES OF INTERNAL MEDICINE

www.medarhive.ru

АПРЕЛЬ 2018 (№ 2(40))

УЧРЕДИТЕЛЬ И ИЗДАТЕЛЬ
Общество с ограниченной ответственностью «Синапс»
109089, Москва, ул. Угрешская, д. 2, стр. 145
Тел.: (495) 777-41-17
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Тираж 3000 экземпляров.

Издание зарегистрировано в Федеральной службе по надзору в сфере связи, информационных технологий и массовых коммуникаций (Роскомнадзор).

Свидетельство о регистрации
ПИ № ФС77-45961 от 26 июля 2011 г.

ISSN 2226-6704 (Print)
ISSN 2411-6564 (Online)

Отпечатано в типографии «Onebook.ru»
ООО «Сам Полиграфист»
г. Москва, Волгоградский проспект, д. 42, корп. 5
www.onebook.ru

Ⓐ авторский материал

Ⓟ публикации на правах рекламы

Журнал включен в Российский индекс научного цитирования (РИНЦ)

Статьи журнала представлены в Российской универсальной научной электронной библиотеке www.elibrary.ru

Подписной индекс в каталоге «Почта России» 87732

DOI: 10.20514/2226-6704-2018-2

Scientific and practical journal for health professionals

Included the List of the Russian reviewed scientific magazines in which the main scientific results of theses on competition of academic degrees of the doctor and candidate of science have to be published.

Архивъ внутренней медицины

THE RUSSIAN ARCHIVES OF INTERNAL MEDICINE
www.medarhive.ru
APRIL 2018 (№ 2(40))

FOUNDER AND PUBLISHER
«SYNAPSE» LLC
109089, Moscow, Ugreshskaya str., 2-145
info@medarhive.ru

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Circulation 3000 exemplars

It is registered by state committee of the Russian Federation on the press

The certificate on registration of mass media ПИ № ФС77-45961, 26 July 2011

ISSN 2226-6704 (Print)
ISSN 2411-6564 (Online)

Printed «Onebook.ru»
«Sam Poligrafist»
Moscow, Volgograd Prospect, 42-5
www.onebook.ru

The journal is included in Russia Science Citation Index (RSCI)

Journal data are published on website of Russian General Scientific Electronic Library www.elibrary.ru

Subscription index in the catalogue «Russian Post» 87732

DOI: 10.20514/2226-6704-2018-2

СОДЕРЖАНИЕ

Лекции

Обзорные статьи

Оригинальные статьи

Разбор клинических случаев

С 2016 ГОДА СТАТЬИ В ЖУРНАЛ ПРИНИМАЮТСЯ ЧЕРЕЗ РЕДАКЦИОННУЮ ПЛАТФОРМУ:
http://www.medarhive.ru/jour/about/submissions#onlineSubmissions

НОВЫЕ ПРАВИЛА ПУБЛИКАЦИИ АВТОРСКИХ МАТЕРИАЛОВ (2017):
http://www.medarhive.ru/jour/about/submissions#authorGuidelines

CONTENT

SINCE 2016, ARTICLES IN THE JOURNAL HAVE BEEN ACCEPTED THROUGH THE EDITORIAL PLATFORM:

http://www.medarhive.ru/jour/about/submissions#onlineSubmissions

NEW GUIDELINES OF PUBLICATION FOR AUTHORS OF ARTICLES (2017):

http://www.medarhive.ru/jour/about/submissions#authorGuidelines

UDC 616.12-008.46-08

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ALGORITHM FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEART FAILURE WITH REDUCED LEFT VENTRICULAR EJECTION FRACTION

Abstract:

Despite a significant number of publications devoted to the management of patients with chronic heart failure (CHF), a practicing doctor is not always easy to navigate in the use of medicines and indications for high-tech methods of treatment in these patients. The largest evidence base is currently accumulated in patients with CHF with a reduced left ventricular ejection fraction (CHFrEF), which is characterized by a significant decreasing in the quality of life, decreased/lost ability to work, disability of patients and high mortality. This article details all the essential medicines used for therapy of CHFrEF, the sequence and practical aspects of their prescribing in accordance with contemporary guidelines. The issue of treating patients with CHF refractory to standard therapy, including with the help of a new class of medicines from the group of angiotensin receptor-neprilysin inhibitors, cardiac resynchronization therapy, implantation of cardioverter-defibrillators and application of devices for mechanical circulatory support and heart transplantation is considered. The publication is illustrated by tables, figures, charts, which makes it accessible for understanding and memorizing.

Key words: *chronic heart failure, ejection fraction, therapy algorithm, drug therapy, cardiac resynchronization therapy, ARNI, prognosis*

For citation: Reznik E.V., Gendlin G.E., Nikitin I.G. ALGORITHM FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEART FAILURE WITH REDUCED LEFT VENTRICULAR EJECTION FRACTION. The Russian Archives of Internal Medicine. 2018; 8(2): 85-99. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-85-99

DOI: 10.20514/2226-6704-2018-8-2-85-99

ICD — implantable cardioverter-defibrillator, MCS — mechanical circulatory support, CHF — chronic heart failure, EF – ejection fraction, CHFrEF — chronic heart failure with reduced ejection fraction, CRT — cardiac resynchronization therapy

Introduction

Despite significant advances in the management of various cardiovascular diseases, the prevalence of chronic heart failure (CHF) continues to increase [1]. A total of 37 million people worldwide are affected by CHF. In European countries, this disease is diagnosed in 1–2.6 % of the population [2], in 2.2 % of the population of the USA [3, 4], and in 7–10 % of the population of the Russian Federation [5, 6], i.e. the prevalence of this disease in our country is much higher than in the European countries and the USA.

In Europe, CHF accounts for 5 % of all hospital admissions [7]. In the USA, CHF leads to 1.023 million hospital admissions per year (6.5 million bed-days) [4]. In Russian Federation, CHF is the main cause of admission in 16.7 % of the patients admitted with CVD. This disease is the most common cause of inpatient treatment among people over 65 years old [5, 8]. Moreover, about 50 % of patients with CHF are rehospitalized

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within 6 months; 20–25 % of patients are rehospitalized within 30 days after discharge from the hospital [9]. Seventy percent of rehospitalizations are associated with CHF decompensation [10].

A further increase in the number of patients with CHF is expected due to an increase in the prevalence of cardiac risk factors, an improvement in the survival of patients with various cardiovascular disorders, and the aging of the population in future [8]. By 2030, the number of patients with CHF is expected to increase by 46 % [4].

The cost of CHF treatment amounted to 30.7 billion dollars in the USA in 2012 [3]. By 2030, it is expected to increase by 127 % to 69.7 billion dollars per year [3, 4].

CHF progression is accompanied by a significant decrease in life quality, decreased/lost ability to work, disability of patients, and increased mortality. The loss of working-age population due to cardiovascular morbidity and mortality in the European Union is 45 billion euro per year [11].

CHF is the leading cause of cardiovascular mortality. The mortality in patients with CHF is 4–10.3 times higher than that in the general population of the corresponding age, and is comparable to, or even in excess of, the mortality rate for a number of oncological diseases. The five-year mortality rate in patients with CHF from the moment of diagnosis was 60–70 % of patients until the 1990's. In recent years, a small but significant decrease to 50 % was recorded [12]. The annual CHF mortality is 17.4–33 % [13]: in the USA, accounting for 250,000 people per year, and in the Russian Federation 612,000 people die per year from the disease [6]. Mortality in patients with CHF with reduced ejection fraction (CHFrEF, EF < 40 %) is higher than in patients with CHF with preserved EF (CHFpEF, EF ≥ 50%) regardless of the age, gender, and etiology of CHF [14]. The hospital mortality in patients with CHF is 2–20 %. The 30-day post-discharge mortality is 11.3 % [15].

Due to this, the objective of healthcare is to significantly improve the quality of medical care for patients with CHF. This lecture presents a procedure and practical recommendations for the treatment of patients with CHF, and in particular those with CHFrEF, from the standpoint of current local and international guidelines.

The Goal of CHF Management

The goal of treatment for patients with CHF is to improve their clinical status, functional ability, and prognosis [16].

The Objectives of CHF Management

1. Decrease the severity of clinical symptoms.
2. Increase the exercise tolerance.
3. Improve the quality of life.
4. Prevent disability.
5. Prevent the progression of CHF.
6. Improve hemodynamics and organ perfusion and reverse target organ damage.
7. Decrease the frequency of decompensation and the number of hospitalizations.
8. Prevent thromboembolic and other complications.
9. Increase life expectancy, and reduce mortality in patients with CHF [17].

CHF and EF Treatment Procedure

All medications for the treatment of CHF and decreased EF can be divided into two main categories in accordance with the strength of evidence. The first is medicines that reduce mortality in patients with CHF. The second is medicines that do not affect the prognosis for patients with this disease (Figure 1) [17]. While taking into consideration the goals and objectives of treatment, it is necessary to primarily prescribe medicines that have been proven to be able to reduce mortality, i.e., to prolong the life expectancy of patients with CHF.

The procedure for managing patients with CHF and EF is presented in Figure 2.

Angiotensin Converting Enzyme (ACE) Inhibitors

ACE inhibitors should be prescribed to a patient with heart failure with reduced ejection fraction of the left ventricle (CHFrEF). It is necessary to begin treatment with the starting dose and gradually

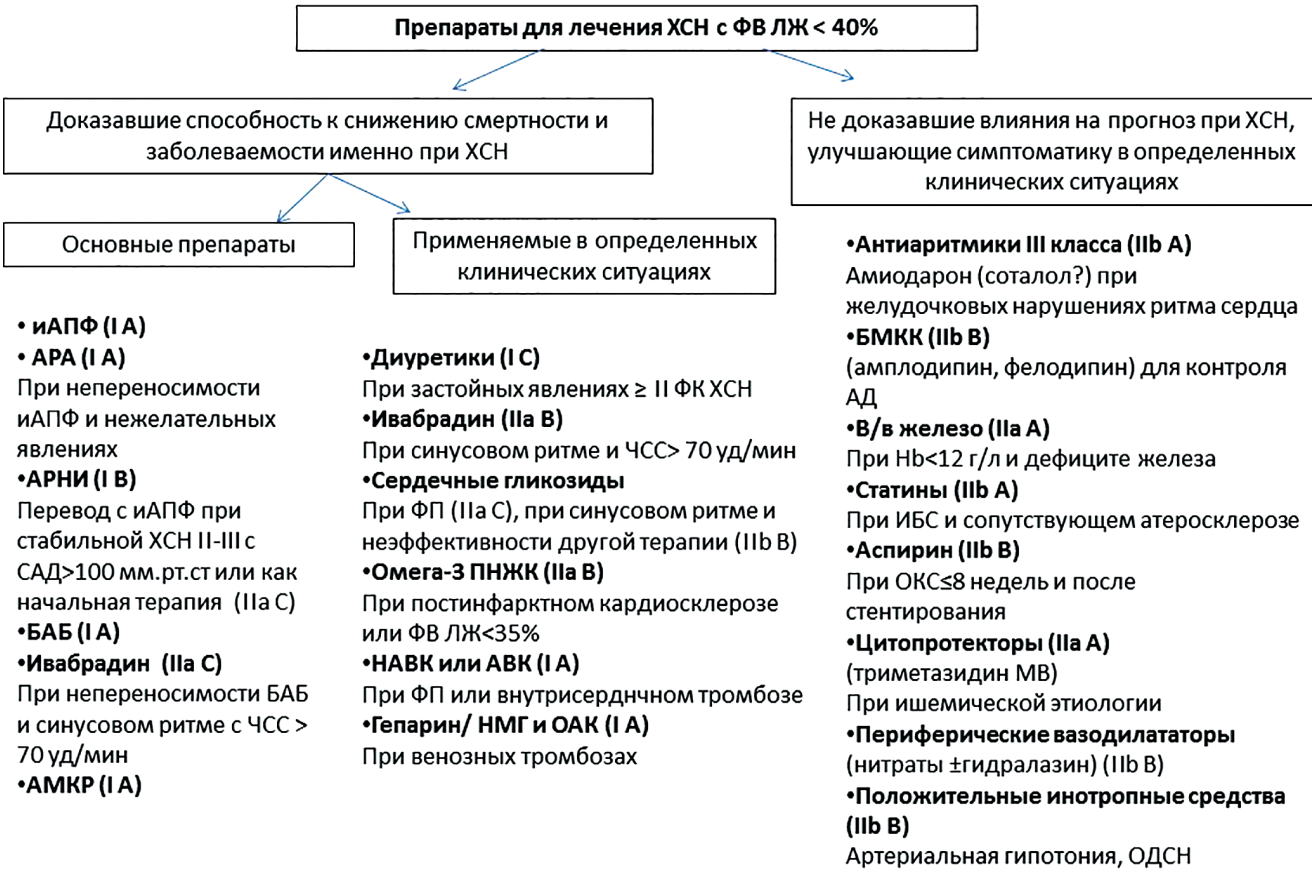

Figure 1. *Medicines for treatment of CHF with EF <40% [17].*

Caption to Figure 1. The classes of recommendations and the levels of evidence are graded in parentheses. Classes of recommendations: I — **sufficient evidence** and/or the general agreement that a given treatment or procedure is **beneficial, useful and effective — such a course of treatment must be prescribed;** IIa — weight of evidence/opinion is in favor of usefulness/efficacy of treatment (**the benefit** of the procedure/treatment **exceeds the risk** of adverse events, but further studies are needed) **— it is reasonable to prescribe such a course of treatment;** IIb — usefulness/efficacy is less well established (**the benefit** of the procedure/treatment **is either somewhat greater than or equal to the risk** of adverse effects; additional studies are needed to clarify the appropriateness of the prescribing the course of treatment) — **may be prescribed if clinically indicated**; III — **sufficient evidence** and/or general agreement that the given treatment or procedure **is not useful/effective** and in some cases **may be harmful — such a course of treatment should not be prescribed**.

Levels of evidence: A — the recommendation is based on the results of **multiple, randomized** clinical trials or meta-analyses; B — the recommendation is based on results of **a single randomized** clinical trial **or several** large **non-randomized** clinical trials; C — the recommendation is based **on the opinion of experts** and/or small studies, retrospective studies, and register data.

increase the dose to the target (optimal) value while monitoring blood pressure (BP), serum creatinine and potassium values (Table 1).

ACE inhibitors reduce the risk of death by 44 %. In this regard, they should be used in all patients with CHFrEF to reduce the risk of death and rehospitalization and improve the medical state of the patient. Not prescribing ACE inhibitors to patients with low EF cannot be considered justified at a SBP > 85 mm Hg, and leads to an increased risk of death in patients with CHF (Class of recommendations Ia, Level of evidence A). ACE inhibitors have not yet proven their ability to improve the prognosis in patients with CHF with a midrange left ventricular ejection fraction (CHFmrEF, EF 40–49 %). However, due to improvement of the functional status of patients and the reduction in the frequency of hospitalizations, ACE inhibitors are indicated for all patients with CHF and midrange EF [16, 17].

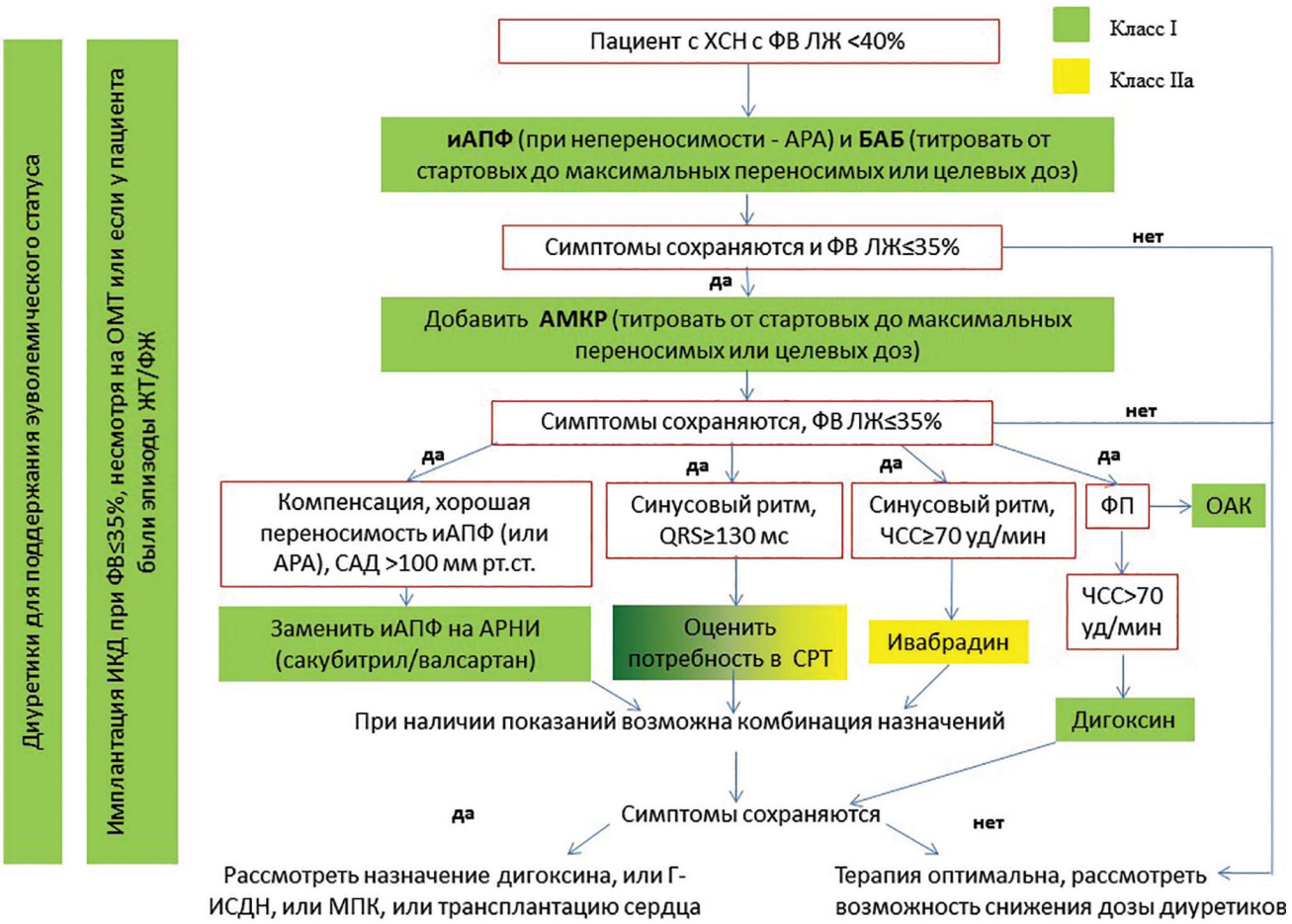

Figure 2. *The algorithm of patients management with CHFrEF (EF <40%) [16, 17]*

Table 1. *Angiotensin-converting enzyme (ACE) inhibitors and their doses used in CHF [16, 17]*

ACE inhibitors	Starting dose (mg)	Target dose (mg)
Captopril	6,25/t.i.d.	50/t.i.d.
Enalapril	2,5/b.i.d.	10-20/b.i.d.
Lisinopril	2,5-5,0/o.d	20-35/o.d.
Ramipril	2,5/o.d.	10/o.d.
Trandolapril	0,5/o.d	4/o.d.

Note: b.i.d. — bis in die (twice daily); o.d. — omne in die (once daily); t.i.d. — ter in die (three times a day).

Table 2. *Angiotensin receptor blocker (ARBs) and their doses used in CHF [16, 17]*

ARB	Starting dose (mg)	Target dose (mg)
Candesartan	4-8/o.d.	32 * 1 р/сутки/o.d.
Valsartan	40/b.i.d.	160 * 2 р/сутки/b.i.d.
Losartan	50/o.d.	150 * 1 р/сутки/o.d.

Table 3. *Beta-blockers and their doses used in CHF [16, 17]*

Beta-blocker	Starting dose (mg)	Target dose (mg)
Bisoprolol	1,25 o.d.	10 o.d.
Carvedilol	3,125 b.i.d.	25 b.i.d.
Metoprolol succinate (CR/XL)	12,5-25 o.d.	200 o.d.
Nebivolol	1,25 o.d.	10 o.d.

Contraindications to ACE inhibitors and ARBs:
1. Allergic reaction (angioedema, rash, etc.).
2. Bilateral stenosis of the renal arteries or stenosis of the renal artery of a single kidney.
3. Pregnancy.
4. Clinically apparent hypotension (SBP < 85 mm Hg).

Angiotensin II Receptor Blockers (ARBs)

If the patient is intolerant to ACE inhibitors due to allergic reactions or cough, ARBs should be prescribed to reduce the combination of risk of death and hospitalization (Table 2). It is necessary to begin treatment with the starting dose and gradually increase the dose to the target one while monitoring BP, serum creatinine and potassium values. Contraindications to ARBs are the same as for ACE inhibitors (Table 2).

Beta-Adrenergic Blockers

β-blockers as well as ACE inhibitors should be used in all patients with CHFrEF to reduce the risk of death and rehospitalizations because they reduce mortality by 34–35 %. This has been proved only for 4 β-blockers. These β-blockers should be prescribed to patients with CHF (Table 4). Patients with CHF with midrange EF and CHFpEF can be prescribed β-blockers to reduce heart rate and severity of LVH. Nebivolol is also able to reduce the risk of hospitalization and death in patients with CHF with midrange EF [16, 17].

Treatment with β-blockers in CHF should begin cautiously, starting with an initial dose, which is ⅛ of the therapeutic dose. Doses should be increased (titrated) slowly (not more than once every 2 weeks, and in case of doubtful tolerability and excessive decrease in blood pressure — once per month) until the optimal dose is achieved. In each patient with CHF and sinus rhythm, the optimal dosage of β-blockers is defined as the one that will decrease heart rate to < 70 beats per minute. For every 5 beats that the heart rate is decreased, the risk of CHF death is reduced by 18 %. Patients who are receiving treatment with non-recommended β-blockers (most often atenolol or short-acting metoprolol tartrate) should be prescribed with the recommended β-blockers (Table 3) [16, 17].

Contraindications to β-blockers:
1. Asthma. COPD is not a contraindication to β-blockers. The physician must make an attempt to prescribe them, starting with small doses and titrating slowly. Treatment with β-blockers should be avoided only in case of exacerbation of bronchial obstruction symptoms when being on β-blockers treatment. The drugs of choice in this situation are highly selective β_1-blockers, bisoprolol and nebivolol.
2. Clinically apparent bradycardia (< 50 bpm)
3. Clinically apparent hypotension (SBP < 85 mm Hg)
4. AV block 2 or 3.
5. Severe obliterating endarteritis and atherosclerosis of the lower extremities.

In case of intolerance and contraindications to β-blockers in patients with CHFrEF with sinus rhythm and heart rate > 70 beats per minute, the physician should consider prescribing the I_f inhibitor ivabradine to reduce the risk of death and hospitalizations.

Diuretics

If congestion signs are present (edemas, fine crackles in the lower lung fields, jugular vein distention, hydrothorax, hydropericardium, ascites, etc.), the prescription of diuretics is necessary for patients with CHF in addition to ACE inhibitors/ARBs and β-blockers to improve clinical symptoms and reduce the risk of rehospitalization (Figure 3, Table 4) [16, 17].

Treatment with diuretics should begin with small doses (especially in patients who have not received any previous diuretics). Afterwards the dose should be chosen in accordance with the principle of quantum satis — as much as necessary. A careless approach to dehydration will only cause side effects and rebound fluid retention [16, 17].

There are 2 phases of diuretic therapy in CHF:

1. Active phase (if congestion signs are present): the amount of urine excreted should be 1–1.5 liters per day more than the amount of fluid taken, weight

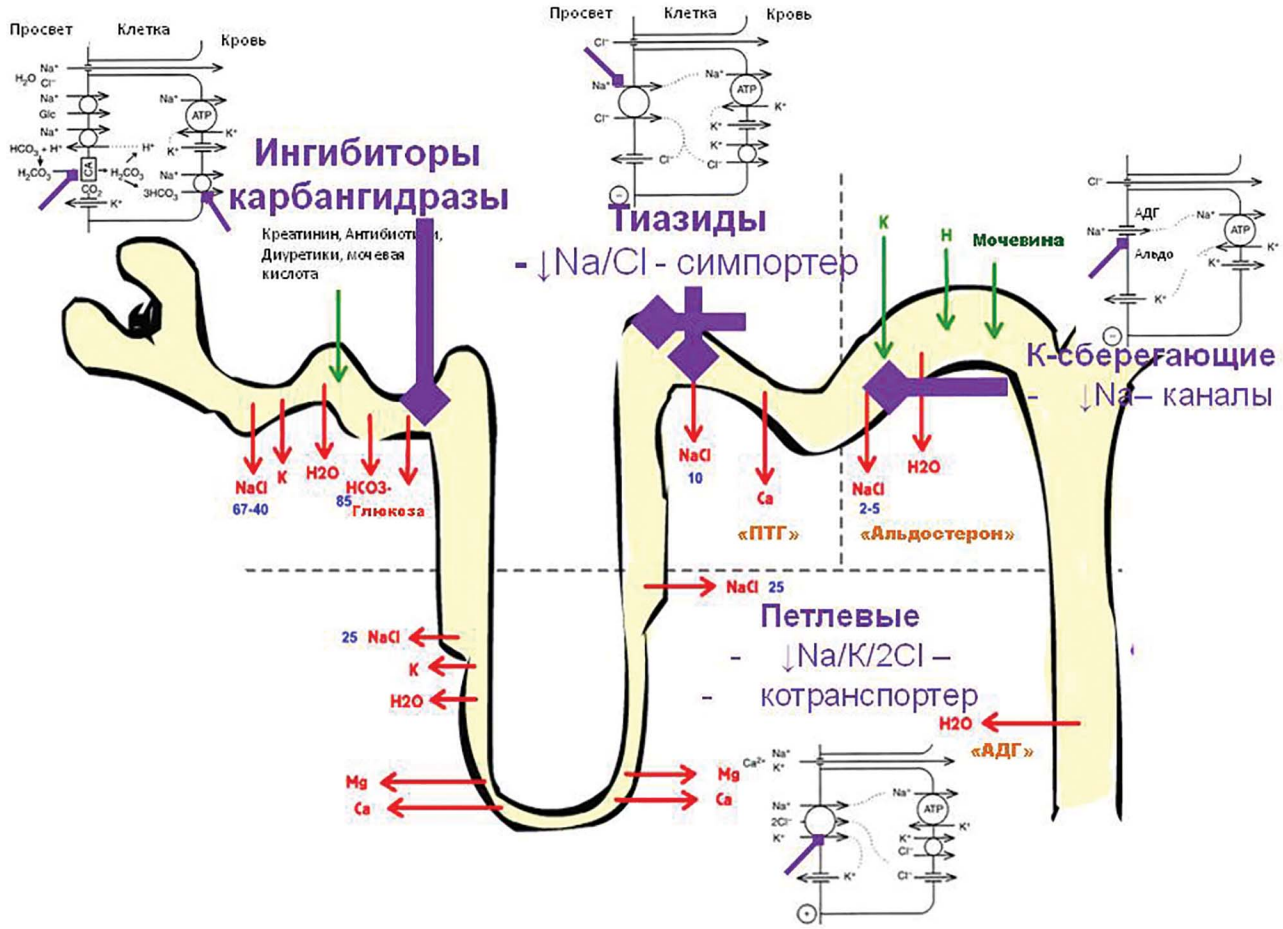

Figure 3. *Groups of diuretics recommended for the treatment of CHF, localization and mechanism of their action*

Table 4. *Diuretics and their doses used in CHF [16, 17]*

Diuretic	Starting dose (mg)		Target dose (mg)	
Loop diuretics				
Furosemide	20-40		40-240	
Bumetanide	0,5-1,0		1-5	
Torasemide	5-10		10-20	
Thiazides and non-thiazide sulfonamide				
Bendroflumethiazide	2,5		2,5-10	
Hydrochlorothiazide	25		12,5-100	
Metolazone	2,5		2,5-10	
Indapamide	2,5		2,5-5	
Potassium-sparing diuretics				
	+ACE-I/ARB	**-ACE-I/ARB**	**+ACE-I/ARB**	**-ACE-I/ARB**
Spironolactone/eplerenone	12,5-25	50	50	100-200
Amiloride	2,5	5	5-10	10-20
Triamterene	25	50	100	200

Table 5. *Mineralcorticoid receptor antagonists (MRA) and their doses used for the treatment of CHF [16, 17]*

MRA	Starting dose (mg)	Target dose (mg)
Eplerenone	25 o.d.	50 o.d.
Spironolactone	25 o.d.	50 o.d.

loss of ~1 kg per day. More rapid dehydration leads to excessive hyperactivation of neurohormones, rebound fluid retention in the body as well as the development of electrolyte, hormonal, arrhythmic and thrombotic complications. Loop diuretics torasemide or furosemide are combined with a diuretic dose of mineralocorticoid-receptor antagonists (MRA) 100–300 mg/day. Torasemide has advantages over furosemide in terms of the strength of its effect, degree of absorption (ease of ingestion), duration of effect (better tolerability, lower incidence of urination), and positive effect on neurohormones (fewer electrolyte disturbances, decreased progression of myocardial fibrosis, and improved diastolic filling of the heart). It also reliably reduces the risk of rehospitalizations that are necessitated due to exacerbation of CHF. For severe cavitary and refractory edemas, additional mechanical evacuation of fluid from the cavities (para-, pleuro- or pericardiocentesis) or isolated ultrafiltration are also possible solutions [16, 17].

2. Maintenance phase (to maintain a euvolemic state after achieving compensation for CHF events): the amount of fluid excreted should be 150–200 ml per day more than the amount of consumed/injected fluid (diuresis + 150–200 ml per day) and body weight should remain stable during the period of daily intake of diuretics. After the patient reaches euvolemia, diuretics should be prescribed on a daily basis in minimal doses, which make it possible to maintain a balanced diuresis. When diuretics are prescribed occasionally (bolus doses once every 3–4–5–7 days), the impact quality on life and the prognosis may be negative. Four to five day courses of the carbonic anhydrase inhibitor acetazolamide (0.75/day) are recommended once every 2 weeks to maintain optimal acid-base balance, preserve sensitivity to loop diuretics and normalize renal blood flow [16, 17].

When prescribing diuretics, it is necessary to remember that you cannot use thiazides if GFR is less than 30 mL/min/1.73 m^2, with the exception of some cases when they are prescribed together with loop diuretics to overcome diuretic resistance.

If the patient with CHF is prescribed a combination of 3 drugs (**ACE inhibitor / ARBs, β-blocker and diuretic), and there are no clinical symptoms of CHF** (dyspnea, weakness, fatigue, palpitations, and swelling)**,** then **it is necessary to continue treatment with these drugs!!!** You can try to reduce the dose of diuretics over time. If clinical symptoms appear after that, return to the initial dose of the diuretic [16, 17].

Mineralocorticoid Receptor Antagonists (MRA)

If, in spite of the fact that the patient with CHFrEF is treated with a 3 drug-combination (**ACE inhibitor / ARBs, β-blocker and diuretic) clinical symptoms of CHF** (dyspnea, weakness, fatigue, palpitations, and swelling) **persist,** then **it is necessary** to add **a mineralocorticoid receptor antagonist** (aldosterone antagonists, MRA, Table 5) to this combination to reduce the risk of death and rehospitalization as well as to improve the medical state. MRA can be prescribed for patients with CHFpEF and CHF with midrange EF to reduce the number of hospitalizations due to CHF [16, 17].

Contraindications to MRA:

1. GFR < 30 ml/min/1.73 m^2, especially in combination with another RAAS blocker, because of the risk of developing kidney dysfunction and hyperkalemia.
2. Hyperkalemia > 5.5 mmol/L

In cases when **clinical symptoms of CHF** (dyspnea, weakness, fatigue, palpitations, and swelling) **persist** despite the 4 drug-combination (**ACE inhibitor / ARBs, β-blocker, diuretic, and MRA**) and EF is 35 % or below, it is necessary to consider the following 3 options of patient management:

1. In case of good tolerability of ACE inhibitors or ARBs, replace ACE inhibitor or ARBs with an angiotensin receptor-neprilysin inhibitor (ARNI).
2. If there is sinus rhythm, and the heart rate is 70 beats per minute or higher, add ivabradine to the 4 drug-combination.
3. If the rhythm is sinus and QRS duration is 130 ms or more, cardiac resynchronization therapy (CRT) should be considered.

Angiotensin Receptor-Neprilysin Inhibitors (ARNI)

If, in spite of the fact that the patient with CHFrEF is prescribed a 4 drug-combination (**ACE inhibitor / ARBs, β-blocker, diuretic, and MRA) clinical symptoms of CHF persist,** then **ACE inhibitor / ARBs should be replaced** with **angiotensin receptor-neprilysin inhibitor** (ARNI).

Neprilysin is a neutral endopeptidase that cleaves natriuretic peptides, bradykinin, and other peptides. Inhibition of neprilysin leads to an increased level of natriuretic peptides in the blood, increased diuresis, natriuresis, improved relaxation of the myocardium, and a decrease in the secretion of renin and aldosterone. Currently, there is one drug in the ARNI group, which is a cross-linked molecule of valsartan (ARBs) and sacubitryl (neprilysin inhibitor). It is able to reduce mortality by 20 % better than an ACE inhibitor (enalapril).

ARNI is recommended for patients with stable CHFrEF (without decompensation, intravenous administration or doubling of the dose of oral diuretics and SBP > 100 mm Hg), and in case of intolerance to ACE inhibitors (or ARBs). This category of patients is moved onto ARNI (at the starting dose of 100 (49/51) mg b.i.d., no earlier than 36 hours after the last dose of ACE inhibitors (ARBs), followed by titration of the dose to the optimal 200 (97/103) mg b.i.d.) to further reduce the risk of death and subsequent hospitalizations for CHF. The use of ARNI in patients with stable CHFrEF can be considered as an initial therapy (instead of ACE inhibitors) to reduce the risk of death and hospitalizations. A combination of two RAAS blockers (excluding MRA) is not recommended for the treatment of patients with CHF due to a significant increase in serious adverse events, including hypotension and impaired renal function [16, 17].

Triple neurohormonal blockade: ACE inhibitors (in case of ARBs intolerance) or ARNI (in case of stable CHF with SBP > 100 mm Hg) in combination with β-blocker and MRA provide the basis of therapy for CHFrEF and reduce the mortality rate of patients with CHF by a total of 45 % [16, 17].

Ivabradine

If, in spite of the fact that the patient with CHFrEF is prescribed a 4 drug-combination (**ACE inhibitor / ARBs, β-blocker, diuretic, and MRA) clinical symptoms of CHF** (dyspnea, weakness, fatigue, palpitations, and swelling) **persist,** then, in case of sinus rhythm with heart rate of 70 beats per minute and more **ivabradine should be added to the prescribed combination** to reduce the risk of death and rehospitalizations. The starting dose is 5 mg b.i.d., the target dose is 7.5 mg b.i.d. [16, 17].

Ivabradine slows heart rate by inhibiting If-channels in the sinus node, and therefore it should be used only for patients with a sinus rhythm [16].

Cardiac Resynchronization Therapy (CRT)

If, in spite of the fact that the patient with CHFrEF is prescribed a 4 drug-combination (**ACE inhibitor / ARBs, β-blocker, diuretic, and MRA) clinical symptoms of CHF** (dyspnea, weakness, fatigue, palpitations, and swelling) **persist**, then in the case of a sinus rhythm with QRS of 130 ms and more **the physician should consider the need for cardiac resynchronization therapy (CRT)** [16, 17].

CRT is a method of restoring the heart function by means of correction of impaired intracardiac conduction. The simplest indicator of impaired intracardiac conduction (interventricular dyssynchrony) is a wide QRS complex or bundle branch block on the ECG. Besides that, interventricular and intraventricular dyssynchrony are detected using doppler ultrasound and/or myocardial perfusion scintigraphy synchronized with ECG. CRT includes setting the electrode in the right atrium and biventricular stimulation that synchronizes the work of the ventricles. Indications for CRT are given in Table 6. CPT is contraindicated when QRS duration is less than 130 ms. In many cases, devices that combine the ability to resynchronize the rhythm and cardioverter-defibrillator (CRT-D) functions are used [16, 17].

Table 6. *Indications for cardiac resynchronization therapy (CPT) in patients with CHF [16, 17]*

Recommendations	Class	Level
CRT is recommended for symptomatic patients with HF in sinus rhythm with a QRS duration ≥150 msec and LBBB QRS morphology and with EF ≤35% despite OMT in order to improve symptoms and reduce morbidity and mortality	I	A
CRT should be considered for symptomatic patients with HF in sinus rhythm with a QRS duration ≥150 msec and non-LBBB QRS morphology and with EF ≤35% despite OMT in order to improve symptoms and reduce morbidity and mortality	IIa	B
CRT is recommended for symptomatic patients with HF in sinus rhythm with a QRS duration of 130–149 msec and LBBB QRS morphology and with EF ≤35% despite OMT in order to improve symptoms and reduce morbidity and mortality	I	B
CRT may be considered for symptomatic patients with HF in sinus rhythm with a QRS duration of 130–149 msec and non-LBBB QRS morphology and with EF ≤35% despite OMT in order to improve symptoms and reduce morbidity and mortality	IIb	B
CRT rather than RV pacing is recommended for patients with HFrEF regardless of NYHA class who have an indication for ventricular pacing and high degree AV block in order to reduce morbidity. This includes patients with AF	I	A
CRT should be considered for patients with EF ≤35% in NYHA Class III–IVd despite OMT in order to improve symptoms and reduce morbidity and mortality, if they are in AF and have a QRS duration ≥130 msec provided a strategy to ensure bi-ventricular capture is in place or the patient is expected to return to sinus rhythm	IIa	B
Patients with HFrEF who have received a conventional pacemaker or an ICD and subsequently develop worsening HF despite OMT and who have a high proportion of RV pacing may be considered for upgrade to CRT. This does not apply to patients with stable HF	IIb	B
CRT is contra-indicated in patients with a QRS duration < 130 msec	III	A

Note: LBBB — left bundle branch block; OMT — optimal medical therapy; RV — right ventricular

Implantable Cardioverter-Defibrillator (ICD)

The implantation of a cardioverter-defibrillator (ICD) is recommended for all patients with CHFrEF, who had hemodynamically significant ventricular tachycardia or ventricular fibrillation. For the purpose of primary prevention of sudden cardiac death, the ICD is indicated in patients with CHFrEF and persisting clinical symptoms despite having received optimal medical therapy for 3 months if the life expectancy with a good NYHA class is more than one year and they have IHD (ischemic heart disease) or DCM (dilated cardiomyopathy). ICD is not recommended for 40 days after MI (myocardial infarction). ICD is not recommended for patients with NYHA IV with the exception of candidates for CRT, implantation of LVAD (left ventricular assist device), or heart transplantation (Table 7) [16, 17].

Cardiac Glycosides in Patients with CHFrEF

To reduce the risk of rehospitalizations, it is useful to prescribe digoxin for patients with CHFrEF and sinus rhythm and persisting clinical symptoms despite optimal drug therapy, including all the approaches described above, and who have also experienced several episodes of CHF decompensation during the year, low EF ≤ 25 %, LV dilatation and high NYHA class (III–IV), if CHF is compensated [16, 17].

In patients with CHFrEF, the prescription should be considered for tachysystolic form of atrial fibrillation (AF) [16, 17]. Oral β-blockers are safe for use in patients with I–III NYHA class, and therefore they are recommended as a **first-line** therapy for monitoring ventricular rate (VR) in AF. The use of digoxin should be considered in patients with CHF, if, despite the use of β-blockers, high VR persists or in the case of resistance or contraindications to β-blockers [16, 17].

Table 7. *Recommendations for implantable cardioverter-defibrillator (ICD) in patients with CHF [16, 17]*

Recommendations	Class	Level
Secondary prevention: An ICD is recommended to reduce the risk of sudden death and all-cause mortality in patients who have recovered from a ventricular arrhythmia causing haemodynamic instability, and who are expected to survive for >1 year with good functional status.	I	A
Primary prevention: An ICD is recommended to reduce the risk of sudden death and all-cause mortality in patients with symptomatic HF (NYHA Class II–III), and an EF ≤35% despite ≥3 months of OMT, provided they are expected to survive substantially longer than one year with good functional status, and they have: — Ischemical Heart Disease	I	A
— Dilated cardiomyopathy	I	B
ICD implantation is not recommended within 40 days of an MI as implantation at this time does not improve prognosis	III	A
ICD therapy is not recommended in patients in NYHA Class IV with severe symptoms refractory to pharmacological therapy unless they are candidates for CRT, a ventricular assist device, or cardiac transplantation	III	C
Patients should be carefully evaluated by an experienced cardiologist before generator replacement, because management goals and the patient's needs and clinical status may have changed	IIa	B
A wearable ICD may be considered for patients with HF who are at risk of sudden cardiac death for a limited period or as a bridge to an implanted device	IIb	C

An optimal ventricular rate (VR) for patients with HF and AF has not been established, but most of the data suggest that strict VR control may be harmful. Heart rate at rest should be considered in the range of 60–100 bpm [16].

Digoxin should be prescribed when the level of the drug in the blood is controlled (a dose reduction is necessary at a concentration of more than 1.1–1.2 ng/ml), both in case of sinus rhythm and AF (optimal digoxin concentration in blood is less than 0.9 ng/ml) if contraindications are absent. If it is not possible to determine the digoxin concentration, the use of the drug can be continued in small doses (0.25–0.125 μg) if there is no data on glycoside poisoning (at a dose of not more than 0.125 mg with a body weight of less than 60 kg (especially in women) aged 75 years and more and with GFR of less than 60 mL/min/1.73 m^2) [16, 17].

Oral Anticoagulants (OAC)

The CHA2DS2-VASc and HAS-BLED scales are recommended for assessing the risk of TC (thromboembolic complications) and bleeding (Appendix). OAC (Figure 4, Table 8) should be prescribed to reduce the risk of death and hospitalization for patients with CHF with paroxysmal, persistent and permanent AF with a score according to the CHA2DS2VASc scale ≥ 2 or intracardiac thrombosis. For patients with CHF and non-valvular AF who have indications to anticoagulant therapy, the prescription of new oral anticoagulants (non-vitamin K antagonist oral anticoagulants, NOACs) should be preferred over vitamin K antagonists (VKA), given the fact that they are better able to reduce the risk of death and thromboembolic complications while also lowering the risk of bleeding, including intracranial hemorrhage in particular, at the same time. The use of NOACs is contraindicated in the presence of mechanical valves, mitral stenosis, GFR of less than 30 mL/min/1.73 m^2 [16, 17].

Heparin

Prescription of heparin or low-molecular-weight heparin (LMWH) for a minimum of 7 days should be considered in patients with CHFrEF in the presence of venous thrombosis, PE (pulmonary

embolism) or decompensation requiring bed rest (≥ 3 days) to reduce the risk of thromboembolism, improve prognosis, and reduce the risk of hospitalization followed by transfer to the VKA (with INR control) or NOACs [16, 17].

In case of venous thrombosis and PE in patients with CHF, alternative therapy with oral Xa factor inhibitors is possible in place of heparin: apixaban at 10 mg b.i.d. for 7 days followed by a transfer to 5 mg b.i.d., or rivaroxaban at 15 mg b.i.d. for 21 days with a transfer to 20 mg once daily [16, 17]. The duration of anticoagulant therapy for patients who have experienced a single episode of venous thrombosis or PE is up to 3 months, and for those who have experienced repeated episodes it should be longer; NOACs should be preferred in these cases. If anticoagulant therapy is not possible, acetylsalicylic acid can be prescribed [16, 17].

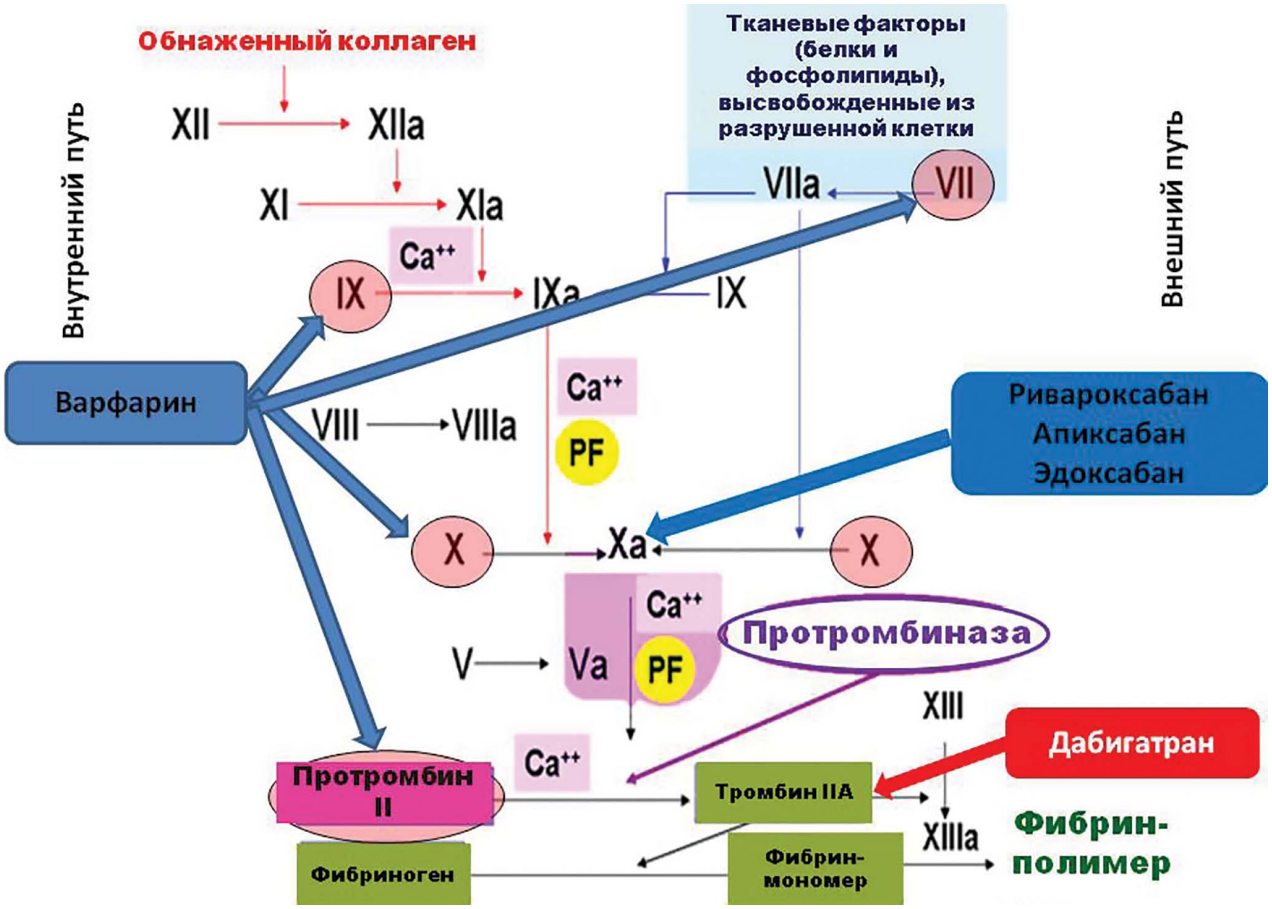

Figure 4. *Effect of oral anticoagulants on coagulation*

Table 8. *Oral anticoagulants*

	Vitamin K antagonist	Nonvitamin K antagonist (NOAC)	
Drugs	Warfarin	Dabigatran	Rivaroxaban, Apixaban, Edoxaban
Effect	Blockade of the synthesis of II, VII, IX, X coagulation factors in the liver	Inhibition of factor II coagulation — thrombin	Antagonist Xa factor
Indication	1. Atrial Fibrillation in mechanical heart valves or at least moderate mitral stenosis 1. CKD 3-4 (GFR <60 ml/min/1.73 м^2)		
Control	INR 2-3, in mitral valve disease >2,5		

Acetylsalicylic Acid (ASA)

The prescription of ASA does not affect the prognosis in patients with CHF and can weaken the effect of ACE inhibitors and other essential drugs. Therefore, the prescription of ASA can only be considered for patients who had ACS within the last 8 weeks and who underwent percutaneous coronary intervention in the last year [16, 17].

Peripheral Vasodilators

The use of peripheral vasodilators (hydralazine and/or nitrates) can be considered only for the management of angina pectoris and when all other methods of treatment described above are ineffective.

Circulatory Assistance Devices: MCS (Mechanical Circulatory Support), LVMSD (Left Ventricle Mechanical Support Device), LVAD (Left Ventricular Assist Device)

If all of these strategies for the CHF treatment are ineffective, mechanical circulatory support can be considered (Table 9, 10).

Heart Transplantation

Heart transplantation is a common treatment method for end-stage HF. Although no controlled studies have been conducted, it is believed that heart transplantation (if the patient selection criteria are

Table 9. *Terms describing various indications for mechanical circulatory support [16, 17]*

Bridge to decision (BTD)/ Bridge to bridge (BTB)	Use of short-term MCS (e.g. ECLS or ECMO) in patients with cardiogenic shock until haemodynamics and end-organ perfusion are stabilized, contra-indications for long-term MCS are excluded (brain damage after resuscitation) and additional therapeutic options including long-term VAD therapy or heart transplant can be evaluated
Bridge to Candidacy (BTC)	Use of MCS (usually LVAD) to improve end-organ function in order to make an ineligible patient eligible for heart transplantation
Bridge to transplantation (BTT)	Use of MCS (LVAD or BiVAD) to keep patient alive who is otherwise at high risk of death before transplantation until a donor organ becomes available
Bridge to recovery (BTR)	Use of MCS (typically LVAD) to keep patient alive until cardiac function recovers sufficiently to remove MCS
Destination therapy (DT)	Long-term use of MCS (LVAD) as an alternative to transplantation in patients with end-stage HF ineligible for transplantation or long-term waiting for heart transplantation

Abbreviations: BiVAD — biventricular assist device; BTB — bridge to bridge; BTC — bridge to candidacy; BTD — bridge to decision; BTR — bridge to recovery; BTT — bridge to transplantation; DT — destination therapy; ECLS — extracorporeal life support; ECMO — extracorporeal membrane oxygenation; LVAD — left ventricular assist device; MCS — mechanical circulatory support; VAD — ventricular assist device.

Table 10. *Patients potentially eligible for implantation of a left ventricular assist device [16, 17]*

Patients with >2 months of severe symptoms despite optimal medical and device therapy and more than one of the following:
EF <25% and, if measured, peak V_{O2} <12 mL/kg/min
≥3 HF hospitalizations in previous 12 months without an obvious precipitating cause
Dependence on i.v. inotropic therapy
Progressive end-organ dysfunction (worsening renal and/or hepatic function) due to reduced perfusion and not to inadequate ventricular filling pressure (PCWP ≥20 mmHg and SBP ≤80–90 mmHg or CI ≤2 L/min/m^2)
Absence of severe right ventricular dysfunction together with severe tricuspid regurgitation

Abbreviations: SBP — systolic blood pressure, SI — cardiac index, HF — heart failure, EF — left ventricular ejection fraction, PCWP — wedge pressure in pulmonary capillaries

met) significantly increases the patient survival rate, and it also improves exercise tolerance, quality of life, and the ability to return to work as compared to conventional treatment [16].

The main problems in heart transplantation are the lack of donor hearts, the consequences of the limited effectiveness of the method and the complications of immunosuppressive therapy over the long term (for example, antigen-antibody mediated rejection of the transplant, infectious complications, hypertension, kidney failure, malignancy, and vasculopathy of the coronary arteries) [16].

Indications for heart transplantation [16]:
1. The end-stage of HF, severe clinical symptoms, unfavorable prognosis, and inability to use alternative therapies.
2. Motivated, well-informed, emotionally stable patients.
3. Ability of a patient to comply with a course of intensive treatment in the postoperative period.

Contraindications to heart transplantation [16]:
1. Active infection.
2. Severe damage to peripheral and/or cerebral arteries.
3. Pharmacologically irreversible pulmonary hypertension.
4. Cancer (cooperation with oncologists is necessary to assess the risk of tumor recurrence).
5. Irreversible kidney injury (e.g., creatinine clearance < 30 mL/min).
6. Systemic diseases involving multiple organs.
7. Other comorbidities with poor prognosis.
8. BMI (body mass index) > 35 kg/m^2 (weight loss is recommended to achieve BMI < 35 kg/m^2).
9. Alcohol and drug abuse.
10. Patients with insufficient social support.

It should be considered that some contraindications are temporary. In patients with potentially reversible or compensable comorbidities, such as obesity, kidney failure, pulmonary hypertension, the use of MCS, particularly LVMSD, should be considered, followed by a reassessment of indications and contraindications for heart transplantation [16].

Drug Products That Can Harm Patients with CHFrEF

In addition, the use of drugs that can harm patients with CHF should be avoided in these patients (Table 11).

Thus, currently, a clear procedure for managing patients with CHFrEF has been developed on the evidence-based data. Unfortunately, in real clinical practice, patients rarely follow this procedure sufficiently closely to obtain tangible benefits. In addition, patients often fail to adhere to the treatment regimen and do not take prescribed medications even when a course of therapy has been properly prescribed. It is necessary to have a clear understanding of the procedures for managing patients with CHF and to follow them in real clinical practice. This will make it possible to achieve the set goals and resolve the specified objectives for managing patients with CHF.

Table 11. *Treatments that may cause harm in patients with CHFrEF [16, 17]*

Recommendation	[a] Class	[b] Level
Thiazolidinediones (glitazones) are not recommended in patients with CHF, as they increase the risk of CHF worsening and CHF hospitalization	III	A
Non-steroidal anti-inflammatory drug and cyclooxygenase-2 inhibitor are not recommended in patients with CHF, as they increase the risk of CHF worsening and CHF hospitalization	III	B
Diltiazem or verapamil are not recommended in patients with CHFrEF, as they increase the risk of CHF worsening and CHF hospitalization	III	C
The addition of an ARB (or renin inhibitor) to the combination of an ACE-I and an MRA is not recommended in patients with HF, because of the increased risk of renal dysfunction and hyperkalaemia	III	C

Appendices

Приложение. Шкала CHA_2DS_2-VASc для оценки риска ТЭО:
Congestive heart failure or left ventricular dysfunction, Hypertension, Age ≥75 (doubled), Diabetes, Stroke (doubled)-Vascular disease, Age 65–74, Sex (female)

Факторы риска	Балл
Клиника ХСН или ФВ ЛЖ≤40%	1
Артериальная гипертония	1
Возраст ≥75 лет	2
Сахарный диабет	1
Инсульт / ТИА/ тромбоэболия в анамнезе	2
Сосудистое заболевание (инфаркт миокарда, атеросклероз аорты, периферических артерий)	1
Возраст 65-74 года	1
Женский пол	1

Категория риска	Сумма баллов	Тактика антитромботической терапии
Низкий	0	Нет необходимости
Средний	1	Пероральные антикоагулянты (предпочтительнее) или дезагреганты
Высокий	≥2	Пероральные антикоагулянты

Приложение. Шкала HAS-BLED для оценки риска кровотечений: Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile INR, Elderly, Drugs/alcohol concomitantly

Факторы риска	Балл
Артериальная гипертензия (САД>160 мм.рт.ст.)	1
Нарушение функции почек и печени (1 балл каждое)	1 или 2
Инсульт	1
Склонность к кровотечениям	1
Лабильность МНО (на фоне варфарина)	1
Возраст>65 лет	1
Лекарственные препараты (например, аспирин, НПВС) или злоупотребление алкоголем (1 балл каждое)	1 или 2
При сумме баллов ≥3 необходимо с осторожностью назначать пероральные антикоагулянты и регулярно контролировать МНО	

Conflict of interests
The authors declare no conflict of interests.

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Article received on 12.02.2018
Accepted for publication on 01.03.2018

UDC 616.71-007.234-07

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BONE REMODELING IN NORM AND IN PRIMARY OSTEOPOROSIS: THE SIGNIFICANCE OF BONE REMODELING MARKERSS

Abstract

Osteoporosis is a systemic skeletal disease is characterized by low bone mass and microarchitectural deterioration of bone tissues, leading to fragility fractures. The number of patients with osteoporosis is steadily increasing due to the aging of society. Osteoporosis is an extremely common disease: it affects more than 200 million people worldwide and causes more than 8.9 million fractures. In Russia, among people aged 50 years and older, osteoporosis is diagnosed in 34% of women and 27% of men. The social significance of osteoporosis is determined by its consequences: fractures of bones of the peripheral skeleton and vertebral body fractures, leading to high material costs and causing a high level of disability and mortality. The normal physiological process of bone remodeling involves a balance between bone resorption and bone formation. In osteoporosis, this process becomes unbalanced, resulting in gradual losses of bone mass and density due to enhanced bone resorption and/or inadequate bone formation. Several signaling pathways underlying primary osteoporosis have been identified, such as the osteoprotegerin/receptor activator of nuclear factor kappa-B (RANK) / RANK ligand (RANKL), bone morphogenetic proteins, canonical Wnt-signaling pathway. In addition, genetic disorders are involved in the development of the pathogenesis of osteoporosis. To identify osteoporosis, WHO recommends the use of dual energy X-ray absorptiometry, which allows you to study the quantitative characteristics of bone tissue. Currently, there are various methods for evaluation of the quality of bone (microarchitectonics, the ability of bone tissue to be resistant to fracture), but these methods have limitations such as high cost and limited availability for their widespread using. The study of markers of bone remodeling normally and in pathology helps to assess the quality of bone tissue indirectly, gives prospects in the selection of targeted therapy and improvement of early diagnosis of osteoporosis.

Key words: *osteoporosis, bone remodeling, bone turnover markers*

For citation: Nurullina G.M., Akhmadullina G.I. BONE REMODELING IN NORM AND IN PRIMARY OSTEOPOROSIS: THE SIGNIFICANCE OF BONE REMODELING MARKERS. The Russian Archives of Internal Medicine. 2018; 8(2): 100-110. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-100-110

DOI: 10.20514/2226-6704-2018-8-2-100-110

BMP — bone morphogenic protein; DXA — dual energy X-ray absorptiometry; M-CSF – macrophage colony stimulating factor; CTX — C-terminal telopeptide of type I collagen; PINP – procollagen type I aminoterminal propeptide; OPG — osteoprotegerin; RANK — receptor activator of nuclear factor kappa-B; RANKL — RANK ligand; TRAP 5b — tartrate-resistant acid phosphatase 5b; BMU — basic multicellular unit

Introduction

Osteoporosis is a metabolic skeletal disease, which is characterized by its tendency to decrease bone mass and deteriorate the microarchitecture of bone tissue, leading to fragility fractures [1]. The number of patients with osteoporosis is steadily increasing due to the aging of society. Osteoporosis affects about 200 million people worldwide, and about 8.9 million fractures are fragility fractures [2]. In Russia, among the people aged 50 and older, osteoporosis is diagnosed in 34% of women and

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27% of men. The social significance of osteoporosis is evident in its consequences: fractures of bones of the peripheral skeleton and vertebral body fractures leading to high material costs and causing a high level of disability and mortality [3].

Bone strength is determined by the quantity and quality of bone tissue. Measuring bone mineral density (BMD) using dual energy X-ray absorptiometry (DXA) is the gold standard for studying bone tissue quantity. The bone quality is assessed by its microarchitectonics, presence of microdamage, the remodeling parameters, and the ability of bone tissue resist fracture [4]. Currently, the following different methods for assessment of the bone quality parameters are available: trabecular bone score [5], high-resolution peripheral quantitative computed tomography, high-resolution magnetic resonance tomography [6], microcomputer tomography, bone histomorphometry [7], bone microindentation testing [8]. However, these methods have limitations for their widespread use, such as high cost and limited availability.

Thus, the study of bone remodeling under normal and pathological conditions helps us to indirectly assess the quality of bone tissue, provide perspectives in the selection of targeted therapy, and improve early diagnosis of osteoporosis.

Normal Cycle of Bone Remodeling

Bone remodeling can be divided into 2 types: stochastic (probabilistic) and targeted. Targeted remodeling is activated when microdamages occur in bone tissue and osteocytes die [9]. Stochastic remodeling is regulated by a number of hormones (parathyroid hormone, estrogens, STH, and free T4) [10].

Cycle of Bone Remodeling

Osteocytes of intact bone produce the glycoprotein sclerostin which prevents differentiation of mesenchymal stem cells (progenitor cells) by blocking the Wnt/β-catenin signaling pathway. When microdamage occurs in bone tissue, the osteocytes transmit a mechanical signal (transduction) and a chemical signal (release of prostaglandins, growth factors, and nitric oxide) to the bone lining cells that line the surface of the bone trabecula. In response to incoming signals, the bone lining cells detach from the bone surface, forming so-called "canopy". Cells of the "canopy" are connected to the osteocyte network via gap junctions, to the lining cells on quiescent surfaces [11], and to a capillary, forming a bone remodeling compartment (BRC). BRC blood supply is provided by capillaries either coming from the marrow space (in cancellous bone) or from the central vessel of the Haversian system (in cortical bone) [12]. It is assumed that the osteocytes death as well as the release of osteotropic growth factors and cytokines stimulates angiogenesis. At the same time, the angiogenic factors VEGF (vascular endothelial growth factor) and endothelin regulate the activity of osteoclasts and osteoblasts [13], taking an active part in signaling between blood vessels and bone tissue [14]. Osteoprogenitor cells under the cover of bone lining cells become free from the sclerostin exposure and enter the process of differentiation into preosteoblasts under the influence of growth factors and interleukin-1. Some osteoblastogenesis markers are the transcription factors Runx2 (Runt-related transcription factor 2, and transcription factor 2, which is associated with dwarfism) and Osterix (Osx) [15].

Nearly all stages of osteoblastogenesis are associated with activation of the Wnt signal: differentiation of the mesenchymal stem cell into preosteoblast, as well as survival and continuation of differentiation of preosteoblasts into osteoblasts. Wnt-proteins activate the Wnt/β-catenin signaling pathway, triggering osteoblastogenesis processes. The Wnt/β-catenin signaling pathway consists mainly of Wnt ligands (or wingless-type MMTV integration site family ligands), Fz receptors (Frizzled), LRP5/6 coreceptors (a protein bound to a low-density lipoprotein receptor of types 5 and 6), Dsh protein (Disheveled), β-catenin, GSK-3β (glycogen synthase kinase-3β), Axin protein (axis inhibition protein 1), APC (adenomatous polyposis coli and suppressor protein), CK1 (casein kinase 1), and TCF/LEF (nuclear T-cell transcription factor / lymphoid enhancer factor) [16]. In the absence of the Wnt ligand, a multiprotein complex is formed in the cell cytoplasm, including APC, CK1, Axin, and GSK-3β. This complex facilitates GSK-3β-dependent phosphorylation of β-catenin and its subsequent ubiquitination, which leads to further degradation of β-catenin in the proteasome. Thus, β-catenin does not translocate into the cell nucleus and does not trigger the

osteoblastogenesis processes. Upon activation of the canonical Wnt/β-catenin signaling pathway (cWnt-SP), Wnt ligands bind to Fz (Frizzled) and LRP5/6 to form the Wnt-Fz-LRP5/6 ternary complex. This complex is stabilized by the proteins Dsh and Axin, resulting in the formation of "receptor complex" Wnt-Fz-LRP 5/6 -Dsh-Axin on the cell surface. This inhibits GSK-3β-phosphorylation of β-catenins, which leads to the cessation of their degradation. β-catenins penetrate the nucleus, and they interact with the transcription factors TCF/LEF, resulting in the activation of target genes (Figure 1).

These target genes include genes encoding non-collagen bone matrix proteins (osteocalcin, osteopontin, bone morphogenetic proteins (BMP)), type I collagen, SP7 gene (encodes transcription factor Osterix), and ALPL (determines alkaline phosphatase). Also, Wnt signaling stimulates osteoprotegerin (OPG) secretion [17].

There are 19 Wnt ligands that have been described in humans, and they can activate both canonical and non-canonical signaling pathways. These include Wnt1, Wnt2, Wnt2B, Wnt3, Wnt3A, Wnt4, Wnt5A, Wnt5B, Wnt6, Wnt7A, Wnt7B, Wnt8A, Wnt8B, Wnt9A, Wnt9B, Wnt10A, Wnt10B, Wnt11, and Wnt16 [18]. Sclerostin, DKK-1 (Dickkopf-related protein 1), and Wnt-inhibitory factor 1 (WIF-1) are the antagonists of the Wnt / β-catenin signaling pathway [19].

BMP-2 (bone morphogenetic protein 2) is able to stimulate angiogenesis, enhancing the secretion of the endothelium growth factor by osteoblasts.

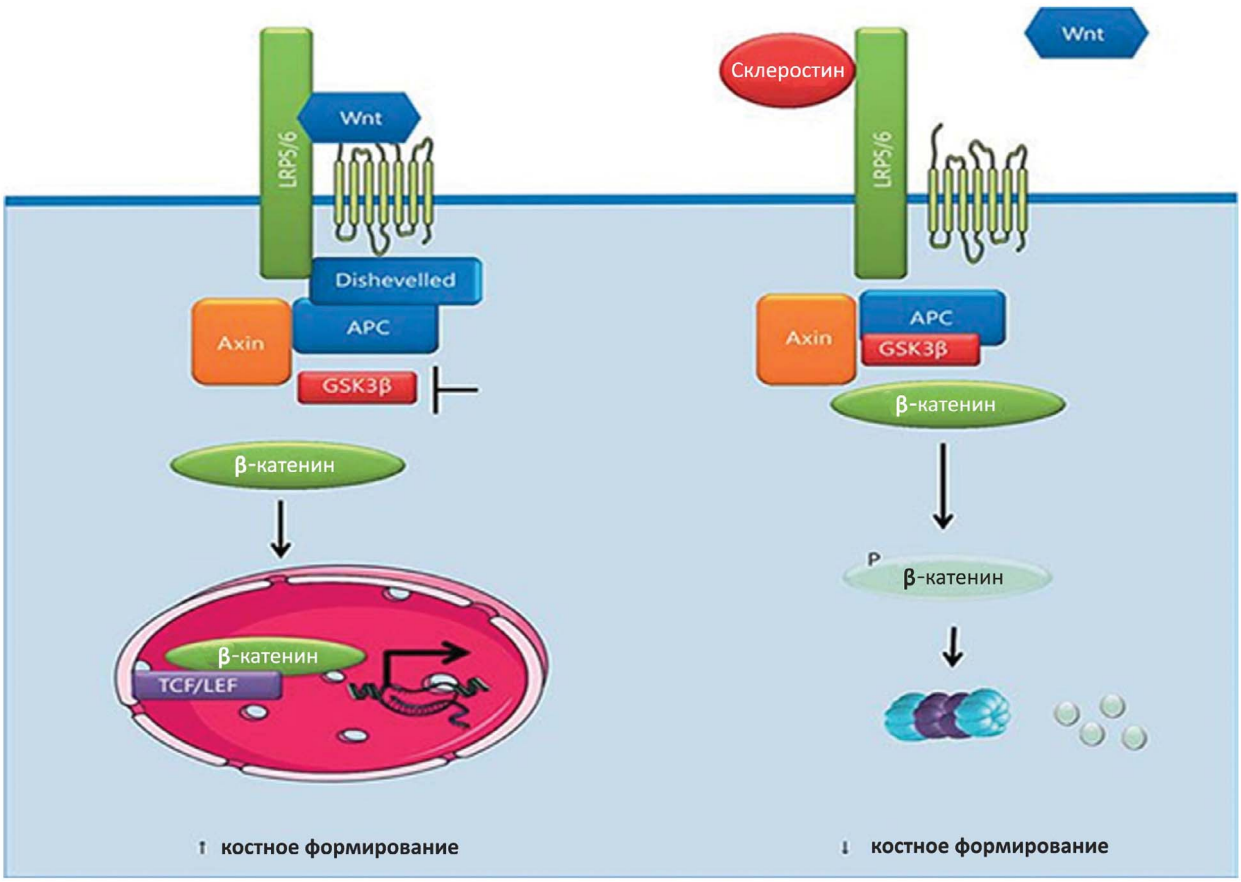

Figure 1. *Copyright © Gerontology 2016;62:618–623 DOI: 10.1159/000446278: Description of the canonical Wnt/β-catenin pathway and its regulation by sclerostin. Wnt binding to Frizzled and LRP5/6 coreceptor promotes the recruitment of Dishevelled that destabilizes the GSK3-Axin complex. Then, GSK3 inhibits the phosphorylation of β-catenin, leading to its intracellular accumulation and to its translocation into the nucleus. Thereafter, β-catenin forms a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) and promotes bone formation. In the absence of Wnt, the cytoplasmic complex containing GSK3 allows the phosphorylation of β-catenin and promotes its ubiquitination. Sclerostin induces an inhibition of the non-canonical pathway. By binding to LRP5/6, sclerostin prevents the association LRP5/6-Frizzled receptor and then inhibits bone formation. APC = Adenomatous polyposis coli.*

BMP-2 induces osteogenesis through enhanced activation of the Wnt / β-catenin signaling pathway. In addition, BMPs belonging to the superfamily of the transforming growth factor beta (TGF-β) proteins are responsible for numerous cellular regulatory processes, including osteogenesis and regulation of bone formation [20]. The BMP ligand binds to serotonin-threonine-kinase receptors of the 1st and 2nd types (BMPR) on the cell surface. Interaction with the receptors leads to activation of intracellular signal proteins Smad-1, -5 and -8 (9), which are transported to the nucleus and act as transcription factors, resulting in the activation of BMP-dependent genes [21, 22]. In particular, BMPR-IA and BMPR-IB are involved in the differentiation of mesenchymal stem cells [23]. BMP-2, BMP-4, BMP-7, BMP-9, and BMP-13 are usually studied in the context of osteoblastogenesis and bone formation [24, 25]. It is noteworthy that BMP-2 stimulates expression of Runx2 in osteoprogenitor cells and expression of Osx and the distal-less homeobox 5 gene (Dlx5 gene) in osteoblasts [26]. BMP-3 is an exception because it inhibits osteogenesis [27]. BMPs function as both autocrine and paracrine factors, and their synthesis is induced by the BMP themselves through local feedback mechanisms [28].

Preosteblasts also release a macrophage colony-stimulating factor (M-CSF), which interacts with its high affinity transmembrane receptor (c-fms) located on macrophage lineage cells in capillary circulation, which leads to their differentiation in preosteoclasts. Preosteoclasts express RANK (receptor activator of nuclear factor kappa-B), and preosteoblasts express RANKL. RANKL belongs to the of tumor necrosis factors family TNFSF11. RANK activation by RANKL binding induces the activation of transcription factors, such as c-fos, NFAT (nuclear factor of activated T cells) and nuclear factor kappa B (NF-kB) in preosteoclasts, which leads to its differentiation into mature osteoclast [29, 30]. M-CSF is a cofactor for RANKL/RANK, which mediates osteoclastogenesis. Experimental data showed that RANKL can stimulate bone resorption in mice which have no M-CSF [31]. Conversely, only M-CSF is not sufficient to activate osteoclasts. Therefore, RANKL plays a decisive role in osteoclastogenesis and is necessary for bone resorption [32].

Osteoclasts with αvβ3 integrins and bone matrix proteins (osteopontin, bone sialoprotein) are fixed on the bone surface and produce cathepsin K, cysteine protease, matrix metalloproteinases, and hydrogen ions [33]. Cathepsin K is a lysosomal cysteine proteinase, which is one of the enzymes which destroy type I collagen, the main component (90%) of bone matrix [34]. Cathepsin K is the main proteolytic enzyme of osteoclasts and, therefore, one of the most specific markers of bone resorption. Due to the action of cathepsin K, large fragments of collagen consisting of N-telopeptides and associated transverse pyridine cross-links (cyclic pyridinolines (PYD) and deoxypyridinolines (DPD)) enter the bloodstream from the bone resorption zone. Matrix metalloproteinases (MMPs) belong to a family of zinc- and calcium-dependent endopeptidases that impact the metabolism of the components of the extracellular matrix. If cathepsin K cleaves the N-terminal portion of the collagen (NTX, Amino-terminal cross-linked telopeptide of type 1 collagen), the MMP forms large fragments in the bone resorption zone, consisting of two C-telopeptides of the same type I collagen molecule (CTX, Carboxy-terminal cross-linked telopeptides of type I collagen), a spiral segment of another collagen molecule, and transverse pyridine cross-link between them. These fragments, which are designated as CTX-MMP, enter the bloodstream and are then excreted in the urine. However, their structure is unstable and destroyed by the action of cathepsin K as well as proteolytic enzymes in the vascular bed, resulting in circulation of various fragments of C-telopeptides in the bloodstream [35].

Due to the action of proteolytic enzymes and acidification, the Howship's lacuna is formed. Transcellular transport of microvesicles containing bone matrix degradation products into the cell is performed by means of tartrate-resistant acid phosphatase 5b (TRACP 5b). This enzyme is synthesized by osteoclasts. The activity of bone resorption processes can be measured by the level of TRACP 5b in blood plasma [36, 37].

IGF-1 (insulin-like growth factor-1), IGF-2, and TGF-β (transforming growth factor β) are released from the bone matrix during resorption [33].

Preosteoblasts, which are completely differentiated into the osteoblast, stop RANKL synthesis on the cell surface and start secreting OPG (osteoprotegerin).

OPG (TNFRSF11B) is a member of the TNF family, which is secreted not only by osteoblasts, but also by bone marrow stromal cells [38] and T cells [39]. OPG protects the skeleton from excessive bone resorption, acting as a soluble receptor, a decoy molecule that can bind to RANKL [40]. The binding of OPG with RANKL subsequently inhibits the binding of RANKL to its RANK receptor [38]. Overexpression of the gene encoding OPG leads to the development of high bone mass and a decrease in the number and activity of osteoclasts [41]. In physiological conditions, the OPG/RANKL ratio is in equilibrium and bone homeostasis is maintained. The OPG/RANKL ratio is an important factor for determining bone mass and skeletal integrity [42]. TNF-α, IL-1, IL-4, and IL-6 modulate the RANKL/RANK ratio by stimulating and enhancing RANKL expression on T cells. The cross-regulation between bone and immune cells is considered as a bone immunological niche [43].

Mature osteoblasts fill the resorption cavity, producing a variety of bone matrix proteins and type I collagen, resulting in organic matrix formation (osteoid) which later becomes mineralized. The osteoid predominantly consists of type I collagen, which is formed from type I procollagen synthesized by fibroblasts and osteoblasts. Procollagen type I has N-terminal and C-terminal propeptides (Procollagen type I C-terminal propeptide, PICP; Procollagen type I N-terminal propeptide, PINP) that are removed by specific proteases when procollagen is converted into collagen. The N-terminal propeptide of type I collagen is released into the intercellular space and the bloodstream when the type I collagen is formed and incorporated into the bone matrix, and it is one of the markers of bone formation [44].

Some osteoblasts extending deep into the osteoid are transformed into osteocytes. Other osteoblasts are differentiated into bone lining cells, and the rest (up to 80%) undergo apoptosis. The cells of a "canopy", which previously formed the tent cover of the resorption zone, return to their original position. Newly formed osteocytes restore the syncytium and begin to secrete sclerostin. As a result, all processes of cell differentiation are fully terminated [45].

Bone remodeling occurs in the basic multicellular unit (BMU), which is a microcavity with osteoclasts, osteoblasts and osteocytes. During normal bone remodeling, the resorbed bone is completely replaced by the new bone in the same amount and location [12]. Factors controlling these processes are molecules excreted by osteoclasts that induce bone formation at the BMU level either by attracting osteoprogenitor cells and osteoblasts or by stimulating their differentiation and activation.

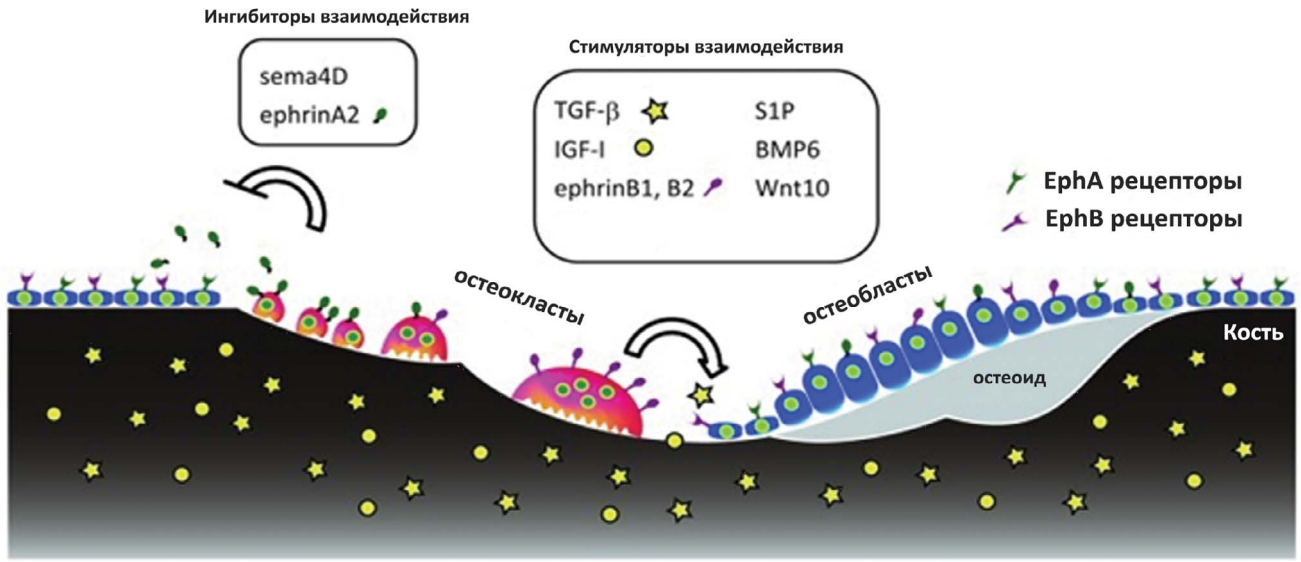

Figure 2. *Cell Adhesion & Migration 6:2, 148–156; March/April 2012; © 2012 Landes Bioscience: Coupling stimulators and inhibitors during bone remodeling. Bone matrix contains TGF-b (yellow stars) and IGF-I (yellow circles), which are released by osteoclastic bone resorption to stimulate coupling. Cells in the osteoclast lineage (red) produce various coupling stimulators and inhibitors that act on osteoblasts or their progenitors (blue).*

Transforming growth factor b (TGF-b) and insulin-like growth factor-1 (IGF-1) are released from the extracellular matrix during bone resorption, while factors such as cardiotrophin-1, sphingosine-1-phosphate, BMP6 and Wnt10b are secreted by osteoclasts [46]. EphrinB1 and ephrinB2 are located on the cell membrane and act only locally for the transition from a bone resorption to an osteogenesis phase in BMU [47].

Eph-receptors belong to the family of receptors-tyrosine kinases, which are activated by ephrin ligands. Both Ephs and ephrins are divided into two groups: A and B. As a rule, EphA receptors (EphA1–A8, A10) interact with ephrinA (ephrinA1–A5), and EphB receptors (EphB1–B6) interact with ephrinB ligands (ephrinB1–B3), with some exceptions [48]. As an exception, EphA4 binds to ephrinB2, ephrinB3, and ephrin As. Eph receptors interact with ephrin ligands on the cell surface, initiating bidirectional signaling: forward signaling through Eph-receptors, and reverse signaling through the ephrin ligand [49]. Bidirectional signaling between the osteoblasts ephrinB2 ligands and the osteoclasts EphB4 receptors inhibits osteoclastic bone resorption and increases osteoblastogenesis by transitioning between two states (Figure 2). Parathyroid hormone (PTH) induces ephrinB2 formation in osteoblasts and enhances bone formation. In contrast to ephrinB2, ephrinA2 acts as an inhibitor of bone formation [50].

Remodeling in cancellous bones lasts on average about 200 days, and resorption lasts for 30-40 days. The period of formation is about 150 days [51]. Remodeling of the cortical layer is faster and takes about 120 days [52].

Specific Aspects of Bone Remodeling in Primary Osteoporosis

Osteoporosis is a multifactorial disease, the development of which depends on lifestyle, genetic predisposition, concomitant diseases, physical activity, medication intake, endocrine status, human aging and individual longevity [53].

Genetic studies have shown that polymorphisms of Wnt10B impact the reduction in bone mass and the risk of osteoporosis [54]. Wnt10b seems to be a modulator of bone regeneration and homeostasis. β-catenin deficiency leads to the arrest of osteoblasts development at an early stage in mesenchymal osteoblastic precursors and impairs maturation and mineralization of osteoblasts [55].

There is evidence that mesenchymal stem cells in patients with osteoporosis have a function disorder, and this damage is associated with BMP signaling [56]. However, BMP antagonists have been described, including noggin (NOG) and gremlin (GREM). Overexpression of NOG, as shown in studies of transgenic mice, leads to a decrease in BMD due to increased inhibition of bone formation [57]. Single nucleotide polymorphisms in the NOG gene are associated with the phenotypes of patients with osteoporosis [58]. GREM is found in the skeleton, and its overexpression causes osteopenia and fractures [59]. Genetic variants of GREM2 are associated with bone mineral density (BMD), and GREM2 is considered a gene that increases the risk of osteoporosis [60].

Osteoporosis with an excessive number of osteoclasts is observed in patients with OPG deficiency [61]. RANKL is upregulated in conditions of osteoporosis, which is associated with downregulation of OPG [62]. In addition, the activity of some cytokines increases in patients with osteoporosis, including, in particular, TNF-α, IL-1, IL-4, and IL-6 [63]. The literature data showed that the malfunctioning of T cells subpopulations and their proinflammatory cytokines is associated with the development of osteoporosis. At the bone level, Th1 and Th2 cells affect the formation and activity of osteoclasts indirectly through secreted cytokines, including RANKL [64]. In addition, Th17-cells, a special line of proinflammatory T-helpers, have recently been identified as a potential T-cell subpopulation that plays a role in bone destruction [65]. The number of Th17 cells was found to be elevated in many bone diseases and in osteoporosis in particular. Th17 cells produce IL-17, which is able to mediate the differentiation of osteoclasts [66]. It is significant that Th17-helper cells also produce RANKL, which directly contributes to bone loss [65]. In addition, the Th17 population pool in the bone marrow and peripheral blood is found to be enlarged in postmenopausal osteoporosis [67]. Together, Th1/Th2/Th17 cells and their cytokines can play a key role as strong pro-osteoclastogenic mediators underlying the pathogenesis of osteoporosis (Figure 3).

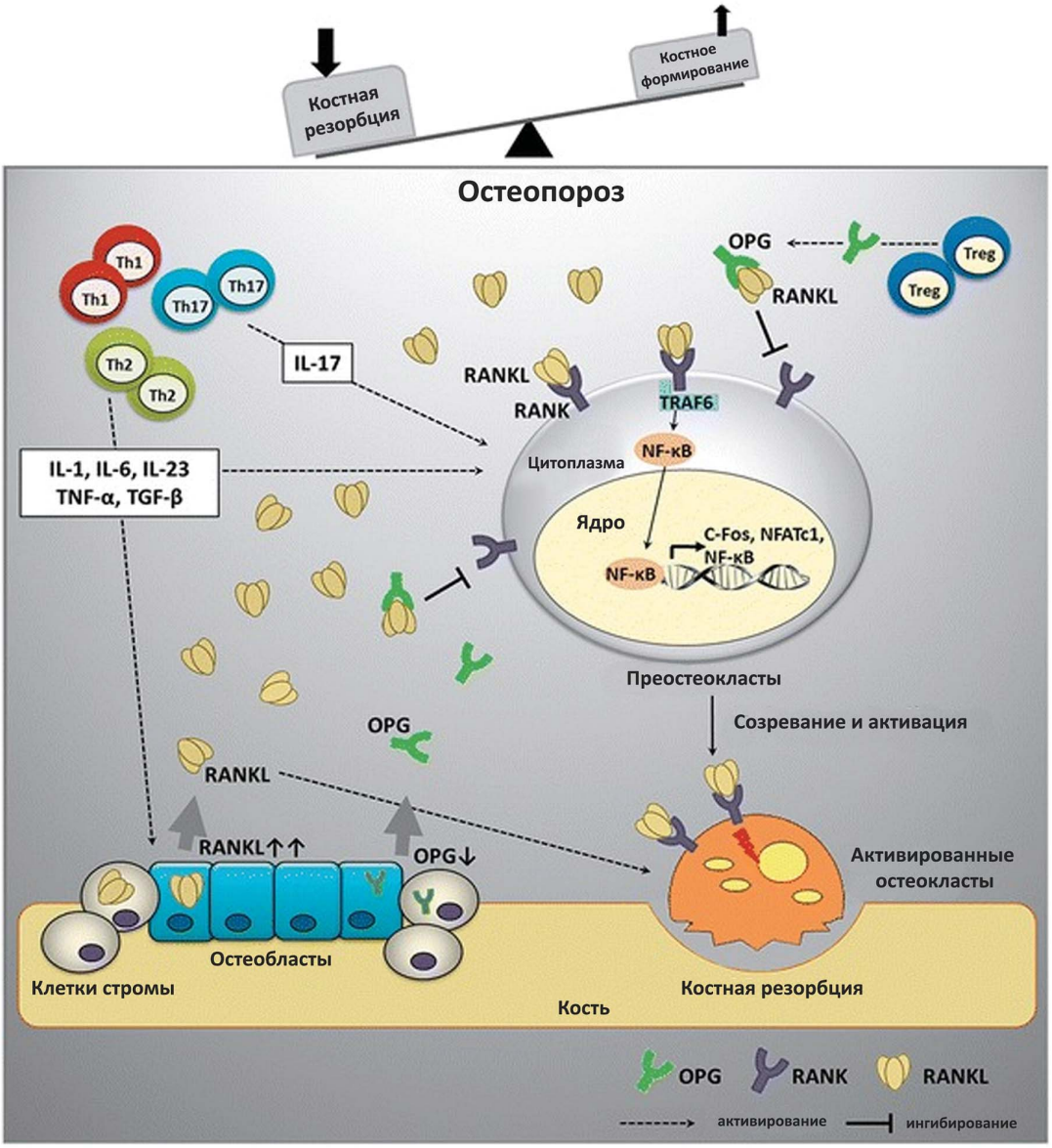

Figure 3. *©Copyright. Phetfong et al. Cellular & Molecular Biology Letters (2016) 21:12. Bone homeostasis regulation by OPG/RANK/RANKL system. RANKL which is secreted by activated T cells functions as an osteoclast-activating factor by binding to its receptor, RANK, which is expressed on preosteoclasts. RANKL-RANK binding induces the activation of several transcription factors in preosteoclasts and initiates several downstream signaling pathways that drive osteoclast differentiation and maturation. OPG which secreted by osteoblasts, bone marrow stromal cells, and T-cells acts as a soluble receptor that can bind to RANKL and subsequently prevents RANKL-RANK binding. Under physiologic conditions, OPG/RANKL is in equilibrium and preserves bone homeostasis. Under osteoporotic conditions, RANKL is upregulated, which is associated with downregulation of OPG. Several proinflammatory cytokines are secreted by T helper cells (Th1/Th2/Th17) stimulating and upregulating RANKL expression and mediating osteoclast formation and activity, which are linked to increased bone resorption.*

Bone Remodeling Markers

The bone turnover markers (BTMs) can be measured in blood or urine, and they reflect the metabolic activity of osteoblasts or osteoclasts, respectively. Bone remodeling markers are not specific to a particular disease.

Bone turnover markers include markers of bone formation, bone resorption, and bone metabolism regulators. Bone metabolism is studied by determination of enzymes, proteins, and by-products during the process of bone remodeling [68].

The markers of bone formation are osteoblast enzymes or by-products of active osteoblasts

produced during different phases of their development. Bone formation markers include total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), osteocalcin (OC), procollagen type I N-terminal propeptide (PINP), and procollagen type I C-terminal propeptide (PICP). PINP has several functional advantages: it has a low intra-individual variability and is relatively stable in serum at room temperature, being the marker of choice. PINP is released as a trimeric structure, but it is rapidly broken down to monomeric form by thermal degradation.

Markers of bone resorption are hydroxyproline (HYP), hydroxylizine (HYL), deoxypyridinoline (DPD), pyridinoline (PYD), bone sialoprotein (BSP), osteopontin (OP), tartrate-resistant acid phosphatase 5b (TRAP 5b), C-terminal telopeptide of type I collagen (CTX or β-CrossLaps), aminoterminal telopeptide of type I collagen (NTX), and cathepsin K (CTSK). The majority of bone resorption markers are degradation products of bone collagen (HYP, HYL, DPD, PYD, CTX, NTX) and osteoclast-derived enzymes (TRAP 5b, CTSK). Early studies of bone metabolism were based on the determination of urinary DPD and PYD, which consisted in the collection of urine over a 24-hour period. That process was cumbersome and time consuming, leading to inaccuracies in their study. Plasma markers of bone resorption have now become available. It is more desirable to study them; CTX is the marker of choice.

The regulators of bone turnover are receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), dickkopf-1 (DDK-1) and sclerostin (SCL). DDK-1 and SCL are produced by osteocytes and inhibit the Wnt signal. Studies of the last decade have shown that osteocytes play a key role in regulating bone turnover due to the ability to detect changes in bone morphology, especially microcracks, due to mechanoreceptors. They regulate bone turnover through direct contact with other bone cells and through the production of different factors such as dickkopf-1 (DDK-1) and sclerostin (SCL), dentin matrix protein 1 (DMP1), and matrix extracellular phosphoglycoprotein (MEPE) [44] (Figure 4).

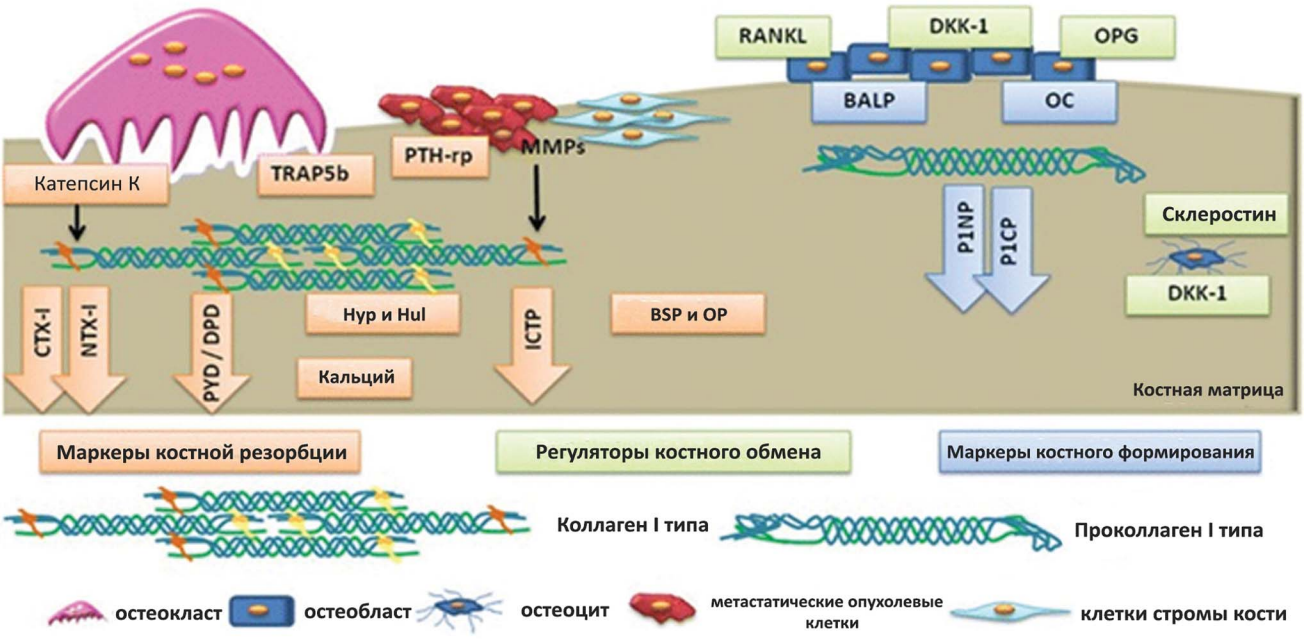

Figure 4. *Copyright © 2015, Nature Publishing Group. Biochemical biomarkers of bone turnover. Blue boxes/arrows represent bone formation markers: bone-specific alkaline phosphatase (BALP); osteocalcin (OC); propeptides of type I procollagen (P1NP and P1CP). Orange boxes/arrows represent bone resorption markers: pyridinoline (PYD); deoxypyridoline (DPD); C-terminal crosslinked telopeptide of type 1 collagen (CTX-1); N-terminal crosslinked telopeptide of type 1 collagen (NTX-1); hydroxyproline (HYP); hydroxylysine (HYL); bone sialoprotein (BSP); osteopontin (OP); tartrate-resistant acid phosphatase 5b (TRAP 5b); cathepsin K (CTSK). Green boxes represent regulators of bone turnover: receptor activator of NF kappa-B ligand (RANKL), osteoprotegerin (OPG), dickkopf-1 (DDK-1) and sclerostin.*

Over the last decade, significant advances have been made in identifying and evaluating specific bone turnover markers for use in clinical trials for medications and monitoring the therapeutic management of osteoporosis. The use of bone turnover markers is usually not recommended for selecting individuals at risk for fractures, partly due to their degree of variability. However, the analysis of the CTX resorption marker is recommended prior to the initiation of antiresorptive treatment, for example bisphosphonates or denosumab, and can be repeated at 3-6 months to check the effectiveness of the treatment and the patient's adherence to therapy. Similarly, a marker for bone formation, PINP, can be used to monitor anabolic treatment. The analysis of bone remodeling markers can also be useful in the monitoring process during drug "vacations" and for deciding when to resume therapy [69].

It is recommended to consider initiation of osteoporosis therapy in postmenopausal women with osteopenia at the level of bone remodeling markers in the upper quarter of the reference interval [3]. Therefore, the combination of a BMD examination using DXA and bone markers has great potential to improve the early diagnosis of individuals at high risk of developing osteoporosis.

Conclusion

The normal physiological process of bone remodeling involves a balance between bone resorption and bone formation. In osteoporosis, this process becomes unbalanced, resulting in gradual losses of bone mass and density due to enhanced bone resorption and/or inadequate bone formation. Several signaling pathways underlying primary osteoporosis have been identified, such as the osteoprotegerin/receptor activator of nuclear factor kappa-B (RANK) / RANK ligand (RANKL), bone morphogenetic proteins, and canonical Wnt-signaling pathway. Polymorphisms of Wnt10B, NOG gene, and GREM2 impact the reduction in bone mass and the risk of osteoporosis. The malfunctioning of T cells subpopulations and their proinflammatory cytokines is associated with the development of osteoporosis.

Currently, analysis of bone remodeling markers allows to study the function of osteoblasts, osteoclasts, osteocytes in health and in primary osteoporosis by noninvasive methods. It allows us to perform an early assessment of the effectiveness of anti-osteoporotic treatment that is conducted; identify individuals at high risk of osteoporosis in combination with the measurement of MBD; and determine the prospects for the selection of a targeted therapy in the future.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 15.01.2018
Accepted for publication on 26.02.2018

UDC 616.24-002.2-073.173

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LEVEL OF ZINC AND ITS FRACTIONS AS REFLECTION OF A CONDITION OF ANTIOXIDANT SYSTEM AT THE CHRONIC OBSTRUCTIVE LUNG DISEASE

Abstract

The objective of the study: To estimate a possibility of use of zinc and its fractions levels as the indicator reflecting a condition of antioxidant system at patients with COPD or in groups of high risk. **Materials and methods**. Patients with COPD, smokers and non-smoking healthy people participated in a study. Spirometry was carried out for all study subjects. Forced expiratory volume$_1$ (FEV_1) was determined followed by calculation of % FEV_1 from the normal value. Taking into account the importance of an oxidative stress in COPD pathogenesis, the level of activity of the main enzyme of antioxidant defense — superoxide dismutase, levels of zinc and its fractions as main component of the enzyme and also albumin level as main metabolically active zinc transporter were determined. The indicator "proportion of bound zinc fraction" was introduced to define zinc fractions changes. **The results of the study**. It is defined that at smokers with minimal impairment of the respiratory function the change of indicators are similar to that at patients with diagnosed COPD. Statistically significant differences in the level of the studied indicators at non-smoking patients are also revealed. A Spearman correlation analysis showed significant correlation between % FEV_1 from normal value and SOD activity; between SOD activity and total and bound zinc levels. This confirms a hypothesis of the possibility to use zinc and its fractions levels in screening examinations as a parameter that reflects the state of antioxidant system in smokers.

Key words: *antioxidant system, superoxide dismutase, zinc, zinc pools, smoking, screening, spirometry*

For citation: Shevcova V.I., Zujkova A.A., Pashkov A.N., Shevcov A.N. LEVEL OF ZINC AND ITS FRACTIONS AS REFLECTION OF A CONDITION OF ANTIOXIDANT SYSTEM AT THE CHRONIC OBSTRUCTIVE LUNG DISEASE. The Russian Archives of Internal Medicine. 2018; 8(2): 111-116. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-111-116

DOI: 10.20514/2226-6704-2018-8-2-111-116

COPD — chronic obstructive pulmonary disease

The new recommendations for the diagnosis and management of patients with chronic obstructive pulmonary disease (COPD) propose a new disease definition in which the GOLD working group focused on certain risk factors, including smoking in particular. Smokers constitute a risk group for COPD and require examinations of pathophysiological changes that are characteristic of this disease [1].

Oxidative stress is one of the components of the COPD pathogenic mechanism. Cigarette smoke contains a variety of substances with the potential to form free radicals. Such substances are also produced by activated inflammatory cells [2, 3]. It is known that peroxidation processes are involved in spasms of smooth muscle cells in the bronchi and condensation of bronchial mucus leading to an aggravated course of the disease [4].

Effects of oxidative stress vary and include: inactivation of antiproteinases, initiation of endothelial dysfunction, remodeling of blood vessels, and fibrosis [5].

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The substances that control peroxidation processes include catalase, superoxide dismutase (SOD), reduced glutathione, glutathione peroxidase, and vitamin E. Their levels drop in case of COPD, and some researchers attribute this to the exhaustion of antioxidant defenses under long-term oxidative stress [6, 7].
The impairment of free-radical oxidation in smokers that can be observed at the preclinical stage is of clinical interest [6–8].
Being one of the components of anti-radical defense, SOD contains zinc [9]. It has been established that zinc deficiency impairs SOD synthesis, thus leading to increased oxidative stress [10].

Therefore, the aim of our study was to determine total zinc and its fractions levels and SOD activity in patients with COPD and in both smoking and non-smoking healthy subjects. The study's objectives included determination of SOD activity level, albumin, total zinc and its fractions levels in study subjects and the establishment of a correlation between the studied parameters.

Materials and methods

The study was conducted at the Federal State Budgetary Institution of Higher Professional Education Voronezh State Medical University named after N. N. Burdenko, Department of Outpatient Treatment and General Practice; Budgetary Healthcare Institution of the Vronezh Region Voronezh City Emergency Clinical Hospital No. 8; OOO Medical Center of Professional Pathology. After completion of the Informed Consent Form, a total of 30 patients with confirmed COPD were enrolled. The patients had no co-morbidities, and 20 of them were males while 10 were females (with a mean age of (55.8 ± 6.78) years). They had been hospitalized between December 2016 and January 2017. A total of 90 healthy subjects who had undergone periodic physical examination also participated in the study: 48 men and 42 women (with a mean age of (43.7 ± 7.17) years). The Study Protocol was approved by the Ethics Committee of the Federal State Budgetary Institution of Higher Professional Education Voronezh State Medical University named after N. N. Budenko.

Spirometry data from patients with verified COPD were obtained using the Diamant spirometer.

Healthy subjects completed a specifically designed questionnaire to determine their smoking status. Then active smokers entered data on the number of cigarettes they smoked per day and smoking duration to calculate the smoking index. Those who did not smoke at the moment answered questions on their smoking history and exposure to secondhand smoke outside, at work or at home. Subjects had to provide information on chronic disorders (those with chronic disorders were excluded from the study in order to verify effects of smoking on the studied parameters). Respiratory function was evaluated using a portable Spirotest USPC-1 spirometer (made in Russia): forced expiratory volume$_1$ (FEV_1) was determined followed by calculation of % FEV_1 from the normal value.
All patients provided blood samples.
Calorimetric measurement of total zinc level using RAL Clima MC-15 instrument and Vital Development Corporation kits. The following was added to 1.0 mL of mono reagent: 0.05 mL of blood serum in the test sample, 0.05 mL of the calibrator in the calibration sample, and 0.05 mL of double distilled water in the blank sample. The samples were then analyzed using photometry at a wavelength of 560 nm. The following equation was used for calculations:

$$C = Atest/Acal \times 30.6\ [\mu mol/L]$$

To measure bound zinc level, proteins were precipitated with trichloracetic acid and then centrifuged. Zinc level was then calculated in accordance with the method described above.
Albumin levels were measured using Vital Development Corporation kits.
SOD activity was determined using Spekol instrument with a chemiluminescent detector, developed by Carl Zeiss Jena. 70 μL of luminol, 70 μL of methionine, 80 μL of riboflavin, and 3 μL of blood serum (3 μL of distilled water for control samples) were added to 2.7 mL of the buffer solution.
The following equation was used for calculations:

$$\% \text{ quenching} = 100 - \text{test value} \times 100/\text{control value}$$

A statistical analysis was conducted using the Kruskal-Wallis H-test in Microsoft Excel 2010 and Statistica 6.0 software, since we had to compare four independent samples. Kruskal-Wallis H-test is a generalized Mann-Whitney test for two or more independent samples. The test does not require a hypothesis of a normal distribution. The null hypothesis H_0 means that only random differences exist between the samples. The alternative hypothesis H_1 means that the differences in the studied parameter that exist between the samples are not random. The differences were considered significant at $p \le 0.05$. The Spearman correlation analysis in conjunction with the rank correlation coefficient (r) was also used; r coefficient was interpreted as follows: very weak correlation ($0 < r \ll 0.3$); weak correlation ($0.3 < r \ll 0.5$); medium correlation ($0.5 < r \ll 0.7$); strong correlation ($0.7 < r \ll 0.9$); very strong correlation ($0.9 < r < 1$).

Results and discussion

After the analysis of questionnaires and spirometry data (Table 1), all subjects were divided into 4 groups: 1 — smokers with diagnosed COPD (N = 35, mean age of (55.8 ± 6.78) years), 2 – smokers with minimal impairment of the respiratory function (FEV_1 is 80–70 % from the normal value) (N = 25, mean age of (45.6 ± 5.79) years), 3 — smokers with preserved respiratory function (N = 30, mean age of (41.8 ± 7.97) years), 4 — passive smokers (N = 30, mean age of (42.4 ± 9.31) years).

Preventive medical examination of five smoking patients with cough revealed % FEV_1 less than 70 % of the normal value. Therefore, these patients were transferred to Group 1 (smokers with COPD).

At the first stage, SOD activity as the main enzyme of antioxidative defense was determined in the study subjects, and albumin levels were determined

Table 1. *Spirometry results of the study subjects*

	Statistics	FEV_1 (l)	% FEV_1 from normal value (%)
Group 1 (smoking patients with COPD)	M±m	1,68±0,61	48,6±10,5
	min-max	0,91-3,20	33,0-69,0
Group 2 (smokers with minimal impairment of the respiratory function)	M±m	2,99±0,45	74,3±3,0
	min-max	2,04-3,59	71,0-79,0
Group 3 (smokers with preserved respiratory function)	M±m	3,01±0,63	84,8±3,6
	min-max	2,10-4,42	80,0-96,0
Group 4 (secondhand smokers)	M±m	2,85±0,57	88,2±3,1
	min-max	82,0-95,0	82,0-95,0

Table 2. *SOD and albumin levels in patients from different groups (μM)*

		Group 1 (smokers with diagnosed COPD)	Group 2 (smokers with minimal impairment of the respiratory function)	Group 3 (smokers with preserved respiratory function)	Group 4 (secondhand smokers)
Superoxide dismutase	M±σ	36,02±2,77	39,85±1,63	43,47±5,06	52,66±2,71*
	min-max	32,0-45,6	36,8-43,7	37,4-55,6	48,9-57,9
Albumin	M±σ	32,57±2,87	37,77±3,10	42,02±4,57	47,88±1,94**
	min-max	32,6-27,6	33,6-43,7	33,1-49,6	42,9-51,9

Note: * — Differences of the SOD level in the studied groups are significant at p=0,001; ** — Differences of the albumin level in the studied groups are significant at p=0,001

as the main transporter of metabolically active zinc fractions that can participate in enzyme synthesis if necessary (Table 2).

To compare the samples, the Kruskal-Wallis analysis of variance was used for several independent groups. We found statistically significant differences in SOD (H = 91.5898 at p = 0.01) and albumin levels (H = 90.812 at p = 0.01) between the groups.

SOD level in Group 1 (smokers with diagnosed COPD) was significantly higher than in other groups due to active pathophysiological changes involving the enzyme. SOD activity levels in smokers (Group 2 — smokers with minimal impairment of the respiratory function, Group 3 – smokers with preserved respiratory function) were not significantly different. Interestingly, SOD activity level in non-smokers from Group 4 (those exposed to secondhand smoke) was significantly lower than in smokers (Groups 2 and 3). These patterns can be interpreted as activation of antioxidant defense involving SOD.

We found the following differences in albumin levels in the blood between the study subjects: albumin levels in healthy smokers from Groups 2 and 3 (smokers with minimal impairment of the respiratory function and smokers with preserved respiratory function) were significantly higher than in Group 1 (smokers with diagnosed COPD). These could be due to the activation of metabolically active zinc transport to meet the need in this trace element for the production of enzymes that mediate pathogenic changes in the respiratory tract.

To confirm the significance of obtained values for pathophysiological reactions during the development of COPD, we performed a Spearman correlation analysis with rank correlation coefficients.

To verify the significance of changes in SOD activity for respiratory tract obstruction accompanied by changes in the respiratory function, we evaluated the relationship between SOD activity and % FEV_1 of the normal value (Figure 1). A strong positive correlation was revealed (r = 0.80 at p = 0.01). The following equation was obtained in the correlation analysis:

$$\% FEV_1 = -11.0021 + 1.9585 \times SOD$$
$$(r = 0.80 \text{ at } p = 0.01),$$

where % FEV_1 is a percentage of forced expiratory volume during the first second from the normal value, SOD is the superoxide dismutase activity

Revealed patterns confirm the role of SOD in pathophysiological changes of the respiratory tract in patients with COPD.

At the next stage, we analyzed zinc level and its fractions. We also determined the proportion of bound zinc fraction to verify changes in zinc pools, namely, the conversion of some unbound zinc to the bound form (which is metabolically active and necessary for producing zinc-containing enzymes) (Table 3).

Statistical analysis of the obtained results revealed significant differences in the studied parameters between the groups (H = 92.322 at p = 0.01 for total zinc level; H = 90.355 at p = 0.01 for the bound

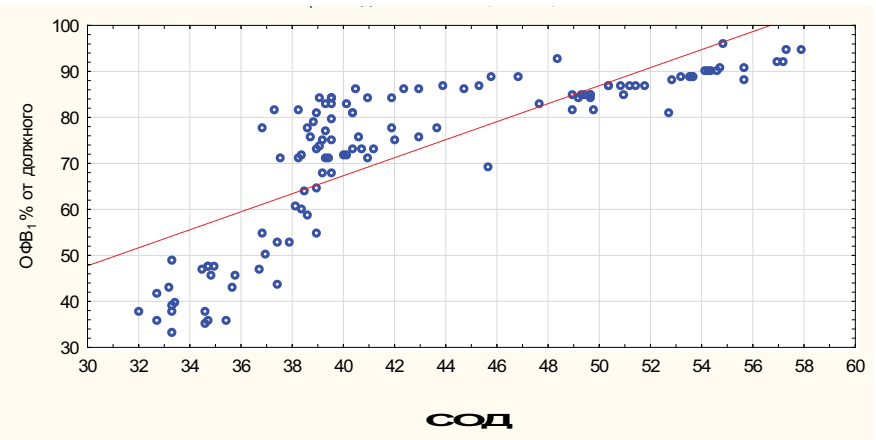

Figure 1. *The scatter plot: % FEV_1 vs SOD*

Table 3. *Total zinc and its fractions levels in study subjects (μM)*

		Group 1 (smokers with diagnosed COPD)	Group 2 (smokers with minimal impairment of the respiratory function)	Group 3 (smokers with preserved respiratory function)	Group 4 (secondhand smokers)
Total zinc	M±σ	11,60±2,62	15,54±1,52	17,50±2,54	22,49±2,17*
	min-max	6,98-18,20	12,30-17,90	13,60-23,40	19,10-27,00
Bound zinc	M±σ	10,73±2,35	14,14±1,37	15,65±2,14	19,74±1,63**
	min-max	6,50-16,63	11,17-16,20	12,28-20,60	17,25-23,27
Unbound zinc	M±σ	0,87±0,28	1,40±0,15	1,86±0,46	2,75±0,56***
	min-max	0,48-1,57	1,11-1,70	1,32-3,23	1,85-3,73
Proportion of bound zinc fraction	M±σ	92,6±0,7	91,0±0,2	89,5±1,2	87,9±1,3****
	min-max	91,4-93,7	90,5-91,4	86,0-90,5	86,2-90,3

Note:* — differences general level of zinc are significant at ρ=0,001; ** — differences related fraction of zinc are significant at ρ=0,001, *** — differences free fraction of zinc are significant at ρ=0,001, **** — differences share of the related fraction of zinc are significant at ρ=0,001

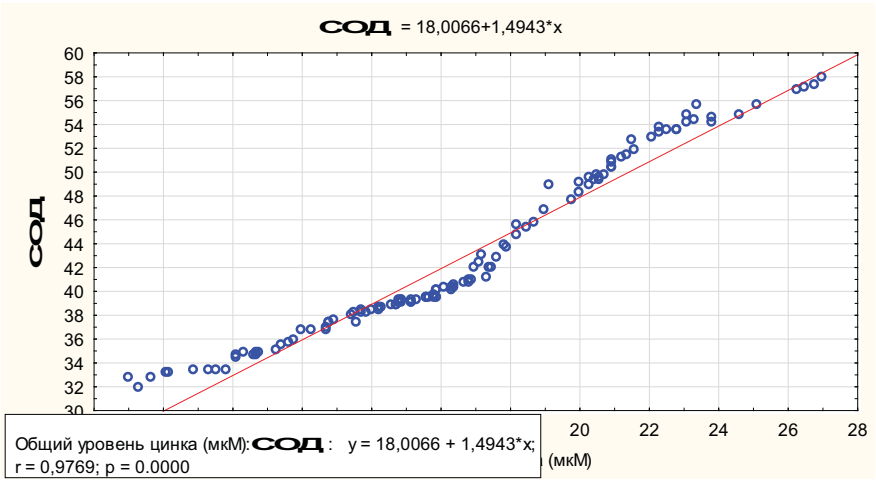

Figure 2. *The scatter plot: total zinc vs SOD*

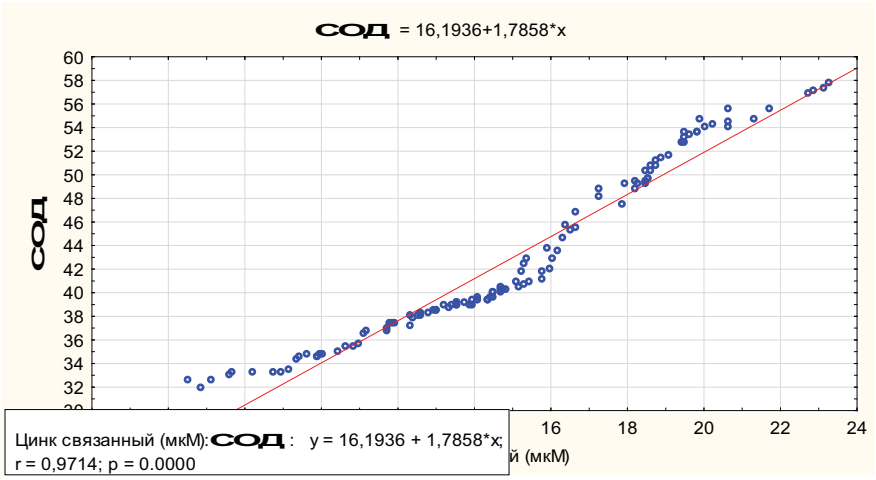

Figure 3. *The scatter plot: bound zinc vs SOD*

fraction; H = 99.748 at ρ = 0.01 for the unbound fraction; H = 104.523 at ρ = 0.01 for the proportion of the bound fraction), with total zinc level decreasing and the proportion of the bound fraction increasing as the % FEV_1 of the normal value decreased (r > 85 % at ρ < 0.05) (Figures 2 and 3).
A Spearman correlation analysis with rank correlation coefficients yielded the following correlation equations:

$$SOD = 18.0066 + 1.4943 \times Zn_{total}$$
$$(r = 0.98 \text{ at } \rho = 0.01),$$

$$SOD = 16.1936 + 1.7858 \times Zn_{bound}$$
$$(r = 0.97 \text{ at } \rho = 0.01)$$

Significant differences in SOD activity, levels of zinc and its pools, and in an additional parameter (the proportion of bound zinc fraction) that were revealed in our study together with strong correlations between the studied parameters confirm that it is possible to use levels of zinc and its fractions as a value reflecting the state of antioxidant system in COPD at-risk groups.

Conclusions

1. The study revealed statistically significant differences in SOD, albumin, total zinc and zinc fractions levels.
2. We obtained correlation equations for the studied parameters and % FEV_1 of the normal value (r > 85 % at ρ < 0.05), which indicated the relevance of the studied parameters for the COPD pathogenic mechanism.
3. Levels of zinc and its fractions can be used in screening examinations as a parameter that reflects the state of antioxidant system in smokers.

Conflict of interests
The authors declare no conflict of interests.

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Article received on 21.12.2017
Accepted for publication on 05.02.2018

UDC 616.891-008.441.12-06:[616.12-008.331.1+616.399]

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ANXIETY SYNDROME IN PATIENTS WITH ARTERIAL HYPERTENSION AND OBESITY AND ITS EFFECT ON CARDIOVASCULAR RISK FACTORSS

Abstract

The objective of the study: To reveal the prevalence of anxiety syndrome in young men (working age) with hypertension, to compare its frequency depending on the presence and absence of obesity (OB), and also to trace its correlation with the Echo-signs of left ventricular remodeling and with eating behavior pattern. **Materials and methods.** 80 patients with hypertension were included in the study. The reasons for hospitalization were the deterioration of the disease course and the routine examination. Depending on body mass index, all patients were divided into 2 groups: 1- patients with obesity, and 2 — the control group. All patients filled out the questionnaires to determine trait and state anxiety, and various types of eating disorders. **Results and discussion.** During the study, it was found out that obesity in patients with hypertension is associated with such pathological mechanisms as anxiety, which in turn leads to the progression of myocardial pathology, in particular ventricular remodeling. It is significant that the anxiety syndrome in patients with obesity is directly associated with restrained and external types of eating disorders. **Conclusion.** Timely detection of a high level of anxiety, as well as abnormalities in eating behavior in patients with obesity offers the opportunity to optimize medical treatment by using psychocorrective methods.

Key words: *hypertension, obesity, anxiety, eating disorders*

For citation: Panova E.I., Kovaleva A.O., Katynova Y.V., Govorkova E.P. ANXIETY SYNDROME IN PATIENTS WITH ARTERIAL HYPERTENSION AND OBESITY AND ITS EFFECT ON CARDIOVASCULAR RISK FACTORS. The Russian Archives of Internal Medicine. 2018; 8(2): 117-122. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-117-122

DOI: 10.20514/2226-6704-2018-8-2-117-122

LVH — left ventricular hypertrophy, BMI — body mass index, BP — blood pressure, TA — trait anxiety, HC — hip circumference, OB — obesity, WC — waist circumference, ABPM — 24-hour ambulatory blood pressure monitoring, CVD — cardiovascular diseases, SA — state anxiety, Echo — echocardiography

Introduction

In cases of hypertension, the patients' prognosis is mostly determined by the condition of the target organs, and in the first place by the presence and severity of ventricular hypertrophy that is assessed as a manifestation of hypertensive cardiomegaly [1]. Hypertension is known to be the cause of atrial fibrillation and chronic heart failure, which is a syndrome of poor prognosis. Hypertension leads to left ventricular hypertrophy in 80 % of cases, which significantly affects the quality of patient's life [2]. It has also been established that hypertension often occurs together with obesity, mainly abdominal obesity. The hypothesis that there is a relationship between excess fat and cardiovascular diseases (CVD) was proposed more than 50 years ago. The well-known clinical physician

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E. M. Tareev wrote in 1948: "The portrait of a patient with hypertension is most frequently associated with an obese hypersthenic and with possible protein metabolism disorders and accumulation of incomplete metamorphosis products in the blood such as cholesterol, uric acid..." [3]. At the same time, the importance of psycho-emotional aspects as an independent risk factor for the development and progression of cardiovascular disease has been increasingly discussed recently. The most significant of these are: depression, anxiety, individual personality traits, social isolation, and stress. Currently, a large amount of data has been accumulated, indicating that anxiety increases the hypertension and coronary artery disease incidence and mortality rates, and it also complicates the course and worsens the prognosis of these diseases [4, 5, 6, 7, 8, 9].

The significance of anxiety disorder in cardiology was confirmed by the results of a prospective 32-year study conducted in the United States by the Centers for Disease Control and Prevention [10], which showed that with an increase in anxiety, the probability of fatal myocardial infarction increases by 1.9 times and the risk of sudden death increases by 4.5 times.

At the same time, numerous socio-epidemiological studies (according to the data of National Research Center for Preventive Medicine) showed a tremendous increase in psychological stress. Thus, about 70 % of people live under medium and high levels of stress [11].

In this regard, special attention is paid to the study of the role of anxiety in the development of hypertension. It is known that hypertension is one of the most common cardiovascular diseases, which affects, in particular, 25–35% of the population of Russia. On the one hand, the major studies have demonstrated the correlation between anxiety or depression symptoms and hypertension development, and, on the other hand, hypertension is considered to be a psychosomatic disorder [12]. At the same time, many authors indicate increased anxiety as one of the risk factors for food addiction and, as a result, overweight and obesity development [13, 14, 15].

The objective of the study was to identify the prevalence of anxiety syndrome in young men (of working age) with hypertension, to compare its frequency depending on the presence or absence of obesity (OB), and to trace its relationship with left ventricular remodeling indicators in accordance with echocardiographic data (Echo) and eating behavior.

Materials and methods

The study included 80 patients with hypertension between the ages of 34 and 58 (mean age of 49 [44; 54]) who were hospitalized either due to the deterioration of the disease course or for routine examination. The examination of patients included a thorough analysis of anthropometric parameters (height, body weight, calculation of BMI, measurement of waist circumference (WC), hip circumference (HC) along with the calculation of waist-hip ratio). Patients were surveyed to evaluate their psychoemotional status. The questionnaire was chosen in conjunction with leading psychologists at the center of psycho-diagnostics at the hospital medical unit of the MIA of Russia in Nizhny Novgorod Region. In order to identify the presence of anxiety syndrome, all patients were interviewed in written form. The Spielberg-Hanin scale of trait and state anxiety as well as the Beck scale (to exclude depression) were used. The Dutch Eating Behavior Questionnaire (DEBQ) was used to determine the presence and type of eating disorders. Additional examination included echocardiography (Echo) with calculation of standard parameters and 24-hour ambulatory blood pressure monitoring (ABPM). Statistical processing of the data was carried out with STATISTICA 6.0 software (StatSoft) using nonparametric methods: the parameters of descriptive statistics: the data obtained are presented as medians and interquartile range (Me [25 %; 75 %]). The Mann-Whitney U test was used to assess the significance of differences between two unrelated samples. The correlation analysis was carried out using the Spearman criterion; $p < 0.05$ was used as the error probability.

Prior to participating in the study, all patients signed an informed consent form. This study was approved by the local ethics committee.

Results

Depending on the BMI, all patients were divided into 2 groups:
Group 1 (main) consisting of patients with OB — BMI ≥ 30 kg/m², 42 patients,
Group 2 (control) consisting of those with BMI < 30 kg/m² — 38 patients.

A comparative anxiety indicators analysis in patient groups with hypertension showed a significantly more pronounced rate of prevalence in patients with OB (Table 1).

The main and control groups were compared based on the presence and level of depression using the Beck scale. It was established that patients with obesity demonstrated no difference in the depression level. On the contrary, their depression scale indicators were more favorable in comparison with normal body weight patients (in patients with obesity: 4.5 [2; 10.5] points, in normal body weight patients: 9 [0; 15] points, p = 0.32).

OB in our patients was accompanied by more pronounced signs of ventricular remodeling (Table 2).

This feature, which is quite logical, corresponded with the ABPM profile, which showed higher BP in patients with OB (Table 3).

At the same time, the presence of a significant difference in the level of anxiety (which was not in favor of patients with OB) dictates the expediency of the question: is there any connection between the anxiety level and Echo indicators of ventricular remodeling? To answer this question, we conducted a correlation analysis of TA and SA severity with Echo indicators in groups of patients with and without OB (Table 4).

Table 1. *Comparison of patients with obesity and normal body weight according to the severity of anxiety*

Characteristic	Group 1 (with obesity) N=42	Group 2 (normal body weight) N=38	P-value
State anxiety (SA), scores	39.5[36;42]	36[28;38]	0,0028
Trait anxiety (TA), scores	39[36;43.5]	33.5[29;36]	0,000008

Table 2. *Comparison of Echo-indices in patients with hypertension in the presence or absence of OB*

Characteristic	Group 1 (with OB) N=42	Group 2 (normal body weight) N=38	P-value
Ejection fraction, %	62[56;65]	65[59;66]	0,09
Left atrium thickness, mm	40[38;45]	36[34;41]	0,0002
Right atrium thickness, mm	32[28;38]	31[28;38]	0,96
Left ventricle end-diastolic diameter, mm	50[48;53.5]	50[47;52]	0,51
Left ventricle end-systolic diameter, mm	33[31;38]	33[30;36]	0,3
Right ventricle thickness, mm	30[23;30]	25[23;28]	0,04
Left ventricle end-diastolic volume, ml	119[110;155]	122.5[113;137]	0,97
Left ventricle end-systolic volume, ml	45.5[41.5;78]	40.5[35;50]	0,08
Interventricular septum thickness, mm	14[12.5;15]	11[11;13]	0,000004
Left ventricle posterior wall thickness, mm	13[11.5;14.5]	11[10;12]	0,00003
Myocardial mass, g	279[233;329]	195[180;228]	0,00001
Myocardial mass index	121[112;128]	99.5[91;116]	0,00008

Table 3. *Comparison of ABPM indices in patients with obesity and normal body weight*

Characteristic	With obesity N=42	Normal body weight N=38	P-value
Mean day SBP	131[126;145]	128[119;136]	0,045227
Mean pulse pressure	46[41;51]	42[38;45]	0,027034
Maximum SBP, day	163.5[151;180.5]	152[142;165]	0,024400
Maximum SBP, night	133[123;144]	100[87;103]	0,049362
Maximum 24-hour SBP	164[151;181]	11.5[9;15.5]	0,036122
Variability of DBP, day	10[8;12]	31[23;39]	0,064068
Variability of DBP, night	10[8;11]	13[9;24]	0,005733

Table 4. *Correlation of TA and SA severity with Echo indicators in patients with and without OB*

Communication indicators	Spearman	P-value
SA & Ejection fraction	0,146654	0,342143
SA & Left atrium thickness	-0,034260	0,825267
SA & Right atrium thickness	0,117759	0,457648
SA& Left ventricle end-diastolic diameter	-0,032788	0,834665
SA& Left ventricle end-systolic diameter	-0,019137	0,904252
SA & Right ventricle thickness	-0,147244	0,352092
SA & Left ventricle end-diastolic volume	0,003433	0,986169
SA & Left ventricle end-systolic volume	-0,002759	0,989104
SA & Interventricular septum thickness	0,101104	0,518859
SA & Left ventricle posterior wall thickness	0,112443	0,472823
SA & Myocardial mass	-0,108641	0,493436
SA & Myocardial mass index	-0,019942	0,900245
TA & Ejection fraction	0,062631	0,686297
TA & Left atrium thickness	0,280526	0,065116
TA & Right atrium thickness	0,205761	0,191118
TA & Left ventricle end-diastolic diameter	0,259936	0,092305
TA& Left ventricle end-systolic diameter	0,203187	0,196851
TA & Right ventricle thickness	0,093394	0,556342
TA & Left ventricle end-diastolic volume	0,298297	0,123118
TA & Left ventricle end-systolic volume	0,237637	0,232649
TA & Interventricular septum thickness	0,356764	0,018855
TA & Left ventricle posterior wall thickness	0,217646	0,160911
TA & Myocardial mass	0,379165	0,013273
TA & Myocardial mass index	0,256466	0,101113

Note: EF — ejection fraction, LA — the left atrium wall thickness, RA — the right atrium wall thickness, LV (EDD) — left ventricular end-diastolic diameter, LV (ESD) — left ventricular end-systolic diameter, RV — the right ventricle wall thickness, EDV — left ventricular end-diastolic volume, ESV — left ventricular end-systolic volume, IVST — the interventricular septum thickness, LPWT — the left ventricle posterior wall thickness, MM — the myocardial mass, MMI — myocardial mass index

As it can be seen from the table, the anxiety syndrome in patients with OB significantly correlated with Echo indicating left ventricular hypertrophy (LVH) — IVST, MM, while in normal body weight patients no correlation was revealed (according to the results of a similar analysis).

Thus, anxiety syndrome, which is detected significantly more frequently in patients with hypertension on the background of obesity has a correlation with left ventricular remodeling indicators.

When the causes of such a high anxiety level in the group of patients with hypertension and obesity were analyzed, close attention was paid to eating disorder, which is known to trigger the development and progression of OB. We have discovered that the restrained and external types of eating behavior were dominant in both groups. The presence and type of eating disorder were assessed using the DEBQ questionnaire. To answer the question "Is there any relationship between anxiety level and eating disorders?" we conducted a correlation analysis (Table 5). Emotional type of eating behavior due to its rare occurrence was not taken into account.

As it can be seen from the table, a direct correlation was established between trait anxiety and eating disorders.

At the same time, according to the Spearman's rank correlation results in the hypertension patients' group with normal body weight patients, there was no correlation between anxiety and eating disorders (Table 6).

On the basis of the conducted study, we are able to conclude that OB in patients with hypertension is associated with the inclusion of additional pathogenesis mechanisms (besides well-known hormonal, metabolic and hemodynamic, pathogenesis mechanisms), which lead to the myocardial pathology progression. In particular, it is anxiety, which relationship to ventricular remodeling was revealed in our study. It is significant that anxiety syndrome in OB patients is directly associated with eating disorders, such as restrained and external types of eating behavior. The question remains unclear: whether eating disorders are primary and lead to anxiety or, on the contrary, anxiety provokes eating disorders.

From a practical standpoint, these disorders should be identified as soon as possible, and measures to correct not only the BP level, but also the patient's psychoemotional status, which may improve the treatment effectiveness, should be conducted.

Table 5. *Correlation of anxiety level with types of eating disorders in patients with hypertension and obesity*

Type of anxiety	Type of eating disorder	Spearman	P-value
SA	Restrained	0,110367	0,475723
SA	External	0,048511	0,757386
TA	Restrained	0,245634	0,010801
TA	External	0,340439	0,025495

Table 6. *Correlation of anxiety level with types of eating disorders in patients with hypertension and normal body weight*

Type of anxiety	Type of eating disorder	Spearman	P-value
SA	Restrained	0,226461	0,197781
SA	External	0,115263	0,516258
TA	Restrained	0,111605	0,529750
TA	External	0,106006	0,550719

Conclusions

1. Patients with hypertension accompanied by OB suffer from an increased level of anxiety in comparison with normal body weight patients.
2. The trait and state anxiety in patients with obesity correlate with left ventricular hypertrophy.
3. A relationship between anxiety syndrome in patients with OB and restrained and external types of eating behavior was revealed.
4. The timely detection of these states in patients with OB by surveying patients makes it possible to optimize treatment using not only medicines (antihypertensive drugs) but also psychocorrective methods.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 29.01.2018
Accepted for publication on 13.02.2018

UDC 618.3-008.6-053.71-084

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NEW METHOD OF PREVENTION OF IRON-DEFENSE ANEMIA IN PREGNANT TEENS

Abstract

The paper presents an assessment of the effectiveness of the method proposed by the authors for the prevention of iron deficiency anemia in pregnant minors. At the first stage of the study, 593 labor and delivery records were retrospectively analyzed (Group 1: minors between the ages of 13–15 (n = 49), Group 2: minors between the ages of 16–17 (n = 434), Group 3: women of average reproductive age (n = 110)). At the second stage, the incidence rate and development of anemia in pregnant women were prospectively analyzed (Group 1: minors between the ages of 13–15 (n = 49), Group 2: minors between the ages of 16–17 (n = 434), Group 3: women of average reproductive age (n = 110)). At the third stage, pregnant minors were divided into two groups: in the 1st (main) group (n = 144), iron deficiency anemia was prevented using the proposed method; in the 2nd (experimental) group, the traditional therapy with iron supplements was carried out after the onset of clinical and laboratory signs of anemia. The essence of the proposed method is that a pregnant minor woman is examined for ferritin in venous blood with the absence of laboratory signs of anemia, and at a value below 35 ng/ml, oral iron supplements are prescribed in conventional preventive doses for a period of 3 months, and if in three months the content of ferritin in the venous blood is again below 35 ng/ml, the intake of iron supplements continues for another 3 months. The use of the proposed method contributed to a significant decrease in the incidence of anemia in pregnant minors. The proposed method of preventing iron deficiency anemia in pregnant minors helps to reduce the incidence and severity of anemia in this complex category of patients.

Key words: *anemia of pregnant women, iron deficiency anemia, ferritin, pregnancy in minors*

For citation: Mikhaylin E. S., Ivanova L. A., Savitsky A. G., Shilo M. M., Berlev I. V. NEW METHOD OF PREVENTION OF IRON-DEFENSE ANEMIA IN PREGNANT TEENS. The Russian Archives of Internal Medicine. 2018; 8(2): 123–126. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-123-126

DOI: 10.20514/2226-6704-2018-8-2-123-126

Introduction

The incidence rate of anemia of pregnant minors exceeds that in women of average reproductive age. According to the literature, it can reach 30–40% [1]. It is argued that the main cause of anemia in minors is malnutrition [2]. Despite the fact that malnutrition is usually associated with a low socio-economic standard of living, the incidence rate of anemia in minors does not tend to decrease in both developing and developed countries [3]. Anemia is accompanied by dystrophic processes in myometrium and placenta, which lead to its hypoplasia and a reduction in hormone levels [4]. It was shown that anemia during pregnancy is associated with various pregnancy complications (pre-eclampsia, placental abnormalities, small for gestational age fetus), birth complications (preterm birth, weak uterine contractions, hypotonic hemorrhage) and complications during the postpartum period (purulent-septic complications) [5, 6]. The well-known method of anemia treatment in pregnant

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women is to prescribe oral iron supplements if there are clinical and laboratory signs of anemia. If the patient suffers from severe anemia, iron infusion is conducted (if anemia is confirmed as iron deficiency anemia).

The important role of subclinical iron deficiency anemia in pregnant minors has been noted since the presence of clinical and laboratory signs of anemia are preceded by absence of iron stores in the human body [1]. The late detection and treatment is fraught with severe complications, which always decompensate in minors suddenly.

The proposed method is aimed at preventing iron deficiency anemia in pregnant minors without waiting for clinic or laboratory signs of anemia. The method helps to avoid dystrophic processes in placenta, placental insufficiency, small for gestational age fetus, and thus avoiding preterm birth and low birth weight babies with a low Apgar score.

The objective of this work was to evaluate the effectiveness of a new method for iron deficiency anemia prevention in pregnant minors.

Materials and Methods

During the first stage, 593 labor and delivery records were retrospectively analyzed (where group 1 consisted of minors between the ages of 13–15 (n = 49), group 2 consisted of minors between the ages of 16–17 (n = 434), and group 3 consisted of women of average reproductive age (n = 110)).

During the second stage, the incidence rate and development of anemia in pregnant women were prospectively analyzed (where group 1 consisted of minors between the ages of 13–15 (n = 17), group 2 consisted of minors between the ages of 16–17 (n = 127), and group 3 consisted of women of average reproductive age (n = 110)).

During the third stage, pregnant minors were divided into two groups: in the 1st (main) group (n = 144), iron deficiency anemia was prevented using the proposed method; in the 2nd (experimental) group, the traditional therapy with iron supplements was carried out after the onset of clinical and laboratory signs of anemia.

The essence of the proposed prevention method is as follows: when they are admitted for prenatal care, all pregnant minors are examined for ferritin in venous blood. When a ferritin value in venous blood was recorded as being below 35 ng/ml (having normal RBC, hemoglobin and hematocrit level), oral iron supplements (iron protein succinylate + calcium folinate) were administered in preventive doses (in accordance with the Instruction for Use) for a period of 3 months. After three months, a check analysis on the ferritin content in venous blood is performed. When the ferritin value in venous blood falls below 35 ng/ml (having normal RBC, hemoglobin and hematocrit level), the oral iron supplement continues to be administered (in the conventional preventive dose according to the Instruction for Use) for three extra months. A patent on the invention "A Method for Preventing Iron Deficiency Anemia in Minor Pregnant Women" has been obtained (Patent of the Russian Federation No. 2616264 dated 13/4/2017) [7].

The statistical analysis was performed using the software STATISTICA v.7.0 (Statsoft Inc., Tulsa, USA).

Results

In the first stage of the present study, it was found that the incidence rate of chronic anemia existing before pregnancy was 2 (2.0%) in minors between the ages of 13–15, 19 (4.3%) in minors between the ages of 16–17, and 4 (3.6%) in women of average reproductive age ($p > 0.05$).

Anemia in pregnant women was observed in minors between the ages of 13–15 2.0 times more often (16 – 32.7%) ($p < 0.05$), and in minors between the ages of 16–17 1.8 times more often (129 – 29.7%) ($p < 0.01$) than in women of average reproductive age (18 – 16.4%).

Wherein mild anemia was observed in minors between the ages of 13–15 2.1 times more often (12 – 24.5%) ($p < 0.01$), and in minors between the ages of 16–17 2.2 times more often (112 – 25.8%) ($p < 0.01$) than in women of average reproductive age (13 – 11.8%).

Moderate anemia was observed in minors between the ages of 13–15 1.1 times more often (2 – 4.1%) ($p > 0.05$), and in minors between the ages of 16–17 1.4 times less often (11 – 5.2%) ($p > 0.05$) than in women of average reproductive age (4 – 3.6%).

Table 1. *Anemia patterns in pregnant women, abs. (%)*

Anemia	Groups		
	Age 13–15 n = 17	Age 16–17 n = 127	Women of average reproductive age n = 110
Anemia of pregnant women:	4 (23.5)	28 (22.0)	18 (16.4)
– iron-deficiency anemia	4 (23.5)	25 (19.7)	16 (14.5)
– B_{12}-deficiency anemia	0	1 (0.8)	1 (0.9)
– others	0	2 (1.6)	1 (0.9)

Note: * — $p_{2-3} < 0.1$.

Table 2. *Evaluation of the effectiveness of the proposed method for iron deficiency anemia prevention, abs. (%)*

Anemia	Groups	
	Main group n = 144	Experimental group n = 339
Anemia of pregnant women:	32 (22.2)	113 (33.3)*
– mild	29 (20.1)	95 (28.0)*
– moderate	3 (2.1)	10 (2.9)
– severe	0	8 (2.4)*

Note: * — $p < 0.05$.

There was a tendency ($p < 0.01$) to observe an increased incidence rate of severe anemia in minors between the ages of 13–15 (2 – 4.1%) compared with women of average reproductive age (1 – 0.9%). The incidence rate of severe anemia in minors between the ages of 16–17 was 6 (1.4%).

The results of the study of the incidence rate and anemia patterns in pregnant women in the II (prospective) stage of this study are presented in Table 1.

Anemia during pregnancy was observed in minors between the ages of 13–15 1.4 times more often ($p > 0.05$), and in minors between the ages of 16–17 1.3 times more often ($p < 0.1$) than in women of average reproductive age. About 90% of the total number of cases of anemia during pregnancy in all groups was iron deficiency anemia.

The evaluation of the effectiveness of the proposed method for iron deficiency anemia prevention is presented in Table 2.

Anemia during pregnancy was recorded 1.5 times less often ($p < 0.05$) in minors who underwent anemia prevention in accordance with the proposed method. The absence of severe anemia in this group should be noted.

Results and Discussion

As the results of the study showed, the rate of incidence of anemia during pregnancy in minors exceeds that in women of average reproductive age, which is consistent with data from the literature [8, 9, 10]. The probable reason is that the body of a pregnant minor is still growing, and the need for iron during pregnancy increases to a much greater extent than in a woman of average reproductive age since a minor needs iron both for her own growth, which is not the case for women of average reproductive age, and for the growth and development of the fetus. Therefore, we assume that one of the key principles of prenatal care for minors is anemia prevention. This study discusses anemia as a pregnancy complication, and it does not concern chronic anemia that may exist before pregnancy.

It should be noted that the decrease in the incidence rate and severity of anemia in pregnant women in cases where anemia prevention was carried out according to the proposed method was due to a decrease in the incidence rate of iron deficiency anemia, which accounts for more than 90% of the total number of cases of anemia in pregnant

women according to our data. The incidence rate of other types of anemia (particularly, pernicious anemia) apparently did not change.

Conclusion

The proposed method for iron deficiency anemia prevention in pregnant minors helps to reduce the incidence rate and severity of anemia in this complex patient population.

Conflict of Interests

The authors declare no conflict of interests.

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Article received on 19.02.2018
Accepted for publication on 26.02.2018

UDC 614.25 (571.6)

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EVALUATION OF LABOR CONDITIONS BY PUBLIC HEALTH WORKERS IN THE RUSSIAN FAR EAST

Abstract

The objective of the study is to analyze the conditions and working practices of health workers in public healthcare organizations in the Russian Far East on the basis of a medical and sociological survey. **Materials and methods.** A questionnaire survey was chosen with 835 people involved. Three groups of respondents were selected to obtain sociological information: 1) top and middle managers, 2) heads of structural units; 3) doctors of various clinical specialties, who are the part of management personnel reserve. The collection of statistical data was conducted in 2015-2017. Statistical analysis of the data was carried out using the methods of calculation of relative values, calculation of mean values, and ANOVA. **Results.** According to the obtained results, all groups of respondents believe that healthcare sector in Russia has achieved a satisfactory level of development. The current state of resources of public health institutions was estimated as rather low. Respondents gave a low assessment of the condition of buildings, the staffing of medical institutions by medical specialists and nursing staff. Among the proposed motivational factors to encourage productivity among healthcare professionals, the respondents agreed and gave a satisfactory assessment of such factors as workplace discipline, workload, observance of occupational safety, ensuring stability of employment, and interpersonal relations with colleagues. Medical workers did not agree on the factors of salary; financial and logistical support at the workplace; volume of document flow; opportunities for professional development, self-realization and career development. Top and middle managers assess these factors as "satisfactory," doctors give an assessment of "unsatisfactory." The heads of departments assess the factors of financial and logistical support at the workplace, salaries and volume of document flow as unsatisfactory, and the opportunity for professional development, self-realization and career growth as "satisfactory". **Conclusion.** Based on the subjective opinion of health workers, the results of the study made it possible to identify a number of economic and organizational aspects of working conditions in public health institutions that require scientific and practical justification in health care and management decisions affecting the development of the industry.

Key words: *Far Eastern Federal District, state medical organizations, health workers, working conditions, satisfaction*

For citation: Kirik Yu.V. EVALUATION OF LABOR CONDITIONS BY PUBLIC HEALTH WORKERS IN THE RUSSIAN FAR EAST. The Russian Archives of Internal Medicine. 2018; 8(2): 127-136. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-127-136

DOI: 10.20514/2226-6704-2018-8-2-127-136

Introduction

The creation of an effective healthcare system with a high level of results achievement and low financial expenditures is a major persistent task for most countries around the world nowadays. Previously created models for healthcare providing are rapidly becoming obsolete due to dynamic and rapid changes in the modern world. Capitalist Russia has long been searching for an effective model of the healthcare system development and conducted reforms to transform state health organizations.

One of the important aspects of studying the problems of a healthcare system is the consistency of the medical care provision to present needs of the population. Previously, we have conducted medical and

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sociological researches to determine whether the population of Russian Far East was satisfied with all stages of healthcare delivery. According to the study results, the population believes that medical care is inaccessible in their region. Among the main reasons that citizens name are low medical service density, inadequate supply and deterioration of equipment, and outdated fixed assets [4, 5]. These results have become prerequisites for a more in-depth analysis and study of the subjective opinion not only of the population itself about the status and development of the modern healthcare system and those who are receiving medical care, but also of health workers who are providing these medical services.

Scientific studies targeted at the problem of the position of the individual within the occupational environment and scientifically examining objective and subjective indicators that impact the attitude that people take towards work have been conducted around the world for many years now. A retrospective and contemporary review of foreign and Russian publications demonstrates that the analysis of this problem involved scientists from different sectors, including engineers, economists, sociologists, psychologists, and physiologists [3, 6–9, 11, 13–21, 23–24].

In the past two decades, empirical research of personal attitude toward work and job satisfaction has focused on certain types of activity. The number of scientific publications and works devoted to the subjective assessment of working conditions by medical workers, both in foreign and Russian literature [1–2, 10, 12, 22], is insufficient, and the studies that have been conducted are not interdisciplinary.

The objective of the study is to analyze the conditions and working practices of health workers in public healthcare organizations in the Russian Far East on the basis of a medical and sociological survey.

Materials and methods

Medical officers and specialists at state medical organizations of all healthcare system levels were selected as the subjects of the present study. Three groups of respondents were selected to obtain sociological information:

1) Chief physicians and their deputies (for medical treatment, for clinical and expert work, for organizational and methodical management).
2) Heads of structural departments.
3) Medical specialists in various clinical fields who are the part of management personnel reserve.

The study was performed during the period from 2015 to 2017. Survey and registration of the subjects were carried out using the selective observation method. The arrangement of the sampling units' extraction from the general population was carried out in accordance with a simple random sampling and serial (cluster) sampling procedure. Respondents from all federal subjects of the Russian Far East were included in the set of observation units.

The sociological survey covered 835 people. An assessment of the representatives of the sample was conducted on the basis of statistical analysis and errors calculation. The number of respondents included in the study was 10 % of the total population, where the deviation in the sample population did not exceed 5 %. The study assumes that the scope of observation of this study is reliable.

The statistical distribution of respondents by occupational groups is presented as follows: chief physicians — 34.1 % (group I); heads of structural departments — 33.7 % (group II); medical specialists — 32.2 % (group III).

The age of the respondents was included as one of the main indicators together with professional characteristics in order to ensure the reliability of information in the sociological study. Age is a fundamental demographic characteristic that defines roles, behavior and norms, and it helps shape the subjective opinion of others. The age differentiation has been assessed in the study. The mean age of chief physicians of public healthcare institutions was 46.7; heads of structural departments were 45.0 on average, and medical specialists were 46.8 on average. All respondents are in the same age group. The mean age of the respondents of all groups was 46.2 years (Table 1).

According to the analysis of the mean age distribution the degree of the empirical skewness values deviation from zero values corresponds to the normal distribution in all the surveyed groups of respondents. The degree of deviation of the empirical kurtosis values (peaked distribution) from zero values in the groups of chief physicians and heads

Table 1. *Characteristics of the respondents mean age*

	M ± δ (years)	m	Minimum (years)	Maximum (years)	Skewness	Kurtosis
Group I	46.7 ± 9.8	1.7	27	66	–0.09	–0.6
Group II	45.0 ± 8.9	1.7	30	61	0.1	–0.9
Group III	46.8 ± 10.3	2.2	32	63	0.1	–1.2
Total	46.2 ± 9.6	1.0	27	66	0.06	–0.9

of structural departments is estimated as being close to a normal distribution, with a tendency to low-peaked (flat-topped) distribution. The same distribution was observed in the group of medical specialists (Table 1).

A questionnaire survey was chosen as the sociological research method. The collection of primary information was performed in the presence of the researcher-interviewer at the place of work and training of respondents. The survey was conducted with the help of individual, group, and audit questionnaires. Anonymity was guaranteed to the respondents included in the sociological survey.

The questionnaire, which was specially designed by the researcher, included 24 questions and consisted of three parts: introductory, basic, and biographical sections. A welcome message to the respondent was presented in the introductory part of the questionnaire; thereafter the purpose of the interview, the conditions of its anonymity, the rules for filling out the questionnaire, and an explanation of how the results would be used were presented. The main part contained questions about the assessments and opinions of respondents on healthcare reforms and development, quality of medical services, and labor conditions at state medical organizations. The biographical part of the questionnaire included questions about the socio-demographic data of the respondents.

The questionnaire consisted of three types of questions: closed-ended, semi-closed, and open-ended. Closed-ended questions (dichotomous, multiple choice and rank order questions) contained a full set of possible answers. The open-ended questions did not contain answers, and they suggested that the respondents formulate the answer themselves in free form. Semi-closed questions contained answer options for the posed question, but the respondents were also allowed to propose their own answer.

The following statistical analysis methods were used in the study: calculation of relative values, calculation of mean values, and calculation of one-way analysis of variance.

During the analysis of variance, the hypothesis about the normal distribution of the studied random value was checked in advance using the Levene test. The null hypothesis of the variance equality was applied when the Levene criterion (L_{cr}) was less than the empirical significance level (L). Differences in the experimental groups were considered significant at $p < 0.05$.

Presence of an influence of the set factor on the studied process was determined using an analysis of variance. The conclusion about the influence of the factor on the mean of the observed value was considered significant in obtaining the Fisher criterion (F_{cr}) higher the empirical significance level (F_{emp}).

Statistical data processing was performed using the computer programs Statistical Package for Social Sciences (SPSS) version 14 and Statistica version 6.

Results and discussion

The first phase of the empirical study examined the opinion of the survey participants on the overall developmental level of healthcare service in the country and the quality of medical care at the institution where they work. According to the results obtained, all respondents, including chief physicians of medical organizations, heads of structural departments, and doctors from management personnel reserve, believe that currently the healthcare sector in Russia has achieved a satisfactory level of development (L_{cr} 0.32 < L 0.72; F_{cr} 0.12 < F_{emp} 0.88) (Figure 1).

Among chief physicians and heads of structural departments, only one in five (20.6 % and 21.4 %, respectively) support the reforms, and every third respondent (33.3 %) does so among doctors. In all study groups, one in ten is against the political

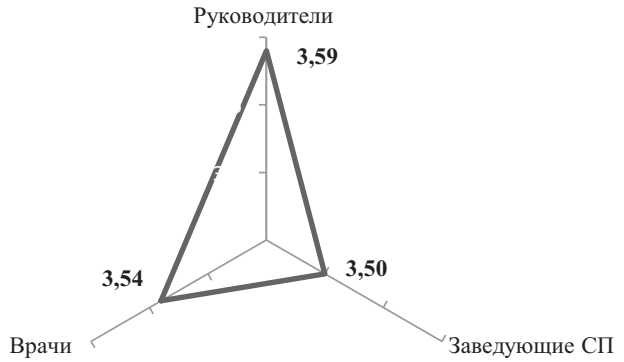

Figure 1. *The assessment of healthcare system development by the respondents (mean score)*

Note: *Руководители – Chief physicians, Врачи – Doctors, Заведующие СП – Heads of structural departments*

changes that have taken place in recent years in Russian healthcare (F_{cr} 0.01 < F_{emp} 0.98).

To ensure the development of the industry in accordance with the order of importance of the identified factors, all respondents stated that the most important objective should be effective healthcare management at every level; the second most important objective is the formation of the necessary volume of financial resources; the third is the availability of a sufficient number of trained medical personnel; and the fourth is the development of infrastructure and technological equipment of medical institutions (F_{cr} 0.14 < F_{emp} 0.86).

The majority of survey participants believe that the healthcare system in the country should receive shared management and funding. At the same time, most chief physicians and doctors believe that primarily public healthcare institutions are developed (76.4 % and 66.6 %, respectively). More heads of structural departments believe that medical organizations mainly within the system of private practice are developed (60.7 %) (F_{cr} 0.73 > F_{emp} 0.31).

No differences were discovered in how various groups of survey respondents evaluated the provision of paid healthcare services to citizens at public healthcare institutions. Respondents on average approved of how paid healthcare services were provided to members of the statistical population at healthcare institutions (F_{cr} 0.14 < F_{emp} 0.96).

More than half of doctors (52.4 %) believe that the quality of medical care in the institution where they work is good. The same opinion is held only by every third head of the department (32.1 %) and every fourth chief physician (26.5 %). More than half of respondents representing groups of chief physicians and heads of structural departments assessed the quality of medical care as satisfactory (F_{cr} 1.55 > F_{emp} 0.21).

Therefore, all healthcare professionals (both in leadership positions at various levels and at the practitioner level) characterize the current state of healthcare sector as satisfactory. A significant share of them does not support the political reforms in the industry that have been carried out in the recent years. According to the opinion of the healthcare professionals, it is necessary to fulfill a number of priority tasks to develop the system: create a highly effective management model at all levels, provide the sector with sufficient financial resources, trained specialists staff, modern infrastructure, and medical and non-medical technologies. Most healthcare professionals believe that the state should administer a mixed socio-economic model of healthcare service provision. But at the same time the top managers of medical institutions and doctors organizationally support the model of healthcare provision based primarily on state medical organizations, while lower level managers (heads of departments) believe that the system should be based primarily on private clinics. Given the current economic conditions, a significant share of healthcare professionals expressed a positive attitude toward the market for paid medical services. The quality of medical care at public institutions is assessed by practitioners higher than by managers at different levels.

The term "quality of medical care" is not a strictly defined concept and does not have a single generally accepted definition. The multidimensionality of the concept allows society to understand and interpret the definition unequally. It can be assumed that in answering the question about the quality of medical care, the surveyed groups of respondents considered the term from the standpoint of their own professional competencies. Participants in the survey were doctors who possess a certain set of medical skills (diagnosis, treatment, rehabilitation, and prevention) and who do not practice management functions (planning, organization, incentivization, and control), assess the quality of medical care in terms of performing

medical functions, and obtain results for individual patients. Chief physicians and heads of departments that ensure the quality of medical care through the consistent implementation of management functions assess a set of characteristics that include the quality of the conditions, the stages of the medical care process, and the results achieved in regard to patient flows within the healthcare institution.

During the second stage of empirical research we studied the satisfaction of respondents with the provision and condition of the medical institution resources (Table 2). During the survey, respondents were asked to assess their satisfaction on a five-point scale where the lowest score was one.
According to the obtained results, it was found that respondents gave a low assessment of the condition of buildings (the average number of points given by members of groups was between 2.54 and 2.79), the staffing of medical institutions by medical specialists (between 2.38 and 2.61 points) and nursing staff (between 2.71 and 2.91 points).

The sanitary condition of buildings and the level of medical and hospital equipment received an average assessment of three points (3.24–3.54 points, 3.01–3.14 points, and 3.05–3.21 points, respectively).

Taking into account the mean values and standard deviations in the conditions of normal distribution, the answers of all groups of respondents were similar. There were no significant differences between the aforementioned assessments of the respondents.

The results of the study showed statistically significant differences in the degree of satisfaction

Table 2. *Satisfaction of respondents with medical institution resources*

Resource	Position*	M**	σ	m	Fisher's test (F_{cr})	Empirical level of significance F_{emp}	$F_{cr} <> F_{emp}$
Technical building condition	Group I	2,79	1,2	0,2			
	Group II	2,54	1,1	0,2	0,43	0,64	$F_{cr} < F_{emp}$
	Group III	2,57	0,9	0,2			
Sanitary building condition	Group I	3,53	1,1	0,1			
	Group II	3,36	1,0	0,2	0,50	0,60	$F_{cr} < F_{emp}$
	Group III	3,24	1,0	0,2			
Supply with medical equipment	Group I	3,01	1,0	0,1			
	Group II	3,04	1,1	0,2	0,49	0,54	$F_{cr} < F_{emp}$
	Group III	3,14	1,2	0,2			
Supply with hospital equipment	Group I	3,21	1,1	0,1			
	Group II	3,14	1,1	0,2	0,13	0,87	$F_{cr} < F_{emp}$
	Group III	3,05	0,9	0,2			
Computer equipment	Group I	2,71	1,0	0,1			
	Group II	3,25	1,0	0,2	2,03	0,13	$F_{cr} > F_{emp}$
	Group III	2,81	1,2	0,2			
Medicines equipment	Group I	2,92	1,1	0,1			
	Group II	3,21	1,0	0,2	0,75	0,47	$F_{cr} > F_{emp}$
	Group III	3,36	0,9	0,2			
Staffing by doctors	Group I	2,38	0,9	0,1			
	Group II	2,61	0,9	0,2	0,46	0,63	$F_{cr} < F_{emp}$
	Group III	2,38	0,9	0,2			
Nursing staff	Group I	2,91	1,1	0,2			
	Group II	2,71	1,1	0,2	0,29	0,74	$F_{cr} < F_{emp}$
	Group III	2,71	1,1	0,2			

Note: * group I — Chief physicians, group II — Heads of departments, group III — Doctors
** 1 point — not satisfied; 2 points — little satisfied; 3 points — not satisfied with everything; 4 points — mostly satisfied; 5 points — fully satisfied

of the participants with regard to provision with computer equipment and medications. Chief physicians were not very satisfied with the provision of both medicines and computer equipment (2.71 and 2.92 points, respectively). Doctors were not satisfied with the computer equipment or with all of the aspects of the medicines supply (2.82 and 3.36 points, respectively). Heads of structural departments were not completely satisfied and gave an average assessment of three points for two types of resources (3.25 and 3.21 points, respectively). The answers of the surveyed groups of respondents differ to a substantial and statistically significant degree (Table 2).

Thus, similar to how healthcare professionals assess the state of healthcare sector, they generally give quite low rating to the current state of resources of public health institutions. According to the results of the survey, healthcare professionals give an unsatisfactory assessment of the physical condition of buildings of medical institutions and the level of staffing of medical institutions with doctors and nurses. At the same time, they believe that the maintenance of the necessary sanitary norms in medical institutions, the provision of facilities with medical and hospital equipment, including functional beds, operating tables, medical clothes and medical products, has been organized to a satisfactory level. All healthcare professionals agree on the low staffing of medical personnel, as well as on obsolete fixed assets that support the process of healthcare service provision.

Health workers have differing opinions concerning the medicines and computer equipment supply. Top and middle managers believe that the current level of provision of these types of resources at healthcare institutions is unsatisfactory. In turn, heads of departments, i.e., the lowest level managers, give these indicators a satisfactory assessment. Doctors assess the current state of provision of computer equipment as unsatisfactory, while they assess current stocking levels of medicines as satisfactory.

So, the differences in the opinions of the three groups can be summarized as follows:

- Chief physicians of top and middle levels of management recognize the problems of insufficient supply of medicines and weak implementation of modern information and communication equipment at institutions.
- Heads of departments do not generally experience difficulties with these two types of resources.
- Doctors believe that they are poorly provided with computers.

The third stage of the empirical research studied the twelve motivational factors that encourage healthcare professionals to work. When choosing factors for this study, we utilized the methods from motivation theories, including F. Herzberg's two-factor theory of motivation [8, 11].

The results of the study show that out of all the factors that motivate employees to work, the respondents gave the highest rating to choosing a profession (from 4.18 to 4.38 points) (Table 3). Being a particular social group within society, the respondents were mostly satisfied with their professional activity.

All groups of respondents evaluated the following working conditions by assigning a score according to a three-point scale: discipline (between 3.07 and 3.18 points), relations on the team (between 3.62 and 3.79 points), workload (between 3.01 and 3.15 points), observance of occupational safety (between 3.52 and 3.71 points), and stability of employment (between 3.48 and 3.68 points). There were no significant differences in evaluation scores that survey participants gave.

The results of the study showed statistically significant differences in the degree of satisfaction of respondents with a number of factors. According to the data obtained (Table 3), chief physicians at medical organizations rated the following factors on a three-point scale (within a range of 3.00 to 3.68 points): financial and logistical support at the workplace; salary; volume of document flow; and opportunities for professional development, self-realization, and career development.

In turn, doctors evaluated all of the above factors and working conditions within a range of two points (2.19–2.96).

Heads of structural departments gave assessments in the range of two points (2.14–2.96) for financial

Table 3. *Satisfaction of respondents with working conditions*

Resource	Position	M ± σ **	σ	m	Fisher's test (F_{cr})	Empirical level of significance F_{emp}	F_{cr} < > F_{emp}
Financial and logistical support at the workplace	Group I	3,24	0,8	0,1	0,90	0.41	$F_{cr} > F_{emp}$
	Group II	2,96	1,3	0,2			
	Group III	2,86	1,0	0,2			
Discipline	Group I	3,18	0,9	0,1	0,08	0,91	$F_{cr} < F_{emp}$
	Group II	3,07	1,1	0,2			
	Group III	3,10	1,0	0,2			
Relations on the team	Group I	3,62	0,8	0,1	0,31	0,73	$F_{cr} < F_{emp}$
	Group II	3,79	0,9	0,2			
	Group III	3,76	0,8	0,2			
Professional development opportunities (courses, seminars, etc.)	Group I	3,35	1,1	0,1	1,13	0,32	$F_{cr} > F_{emp}$
	Group II	3,39	0,9	0,1			
	Group III	2,96	1,2	0,2			
Salary	Group I	3,02	1,0	0,1	1,55	0,10	$F_{cr} > F_{emp}$
	Group II	2,68	1,1	0,2			
	Group III	2,19	0,8	0,1			
Workload	Group I	3,15	1,1	0,1	0,39	0,57	$F_{cr} > F_{emp}$
	Group II	3,04	1,0	0,2			
	Group III	3,01	1,2	0,2			
Volume of document flow	Group I	3,00	1,1	0,2	1,78	0,13	$F_{cr} > F_{emp}$
	Group II	2,14	1,2	0,2			
	Group III	2,38	1,3	0,3			
Observance of occupational safety	Group I	3,71	0,9	0,1	0,29	0,74	$F_{cr} < F_{emp}$
	Group II	3,54	1,1	0.2			
	Group III	3,52	0,9	0,2			
Self-realization opportunities	Group I	3,38	0,9	0,1	0,87	0,46	$F_{cr} > F_{emp}$
	Group II	3,29	1,1	0,2			
	Group III	2,94	1,3	0,3			
Career development opportunities	Group I	3,65	0,9	0,1	2,30	0,10	$F_{cr} > F_{emp}$
	Group II	3,43	1,1	0,2			
	Group III	2,91	1,2	0,2			
Stability of employment	Group I	3,68	0,9	0,1	0,32	0,72	$F_{cr} < F_{emp}$
	Group II	3,46	1,2	0,2			
	Group III	3,62	1,0	0,2			
Profession	Group I	4,18	0,9	0,1	0,48	0,61	$F_{cr} < F_{emp}$
	Group II	4,36	0,8	0,1			
	Group III	4,38	0,7	0,1			

Note: * group I — Chief physicians, group II — Heads of departments, group III — Doctors
** 1 point — not satisfied; 2 points — little satisfied; 3 points — not satisfied with everything; 4 points — mostly satisfied; 5 points — fully satisfied

and logistical support at the workplace as well as salary and volume of document flow, while they gave assessments in the range of three points (3.29–3.43) for opportunity for professional development, self-realization, and career development.

The results of the study allow us to conclude that healthcare professionals highly assess their career decision to go into professional medical practice. Among the proposed motivational factors to encourage productivity among healthcare

professionals, the respondents agreed and gave a satisfactory assessment of such factors as workplace discipline, workload, observance of occupational safety, ensuring stability of employment, and interpersonal relations with colleagues.

Medical workers did not agree on the factors of salary; financial and logistical support at the workplace; volume of document flow; opportunities for professional development, self-realization and career development. Executive top and middle level managers assess the listed factors as satisfactory, while the doctors give an assessment of "unsatisfactory". The heads of departments, who have been assigned medical and tactical management functions, assess the factors of financial and logistical support at the workplace, salaries and volume of document flow as unsatisfactory, but their assessment of opportunities for professional development, self-realization, and career development is satisfactory.

An analysis of the data obtained shows that healthcare professionals poorly assess the proposed motivational factors. But, at the same time, the leaders of medical organizations, who have been delegated decision-making powers, are socially active and open, and are the most satisfied with work. The heads of the structural departments have the greatest need for material factors. Doctors who are members of the management personnel reserve expressed a need for both material and personal development factors.

The last stage of the empirical study investigated how respondents assessed the subjective estimation of their own work activity. According to the results, 66.7 % of chief physicians believe that the amount of work performed in the healthcare organization exceeds the essential standards (in fact, staff works overtime hours). A total of 89.3 % of heads of departments and 73.4 % of doctors agree with this assessment (F_{cr} 1.56 > F_{emp} 0.22).

There were no differences between the groups of respondents in how they assessed the efficiency and performance of their work. The surveyed population on average stated that functional duties are performed well in many respects, but some aspects of the work require improvement to achieve an excellent result (F_{cr} 0.09 < F_{emp} 0.91).

Each fourth chief physician (23.6 %) and head of the structural department (25 %), and every fifth doctor (19.0 %) like their work and are satisfied with their salary level. More than a half of chief physicians (52.9 %) and doctors (57.1 %) and less than a half of heads of departments (46.4 %) believe that their salaries are too small, but they like their work, which brings them professional satisfaction (F_{cr} 2.9 > F_{emp} 0.05).

In addition to the main place of work, half of chief physicians (50 %) and more than a half of heads of structural departments (67.9 %) and doctors (61.9 %) take on additional paid work. Every third executive (32.4 %), every second head of department (42.9 %), and every fourth doctor (23.8 %) pursue internal secondary employment (F_{cr} 0.79 > F_{emp} 0.45). The ratio of the place of work for external secondary employment within a public medical organization to a place of work at a private clinic for chief physicians is 1:0.5, and for heads of structural departments and doctors it is 1:1.3 and 1:1.7, respectively (Figure 2).

We did not discover any differences in the assessments between the groups of respondents about the attitude to the fee-based method of payment for healthcare professionals or material remuneration for the provision of medical services. Just over half of the survey respondents support this method of payment (F_{cr} 0.52 < F_{emp} 0.65).

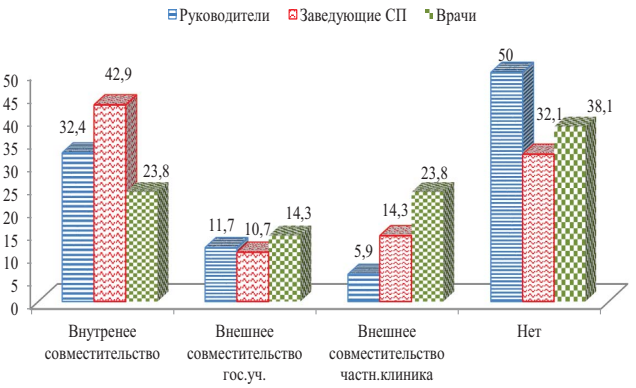

Figure 2. *Availability of additional paid work (%)*

Note: руководители/Chief physicians, заведующие СП/Heads of departments, врачи/Doctors; внутреннее совместительство/internal secondary employment, внешнее совместительство гос.уч./external secondary employment within a public medical organization, внешнее совместительство частн.клиника/external secondary employment at a private clinic, нет/no additional work

In all investigated groups more than half of respondents are thinking about changing their job for various reasons. At the time of the sociological study every fourth chief physician and doctor (23.5 % and 23.8 % respectively) and each third (32.1 %) head of structural department (F_{cr} 2.52 > F_{emp} 0.08) were not thinking about changing their job.

In all groups, those survey participants who are thinking of changing their job have indicated the prospect of achieving a high salary as the main basis for their decision. Chief physicians of medical organizations and doctors indicated the prospect of achieving more interesting professional work within their specialty as the second most important reason, and heads of departments named comfortable conditions and good organization of work at their new job. As the third most important reason chief physicians named comfortable working conditions, good organization of work, and stability of employment, whereas the heads of structural departments indicated more interesting professional work within their specialty and a change of place of residence, and doctors specified the opportunity to achieve self-realization in a new place and growth prospects (F_{cr} 0.92 > F_{emp} 0.07).

Thus, most healthcare professionals agree that the workload that they must shoulder in the course of the performance of their duties is now reaching a high level. Among all healthcare professionals, a large proportion of the heads of structural departments noted in particular experiencing an excess workload. It should be noted that the availability of additional paid work on both internal and external part-time work terms is recognized by the majority of healthcare professionals. Managers at all levels mainly pursue internal secondary employment, while ordinary doctors pursue external opportunities. Among all healthcare professionals who have secondary employment on an external part-time basis, the heads of departments and doctors most often choose a private clinic as a place of work.

Most healthcare managers and doctors appreciate carrying out their professional duties. In general, the work brings them satisfaction. However, healthcare professionals believe that in order to get a better result, it is necessary to improve working conditions. The number one problem for most of them is the low level of salary. Secondary and tertiary problems include workplace organization conditions, interpersonal relations, and personal development factors.

Conclusion

In completing our analysis of the medical and sociological analysis of the subjective opinion of healthcare professionals of the Russian Far East on the conditions and organization of work at state medical institutions, we are able to draw the following conclusions:

1) The overall assessment by healthcare professionals of the development of the healthcare service provision system in general, and at the individual institution level in particular, is the same, and is assessed by them as satisfactory. Characterizing the state of the resources of the medical institution, healthcare professionals believe that the following factors are of major concern: low availability of medical personnel, the use of obsolete fixed assets, and the low level of implementation of modern and effective medical and information technologies.
2) According to the high assessments that respondents provided of their chosen profession, the chosen career of healthcare is important to employees at public healthcare institutions. However, the overall level of satisfaction with working conditions is low. The status of employees has had an impact on assessments of motivational factors of work activity. The higher the status of the respondents, the higher the degree of their satisfaction with the work.
3) Among the proposed external motivational factors for healthcare professionals, salaries, workload and document flow, and financial and logistical support at the workplace have a significant impact. Doctors who are members of the management personnel reserve also feel the need to pursue personal development factors – the possibility of professional development, self-realization, and career development.
4) The high evaluation by healthcare professionals of their own functional duties and the results of their work demonstrates the high potential of internal motivational factors that influence the results of the labor process and the quality of medical services.

5) Based on the subjective opinion of healthcare professionals, the results of the study revealed a number of economic and organizational aspects of working conditions in public healthcare institutions, which require scientific and practical justification in healthcare service activities and adoption of strategic, prospective and operational management decisions affecting the development of the sector.

Acknowledgments

The author is grateful to Ratmanov P.E., Doctor Med. Sci., Prof., Far Eastern State Medical University, the Department of Public Health and Health Management (Khabarovsk, Russia), for assistance in the preparation of this publication.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 09.02.2018.
Accepted for publication on 22.02.2018.

UDC 616-006-085.28-06:616.85-03

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CLINICAL SIGNS OF CHEMOTHERAPY-INDUCED NEUROPATHY IN CANCER PATIENTS AND PHARMACOTHERAPY CORRECTION OPTIONS

Abstract

The objective of the study was to analyze the clinical signs of chemotherapy-induced neuropathy (CIPN) in cancer patients and explore pharmacotherapy correction options using vitamin B supplements. **Materials and methods.** During the first stage that lasted from May to September 2017 after the screening period, 219 patients (mean age of (50.4 ± 6.9) years) were selected for the study; 105 (46.7%) of them were female patients undergoing chemotherapy treatment cycles at Samara Regional Oncological Clinic. The methods of standard neurological examination and patient questioning established clinical signs of polyneuropathy: its localization, the main manifestations, including sensory and/or pain disorders. The patients were further randomized into two groups: group 1 received vitamin B supplements, and group 2 received no vitamin B supplements. The patients were observed for 60 days. **Results.** The incidence of polyneuropathy in oncology patients receiving chemotherapy turns out to be very high. The phenotype of clinical signs and their severity and localization is probably related to the type of the drug agent used. Our study demonstrated the effectiveness of the use of step-by-step therapy with group B supplements in order to reduce the clinical manifestations of polyneuropathy.

Key words: *polyneuropathy, chemotherapy, oncology*

For citation: Zolotovskayaia I.A., Davydkin I.L., Lokshtanova T.M., Orlov A. E. CLINICAL SIGNS OF CHEMOTHERAPY-INDUCED NEUROPATHY IN CANCER PATIENTS AND PHARMACOTHERAPY CORRECTION OPTIONS. The Russian Archives of Internal Medicine. 2018; 8(2): 137-144. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-137-144

DOI: 10.20514/2226-6704-2018-8-2-137-144

VAS — visual analog scale, QOL — Quality of Life, BMI — Body Mass Index, ICD-10 — International Classification of Diseases, 10th Edition, MP — medicinal preparation, FOLFOX — folinic acid / 5-fluorouracil/oxaliplatin, SD — standard deviation

Recently, the issue of the complications that cancer patients face who are either undergoing or have completed chemotherapy cycles has become extremely pressing for specialists of outpatient clinics. The number of cancer patients being treated with chemotherapeutic drugs is rising due to their high therapeutic effectiveness and survival rate. However, this type of treatment increases the risks of acute and delayed side effects, including damage to the peripheral nervous system in the form of polyneuropathies.

To date, this problem has not been the focus of attention of oncologists. Rather, it has been more widely studied by specialists in internal medicine (neurologists, therapists, general practitioners) who perform follow-up patient monitoring on an outpatient basis. The polyneuropathy induced by chemotherapeutic drugs leads to a deterioration in the quality of life (QOL) of cancer patients, causing them severe limitations of motor and sensory functions in the upper and lower extremities [1–4]. It is currently known that polyneuropathy develops in

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almost 90 % of patients of this category, and subsequently more than 30 % of patients exhibit some symptoms of damage to peripheral nerve fibers after completion of the treatment [5].

The development of polyneuropathy with clinical manifestations is described as one of the side effects in the product label of the following most commonly used chemotherapeutic drugs: platinum-based (cisplatin, carboplatin and oxaliplatin), bortezomib, taxanes, vinca alkaloids (vincristine), thalidomide, and lenalidomide [6–11]. This prompts the need to discuss a number of issues related to the development of new MP without possible pronounced side effects and the introduction of preventive strategies aimed at reducing the risk of polyneuropathy. The decrease in clinical manifestations of polyneuropathy not only improves the QOL of cancer patients, but it also increases their survival rate [12]. The follow-up of such patients requires knowledge of algorithms for early diagnosis of the peripheral nervous system damage and opportunities to administer medication therapy to minimize its manifestations.

The objective of our study is to investigate the clinical manifestations of polyneuropathy in cancer patients who are undergoing chemotherapy and the ability to treat them with vitamin B supplements.

Materials and methods

The study was registered as a monitoring program, "Polyneuropathy: risks of early and late complications of chemotherapy in cancer patients (**POSEIDON**)", was conducted in two stages. (The acronym POSEIDON is based on the original Russian wording for the procedure.)

During the first stage that lasted from May to September 2017 after the screening period, 219 patients (mean age of (50.4 ± 6.9) years) were selected for the study through the method of sequential inclusion, of which 105 (46.7 %) were female patients undergoing chemotherapy at the State Budgetary Healthcare Institution Samara Regional Clinical Oncology Dispensary (SBHI SRCOD). Patients were divided into the following groups by the localization of the tumor: 52 patients (the 1st group) with colon cancer (C18.9), 94 patients (the 2nd group) with breast cancer (C50.0), 52 patients (the 3rd group) with lung cancer (C34.0), and 21 patients (the 4th group) with prostate cancer (C61.0). The inclusion criteria were the following: 1. A verified cancer that was not older than 12 months; 2. Chemotherapy treatment. The exclusion criteria were the following: 1. The history of polyneuropathy before the chemotherapy cycle initiation; 2. The patients' written refusal to participate in the study. The methods of standard neurological examination and patient questioning established clinical signs of polyneuropathy: its localization, the main manifestations, including sensory and/or pain disorders.

During the second stage, a subanalysis of patients with three or more clinical manifestations of polyneuropathy was performed. The patients were further randomized into two groups using the sealed envelopes method in accordance with the following pharmacotherapy procedures: in the 1st group (main, n = 30) patients received vitamin B supplements, and in the 2nd group (comparison, n = 30) this medicinal therapy was not administered. The duration of the second stage was 60 days, and during this period three visits (V) were performed, of which V_1–V_2 during the period of therapy with vitamin B supplements (for the main group of patients), and V_3 — during the control period. According to the protocol, the time of visits was determined as follows: V_1 — initially, when included in the study and the therapy started, V_2 — after 31 days, and V_3 — after 60 days. For patients in the 1st group, intramuscular injection of a combined MP containing vitamins B_1, B_6, B_{12} was chosen as a therapy regimen for 10 days, and then they were orally administered for 3 weeks. It should be noted that patients were not prescribed analgesics, anticonvulsants or antidepressants to reduce manifestations of polyneuropathy. This step in the study was undertaken in order to exclude the influence of other MP on the symptoms of polyneuropathy. At all visits, the severity of the studied clinical manifestations of polyneuropathy was assessed using a visual analog scale (VAS) in centimeters. Within the V_1, before prescribing group B vitamins, the patients were thoroughly familiarized with the design of the study in a step-by-step fashion, and they were informed about all possible side effects. Patients signed an informed consent to the

use and processing of their personal data and to the participating in the study. In addition, all patients were informed that at any moment they could abandon the study for any reason.

Statistical analysis of the obtained data was performed using the IBM SPSS Statistics 21 software package (License No. 20130626-3). In order to compare independent groups, a one-way analysis of variance (one-way ANOVA) was used. In cases of deviation from the zero-hypothesis of equality of means, post hoc analyses (group comparisons in pairs) were performed in accordance with Tukey's test. The description of normally distributed quantitative features is given, including indication of the mean value of the feature and the standard deviation ($M \pm SD$). Descriptive statistics were used using the parametric criterion (Student's t-test) to perform the analysis. The median, upper (25th) and lower (75th) quartiles — Me (Q25–Q75) were indicated to describe the characteristics with a different distribution than the normal one. Differences between the studied parameters were considered statistically significant at $p < 0.05$.

The study was performed in accordance with the standards of Good Clinical Practice and principles of the Declaration of Helsinki. The Research Protocol has been approved by the Ethical Committee of the Samara State Medical University.

Results

During the first stage, we performed an analysis of the entire group of patients in accordance with their main clinical and demographic characteristics, which are detailed in Table 1.

The mean duration of the underlying disease was (5.5 ± 3.2) months, during which time (3.8 ± 1.7) cycles of chemotherapy were performed. Polyneuropathy was revealed in 77.2 % of cases, with a mean duration of (2.9 ± 1.8) months. Most patients had distal polyneuropathy with localization in the area of the hands (55.0 %) and feet (42.0 %). Sensory disorders were represented by a variable range of complaints: 89.9 % of cases reported numbness, 52.0 % reported a feeling of tingling, and 40.8 % reported creeping sensation. Neurological examination showed an isolated decrease

Table 1. *Clinical and demographic characteristics of patients at study enrollment*

Characteristics	Whole group, n=225
Age, years	50,5 (42,5–65,0)
BMI, kg/m²	22,5 (20,8–25,6)
Principal Diagnosis (ICD-10)	
Malignant neoplasm of colon (C18.9), n/%	52/23,1
Malignant neoplasm of breast (C50.0), n/%	94/41,8
Malignant neoplasm of lung (C34.0), n/%	52/23,1
Malignant neoplasm of prostate (C61.0), n/%	21/9,3
Duration of underlying disease, months, m±SD	5,5±3,2
Number of chemotherapy cycles, n, m±SD	3,8±1,7
Polyneuropathy, n (%):	169/77,2
Duration, months, m±SD	2,9±1,8
Localization	
Lower limbs, n/%:	
Distal	71/42,0
Proximal	59/34,9
Distal and proximal	25/14,8
Upper limbs, n/%:	
Distal	93/55,0
Proximal	58/34,3
Distal and proximal	25/14,8
Lower and upper limbs, n/%:	30/17,8
Type of sensory impairment, n/%	
Burning pain	36/21,3
Pressing pain	0/0
Shooting pain	23/13,6
Dull pain	52/30,8
Aching pain	3/1,8
Tingling	88/52,0
Burning sensation	60/35,5
Freezing sensation	66/39,0
Sensation similar to electric shock	28/16,6
Creeping sensation	69/40,8
Numbness	152/89,9

Note. Acronyms used in tables 1-3: BMI — body mass index, FOLFOX — folinic acid/5 fluorouracil/oxaliplatin, ICD-10 — International Classification of Diseases, 10th revision, SD — standard deviation.

Table 2. *Polyneuropathy characteristics in groups of patients by tumor localization and chemotherapy agents used*

Characteristics	Group 1 (ICD10 code: C18.9), n=52	Group 2 (ICD10 code: C50.0), n=94	Group 3 (ICD10 code: C34.0), n=52	Group 4 (ICD10 code C61.0), n=21
Age in years	59,7 (57,0-62,5)	47,0 (36,5-51,0)	47,5(42,5-53,0)	61,5(58,0-64,5)
Male, n/%	48/92,3	-	44/84,6	21/100,0
BMI, kg/m^2	22,3 (20,6-23,1)	21,9 (19,5-22,4)	21,8 (20,3-23,4)	21,9 (20,8-23,2)
Duration of underlying disease, m±SD	5,5	5,6	5,8	6,5
Number of chemotherapy cycles, m±SD	3,7	3,3	3,6	2,9
Treatment, n/%				
FOLFOX protocol	52/100	-	-	-
Doxorubicin	-	61/64,9	-	-
Taxotere	-	12/12,8	-	-
Docetaxel	-	2/2,1	-	16/76,2
Paclitaxel	-	19/20,2	12/23,0	-
Cisplatin	-	-	16/30,8	-
Carboplatin	-	-	5/9,6	-
Gemcitabine	-	-	19/36,5	5/23,8
Polyneuropathy, n (%):	51/98,0	59/62,8	40/76,9	19/90,5
Duration, months, m±SD	2,3	2,0	2,2	2,5
Localization				
Lower limbs, n/%:				
Distal	28/53,8	6/10,0	19/47,5	18/94,7
Proximal	22/42,3	8/13,3	8/20,0	10/52,6
Distal and proximal	14/26,9	1/1,7	6/15,0	6/31,5
Upper limbs, n/%:				
Distal	47/90,4	33/55,0	12/30,0	1/5,3
Proximal	9/17,3	36/60,0	13/32,5	1/5,3
Distal and proximal	8/15,4	10/16,7	6/15,0	1/5,3
Clinical signs of peripheral neuropathy, n/%				
Burning pain	23/44,2	13/21,7	-	-
Pressing pain	-	-	-	-
Shooting pain	22/42,3	1/1,7	-	-
Dull pain	24/46,1	15/25,0	-	13/68,4
Aching pain	-	3/5,0	-	-
Tingling	27/51,9	30/50,5	24/60,0	7/36,8
Burning sensation	23/44,2	-	26/65,0	11/57,8
Freezing sensation	33/63,5	15/25,0	10/25,0	8/42,1
Sensation similar to electric shock	13/25,0	2/3,3	6/15,0	7/36,8
Creeping sensation	23/44,2	36/60,0	10/25,0	-
Numbness	52/100,0	47/78,3	37/92,5	16/84,2

in superficial sensitivity in the lower extremities in 99 patients (45.2 %), and 155 patients (70.8 %) reported this symptom in combination with manifestations in the hands.

At this stage, we examined the manifestations of polyneuropathy presented in the Table 2 in detail depending on the localization of the tumor while aiming to clarify the relationship with chemotherapy regimens, which is important to understanding the features of the patient's phenotype.

Patients in all groups did not have statistically significant differences in BMI, duration of the disease, number of cycles of chemotherapy, and duration of polyneuropathy in comparison by ANOVA. As far as drug therapy is concerned, we found that all patients diagnosed with C18.9 received chemotherapy treatment in accordance with the FOLFOX protocol. Most patients (64.9 %) diagnosed with C50.0 received doxorubicin. Patients diagnosed with C34.0 received the following basic chemotherapeutic drugs: paclitaxel (23.0 %), cisplatin (30.8 %), and gemcitabine (36.5 %), and 76.2 % of those in the group of patients who were diagnosed with C61.0 received docetaxel. In all groups there was a high frequency of occurrence of polyneuropathy, but in patients diagnosed with C50.0 it was significantly lower than in patients diagnosed with C18.9 ($p = 0.029$), C34.0 ($p = 0.041$), and C61.0 ($p = 0.035$).

We noted that each of the studied diseases was characterized by specific features of damage to the peripheral nervous system. Thus, patients diagnosed with C18.9 commonly exhibit signs of polyneuropathy mainly in the distal segments (90.4 %) of the upper extremities. In patients with breast cancer, the phenotype of polyneuropathy is characterized mainly by damage to the upper extremities, both distally and proximally. In patients with lung cancer, the injury of the upper and lower extremities (mainly distally) was recorded without significant differences ($p < 0.05$). A special phenotype is demonstrated by the clinical pattern of patients with prostate cancer, where in 94.7 % of cases distal damage of the lower extremities was present. Therefore, after carrying out this stage of the study, we can conclude that it is most likely that the combination of cancer and the applied chemotherapy regimen determines the patient's phenotype. The clinical pattern of this phenotype is dominated by a particular localization of the peripheral nervous system.

During the second stage of the study, the influence of the pharmacotherapy regimens on the regression of the neurological deficit was studied. The obtained data are presented in the Table 3.

Patients from the 1st and 2nd groups had comparable main clinical and demographic indicators: gender ($p = 0.75$), age ($p = 0.69$), mean duration

Table 3. *Changes in signs of peripheral neuropathy at V1-V3*

Clinical signs of peripheral neuropathy	Study group, n=30/30/25			Control group, n=30/30/26			ANOVA p
	V_1	V_2	V_3	V_1	V_2	V_3	p_1- p_2
1. Tingling sensation, VAS, cm, m±SD	3,8±1,5	3,1±1,1 p_{V1}-p_{V2}=0,047	3,1±1,2 p_{V2}.p_{V3}=0,898 p_{V1}.p_{V3}=0,047	3,6±1,2	4,2±1,5 p_{V1}-p_{V2}=0,046	4,7±1,2 p_{V2}-p_{V3}=0,055 p_{V1}-p_{V3}=0,031	0,562
2. Creeping sensation, VAS, cm, m±SD	4,0±1,1	3,2±0,8 p_{V1}-p_{V2}=0,043	3,1±1,0 p_{V2}-p_{V3}=0,799 p_{V1}-p_{V3}=0,043	3,8±1,0	4,5±1,2 p_{V1}-p_{V2}=0,41	5,1±1,0 p_{V2}-p_{V3}=0,065 p_{V1}-p_{V3}=0,037	0,398
3. Numbness, VAS, cm, m±SD	4,4±1,2	3,6±1,3 p_{V1}-p_{V2}=0,038	3,5±1,4 p_{V2}-p_{V3}=0,681 p_{V1}-p_{V3}=0,037	4,3±1,3	4,5±1,3 p_{V1}-p_{V2}=0,52	4,9±1,2 p_{V2}-p_{V3}=0,056 p_{V1}-p_{V3}=0,033	0,6350
4. Freezing sensation, VAS, cm, m±SD	4,1±1,1	3,5±0,5 p_{V1}-p_{V2}=0,046	3,0±0,5 p_{V2}-p_{V3}=0,09 p_{V1}-p_{V3}=0,054	4,0±1,0	4,7±1,0 p_{V1}-p_{V2}=0,45	5,3±1,0 p_{V2}-p_{V3}=0,052 p_{V1}-p_{V3}=0,021	0,989

Notes: Acronyms: VAS — Visual Analog Scale

of the underlying disease (ρ = 0.092), mean duration of polyneuropathy (ρ = 0.38), as well as the studied clinical manifestations of polyneuropathy. During the follow-up period, we observed the following trend: a significant improvement in all the symptoms studied in the group of patients taking vitamins with a decrease in numbness as well as a subsidence of the painful sensation of cold, tingling, creeping sensation during V_3. At the same time, in the comparison group, an increase in all studied clinical symptoms was observed during V_2 with an increase in the VAS score, including during V_3.

Discussion

To date, most researchers working with the problem of polyneuropathy induced by chemotherapy in cancer patients note, first of all, its negative impact on QOL, limiting the patient's performance of everyday household functions while experiencing severe pain, numbness, and convulsions in the upper and/or lower extremities at the same time [13–15]. Our observations show that the number of patients experiencing acute and painful sensations is highly significant among all patients receiving chemotherapeutic drugs, regardless of the tumor localization. At the same time, the symptoms of polyneuropathy are extremely variable, which is due to the individual response of nerve fibers to the damaging effects of chemotherapy. Nevertheless, the main manifestations of polyneuropathy were represented by sensory disorders and pain sensations of a neuropathic nature. According to our data, the majority of patients have presented complaints that are consistent with damage to sensory fibers, including numbness and burning that is generally symmetrical. It is known that the symptoms of polyneuropathy, in some cases, may have a tendency to spontaneously subside after the completion of chemotherapy. However, in a number of cases, the symptoms increase and worsen [16]. This is especially true when the patient is taking such drugs as paclitaxel and thalidomide [17–19]. In our study, patients with breast and lung cancer who received paclitaxel reported acute symptoms of damage to the peripheral nerve fiber with a subsequent deterioration on the VAS in the 2nd group of patients. It was discovered that in the patients with colorectal cancer under the FOLFOX regimen, there is an extremely high incidence (from 70 to 90 %) of symptoms reflecting peripheral nerve fiber damage [20]. Our data also demonstrate the high incidence of polyneuropathy in this category of patients with predominant distal damage of the upper extremities. Moreover, it was noted that in patients undergoing chemotherapy cycles within the FOLFOX regimen, the appearance of a sensory deficit in the upper and lower extremities was noted quite early, starting from the initial courses of treatment. This result is also consistent with the results of previous studies [20]. The results of our study coincide with the data of a number of authors, which associate specifically oxaliplatin administration with the early onset of polyneuropathies, distal dysesthesia, allodynia, and burning pains.

In general, it can be said that manifestations of polyneuropathy in patients receiving chemotherapeutic drugs vary significantly, both in the severity of symptoms and in duration [21–23]. We noted significant differences in the manifestation of qualitative and quantitative characteristics of polyneuropathy with their level of variation in each patient, which is also reflected in the works of other authors [24]. The results of our study prove that the chemotherapy-induced polyneuropathy has a number of phenotypic features.

The second stage of the study was undertaken to directly study the possibility of correcting chemotherapy-induced disorders. According to the publications, methods to treat polyneuropathy induced in cancer patients by chemotherapy have not been sufficiently developed. Despite the fact that a considerable number of observations of such patients has been accumulated, the data on effective regimens for the treatment of polyneuropathy are limited and contradictory [25]. This may be due to the fact that each MP damages the peripheral nerve tissue in its own way, and may also be related to the individual characteristics of the patient. Vitamin B supplements have been proven to be highly effective in clinical practice for the treatment of polyneuropathies, including those primarily of diabetic and alcoholic origin. Few studies on the use and treatment results of this class of drugs in patients with chemotherapy have been conducted. The functional significance of vitamin B for human health is known and has been well studied. The

basic metabolic processes, the synthesis of neurotransmitters, and the activation of enzymatic processes are impossible in the central and peripheral nervous system without vitamin B complex. Deficiency of B_1, B_6 is directly associated with nervous dysfunction and nerve damage [26, 27]. The most significant connection is to vitamin B_{12} deficiency. Thus, the study presented by Soloman L. in 2016 demonstrated the association of peripheral neuropathy and neuropathic pain development in patients with a history of cancer [28]. Recent studies have shown that for patients undergoing chemotherapy, vitamin B_{12} deficiency rapidly increases during a short time from the start of treatment [29]. We have confirmed that patients receiving chemotherapy experience the following positive change: a decrease in manifestations of polyneuropathy while undergoing therapy with group B supplements followed by a sustained effect for two months. While in the comparison group there was a statistically significant increase in all studied clinical symptoms of polyneuropathy during V_2–V_3. In the absence of timely therapy, manifested sensory and painful disorders in the extremities were observed in patients in the 2nd group with delay, which indicated an increase in damage to the peripheral nervous system.

Further studies aimed at discovering the effective doses and therapy regimens for vitamin B supplements are underway. Nonetheless, we already possess the data on both meta-analyses and individual studies on the effectiveness of vitamin therapy in this group of patients. For example, the authors of the recently published review note that the vitamins B_3, B_6 and B_{12} demonstrate potential for helping patients to protect themselves from the development of polyneuropathy, and they indicate that further research is required [30]. Our observations represent an attempt not only to clarify the prevalence, localization, and variability of clinical manifestations of polyneuropathies in patients receiving chemotherapy, but they also indicate that vitamin B complex administration may have a positive clinical effect.

Conclusion

We recognize that our study has had some limitations due to the small sample group of patients. However, we found that the incidence of polyneuropathy among cancer patients receiving chemotherapy is extremely high. The phenotype of clinical manifestations and the severity of their symptoms and localization are probably determined by the used MP. When such patients are followed-up by a doctor at the outpatient stage of treatment, it is necessary to focus on the need for the use of pathogenetic therapy aimed at restoring the function of peripheral nerves. Our study demonstrated the effectiveness of the use of step-by-step therapy with group B supplements in order to reduce the clinical manifestations of polyneuropathy. The obtained data confirm that the problem is topical. It requires further study with a larger sample group of patients over a longer period of time. A study of such scope would allow us to make conclusions that would be valid for the general population about the early and late manifestations of polyneuropathy.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 06.02.2018.
Accepted for publication on 26.02.2018.

ИНФОРМАЦИОННОЕ ПИСЬМО

Главное военно-медицинское управление МО РФ;
Военно-медицинская академия имени С.М. Кирова
Научно-практическое общество баротерапевтов
Санкт-Петербурга и Ленинградской области

17 — 18 мая 2018 года проводят

Юбилейную X Всеармейскую научно-практическую конференцию «БАРОТЕРАПИЯ В КОМПЛЕКСНОМ ЛЕЧЕНИИ И РЕАБИЛИТАЦИИ РАНЕНЫХ, БОЛЬНЫХ И ПОРАЖЁННЫХ»

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На конференции предполагается рассмотреть теоретические и прикладные вопросы лечения раненых, больных и пораженных; проблемы реабилитации человека со сниженной работоспособностью различными видами и методами баротерапии; теоретические и практические положения гипербарической физиологии и водолазной медицины.

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Иванов И.И.

ИНДИВИДУАЛЬНАЯ ОПТИМАЛЬНАЯ ДОЗА КИСЛОРОДА ПРИ ОДНОМ СЕАНСЕ ГБО (ОДНОРАЗОВАЯ ДОЗА)

Военно-медицинская академия имени С.М. Кирова, Санкт-Петербург
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В исследовании по проблеме оптимальной дозы кислорода при гипербарической оксигенации принимали участие 88 практически здоровых мужчин в возрасте 24-34 лет...

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Для участников конференции 18 мая планируется культурная программа.

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UDC 616.131-008.331.1-07

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CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSIA AT THE YOUNG PATIENT. CLINICAL OBSERVATION

Abstract

The objective of the study: To report a case of onset and course of chronic thromboembolic pulmonary hypertension (CTEPH) at the patient of young age. **Materials and methods**. The patient P., 26 years, was admitted with complaints of dyspnea during mild exercise exertion and at rest, edemas of the lower extremities. Patient had recurrent PE, subclavian and brachial venous thrombosis on the right side in her medical history. **Results**. Echo and ECG signs of pulmonary hypertension were obtained. Repeated MSCT pulmonary angiography showed a dissolution of thrombotic masses in a lumen of a pulmonary artery. No data for thrombophilia or systemic vasculitis were obtained. **Conclusion.** The present clinical observation demonstrates the formation and course of CTEPH in a young patient with recurrent pulmonary embolism. The young age, idiopathic and recurrent character of pulmonary embolism were the predisposing risk factors for CTEPH development.

Key words: *pulmonary embolism, chronic thromboembolic pulmonary hypertension*

For citation: Kosheleva N.A., Gamayunova K.A. CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSIA AT THE YOUNG PATIENT. CLINICAL OBSERVATION. The Russian Archives of Internal Medicine. 2018; 8(2): 146-149. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-146-149

DOI: 10.20514/2226-6704-2018-8-2-146-149

VTE — venous thromboembolism, DS — duplex study, mPAP — mean pulmonary arterial pressure, MSCT — multislice computed tomography, PE — pulmonary embolism, CTEPH — chronic thromboembolic pulmonary hypertension, Echo — echocardiography

Introduction

The definition of venous thrombosis and thromboembolism comprises deep venous thrombosis and pulmonary embolism. Pulmonary embolism is the third most common cardiovascular disease with prevalence in the range of 100–200 cases per 100,000 population per year. It is characterized by a high mortality rate. [1]. The most significant predisposing risk factors for females are traumas, obesity, prolonged immobility, use of oral contraceptives, antiphospholipid syndrome, infections, and thrombophilia. Pulmonary embolism can also occur in the absence of any known risk factor [2].

The delayed complication of acute pulmonary embolism (PE) is chronic thromboembolic pulmonary hypertension (CTEPH). Some patients with prior PE do not experience complete recanalization of the pulmonary vascular bed. In these patients embolic masses are dissolved only partially, and they are replaced by connective tissue. These embolic masses change the lumen of the pulmonary vessels, which leads to CTEPH formation [3].

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CTEPH is a rare disease, the prevalence of which is about 5–10 cases per 1 million people per year. The mean age of Russian patients at diagnosis is 45.8 ± 13.7 years according to the National Registry [4]. The prevalence rate is considered equal in male and female populations. When untreated, CTEPH prognosis is unfavorable and depends on the degree of pulmonary hypertension. According to foreign studies, the ten-year survival rate of patients with inoperable CTEPH at a mean pulmonary artery pressure (mPAP) in the range of 31–40 mm Hg is 50 %; at mPAP from 41 to 50 mm Hg it is 20 %; and at mPAP of more than 50 mm Hg it is 5 % [5]. Since the disorder is rare in female young patients, we would like to provide our clinical observation of such case.

Case Report

Female patient P., aged 26, was admitted to the Department of Cardiology of the Regional Clinical Hospital in December 2016. Her chief complaints were mixed dyspnea during mild exercise exertion and at rest, hypotension of 80/60 mm Hg, fatigue, and leg swelling up to knees. The history of present illness: in October 2015, the patient firstly noticed coldness, cyanosis and swelling of her right upper limb. A duplex study of upper extremity veins confirmed subclavian and brachial venous thrombosis on the right side. Treatment with heparin and detralex was prescribed by the vascular surgeon, which had a positive effect in reducing the upper limb swelling. Since thrombosis was revealed screening for its reasons was not carried out. In August 2016, the patient noted hypotension to 80/60 mm Hg, an episode of syncope, mixed dyspnea when walking 3 floors upstairs. Exercise tolerance was further decreasing. In November 2016, a second episode of syncope, hemoptysis, aggravation of the mixed dyspnea on exertion and its onset at rest, and leg swelling up to knees were all noted. According to the past medical history, there were no injuries, prolonged immobility, use of oral contraceptives, infectious diseases. According to the patient's words, she does not smoke or take drugs, including intravenous forms. A chest X-ray showed no cervical rib.

At admission, the patient's condition was severe, due to cardiac and respiratory failure. Mixed dyspnea at rest and aggravated on minimal exertion was noted. Leg swelling was up to knees. The patient was active. BMI was 23 kg/m^2, HR — 102 bpm, RR — 28 per minute, BP — 100/70 mm Hg; oxygen saturation — 88 %. The skin was pale, and acrocyanosis was noted. The heart sounds were muffled, and the rhythm was regular. Accent of S2 over the pulmonary artery and Graham Steele murmur were found. Breathing in the lungs was vesicular, and rales were not heard. The abdomen was soft and nontender. The liver was enlarged, palpable 2 cm below the costal margin. The urine output was decreased. The ECG revealed a sinus tachycardia at 110/min, right axis deviation, "SI, QIII, TIII" pattern, inverted T-wave in V1–V6, and right atrial hypertrophy. Echocardiography (Echo) showed high pulmonary hypertension and signs of right heart overload (Table 1).

MSCT pulmonary angiography scanning showed thrombosis of the lower branch of the right pulmonary artery, and pulmonary infarction (S10) (Figure 1).

A duplex study (DS) showed post-thrombotic changes of the right subclavian vein. D-dimers were negative, no specific findings in the coagulogram. Taking into account negative anti-dsDNA antibodies, antineutrophil cytoplasmic antibodies,

Table 1. *Echo indices in dynamics*

Indices	December, 2016	February, 2017
Right atrium, cm	4.54	5.07
Right ventricle, cm	4.12	4.64
RV anterior wall, cm	0.6	0.78
Pulmonary artery systolic pressure, mm Hg	90	80
Tricuspid regurgitation	3 degree	3-4 degree
Ejection fraction, %	63	63

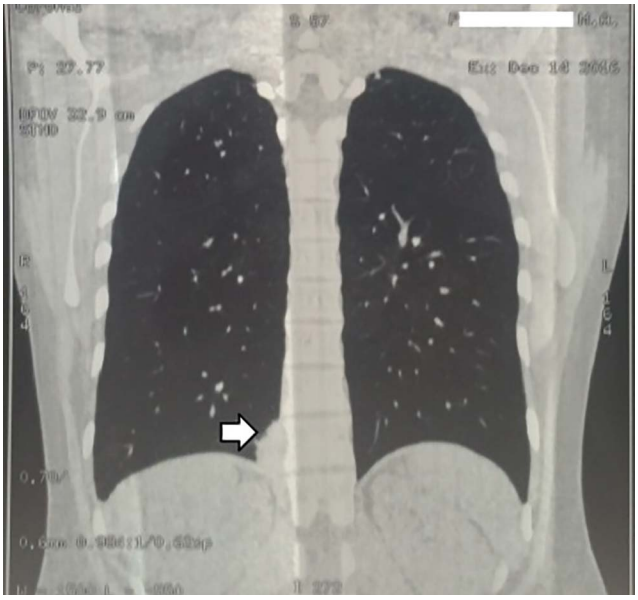

Figure 1. *MSCT: the focus of increased lung density in S10 of the right lung, triangular in shape, widely adjacent to diaphragmatic pleura.*

antinuclear antibodies, lupus anticoagulant, and anticardiolipin antibodies, data for antiphospholipid syndrome or systemic vasculitis have not been revealed. During the examination no data for cancer were received. To exclude primary thrombophilia, the genes were examined (*F5* gene coding the coagulation factor V (Factor V Leiden), *F2* gene coding the coagulation factor II or prothrombin, and MTHFR gene coding methylenetetrahydrofolate reductase, a key enzyme in the conversion of homocysteine amino acid), but no clinically significant mutations were determined.

The principal diagnosis was: Pulmonary embolism (August 2016), recurrent PE (November 2016). Thrombosis of the lower branch of the right pulmonary artery. Pulmonary infarction (S10 of the right lung). Subacute cor pulmonale. 3rd degree of functional tricuspid regurgitation. 3rd degree of pulmonary hypertension. Thrombosis of subclavian and brachial veins on the right in October 2015. Post-thrombotic syndrome of the right upper limb. Complications: respiratory failure of the 2nd degree. Chronic heart failure, NYHA 4. Hemoptysis in November 2016.

The following treatment was provided at the Department of Cardiology: oxygen therapy, enoxaparin 1 mg/kg b.i.d., amlodipine 2.5 mg/day, bisoprolol 2.5 mg/day, torasemide 10 mg/day, and verospiron 50 mg/day. On day 7 rivaroxaban was initiated instead of enoxaparin: 15 mg b.i.d for 3 weeks, then 20 mg/day was prescribed for long-term administration. The patient complied with the recommendations.

In February 2017, the patient was admitted for follow-up. Her state of health has slightly improved: dyspnea when walking 3 floors upstairs decreased (no dyspnea at rest), and no swelling was observed. The lab results, ECG and lower extremities DS data did not show any significant changes. According to Echo, mPAP did not change significantly, and the right heart dilatation increased (Table 1). Repeated MSCT pulmonary angiography showed no thrombotic masses in the pulmonary trunk and pulmonary arteries. Two areas of increased lung density in S9 and S10 of the right lung were revealed, triangular in shape, widely adjacent to diaphragmatic and costal pleura.

The patient was consulted by a cardiac surgeon of the FSBI A. N. Bakulev National Medical Research Center of Cardiovascular Surgery via telemedicine technology. Due to the presence of thrombosis and episodes of PE in the past medical history, the chronic pulmonary heart disease progression, no significant change of mPAP on the background of the adequate three-month anticoagulant therapy, chronic thromboembolic pulmonary hypertension (CTEPH) was proposed. It was recommended to continue the ongoing therapy, including anticoagulants, and sildenafil (20 mg t.i.d.) was added. The follow-up to decide the issue of surgical treatment was recommended in case of no improvement in the patient's state. No significant improvement (according to clinical and instrumental data on the background of adequate therapy) was revealed during the case follow-up with the participation of cardiac surgeons from FSBI A. N. Bakulev National Medical Research Center of Cardiovascular Surgery. In February 2018, the patient was admitted to the Pulmonary Hypertension Department of the Federal Center, and on March 2, 2018, she underwent successful pulmonary thromboendarterectomy.

Discussion

This clinical case shows the formation and further course of CTEPH in a young female patient after recurrent PE. Why do some patients who suffer

from PE develop CTEPH, while others do not? Dissolution of thrombi occurs with the help of local thrombolysis with a full restoration of the pulmonary vascular bed patency. However, in some cases, for unexplained reasons, resorption does not occur and the emboli are converted into organized clots inside the pulmonary artery. Perhaps this process is aggravated by the hemostasis or fibrinolysis disturbance, as well as by recurrent embolism. Currently, scientists continue to investigate congenital and acquired coagulation anomalies in patients with venous thromboembolism (VTE) and CTEPH [6]. Among the coagulation system pathology findings in patients with venous thromboembolism and those who later develop CTEPH, the following are the most often detected: lupus anticoagulant (10 %), antiphospholipid antibodies (20 %), increased factor VII activity (39 %), and fibrinogen genes mutations [7]. In addition to the impairment of coagulation, the following potential risk factors may be involved in the formation of CTEPH: recurrent embolism, large perfusion defect, young age of patients, and idiopathic character of pulmonary embolism [8].

The treatment of choice for the CTEPH management is tromboendarterectomy. It tends to decrease dyspnea, to improve the functional class of CHF, and to prolong the life expectancy of patients. If surgical treatment is not possible and in case of residual pulmonary hypertension, the use of PAH-specific therapy is allowed. Modern PAH-specific medicines possess not only a vasodilating ability, but a number of additional properties, including cytoprotective, antiproliferative, antiaggregational, etc. They affect the targets of the disease pathogenesis: the endothelin system excessive activation (endothelin receptor antagonists), the deficiency of endogenous prostacyclin (prostacyclin analogues — prostanoids) and of nitric oxide (phosphodiesterase type 5 inhibitors, guanylate cyclase stimulants) [9].

Conclusion

The present clinical observation demonstrates the formation and course of chronic thromboembolic pulmonary hypertension in a young patient with recurrent pulmonary embolism. The predisposing risk factors for CTEPH development were young age and the idiopathic and recurrent nature of pulmonary embolism. The decision to conduct a thromboendarterectomy and/or to prescribe PAH-specific therapy is taken jointly with a surgeon at a specialized center.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 12.02.2018.
Accepted for publication on 12.03.2018.

UDC 616.36-044-122.6-089.843-018.46

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MONITORING OF MORPHOLOGICAL EFFECTS AUTOLOGICAL MESENCHIMAL STEM CELLS, TRANSPLANTED IN LIVER WITH VIRUS CYRROSIS (CLINICAL OBSERVATION)

Abstract

Introduction. The importance of the HCV-infection is determined by the wide spread, progressive course, the formation of liver cirrhosis (LC) and hepatocellular carcinoma. The mechanisms of the effect of the virus on hepatic cells, the processes of fibrogenesis and fibrolysis, mechanisms of the reverse development of the LC remain unexplored. There is no effective pathogenetic therapy. **The objective of the study:** to determine the effectiveness and safety of intrahepatic transplantation of mesenchymal stem cells (MSCs) in chronic HCV-infection at the stage of LC. **Materials and methods.** A patient with HCV-LC who has a secondary hemorrhagic vaculities who underwent autologous MSCs transplantation into the liver tissue. The liver biopsy specimens were studied in dynamics by light and electronic microscopy and by immunohistochemistry. **Results.** The transplantation and posttransplantation periods proceeded without complications. After the introduction of MSC the signs of micronodular LC remained. In some parts of the samples, the septa looked thin, sometimes perforated, indicating a resorption in this place of fibrous tissue. There was a decrease in the degree of transdifferentiation of stellate cells into myofibroblasts, a decrease in the number of fibrocytes and fibroblasts, there were no immune reactions in the form of deposition of amorphous and fibrous masses of moderate electron density along the sinusoidal capillaries that were significantly expressed in the primary biopsy. These changes were combined with the appearance of hepatocyte heterogeneity in the density of the cytoplasmic matrix, the state and quantity of organelles and inclusions, and the structural improvement of intracellular organelles. **Conclusion.** Autologous transplantation of mesenchymal bone marrow stem cells reduces the degree of destructive changes in hepatocytes, the severity of fibrosis and contributes to the improvement of the morpho-functional state of the liver, and therefore, it can be recommended as an important component of medical interventions.

Key words: *Cirrhosis, hepatitis C virus, liver damage, mesenchymal bone marrow stem cells*

For citation: Lukashyk S.P., Aleinikova O.V., Tsyrkunov V.M., Isaikina Y.I., Kravchuk R.I. MONITORING OF MORPHOLOGICAL EFFECTS AUTOLOGICAL MESENCHIMAL STEM CELLS, TRANSPLANTED IN LIVER WITH VIRUS CYRROSIS (CLINICAL OBSERVATION). The Russian Archives of Internal Medicine. 2018; 8(2): 150-160. [In Russian]. DOI: 10.20514/2226-6704-2018-8-2-150-160

DOI: 10.20514/2226-6704-2018-8-2-150-160

HCV — hepatitis C virus, GGT — gamma-glutamyl transpeptidase, HSC — hepatic stellate (Ito) cells, IHCR — immunohistochemical reaction, MSCs — mesenchymal stem cells, Mh — mitochondrion, LC — liver cirrhosis, ALP — alkaline phosphatase

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Introduction

The chronic infection caused by the hepatitis C virus (HCV) has been studied for more than 25 years since the discovery of the pathogen. Since then, the structure and life cycle of the virus as well as the natural course and epidemiology of the disease have been studied, the economic burden of the infection on society has been convincingly demonstrated, and antiviral therapy drugs have been developed. At the same time, the persisting significance of the disease has been determined by a number of components: data on the high prevalence and progressive course of the disease, including the development of liver cirrhosis (LC) and hepatocellular carcinoma as well as the possibility of viral replication not only in hepatocytes, but also in B lymphocytes [7, 11,17]. The mechanisms by which the virus affects hepatic cells, the regeneration of hepatocytes, the processes of fibrogenesis and fibrolysis, including those in patients with hepatic and extrahepatic viral replication, remain underexplored, and the mechanisms of already developed LC regression are poorly known. There is no effective pathogenetic therapy that would promote such a regression.

The objective of the study is to determine the effectiveness and safety of intrahepatic transplantation of mesenchymal stem cells (MSCs) in chronic HCV infection at the LC stage.

Materials and methods

In what follows we will present the result of autologous MSCs transplantation in the liver tissue of patient T. with HCV-LC and secondary (caused by HCV) hemorrhagic vasculitis.

The antibodies to HCV (anti-HCV+) were detected in the blood serum of the patient T. for the first time in 1998. She was followed-up by an infectious disease specialist at the community-based facility with a diagnosis of chronic hepatitis C (RNA HCV+) that was moderate in its degree of clinical and biochemical activity. She never received antiviral therapy. Follow-up laboratory blood tests revealed signs of active chronic hepatitis with an increase of the aminotransferases values that were 3–4 times in excess of the upper limit of the normal range, signs of kidney injury (erythrocyturia of up to 40 cells per field of view), and increased rheumatoid factor activity. No evidence of hematological abnormalities was revealed when the patient was examined by a hematologist. Vasculitis affecting the skin and kidneys was diagnosed. The patient started taking prednisolone at 40 mg/day in 2007. Periodic exacerbations of vasculitis were recorded when the drug dose was reduced. The patient refused treatment with antiviral therapies.

Since 2005, the following principal diagnosis was made on the basis of the presence of the process activity and the progressive course of the disease while taking into account the changes revealed in the examinations results: LC of viral (HCV) etiology (anti-HCV+, RNA HCV+, HCV genotype 1), Child-Pugh class A. Intrahepatic portal hypertension. Splenomegaly. Thrombocytopenia. Esophageal varices, grade 1. Vasculitis affecting the skin and kidneys (unconfirmed membranoproliferative glomerulonephritis). Secondary diagnosis: chronic pancreatitis, gallstones, chronic calculous cholecystitis. In 2009, the patient was invited to participate in an ongoing clinical study on transplantation of autologous MSCs from the bone marrow. The study design proposed an initial period of hospitalization in order to conduct a comprehensive examination and harvest MSCs from the bone marrow. Diagnostic tests and instrumental methods included a biochemical blood test with determination of total bilirubin, AST, ALT, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), creatinine, urea, and alpha-fetoprotein values; complete blood count and urinalysis; prothrombin time and INR tests; ultrasound examination of the abdominal organs; blood HCV RNA PCR test; and percutaneous liver biopsy with a complex morphological study of the biopsy specimen in order to establish possible regression of liver fibrosis. The above mentioned tests were subsequently used to monitor the patient's status in the post-transplant period. Rehospitalization was provided 1 month later for the purpose of transplanting MSCs into the liver tissue and to assess the safety and tolerability of the procedure. The purpose of the follow-up hospitalization 6 months after transplantation was to monitor the effectiveness and to assess the long-term effects of therapy.

In August 2009, the patient was admitted to the hospital of the Belarusian Research Center for Pediatric Oncology, Hematology and Immunology.

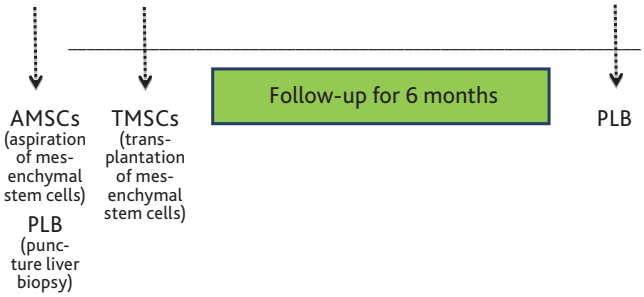

Figure 1. *Study design*

Note: AMSC — autologous of mesenchymal stem cells; PLB — puncture liver biopsy; TMSCs –transplantation of mesenchymal stem cells

Clinical examination confirmed an infection caused by the HCV genotype 1, with moderate biochemical disease activity at the stage of formed LC. The blood PCR test revealed the replication activity of the virus with HCV RNA value of 3.39×10^5 IU/ml. MELD score was 13. Signs of vasculitis were recorded: vascular purpura in the area of the shins, kidney injury (erythrocyturia), and arthralgias. Blood test for autoimmune diseases, including anti-mitochondrial M2 antibodies (AMA-M2), antinuclear antibodies (ANA), anti-liver/kidney microsomal antibodies type 1 (LKM-1), produced negative results. HIV infection and HBV infection were ruled out by the blood EIA test.

During the initial hospitalization, the bone marrow was harvested using several iliac bone punctures under local anesthesia in aseptic conditions. The MSC autograft was obtained from 35 ml of the patient's bone marrow; 242×10^6 MSC were isolated. Three passages were carried out afterwards to obtain a sufficient number of MSCs, and the duration of cell culture was 42 days. As a result of cell expansion *in vitro*, 113×10^6 MSCs were derived, which made it possible to obtain the MSC autograft at a mean dose of 1.8×10^6/kg of the patient's weight. The fact that it took 3 passages to derive this amount of cell mass indicates the high proliferative activity of the cells. The results of immunophenotypic analysis of expanded MSCs *in vitro* indicated that the CD90 antigen was present in 98 % of cells, the CD105 antigen was in 96 % of cells, and the CD44 antigen was in 98 % of cells. At the same time, the number of cells with antigens CD45 and CD34 on their surfaces, which are hemopoietic markers, was less than 1 %, and no cells expressing the CD14 antigen were identified. This indicated that the biotransplant for the infusion consisted of MSCs, and there was no contamination with myeloid cells. The viability of cells in the MSCs autograft was 99 %. A freshly prepared culture of MSCs was used for infusion; the preparation time for the transplant of MSCs was 2 hours prior to administration. Before the transplantation, the cells were tested for HCV RNA using the PCR method. The result was negative.

Morphological verification of the diagnosis was performed by percutaneous biopsy with the examination of the biopsy specimen concurrently with the bone marrow harvesting procedure. To exclude the limitations of a possible sampling error, a 20 mm tissue specimen containing 12 portal tracts was obtained with biopsy.

Initially, the biopsy specimen was assessed using light microscopy. There have already been discussions in the literature of specific features of the abnormalities in the liver tissue of patients with HCV-caused LC and extrahepatic manifestations of infection when using this method. In this study, we used a complex morphological approach, in which the immunohistochemical method was additionally introduced, that allowed assessment of the extent of the sinusoidal capillarization phenomenon (using CD34 expression) and the state of myofibroblast transdifferentiation (using expression of alpha smooth muscle actin), as well as electron microscopy together with the evaluation of non-parenchymal and parenchymal compartments of the liver.

The following symptoms were assessed in the biopsy specimens: fibrous septa, including their thickness and location; the presence and size of regenerative nodes; intralobular infiltrates; the nature and severity of liver cell damage. This allowed us to determine the depth of the structural changes that impact the disease course in a patient with systemic manifestations of HCV infection. The morphological changes revealed were subsequently used to specify the time-related changes of the disease 6 months after autologous transplantation of MSCs.

The results of a morphological study based on the light microscopy data obtained before the transplantation of MSCs showed the abnormalities typical of LC stage 4B according to the Laennec

fibrosis scoring system in the patient's liver specimens [2]. Damage of the hepatic lobular structure and formation of regenerative nodes were observed. Small regenerative nodes were observed in less than half of the tissue of the examined specimen. Growth of fibrous tissue presented as narrow and wide septa was discovered around regenerative nodes. The observed changes were indicative of an increased gradient of portal pressure and corresponded to the clinical signs of portal hypertension revealed in the patient. Inflammatory infiltrates were manifested irregularly with a predominance of lymphocytes penetrating deep into the lobules in some areas (Figures 2a, 2b).

The immunohistochemical study allowed us to detect moderate "capillarization" of sinusoids in the specimens along with significant transdifferentiation of Ito cells into myofibroblasts (Figures 3a and 3b.)

The data that were obtained in the study of the biopsy specimen using electron microscopy made a significant contribution to our understanding of the pathological process occurring in the liver. Advanced destructive abnormalities associated with destruction of the cytoplasmic membranes were observed in microvasculature. Aggressive lymphocytes were discovered. Strong immune reactions were revealed with deposition of material with increased electron density along the pathways of the sinusoidal capillaries, which confirmed and supplemented the data of the immunohistochemical study (Figures 4a and 4b).

Alteration of hepatocytes followed by their death through necrosis or apoptosis was discovered over the entire area of the biopsy specimen. As a rule, cell borders could not be detected. Fibrous structures (Figures 4c and 4d) formed in the cytoplasm of a large number of cells, probably representing fibrils

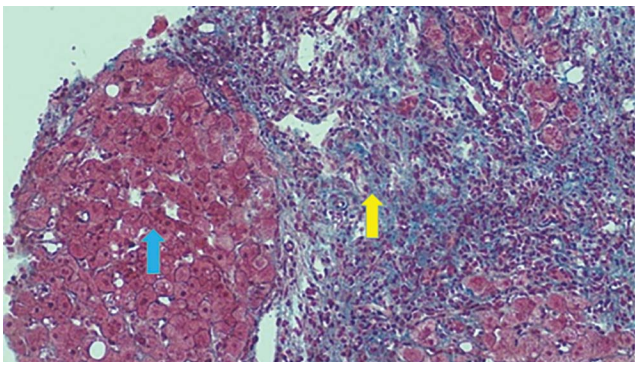

Figure 2a. *Formed micronodular cirrhosis. Regenerator node with proliferation of fibrous tissue (yellow arrow), false segment (blue arrow). Masson coloring. – ×126*

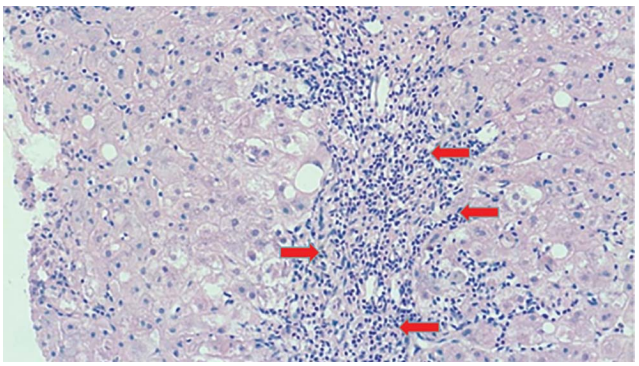

Figure 2в. *Inflammatory lympho-macrophage infiltration in fibrotic septa and periportal region (red arrows). Stained with hematoxylin-eosin. – ×63.*

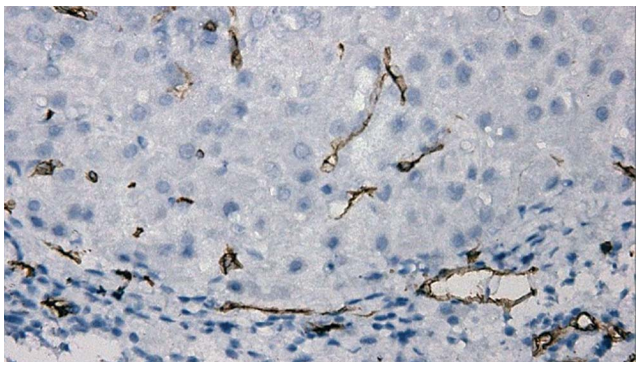

Figure 3a. *IHR (immunohistochemical reaction): CD34. × 400. Moderate capillarization of sinusoids*

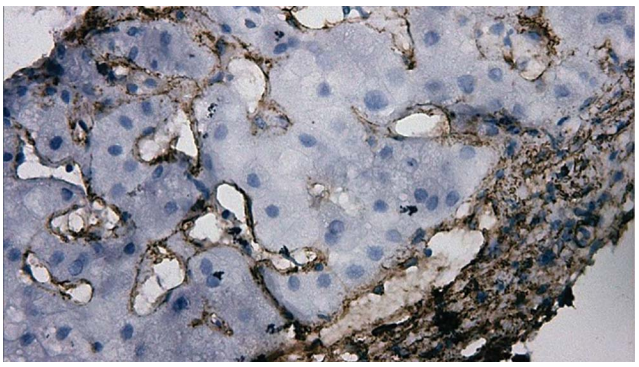

Figure 3в. *IHR (immunohistochemical reaction): α-SMA. × 400. The pronounced transdifferentiation of Ito cells into myofibroblasts*

of abnormal protein located in the cytoplasm of the hepatocytes without any surrounding membrane.

Significant destructive abnormalities were observed in the mitochondria (Mh), which was probably due to osmotic disequilibrium in the intermembrane space. Condensed and abnormally shaped mitochondria were discovered. Often, their condition was exacerbated by disintegration of the outer membrane and detachment of the inner membrane together with formation of bubbles and the destruction of the mitochondrial ultrastructure (Figure 4f). Expansion of intercristal spaces and fragmentation of Mh with the appearance of microclasmatosis were observed.

Previously, similar structural changes in the organelles that are associated with some pathological conditions have been described in the literature. According to a number of authors, the respiration and phosphorylation level drops as a result of these rearrangements, since the amount of endogenous ATP in condensed Mh is several times higher than in "typical" ones, and their ability to synthesize ATP for "export" is three times lower [1]. At the same time, swollen Mh with enlightened matrix (Figure 4g), which are characterized by a low level of energy supply, were found in the biopsy specimen. There were lysis of the cristae, homogenization of the matrix, and an increase in the number of large mitochondrial granules in these organelles. The latter is due to an impairment of the function responsible for the exchange of divalent cations, including Ca.

The agranular endoplasmic reticulum was characterized as sufficiently developed, and its profiles, typically, were significantly dilated. Isolated vacuolated membranes of rough endoplasmic reticulum (RER) were detected at some sites in the hepatocyte cytoplasm. Polymorphic residual bodies were discovered quite often. Bile-containing lysosomes were discovered in some hepatocytes, and local or total cytolysis was also recorded.
Numerous large transparent or fine-grained vacuoles were detected in the hepatocytes cytoplasm as well as in the lumens of sinusoidal capillaries. Large lipid inclusions were found in some hepatocytes.
Round nuclei in the surviving hepatocytes with fine-grained chromatin typically contained a compact nucleolus with a predominantly fibrillar component, which corresponded to their inactive state.

In October 2010, the patient underwent transplantation of MSCs into the liver tissue. The surgery and the postoperative period were uncomplicated. The patient was discharged from the hospital on the 8th day of the hospitalization. After that, the patient underwent outpatient monitoring.

The follow-up results demonstrated clinical improvement already during the 1st month after transplantation of MSCs: general weakness and manifestations of the dyspeptic syndrome disappeared, and appetite improved. There were no recent elements of the shin rash; brown spots were noted. They had developed due to hemosiderin deposition after the previously existing purpura had disappeared.

The patient was rehospitalized to evaluate the results of therapy 6 months after MSCs transplantation. The patient presented no complaints at the time of admission. Brown pigmentation was discovered on the skin of the lower limbs. The sclerae were icteric. Regional lymph nodes were not enlarged. Breathing sounds were vesicular. RR was 16 per minute. The heart sounds were regular and soft. HR was 80 bpm. BP was 130/80 mm Hg. The tongue was moist and white-coated at the tongue root. The abdomen was not enlarged, and it was soft and nontender. The liver protruded from the costal margin by 1 cm. The enlarged spleen was palpated. Stool was regular and had a normal color. Urination was comfort and painless, up to 5 times per day. Urine color was normal. There was a tendency for the platelet count to increase (from 38,000 to 49,000 units/μL) in the complete blood count. Other parameters of the complete blood count that did not change before the transplantation were within normal values throughout the entire post-transplant follow-up period. In the biochemical blood test, there was a tendency for the values of bilirubin (from 24 to 21.7 μmol/L), ALT (from 115 to 106 IU/mL), serum iron (from 32 to 17.9 μmol/L), and amylase (from 156.3 to 88 IU/mL) to decrease. Increased ALP levels persisted (276 U/L before and 280 U/L after transplantation). Albumin, cholesterol, urea, and creatinine values did not change

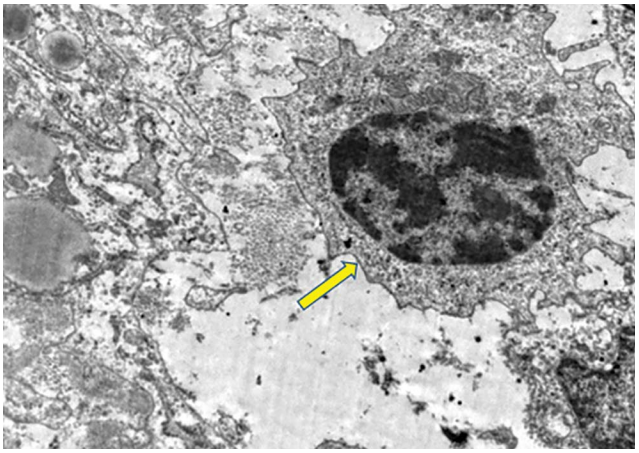

Figure 4a. *Aggressive lymphocyte in the lumen of the sinusoidal capillary (arrow). × 20 000*

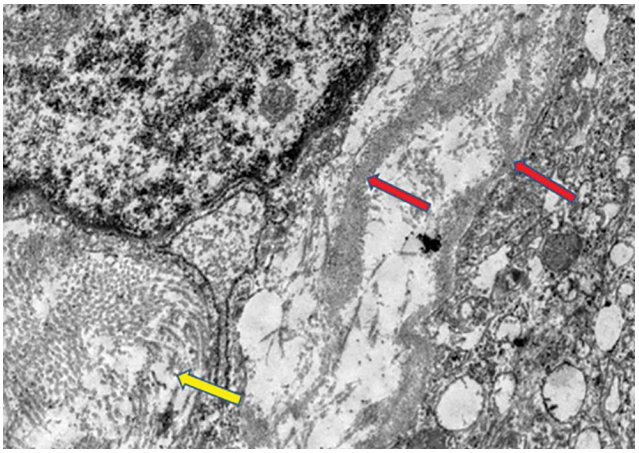

Figure 4в. *Immune reaction (red arrow) and a large bundle of collagen fiber fibrils in the pericapillary space (yellow arrow). – × 20 000*

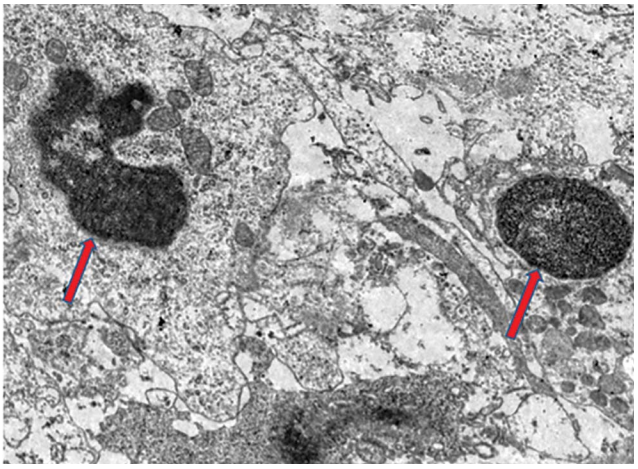

Figure 4c. *Apoptotic nuclei of hepatocytes (arrows). – × 15 000*

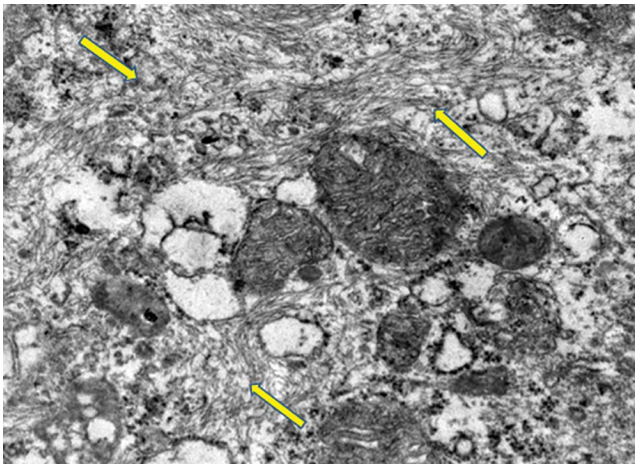

Figure 4d. *Fibrils of pathologically altered protein located in the cytoplasm of the hepatocyte without the membrane bounding them (yellow arrows). – × 20,000*

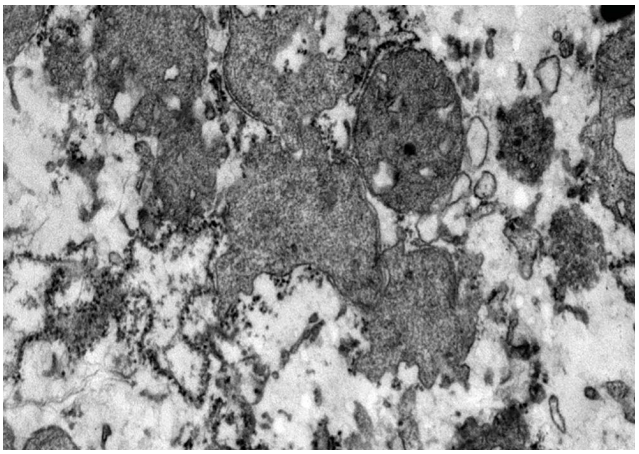

Figure 4f. *Destructively altered condensed mitochondria with loosening of the outer membrane and exfoliation of the inner membrane with the formation of bubbles. × 20 000*

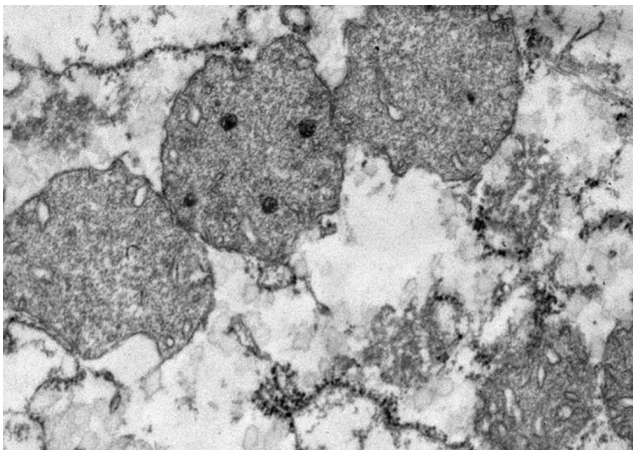

Figure 4g. *Swollen mitochondria with an enlightened matrix corresponding to a low-energy state. × 20 000*

and corresponded to normal values during the entire follow-up period. HCV RNA was detected in blood using the PCR method. Isolated erythrocytes were discovered in the urinalysis.

The signs of the formed micronodular LC with thin and thick septa persisted in the repeat percutaneous biopsy specimens of the liver that were assessed with light microscopy. However, in some parts of the examined specimens, the septa looked thinned, and sometimes perforated, indicating resorption of fibrous tissue at that site instead of its accumulation, which was previously described by a number of authors [23]. Populations of hepatocytes connecting at the sites of septal perforation in the biopsy specimen could be indirect evidence of this process. At the same time, the dilated sinusoids at the site of the connected regenerative nodes appeared to be parts of efferent microcirculation. Integrated parenchymal sites formed with thinning or perforation of the septa could contain several microcirculatory units, each of those being a former cirrhotic node.

Mild inflammatory lymph macrophage infiltration was observed in the fibrous septa and in periportal area (Figure 5a).

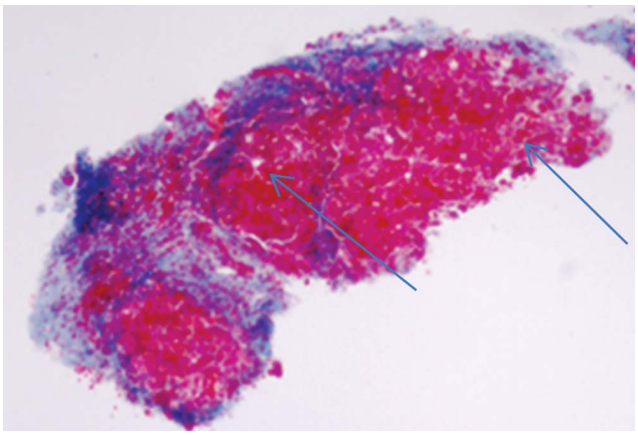

Figure 5a. *Formed micronodular cirrhosis. The cirrhotic node consists of several separate microcirculatory units, each of which, possibly, belonged to the former regenerative node. Painting according to the Mason.– ×32. Long arrows indicate the remnants of thin sept. The expanded sinusoids on both sides of the resorbed septum are parts of the efferent microcirculation*

An immunohistochemical study showed a decrease in intensity of transdifferentiation of stellate cells (HSC) into myofibroblasts while maintaining "capillarization" of sinusoids (Figures 6a and 6b).

Time-related changes of fibrotic abnormalities taking place in the liver tissue were also observed in the course of electron microscopic examination. Pericellular, pericapillary, and intracellular fibrous tissue was observed in the assessed biopsy specimens. Small areas of intralobular infiltration were discovered. Plasma cells, fibrocytes, and fibroblasts were detected occasionally.

A significant improvement was observed in the microvascular system after the transplantation of the MSCs. There were no immune reactions in the form of the deposition of amorphous and fibrous masses with moderate electron density along the

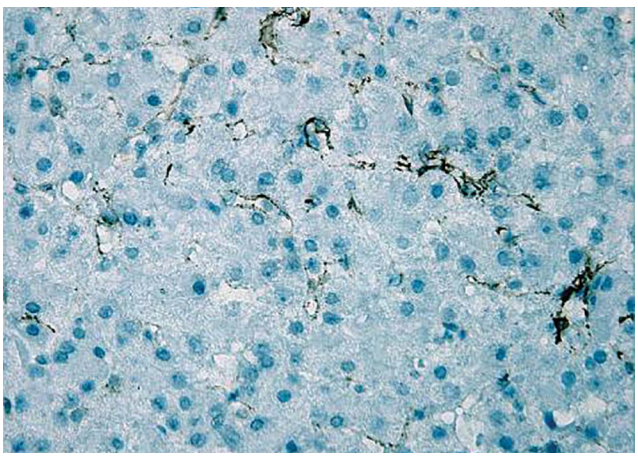

Figure 6a. *IHR (immunohistochemical reaction): α-SMA. × 400. Moderately expressed transdifferentiation of Ito cells into myofibroblasts*

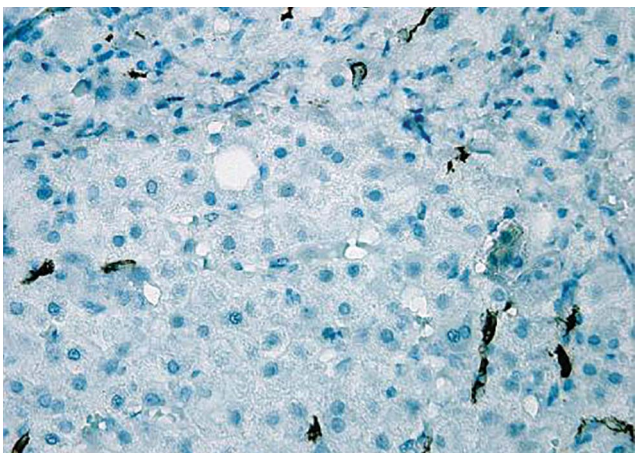

Figure 6в. *IHR (immunohistochemical reaction): CD34. × 400. Moderate capillarization of sinusoids*

sinusoidal capillaries ("capillarization of sinusoids"), which were significantly pronounced in the primary biopsy specimens. No large vacuoles occluding the lumen of the sinusoids were detected. This improvement was combined with the heterogeneity in the density of the cytoplasmic matrix of hepatocytes observed after transplantation, the state and quantity of organelles and inclusions (Figure 7a), and structural improvement of intracellular organelles. Thus, relative normalization of the mitochondrial ultrastructure was observed in the hepatocytes within most of the lobule. A large number of mitochondria were recorded in all hepatocytes. Numerous mitochondria did not have a matrix that was condensed, which would be indicative of a high-energy state (as observed in the primary biopsy), but rather had a moderate electron density, which is typical of an optimally energized status, with distinct cristae oriented predominantly across the long axis of the organelles in part of the cells (Figure 7b). Large intramitochondrial granules were detected in the matrix of the organelles, indicating a change in the exchange of divalent cations, mainly calcium. At the same time, there was no dilation or, especially, excessive dilation of the intercristal spaces with detachment of the inner membrane, as was observed in the primary biopsy. Mitochondria were mainly spread throughout the cells. Concentration of mitochondria around the nucleus was observed in some hepatocytes. Lysis of the cristae and homogenization of the matrix were discovered in the mitochondria of the hepatocytes within another part of the lobule, mainly in those where large lipid inclusions were detected. Mitochondria with a normal ultrastructure (to the left of the nucleus in the figure) and organelles with destructive abnormalities, up to the lysis of the cristae and homogenization of the matrix (to the right of the nucleus in the figure), were frequently discovered in one and the same hepatocyte (Figure 7c).

The morphometric analysis showed that the total mitochondrial area increased by 24 % in the repeated biopsy specimens, which correlated with an increase in the number of organelles by 20 % per slice area unit. However, the mean area of one mitochondria of the primary and repeated biopsy did not change (Table 1).

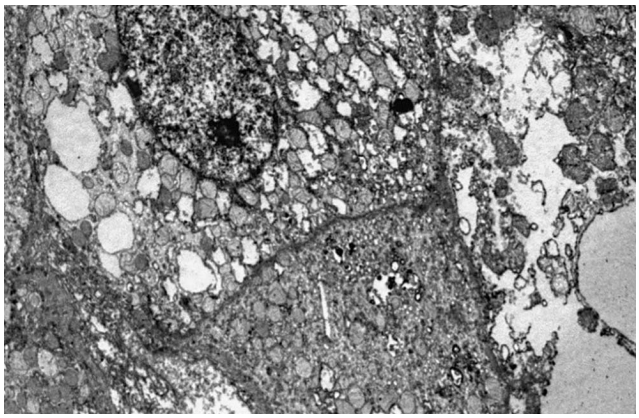

Figure 7a. *The expressed heterogeneity of hepatocytes in the number and degree of changes in the organelles and density of the cytoplasmic matrix. – × 5 000*

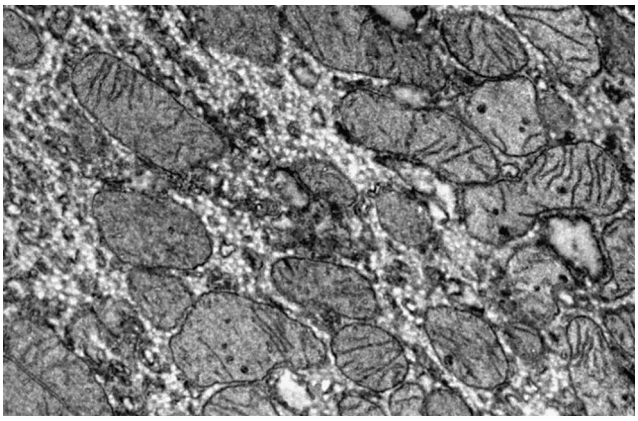

Figure 7в. *Numerous mitochondria characterized by polymorphism, a matrix of moderate electron density, distinct, crystals oriented predominantly across the long axis of the organelles, large intramitochondrial granules. – ×30 000*

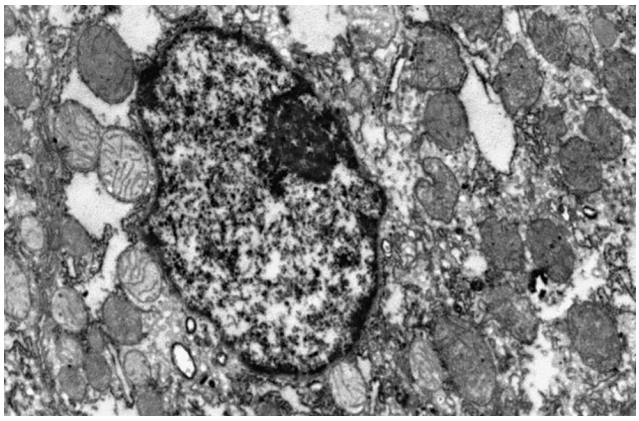

Figure 7c. *Heterogeneity of mitochondria in the hepatocyte. In the figure to the left of the nucleus is the mitochondria with normal ultrastructure, to the right – the lysis of the crista and the homogenization of the mitochondrial matrix. – ×12 000*

Table 1. *Comparative morphometric analysis of mitochondria of primary and repeated biopsy of patient T.*

Period	Area of mitochondria per 100 μm²	Amount of mitochondria per 100 μm²	The average area of one mitochondria μm²
Before transplantation	15,446	60,298	0,256
After transplantation	20,229	75,514	0,268

Single RER cisterns and numerous profiles of the SER being either polygonal or round in shape were identified between the mitochondria, which gave the cytoplasm a micro- or macrovacuolized appearance. The ultrastructural state of the nuclei indicated that they were in an inactive state.

No fibrous formations were detected in any hepatocyte in the repeated biopsy. However, they were detected in a large number of hepatocytes in the primary liver biopsy specimens. In addition, residual bodies were less common. There were no dying hepatocytes with cytoplasm replacement by fibrous structures, as was discovered in the primary biopsy specimens. There were no signs of occlusion of sinusoidal capillaries by large vacuoles, and no large vacuoles were detected in the hepatocytes cytoplasm as compared with the primary biopsy specimens.

Thus, the revealed morphological features indicated positive structural changes in the liver in response to the introduction of MSCs.

Our data are consistent with the commonly held opinion that dense fibrous tissue formed earlier in the process of restructuring in LC cannot undergo reverse development. At the same time, we demonstrated that the process of fibrogenesis is not static, and as a result of treatment of the liver tissue (in our case, with MSCs), the extracellular matrix may resolve and reduce the component of the sinusoids "capillarization" with simultaneous apoptosis of myofibroblasts, which play a key role in the process of fibrogenesis.

At the same time, we managed to demonstrate the positive effect of MSCs on the parenchymal compartment of the hepatic tissue. Structural changes were observed and interpreted as an improvement and even normalization of the mitochondrial ultrastructure, which changed the status of cells and resulted in their being placed in a more optimal energy and biosynthetic state. A decrease in regenerative processes as the result of MSCs was recorded in the hepatocytes, as indicated by the state of the nucleus and RER, which provided indirect evidence of the smaller scale of the damage caused by hepatocytes. At the same time, there were no cells that died with replacement of their cytoplasm by fibrous structures. The occlusion of the sinusoidal capillaries by large vacuoles was leveled.

Discussion

The process of fibrous tissue formation in the liver is a stereotyped response to damage, which is accompanied by the death of hepatocytes. The resulting changes are characterized by the interaction of many types of cells, including those that are resident and recruited into the liver (including bone marrow cells), which contributes to the development of inflammatory signaling pathways and, ultimately, leads to activation of normally resting HSC [6, 12, 14, 16, 21]. The latter are converted into myofibroblasts producing up to about 90 % of all extracellular matrix proteins in the liver [10, 12]. The interactions described above are dynamic and can contribute to both the development and regression of the fibrous tissue, controlling the activity of HSCs and content of the extracellular matrix in the liver tissue. Cell death, inflammation, and fibrosis are the key signs of the events that take place. Due to this observation, these have been proposed as the primary signals in the histological classification systems of Scheuer and Knodell [2, 8, 18]. In the case of acute liver damage, the described scenario of events subsequently leads to the restoration of the architectonics and functional status of the liver: (1) fibrosis ensures mechanical stability; (2) inflammatory cells contribute to the removal of cellular debris; (3) inflammatory signaling pathways play an important role in the development of liver regeneration [13, 19]. However, the same reactions become disadaptive under constant and prolonged exposure to the damaging factor, the continuing death of hepatocytes becomes uncontrolled and prolonged, which leads to chronic inflammation, the progression of the fibrosis, and the development of liver cirrhosis. Cirrhosis was considered irreversible for a long time. However, several

studies with morphological follow-up of biopsy specimens have demonstrated that fibrosis may decrease over time, even at the stage of cirrhosis [3, 9, 15].

We described a patient with LC and active HCV replication in the hepatic and extrahepatic sites, who underwent transplantation of MSCs into the liver tissue, and demonstrated partial improvement and regression of the pathological process in the parenchymal and non-parenchymal compartments. We selected several parameters for analysis, which were studied in more detail: sinusoidal fibrosis, septal fibrosis and the state of hepatocytes. Morphological study of the liver tissue using light microscopy demonstrated that the fibrotic septa separating the regeneration sites became thinner and fragmented six months after the transplantation of MSCs, and it was possible to recognize independently formed and topographically integrated microcirculation systems within the nodes located next to each other and separated by perforated septa. Regenerative nodes in cirrhosis are known to have a separate microcirculation system, and the changes we observed can indirectly indicate that the perforation of the septa followed the development of microcirculation. We also recorded partial resorption of fibrous tissue when studying the liver specimens through electron microscopy: there were no immune reactions in the form of deposition of amorphous and fibrous masses with moderate electron density along the sinusoidal capillaries ("capillarization of sinusoids"), which were significantly expressed in the primary biopsy specimens.

In this regard, it can be assumed that fibrolysis is more active in those parts of the liver where hepatocytes are not yet damaged or are only damaged slightly if the chronic inflammatory process in the liver persists together with LC. If hepatocytes die, the sinusoidal extracellular matrix appears to be able to combine with thin septa and then resolve [20]. At the same time, dense collagen septa that have formed in the course of cirrhosis and having undergone significant architectural restructuring cannot be resorbed completely and persist, which we observed in the case described here after transplantation.

On the contrary, the cirrhotic nodes in the course of fibrolysis can apparently grow larger due to their confluence at the time of lysis of thin septa (Figure 5a) and subsequently appear as large regeneration nodes surrounded by wide septa and areas where hepatic parenchyma have died. This hypothesis requires confirmation.

Another significant interference with complete reversion of cirrhosis is the presence of large regions of destroyed parenchyma [20]. In immunohistochemical studies of liver biopsy specimens, we observed the remaining foci of hepatocyte death, which were visualized as clearly restricted regions with sinusoids lined with CD34-positive endothelial cells (Figure 6b). These areas often contained arteries running separately and dilated sinusoids, which apparently indicated the presence of arteriovenous shunts. Sinusoids lined with CD34-positive endothelial cells may be equivalent to "capillarization", as described by Shaffner, Popper and others [18, 22], and persist as myofibroblast activity declines (Figure 6a).

At the same time, a number of authors described repopulation of damage foci as a result of activation of the so-called reparative complex consisting of proliferating ductules, hepatocytes surrounded with collagen, and CD34-positive sinusoids [20]. In the case of small damage foci, regeneration of hepatocytes occurs in the absence of an extracellular matrix in adjacent sinusoids, which is confirmed in our study by ultrastructural changes in the hepatocytes after transplantation of MSCs.

Our data are consistent with the common opinion that the dense fibrous tissue that formed previously cannot undergo reverse development in cirrhosis. At the same time, we demonstrated that the process of fibrogenesis is not static, and as a result of treatment of the liver tissue (in our case, with MSCs), the extracellular matrix may resolve and reduce the component of the sinusoids "capillarization" with simultaneous apoptosis of myofibroblasts, which play a key role in the process of fibrogenesis.

At the same time, we managed to demonstrate the positive effect of MSCs on the hepatocytes state. Structural changes were observed and interpreted as an improvement and even normalization of the mitochondrial ultrastructure, which changed the status of cells and resulted in their being placed in a more optimal energy state. A decline in

reparative processes under the influence of MSCs was recorded in the hepatocytes, as indicated by the state of the nucleus and RER, which provided indirect evidence that the hepatocytes were not as extensively damaged. At the same time, there were no cells that died with replacement of their cytoplasm by fibrous structures. The occlusion of the sinusoidal capillaries by large vacuoles was leveled.

Conclusions

1. The morphological characteristics of HCV-associated LC together with the time-related changes of fibrogenesis and fibrolysis processes do not depend on the predominant site of virus replication.
2. The transplantation of MSCs from the bone marrow is a promising method of compensating for chronic hepatocellular insufficiency, which contributes to the regression of fibrous tissue in the liver. The effectiveness of its impact depends on the initial severity of the disease and the reserve potential of the recipient liver.
3. The transplantation of MSCs reduces the degree of destructive abnormalities in the hepatocytes as well as the severity of the cirrhosis process, and it contributes to the improvement of the morphological and functional state of the liver. Therefore, it can be recommended as an important component of therapy.

Conflict of interests

The authors declare no conflict of interests.

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Article received on 15.11.2017
Accepted for publication on 09.01.2018