ISSN 2537-6373 (Print)
ISSN 2537-6381 (Online)

The Moldovan Medical Journal

The Publication of the Scientific Medical Association of Moldova

Frequency – 4 per year

Vol. 61, No 2
June 2018

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Index for subscription – 32130

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RESEARCH STUDIES

DOI: 10.5281/zenodo.1299008
UDC: 616.24-002.5:616.379-008.64

The predictors of pulmonary tuberculosis in Xpert MBT/Rif positive and resistant assay patients with diabetes mellitus

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Abstract

Background: One of the most important among risk factors for active tuberculosis development represents diabetes mellitus. The aim of the study was the assessment of the predictive factors for pulmonary tuberculosis in Xpert MBT/Rif resistant assay patients with diabetes mellitus.

Material and methods: A retrospective, selective, descriptive and case-control study was performed. Were enrolled 119 pulmonary drug resistant tuberculosis patients, diagnosed and hospitalized in the Municipal Clinical Hospital of Phthysiopneumology of Chisinau city in the period of 01.01.2013-01.01.2015. The patients were distributed in 2 groups: the 1st group – 34 MDR-TB patients with diabetes mellitus and the 2nd – 85 MDR-TB patients. Investigations were performed according to the National Clinical Protocol – 123.

Results: The biological characteristics of the pulmonary MDR-TB patients with diabetes mellitus were old age with associated diseases, which contributed to a lower treatment outcome. For MDR-TB groups were common social-economic vulnerability, late detection as symptomatic cases and a high treatment success rate.

Conclusions: Patients with MDR-TB and diabetes mellitus need an individualized approach for an early TB detection and prompt initiation of the adequate treatment regimen according of the susceptibility testing results.

Key words: tuberculosis, Xpert MBT/Rif, diabetes mellitus.

Introduction

Tuberculosis is one of the most important challenges for the health care system and was declared a global emergency in 1993 [45, 46, 47]. In 2017 were registered 9 million new cases globally. The Republic of Moldova ranked among 30 countries with the biggest burden of multidrug-resistant tuberculosis (MDR-TB), an estimated disease incidence of 101/100.000 [7]. Health indicators that evaluate the achievement of the sustainable development goals associated with tuberculosis are: HIV prevalence, diabetes prevalence, alcohol use disorders and tobacco smoking prevalence and health expenditure per capita. Diabetes mellitus is an important public health problem, one of four priority non-communicable diseases targeted by the development goals. Diabetes mellitus leads to complications and premature death. In the Republic of Moldova every 10th citizen suffers from disturbances of glucose metabolism and the indices are continuously rising. National capacity to prevent and control tuberculosis is reflected by the early case detection, especially of MDR-TB cases. The incidence of MDR-TB among new and relapsed cases was 19/100.000 and 64.6/100.000 among retreated cases. Patients with both types of diabetes mellitus represent one of the risk groups for tuberculosis and should be annually screened by the chest X-ray. The association of the diabetes mellitus and pulmonary tuberculosis usually occurs in patients where diabetes was the previous diagnosed disease [15]. If both, tuberculosis and diabetes mellitus are detected simultaneously, diabetes worsens the tuberculosis outcome. One half of patients with diabetes mellitus develop tuberculosis in the first three years after the exposure to the infection [5]. Factors associated with the increased risk for tuberculosis are: disturbances of the innate resistance, dysfunction of alveolar macrophages, low cellular immunity response to the specific and non-specific infections, and reduced capacity of the organism to produce antibodies, low levels of interferon gamma, microangyopathy (inclusive pulmonary) and micronutrient deficiency [35].

The first clinical signs of tuberculosis in patients with diabetes mellitus have a low specificity: increased weakness, decreased appetite, loss of the weight, and worsening of the diabetes symptoms [18]. The development of the chronic

forms of tuberculosis – fibro-cavernous type, occurs when the organism's defenses are depleted [29]. The evolution of tuberculosis in diabetes mellitus is unfavorable due to disturbances of glucose metabolism [35]. Late detection and late onset of the therapy, dietary errors and inadequate treatment represent the causes of the worsening of tuberculosis process under the specific treatment. In diabetic patients, blood sugar levels increase, diuresis and glucosuria increase, acidosis may appear, patients have the feeling of dry mouth, thirst, frequent urination and important weight loss [14, 15].

Antidiabetic therapy in tuberculosis patients should be individualized and depends on the patient's state, the tuberculosis extensibility and the severity of diabetes [27,40]. Each patient with diabetes mellitus must be hospitalized. First of all, it is necessary to compensate the metabolic disorders with a physiological diet and optimal doses of antidiabetic drugs. Anti-tuberculosis therapy should be administrated with caution due to high rate of adverse reactions [39].To prevent possible side effects patients must be strictly monitored.

Although, most of the diabetic patients are misdiagnosed regarding tuberculosis, several factors are involved: low specificity of the clinical signs and atypical radiological aspects. The relevant localization of the pulmonary tuberculosis is upper and posterior segments of the lungs: I, II, VI and X, while in diabetic patients tuberculosis is identified in segments III, IV and V. In patients with carbohydrate metabolism disorders predominate the inferior lobe involvement and may be revealed multiple cavities [18].

According to the WHO recommendation microbiological methods remain the golden standard for pulmonary tuberculosis diagnosis**.** Conventional microscopy for identification of acid-fast-bacilli is the first step in TB detection algorithm. The low sensibility of the conventional microscopy diminishes the detection efficiency of TB patients. The long duration of the culture methods delays TB diagnoses. WHO recommends using Xpert MTB/Rif assay in adults, children and persons living with HIV or other risk factors. Xpert MTB/Rif assay represents *in vitro* diagnostic medical device owned by Cepheid Company. Xpert MTB/Rif assay used with Cepheid Xpert MBT/Rif system is a semi-nested, quantitative, real-time polymerase chain reaction testing for the DNA detection of all *Mycobacterium tuberculosis (MBT) complex* species and rifampicin resistance mutations of the *rpoB* gene [23]. Several standard results must be known for appropriate interpretation of Xpert MBT/Rif system: 1. MTB detected & RIF resistance means that MTB target is present and mutation of *rpoB*gene is detected; 2. MTB detected & RIF susceptible means that MTB target is present and no mutation of *rpoB* gene has been detected; 3. MTB not detected – MTB target is not detected within the sample. Despite of clearly defined interpretations the test results must be always correlated with laboratory and clinical data of the investigated patient. Data established that sputum examination through Xpert MBT/Rif assay shows sensitivity among culture positive specimens in an average 97.3% and among smear positive patients – 99.5%. The specificity rate comparing with non-tuberculosis patients was 97.9% [31]. However negative result does not exclude active tuberculosis.

The aim of the study was the assessment of the predictive factors of pulmonary tuberculosis in Xpert MBT/Rif resistant assay patients with diabetes mellitus.

Material and methods

It was performed a retrospective, selective, descriptive and case-control study targeting peculiarities of pulmonary MDR-TB patients, diagnosed and hospitalized in the Municipal Clinical Hospital of Phthysiopneumology of Chisinau city in the period of 01.01.2014-01.01.2016, distributed in two groups. Including criteria in the 1st group: age > 18 years old; patients with pulmonary tuberculosis, established as a new case and diagnosed with diabetes mellitus before tuberculosis; positive and resistant Xpert MBT/Rif assay; including criteria in the 2nd group: age > 18 years old; patients with pulmonary tuberculosis established as a new case without associated diabetes mellitus; positive and resistant Xpert MBT/Rif assay. The total number of 119 cases was distributed in 2 groups: the 1st group (the 1st Group) included 34 patients and the second group (the 2nd Group) included 85 patients. Collection of primary material involved the extraction of data from medical record forms. The individual schedule included information about: anamnesis, clinical examination, results of radiological investigations (chest radiography, high resolution computer tomography), results of microbiological investigations (smear microscopy by Ziehl-Neelson coloration and culture on classic solid medium Lowenstein-Jensen or liquid medium). Investigations were performed according to the National Clinical Protocol – 123 Tuberculosis in adults. Statistical analysis methods used in the study were: comparative, synthesis and discriminant analysis. Mathematic and statistical assessment was carried out by checking the quantitative and qualitative features. Accumulated material was tabled in simple and complex groups. Statistical study was performed using Microsoft Excel XP soft. The predictibility value of each involved factor was calculated using two by two tables. Relative risk and confidence interval was calculated according to the established formula [7]. The interval of 1.2 to 1.6 was assessed as a low predictive factor, 1.6 to 2.4 – as a mild predictive factor, and more than 2.5 – as a high predictive factor.

Results

Assessing general, social and economical peculiarities it was established the statistical predominance of male *vs* female in both groups: 21 (61.8%) *vs* 13 (38.2%) in the 1st group and 61 (71.1%) *vs* 24 (28.3%) in the 2nd group. Comparing the groups it was established a moderate predomination of male in the 2nd group comparing with the 1st group, so male/female ratio=1.6/1 in the 1st group and 2.5/1 in the 2nd group. Assessing the patients according to the age groups it was established the statistical predominance of the young

patients (18-34 years) in the 2nd group 40 (47.1%) comparing with the 1st group 4 (11.8%) and older than 55 years in the 1st group 19 (55.8%) *vs* 15 (17.6%) in the 2nd group. Summing patients in two subgroups: under 44 and older than 44 years, it was identified a statistical difference between the predominance of patients less than 44 years in the 2nd group 52 (61.7%) *vs* 6 (17.6%) in the 1st group and older than 44 years in the 1st group 28 (82.3%) *vs* 25 (29.4%) patients in the 2nd group. Considering that old age represents the specific feature for the group with diabetes and MDR-TB it was assessed as a high risk factor for tuberculosis (OR=11,2 95% CI: 5.8-60). The data are presented in the table 1.

Table 1

Distribution in sex and age groups

Groups	Indices	MDR-TB&DM	MDR-TB	p
		n = 34 (P%)	n = 85 (P%)	
Sex	**Men**	21 (61.8%)	61 (71.1%)	>0.05
	Women	13 (38.2%)	24 (28.3%)	>0.05
18-44 years	**18-24**	0	12 (14.1%)	<0.05
	25-34	4 (11.8%)	28 (33.1%)	<0.05
	35-44	2 (5.8%)	12 (14.1%)	>0.05
>44 years	**45-54**	9 (26.5%)	10 (11.8%)	>0.05
	55-64	12 (35.2%)	12 (14.1%)	<0.05
	>65 years	7 (20.6%)	3 (3.5%)	<0.05

Among patients of both groups a similar distribution from rural and urban areas was established, however homeless were detected only in the 2nd group – 6 (7.1%) patients. Distribution of patients by economic groups was relevant. Due to the old age of the patients with TB-MDR and DM, employed persons statistically predominated in the 1st group 14 (41.2%) *vs* 21 (24.%) in the 2nd group. Socially vulnerable patients were more frequently registered in the TB-MDR group: 52 (61.7%) unemployed patients *vs* 8 (23%) in the 1st group. Low living conditions also predominated in the 2nd group 68 (80.1%) *vs* 21 (61.7%) in the 1st group due to high rate of unemployed patients. Retired patients predominated in the MDR-TB+DM group: 8 (23.5%) *vs* 8 (9.4%) in the 1st group; the same situation was determined for the persons with disabilities: 4 (11.8%) *vs* 7 (8.3%) patients, respectively (tab. 2).

Table 2

Main demographic, social and economical characteristics

Groups	Indices	MDR-TB&DM	MDR-TB	p
		n = 34 (P%)	n = 85 (P%)	
Demographic	Urban	17(50.1%)	41 (48.2%)	>0.05
	Rural	14(41.2%)	38 (44.7)	>0.05
	Homeless	0	6 (7.1%)	>0.05
Economic	Employed	14 (41.2%)	21 (24.7%)	>0.05
	Unemployed	8 (23.5%)	52 (61.7%)	<0.01
	Retired	8 (23.5%)	8 (9.4%)	>0.05
	Students	0	5 (5.9%)	>0.05
	Disease disability	4 (11.8%)	7 (8.3%)	>0.05
Life conditions	Low living conditions	21 (61.7%)	68 (80.1%)	<0.05

The social risk groups with epidemiological role were evaluated. Migrants constituted a similar part in both groups, ex-detained were identified only in the MDR-TB group (13 (15.3%) cases). Exposure to tuberculosis infection (TB contact) statistically predominated in the 1st group – 11 (52.9%) *vs* 25 (29.4%) in the 2nd group and was identified as a low risk factor (OR=1.1 CI 95% 0.5-2.7). All patients in the 1st group and half of the 2nd group had associated diseases. So, the co-morbitities were established as a high risk factor (OR=45; CI 95% 42-48) for developing tuberculosis. In 15 (44.1%) patients of the 1st group diabetes mellitus was diagnosed at the same time as tuberculosis. Regarding the harmful habits, active tobacco smoking and alcohol drinking statistically prevailed in the 2nd group. Tobacco smokers were 63 (74.1%) in the 1st group *vs* 13 (39.2%) in the 2nd group and alcohol abusers were 38 (44.7%) in the 1st group *vs* 7 (20.6%) cases in the 2nd group (tab. 3).

Table 3

Distribution in risk groups

High risk groups	Indices	MDR-TB&DM	MDR-TB	p
		n = 34 (P%)	n = 85 (P%)	
High risk groups	Migrants	6 (17.6%)	16 (18.8%)	>0.05
	Ex-detained	0	13 (15.3%)	<0.05
	TB contact	11(52.9%)	25 (29.4%)	<0.01
	HIV infection	1 (2.9%)	2(2.6%)	>0.05
	Associated diseases	34 (100%)	36 (42.4%)	<0.05
	Psychiatric disorders	1 (2.9%)	8(9.4%)	>0,05
Associated harmful habits	Tobacco smoking	13 (39.2%)	63 (74.1%)	<0.001
	Alcohol abusers	7 (20.6%)	38 (44.7%)	<0.05

By studing the civil status it was identified a statistical higher rate of married patients in the 1st group: 16 (47.1%) *vs* 15 (17.6%) patients in the 2nd group and of the divorced and widowed persons: 10 (29.4%) *vs* 22 (7.1%), respectively. Single persons predominated in the 2nd group due to young age of most of the patients. When assessing the educational level it was established that one half of both groups graduated general school. The incomplete general educational level was more frequently identified in the patients from the 2nd group: 24 (28.2%) *vs* 6 (17.6%) in the 1st group. Higher education level was established in a limited number of cases in both groups. No statistical differences were established between the groups of tuberculosis patients (tab. 4).

Table 4

Main social characteristics

Status	Indices	MDR-TB&DM	MDR-TB	P
		n = 34 (P%)	n = 85 (P%)	
Marital	Single	8 (23.5%)	48 (56.5%)	<0.01
	Married	16 (47.1%)	15 (17.6%)	<0.001
	Divorced/widowed	10 (29.4%)	22 (7.1%)	<0.05

Education	Primary/illiteracy	2 (5.7%)	8 (9.4%)	>0.05
	Incomplete secondary	6 (17.6%)	24 (28.2%)	>0.05
	General	16 (47.1%)	34 (40.1%)	>0.05
	Professional	6 (16.6%)	17 (20%)	>0.05
	Superior	4 (11.7%)	2 (2.4%)	>0.05

Studying case-management it was identified that most of the patients from the 1st group comparing with the 2nd group were detected by high risk group screening, as recommended by the national protocol: 22 (64.1%) *vs* 13 (15.3%), respectively. As to symptomatic cases the patients were detected more often in the 2nd group: 60 (70.6%) *vs* 12 (35.3%) in the 1st group. By direct addressing were detected 12 (14.2%) patients in the 2nd group. Microscopic smear positive results were established in more than two thirds of patients of both groups: 22 (64.1%) in the 1st group and 61 (71.6%) in the 2nd group. Positive culture results were 24 (70.6%) patients in the 1st group and 76 (89.4%) in the 1st group. Positive results of Xpert MTB/Rif assay were more frequently identified in patients from the 2nd group: 76 (89.4%) *vs* 26 (76.5%) in the 1st group (tab. 5).

Table 5

Case-finding detection, microbiological characteristics and treatment outcomes

Characteristics	Indices	MDR-TB&DM	MDR-TB	p
		n = 34 (P%)	n = 85 (P%)	
Case-finding	Detected as symptomatic case	12 (35.3%)	60 (70.6%)	<0.001
	Detected by active screening	22 (64.1%)	13 (15.3%)	<0.001
	Direct addressing to the specialized hospital	0	12 (14.2%)	<0.05
Microbiological positive results	Microscopy	22 (64.1%)	61 (71.6%)	>0.05
	Culture	24 (70.6%)	64 (75.3%)	>0.05
	Xpert MTB/Rif	26 (76.5%)	76 (89.4%)	>0.05

When identifying the radiological characteristics of pulmonary tuberculosis patients it was established lung parenchymal destruction in both groups: 25 (73.6%) in the 1st group and 61 (71.7%) cases in the 2nd group. Disseminated opacities were established at a similar rate in patients of both groups: 21 (61.7%) in the 1st group and 58 (68.2%) in the 2nd group. Both lungs were affected more frequently in patients of the 1st group: 24 (70.6%) *vs* 49 (57.6%) in the 2nd group, but the statistical threshold was not achieved. More than three affected segments had 65 (76.5%) patients in the 2nd group and 22 (64.7%) in the 1st group. Radiologic evolution under the specific treatment with second line antituberculosis drugs assessed as partial resorption was determined in a similar proportion in both groups: 28 (82.3%) in the 1st group and 76 (89.4%) in the 2nd group. Lung infiltrates progression was established in higher proportion in the 1st group: 7 (20.6%) *vs* 6 (7.1%) in the 2nd group, which contributed to the high rate of died patients. Although infiltrative TB form was diagnosed in the majority of patients, the severest forms such as disseminated TB and fibro-cavernous TB were diagnosed more frequently in the 1st group: 6 (17.6%) *vs* 9 (10.6%) in the 2nd group. Data are exposed in the table 6.

Table 6

Case-management characteristics and imagistic features

Characteristics	Indices	MDR-TB&DM	MDR-TB	p
		n = 34 (P%)	n = 85 (P%)	
Imagistic	Destruction	25 (73.6%)	61 (71.7%)	>0.05
	Dissemination	21 (61.7%)	58 (68.2%)	>0.05
	Both lungs	24 (70.6%)	49 (57.6%)	>0.05
	Extensive TB	22 (64.7%)	65 (76.5%)	>0.05
	Partial resorption	28 (82.3%)	67 (78.8%)	>0.05
	Progression	7 (20.6%)	6 (7.1%)	>0.05
Clinical radiological forms	Infiltrative	28 (82.3%)	76 (89.4%)	>0.05
	Disseminated	4 (11.7%)	8 (9.4%)	>0.05
	Fibro-cavernous	2 (5.9%)	1 (1.2%)	>0.05

Treatment outcome was assessed using the standardized indices. The success rate was lower than recommended by WHO (85%) in both groups. The lowest success rate was registered in the 1st group: 20 (58.8%) *vs* the 2nd group 66 (77.6%). Poor outcomes predominated in the 1st group: 14 (41.2%) *vs* the 2nd group 11 (12.9%). The highest rate of died patients was identified in the 1st group: 6 (17.5%) comparing with the 2nd group 3 (3.5%) (tab. 7).

Table 7

Treatment outcome types

Outcome	MDR-TB&DM	MDR-TB	p
	n = 34 (P%)	n = 85 (P%)	
Successfully treated	20 (58.8%)	66 (77.6%)	<0.05
Died	6 (17.5%)	3 (3.5%)	>0.05
Lost to follow-up	5 (14.7%)	8 (9.4%)	>0.05
Failure	3 (8.8%)	5 (5.8%)	>0.05

Considering all above exposed data it was established that the most relevant general and biological characteristics of the pulmonary MDR-TB patients associated with diabetes mellitus were old age and comorbid state. They were more frequently divorced or widowed and were detected by active screening according to the national recommendations (tab. 8).

The most relevant social-economic characteristics of the pulmonary MDR-TB patients were economical disadvantaged state, low living conditions, single civil state and life history of imprisonment. They were more frequently detec-

Table 8

Odds Ratio assessing factors associated with diabetes mellitus and MDR-TB in patients with Xpert MBT/Rif resistant results

Factors	Odds Ratio
Old age (more than 55)	11.2 (95% CI: 5.8-60), p<0.001
Comorbid state	45 (95% CI: 42-48), p<0.001
Detected by active screening	11.2 (95% CI:11.7-11.4),p<0.05
Divorced or widowed	1. (95% CI: 1.16-1.3), p<0.05

ted as to symptomatic cases and were directly addressedto the specialised hospital. Despite the low social-economic state and late detection associated with the passive way of detection they had a high treatment success, demonstrating the strong impact on the disease outcome of the diabetes mellitus (tab. 9).

Table 9

Odds Ratio assessing factors associated with MDR-TB in patients with Xpert MBT/Rif resistant results

Factors	Relative Risk
History of imprisonment	5.6 (95% CI: 5.3-5.9), p<0.05
Economicaly disadvantaged state (unemployment)	5.12 (95% CI: 2.9-13.6), p<0.001
Low living conditions	2.5 (95% CI: 1.5-3.8), p<0.001
Single-civil state	3.9 (95% CI: 3.7-4.1), p<0.01
Detected as symptomatic case	4.2 (95% CI: 4.1-4.3), p<0.001
Detected by addressing to the specialized hospital	5.3 (95% CI: 4.9-5.7), p<0.05
Successfully treated	1.3 (95% CI: 1.25-1.37), p<0.05

Discussion

Association of tuberculosis and diabetes represents an epidemiological challenge and important problem for the health system in the Republic of Moldova. It was established that the tuberculosis prevalence rate among patients with diabetes is 1.8–9.5 times higher than in the general population [48].

In the Republic of Moldova 12.3% of the population have diabetes or reduced tolerance to glucose and 409 patients died due to diabetic complications in 2015 [7]. Since the tuberculosis incidence in the Republic of Moldova slowly decreased, the rate of MDR-TB increased. MDR-TB represents another serious threat to the global disease control. In clinical study was established a strong association between the risk factors and MDR-TB. There are several risk factors which increase the risk for MDR-TB in patients with diabetes: previous treatment, young age, HIV associated infection, smoking, alcohol and other substances abuse [14]. Some clinical studies denoted a high rate (10-23%) of MDR-TB among patients with diabetes [15, 20, 40, 42]. Other cited factors were: HIV co-infection, age older than 45, overweight, and male sex [13, 20].

If tuberculosis is detected earlier a more favorable outcome can be achieved. A severe course of tuberculosis with a tendency to the rapid progression and lung parenchyma destruction occurs mainly in patients with untreated diabetes mellitus or in late detected tuberculosis [6].

Our study demonstrated a strong influence of diabetes on tuberculosis outcome. Obtained results were similar to other studies, which determined a high rate of failure and death among patients with tuberculosis and diabetes [5, 10, 24, 29, 38]. Poor treatment outcomes could be explained by the co-morbidities such as diabetes, HIV infection, and social determinants of health (unemployment, educational level, income distribution, social vulnerability, health services accessibility) [10]. Nowadays, in the Republic of Moldova the global prevalence of tuberculosis among patients with diabetes is high and reflects the general epidemiological situation.

Conclusions

The treatment success rate among patients with drug resistant tuberculosis and diabetes was low due to following contributing factors: old age and comorbid state.

More frequently patients with drug resistant tuberculosis and diabetes were detected by active screening and had a civil unfavorable state (divorced and widowed), associated with old age.

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DOI: 10.5281/zenodo.1299010
UDC: 616.12-008.331.1-084:613.291:641.881

Informing national salt reduction strategy

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Abstract

Background: Excessive salt intake is associated with noncommunicable diseases, especially with hypertension, cardiovascular disease and stroke. Implementation of comprehensive and effective strategies can reduce salt intake and related disease burden. The aim of the present research was to analyse the current situation and existing evidence to inform national salt reduction strategy.
Material and methods: An analysis of current status of national salt reduction initiatives and hypertension prevention, as well as of scientific publications and governmental websites was done to identify the most effective interventions applicable in the Republic of Moldova.
Results: Four out of 10 individuals have raised blood pressure in the Republic of Moldova and the prevalence of hypertension and hypertension linked disease has tripled over the last 15 years. There is a strong link between salt consumption and hypertension. Currently, salt consumption in almost every country is too high. Three out of nine targets on noncommunicable disease prevention and control, which were internationally and nationally assumed, provide the baseline to reduce the salt intake in the Republic of Moldova. The main strategies applied in different combinations at the international level to reduce salt intake are regulations, food reformulation, food labelling, colour coding based on salt content, taxation and education campaigns.
Conclusions: The biggest reduction in salt consumption can be achieved by comprehensive multicomponent strategy involving legal measures, mandatory reformulation, nutritional labelling, efficient enforcement and a good leadership.

Key words: cardiovascular diseases, hypertension, health policy, salt reduction strategy.

Introduction

Worldwide, cardiovascular disease (CVD) is the main cause of death. Hypertension is one of the main risk factor for CVD and stroke, and other noncommunicable diseases (NCDs) [1]. Prevalence of hypertension continues to go up, the growth being more pronounced in low and middle-income countries compared to high income countries. A "diet high in sodium" is the second most important dietary risk factor for health loss identified in the Global Burden of Disease Study 2013 [2]. Salt consumption is strongly linked with high blood pressure and the reduction in salt intake can reduce the level of blood pressure and total cardiovascular risk [3, 4, 5]. Currently, salt consumption in almost every country is too high. It is estimated that suboptimal systolic blood pressure (>115 mmHg) contributes to the development of 49% of total cardiovascular ischemic heart disease and 62% of stroke [6]. Reduction of salt consumption from the actual 9-12 grams per day, the actual worldwide mean consumption to a 5 gram per day, the World Health Organization (WHO) recommended level, is estimated to contribute to a 23% reduction in stroke and 17% in CVD, preventing annually 4 million of deaths at the global level [7, 8]. The disease burden associated with hypertension increased substantially at the global level during 1990-2015. The number of disability adjusted life years (DALY) associated with systolic blood pressure ≥140 mmHg increased during this period from 5.2 million to 7.8 million. Most of the systolic blood pressure related deaths are due to ischemic heart disease (4.9 mil., 54.5%), haemorrhagic stroke (2.0 mil., 58.3%) and ischemic stroke (1.5 mil., 50.0%) [9].

Treatment of hypertension and its complications involves high health care costs, work place absenteeism and income loss, imporoverishng families and contributing to poverty perpetuation. At the same time, hypertension can be prevented by modestly investing in prevention. Systematic analysis of randomized clinical trials concluded that sodium reduction interventions applied at population level contribute to the reduction in blood pressure among adults with or without hypertension [2, 10, 11].

World leaders become aware of the burden of CVD, one of the four main NCDs and committed to fight against it. WHO member-states adopted in 2013 nine voluntary targets on NCDs and their risk factors reduction to be met by 2025 [12]. A 25% relative reduction in premature death due to NCDs (CVD, cancer, diabetes and chronic obstructive pulmonary disease) is dependent on the meeting other two interrelated targets: a 30% relative reduction in sodium/salt consumption and 25% relative reduction in raised blood pressure.

WHO recommended reduction in salt consumption as well as of salt content in food. In its report on global situation of noncommunicable diseases 2010, WHO mentioned the sufficient evidence that salt reduction strategies are the best-buys, recognizing that this is the most cost-effective and feasible approach to prevent NCDs [1, 13].

World states leaders become aware of the importance of reduction of hypertension and high mortality caused by

it, implementing complex strategies to reduce salt intake. The research done supports salt reduction. Evaluation of salt reduction strategies implemented in Argentina, Great Britain, Ireland, Finland, Hungary, Japan, Turkey and other countries worldwide, showed its feasibility and contribution to the salt reduction at population level, and implicitly the reduction of mean blood pressure and of its consequences for health [14, 15, 16, 17].

In the Republic of Moldova, CVD death accounts for more than 50% of total deaths, and prevalence of hypertension is increasing [18]. The Government of the Republic of Moldova is aware of the great burden imposed by high blood pressure as the main risk factor for CVD and committed to reduce salt intake. The national food and nutrition programme and its action plan provide for the development and implementation of a salt reduction strategy in order to achieve the three interrelated NCDs targets, assumed nationally and internationally [19, 20].

We analysed the national hypertension trends and current salt reduction initiatives and reviewed the existent evidence to inform the national salt reduction strategy.

Material and methods

Relevant publications have been identified by electronic search in databases related to medical, public health and social sciences, health policy, nutrition, WHO regional databases. Electronic search included governmental webpages belonging to the ministries of health and national public health institutions, European Commission, with the aim to identify national salt reduction policies or additional initiatives. The study includes also a documentary policy analysis. National policy documents were retrieved from official government webpages. Search has been done on the webpages, of National Register of Normative Acts, Ministry of Health, Labour and Social Protection, National Center of Public Health, National Center of Health Management and other sources.

To resume the countries and their national experience, key words have been used, such as hypertension, salt, salt reduction, salt reduction strategies, successful salt reduction strategies and others in order to collect all the articles, policy documents and other available useful documents published within country and outside.

Results

Hypertension and salt consumption in the Republic of Moldova

Noncommunicable diseases are responsible for more than 80% of deaths annually and about half of them are caused by the diseases of circulatory system [18]. In 2015, the unconditional probability of death between the age of 30 and 70 years due to NCDs was 31%, being the highest in the world. Cardiovascular mortality at the working age has increased slightly (+3.3%) over the last fifteen years (2003-2016). At the same time, cardiovascular mortality increased significantly (+39.6%) in younger population group (18-39 years) and particularly among men (49.1%) and less among women (+14.3%) [21].

The incidence of hypertensive disease, ischemic heart disease with hypertensive disease and cerebrovascular disease with hypertension (hypertension and hypertension linked diseases) was 120.6 per 10 000 population (2016), accounting for about 70% of the total incidence of circulatory system disease. Over the last fifteen years, a slight decline in incidence has been reported. The prevalence of hypertension and hypertension linked diseases has steadily increased, tripling between 2004 (512.1 per 10 000 population) and 2016 (1426.4 per 10 000 population) [18].

A national survey on NCDs risk factors, STEPS, conducted in 2013 revealed that four out of ten adults had raised blood pressure (≥140/90 mmHg) with no significant difference between the sexes. More than three quarters (76.2%) of those with hypertension did not take medication to control their blood pressure, and other 19.7% took medication but had increased blood pressure (≥140/90 mmHg) [22]. Raised blood pressure is ranking the second within the disease burden attributable to the main risk factors and has contributed to the loss of 18.7% of the total DALY (disability adjusted life years) in 2015 [23].

Salt consumption is the main contributor to the raised blood pressure. Some of traditional food habits of the population are less healthy, and specifically consumption of salty foods, such as pickles, sheep cheese, salty spices prepared in the household and salt added at the table, before or while eating. A quarter of adult population is adding salt always or often to their meal [22]. With globalization and international trade, a broad spectrum of salty foods such as salty snacks, sauces, processed meat, fish and dairy products has been added to the traditional food consumption. A third of population (32.4%) is often consuming processed food high in salt [22]. The Republic of Moldova imports all food grade salt and estimation shows that the mean consumption in Moldovan's population is about 11-13 g of salt per day. Generally, population (nine out of 10 individuals) is aware that high salt intake can cause serious health problems, and three quarters (75.1%) consider that reduction of salt consumption is very important for health. At the same time, almost the same proportion of adult population (72.9%) think they are consuming 'just the right amount' and only 15.0% perceive they are consuming far too much or too much salt [22].

Excessive salt intake increases the hypertension levels and the risk for stroke and CVD, in conditions of low potassium intake due to low consumption of fruit and vegetables. Only a third of adult population of the Republic of Moldova is consuming adequate quantities of fruits and vegetables per day (five portions and more) in the period of the presumed highest availability, autumn season [22].

National efforts to reduce salt intake and prevent hypertension

Ministry of Health, Labour and Social Protection has been aware of the high burden of NCDs risk factors and has decided on prevention and control measures. Some prevention measures are already provided at the individual level. Measurement of blood pressure in adult population is done

annually starting from 2008 within the primary health care institutions. Less than one in ten individuals reported in 2013 that their blood pressure had never been measured [22]. Two clinical protocols for family doctors have been developed, on global cardiovascular risk evaluation and global cardiovascular risk management, and are being implemented from 2011 onward [24, 25]. Primary health care professionals provide lifestyle advice, including for salt reduction. A total of 57.8% of adult population has been advised to reduce salt in their diet [22]. Antihypertensive medication is partially compensated within the healthcare insurance. Prevention of hypertension is part of the performance indicators, applied as criteria for bonification within the primary health care [26].

In line with the international recommendations, the Government adopted the National Strategy on Prevention and Control of Noncommunicable Diseases 2012-2020 and its Action Plan [20]. Three out of nine targets that the Government is committed to meet by 2020 are directly related to the relative reduction of salt intake, hypertension and mortality [20]. Relative reduction by 30% of salt consumption by 2020 is a component of the objective # 4 of the National Food and Nutrition Programme [19].

Food legislation was recently amended and modified, providing for the mandatory display of the salt content on the main area of the packaged food and the provisions will come into force in January 2019 [27]. Preparation, selling and distribution of unhealthy food, including high in salt was prohibited within the school and the perimeter of 100 metres starting from 2012 [28]. A general message on salt reduction has been running for several years on television channels.

It should be mentioned that iodine deficiency is also a public health problem in the Republic of Moldova and the Government decided to eliminate iodine deficiency disorders through salt iodisation, intervention that is continuously implemented since 1998.

National Public Health Agency is responsible for the surveillance of population health, including risk factors, and for the development and implementation of public health measures to reduce the disease burden. A national salt survey was conducted in 2016 to establish the baseline salt consumption based on urinary sodium excretion. The survey report will be published soon.

Salt reduction measures implemented at the individual level can contribute to the reduction in salt intake and, irrespectively of hypertension, but the most efficient are complex measures implemented at the population level. For this reason and based on the analysis of the existing salt reduction initiatives using population-based strategy, applied in different countries, we are proposing some considerations for the national salt reduction strategy.

Efficiency and effectiveness of salt reduction strategies

The salt reduction strategy is one comprehensive involving multiple components and population-wide interventions. It can comprise, totally or partially the following types of actions: food reformulation to reduce the salt content; limit of the salt content in food purchased for use in public institutions; nutritional labelling on the main area of the packaging and use of symbols and warnings to identify high salt foodstuffs; applying of taxes; and consumer's education.

Food reformulation to reduce salt content. Most of the identified strategies and interventions on salt reduction are those applied in developed countries, although during the last years the number of developing countries applying such strategies is increasing. Australia, Canada, Finland, France, Ireland, Japan, Great Britain and US have reduced the salt consumption at population level applying voluntary reformulation strategies [13, 14, 15, 29]. By reformulation we mean actions to change the composition of processed food with the goal to obtain a healthy food. Foodstuffs reformulation by limiting some unhealthy components such as salt/sodium is a public health action applicable at the population level with the aim to promote health and prevent disease [13, 30]. Process consists of gradually implemented reformulation with progressive reduction of salt/sodium content. Reformulation involves the establishment of targets that should be achieved by type or category of food, willingness and public engagement of the food industry and its participation, public health authorities working with industry to convince it to participate, as well as monitoring and evaluation [14, 15, 29]. However, the shortcoming is that a voluntary initiative cannot oblige the food industry to participate and therefore the reduction in salt can be limited to the number of participating industry as is the case of Australia or Argentina [14, 31]. Industry engagement, although publicised and monitored, is not mandatory [15, 29].

Japan initiated a comprehensive salt reduction campaign in the 1960s. This contributed to the subsequently decreasing in salt intake from 14.5 g per day in 1972 to 10.6 g per day in 2010, a fall of almost 4 g per day. Stroke mortality was predicted to fall by 80% [17].

Great Britain launched in 2003 an initiative on foodstuffs reformulation with the aim to reduce salt intake. The initiative was launched in cooperation with the food industry. Targets on salt reduction have been established for 85 types of food [29]. Concomitantly, Food Standard Agency developed a traffic light labelling system, to support consumers to make a healthy choice and launched consumers' information and awareness campaigns on health problems caused by excessive salt intake.

Monitoring and evaluation done in Great Britain have established a mean reduction in salt consumption from 9.5 g per day to about 8.1 g per day between 2001 and 2011 [30]. As a result of this change of the diet, during the abovementioned period of time the incidence of stroke (42%) and ischemic heart disease (40%) significantly decreased [29]. Great Britain model has been mentioned as a successful government initiative, being recommended for other countries [14].

In Australia, food reformulation was decided in 2009 by the establishment of an official public-private dialogue between governmental health institutions and food industry. Subsequently, dialogue parties convened to establish food

groups that should be reformulated, targets for salt reduction, as well as the timeframe for reformulation. The industry was to report annually on the progress of engagements assumed in the frame of dialogue [14]. Although the salt content has been reduced in some foods, the overall goal to reduce the salt intake has not been met. The voluntary character of participation in the dialogue had as a consequence nonparticipation of all food production companies and the efforts of the participants to the reformulation have been significantly different. Thus, many of the foods remained outside the scope of the reformulation initiative. Unlike Great Britain, reformulation efforts have not been supported by a communication and education campaign for consumer. Among significant limits of the initiative implemented in Australia, was the lack of monitoring of change in salt content of foodstuffs, subject to reformulation by responsible governmental authorities, as well as lack of transparency and responsibility, and non-disclosure of industry reports on the progress in meeting the targets. Therefore, this public-private dialogue concerning the reduction of NCDs burden failed [32].

Modelling research on health benefit of elimination of 15-25% of sodium from processed food concluded that between 5800 and 9700 cases of myocardial infarction and between 4900 and 8200 of stroke can be prevented in Australia in a 10-year timeframe period, concomitantly preventing between 2000 and 3400 deaths [33]. Australian National Heart Foundation estimated that mandatory reformulation and reducing of sodium content in bread and other foodstuffs to established criteria can prevent 18% of disease burden associated with excessive salt intake [14].

Argentina created in 2010 the National Sodium Reduction Commission on the initiative of the Ministry of Health. Ministry of Agriculture collaborated with the Ministry of Health and led the communication activities both with food industry and general population. National Institute for Industrial Technology provided technical assistance to small and medium enterprises to set baseline sodium levels and reduction targets. National Food Authority led the design and implementation of the monitoring strategy. An agreement on reducing sodium content in foods has been signed between Ministry of Health and Ministry of Agriculture on the one hand and the Association of Food Industry on the other hand. No penalties were mentioned in the agreement [31].

Only larger company actively participated in this initiative with small and medium enterprises declining their participation. This voluntary reformulation was the first step before introducing a regulation in 2014. The regulation includes maximum levels similar to the values set in the voluntary agreement for the main food groups. The food industry saw the agreement as the first and necessary step toward legislation [31]. Health authorities in Croatia, Belgium, Austria, Check Republic, France, Israel and Italy initiated in 2009-2011 strategies to reduce salt intake through voluntary agreements with food industry [34]. Analysis of countries' experience on strategies of voluntary reformulation shows that authorities' monitoring and evaluation on meeting of the assumed engagements as well as ensuring transparency are essential to reduce salt consumption. Auto-regulation involves an independent monitoring and evaluation of 'clearly defined, quantifiable targets, with a clear timeframe of implementation and with clearly specified start point for comparison [35]. In the absence of consequences for non-performance or non-participation, few levers exist through which enterprises can be obliged to meet voluntarily targets [29]. Strengthening performance of voluntary initiative on salt intake reduction can significantly reduce the CVD burden, as it has shown the experience of Great Britain and other countries where these programs are operational. But, voluntary strategy does not address social determinants of health, and as it is seen in Australia or Argentina and other countries, it is not sustainable.

In order to reduce NCDs burden many countries have adopted a legislative approach to reduce salt intake. Most countries, especially in Europe (Belgium, Bulgaria, Greece, Hungary, Holland, Portugal, Turkey and Slovak Republic) established the maximum limits for salt or sodium content in bread. Besides the bread the salt/sodium content has been regulated in meat products, cheese and other staple foods (Bulgaria, Hungary, Turkey, Greece and Argentina) [15, 32, 35]. Modelling studies show that legislation regulating the salt content of foodstuffs is efficient and cost-effective, especially when combined with complementary strategies, such as health promotion through mass-media [17]. Mandatory reformulation could consistently achieve bigger salt reductions than voluntary reformulation; 1.6 g per day compared with 1.2 g per day; and 1.4 g per day versus 0.5 g per day. Mandatory reformulation might also achieve higher reductions in disability-adjusted life years (DALYs) and quality adjusted life years (QALYs) compared to voluntary reformulation [17].

Regulation of salt/sodium content in foodstuffs purchased by public institutions. Within the frame of the initiative to reduce salt intake at the level of European Region many of the member-states adopted and implemented special nutritional policies, aimed to promote a healthy environment within the settings financed by public budget. Thus, Bulgaria, Latvia, Greece, Finland and Romania have regulated the sodium/salt content in foodstuffs intended for use in kindergartens, schools and hospitals, in accordance with healthy eating regulations adopted for these settings [8, 15].

Consumer's information by labelling. The system of presentation of nutritional information through labelling of foodstuffs is used in many countries, like Canada, US, Israel, Great Britain, EU member-states and others. In some of these countries, like Israel, Great Britain and EU member-states, part of nutritional information, including salt content, should be displayed on the main area of the packaging. The Moldova's food labelling law that will come into force since 2019 contains similar provisions [27]. However, research done by the US Centres for Disease Control and Institute of Medicine concluded that presentation of nutritional information on the main area of packaging does not offer clear guidelines on how healthy the food is. Research-

ers proposed to evaluate foodstuffs using a scoring system that will encourage food producers to develop more healthy foods and consumers to find healthier food faster and easier, when shopping [36].

Countries like Great Britain and Australia have more experience in implementing the nutritional labelling system that uses a colour code classification system. Starting from 2006, Great Britain uses a traffic light labelling indicator system that presents the content of fat, sugar and salt and specifies the rate at which the food should be consumed. Thus, red colour (raised) indicates that food should be consumed with caution, yellow (medium) that the food should be consumed, being aware of raised health risk and green (reduced) that these nutrients do not contribute to any risks when consuming standard portions of irrespective foods [29].

France introduced recently a five colours nutritional information system on the main area of the packaging. Nutri-Score system was developed based on traffic light system, used in Great Britain and adapted to the French context. They use a nutritional profile system that classifies foods and beverages in five categories accordingly the nutritional quality, specified by a colour scale varying from green (class A) to red (class E) [37].

A legally imposed warning on food high in salt in Finland stimulated the food industry to reformulate foods. A Finish study reported a reduction by 40% of salt food intake during the last 30 years [8].

Using price policy. The strategy of price manipulation (through application of taxes to reduce consumption or subsidies to increase it) is one that is successfully applied to reduce the health risk factors. Tobacco and alcohol are two products for reduction of which the strategy of price increasing has proven to be the most cost-effective intervention, especially in low and middle-income countries. It has been demonstrated, that food consumption correlates with food prices and directly influences health parameters [38]. Fiji, Portugal and Hungary are currently countries that apply taxation of food high in salt [39].

'Product public health tax' is applied in Hungary since 2011 and an evaluation done after three years of its introduction reported a reduction in sales and consumption of salty snacks by 26%. Increase in price has been reported to be the main cause of consumer's behaviour change followed by the increasing of the level of awareness on negative effects of salt on health. Tax application contributed to the decreasing of salt content in many foods, in some up to 85%. Of all individuals who substituted the salty snacks, 86% have been substituted by fresh fruit and vegetables [16].

Population education and awareness raising campaigns. Food reformulating strategies implemented in the majority of countries have been accompanied by wide campaigns of education and awareness raising of the population.

Discussion

Hypertension is a common risk factor for stroke, coronary heart disease, renal disease, being the main preventable risk factor for death worldwide [1]. A large number of evidence demonstrates the strong association between salt intake and blood pressure and WHO is promoting salt intake reduction as the 'main best-buy' [1, 40]. Taking into account that both global and national action plans on prevention and control of NCDs comprise a target that will have provided for a 30% relative reduction in population mean salt intake by 2025, the need for identification of optimal strategies to reduce salt intake at population level is one intransigent [19, 20].

An important aspect of salt reduction policies, adopted by the majority of countries, is the comprehensive approach with population level interventions. Comprehensive national initiatives on salt reduction are more effective than the unidimensional. In Finland the mean reduction of salt intake by 3 g per day was achieved by reformulating processed foods, mandatory labelling of sodium and awareness increasing campaigns initiated in the yearly 90s. During the same period of time the reduction of both systolic and diastolic blood pressure (a mean measured reduction by 10 mmHg at the population level) was observed as well as a reduction by 60-80% of mortality due to stroke and CVD. Although, in Finland during this period of time there were strategies of primary prevention, reduction in blood pressure was attributed to significant reduction in salt intake [8].

Taking into account the experience of Finland, measures to prevent hypertension done actually at the level of primary health care in the Republic of Moldova should be complemented by a large spectrum of population-based interventions. Population approach on disease reduction involves an intersectoral collaboration, supported by a strong leadership of decision makers, advocacy experts and health care professionals. The pragmatic tool on evaluation and management of the global cardiovascular risk offers guidelines for cost-effective medical interventions. For developing countries, as is the case of the Republic of Moldova, individual management of a large number of patients with a reduced risk for CVD is merely unaffordable. As was elucidated by the epidemiologist Geoffrey Rose, individuals with a lower risk and not those with a higher risk face the largest proportion of the total burden of disease [41]. Prevention and management of CVD should be moved to the population level strategies that approach the main CVD risk factors, and the salt reduction strategy is a good example in this sense [34].

The European network initiative on salt actions contributed to the significant increase in the number of countries that have adopted and implemented public policies in this area [15]. WHO promoted salt reduction as the "best-buy", because it is not only a cost-effective intervention but also an affordable, feasible and culturally accepted for the implementation regardless the level of resources and making it a very convincing proposal for low-income countries [1].

Although the voluntary approach applied in Great Britain and in other developed countries has undoubtedly an impact, some experts and the existing evidence declines the sustainability of voluntary actions for the majority of countries, arguing the need for the legal regulation of ac-

tions to reduce salt intake [14, 17, 42]. The evidence shows that the results are better when actions on reformulation are imposed by law than in case of voluntary reformulation. A modelling study conducted in Australia showed that the impact from mandatory reformulation of sodium is 20 times higher than the health gain from the voluntary reformulation initiatives [14].

The introduction of legal limits for salt in food is dictated by the urgent need to approach the increasing burden of NCDs in the Republic of Moldova. Unlike developed countries where infectious diseases are well controlled, the Republic of Moldova faces the double burden of diseases, that comprise HIV/AIDS and tuberculosis on the one hand and NCDs and injuries on the other hand [18]. Bread and bakery products are among the staple foods, contributing to the highest salt intake in the Moldovan's diet. The mean bread consumption in the Republic of Moldova varied in 2006-2015 between 300 and 350 g per day per person. Establishing the maximum limits for salt content and introducing mandatory reformulation of bread and bakery products may be a start point for the mandatory reformulation [19].

WHO European Food and Nutrition Action Plan for 2015-2020 calls member-states to develop and enforce labelling systems on the main area of packaging, easy to understand and offering an additional interpretation of nutritional information to consumer. By directing consumers towards healthier choices and stimulation food business operators to reformulate their foods, labelling can contribute to the improvement of nutritional quality of the diet. The Parliament of the Republic of Moldova adopted recently the law on consumer's information [27]. Amending the legal provisions by mandatory application of a logo or a colour symbol classifying foods based on their salt content according to Great Britain's, French or Australian models or imposing a warning for the high salt foods as it is done in Finland, will help consumers to make a healthier choice at a glance and thus will contribute more and more efficiently to meet the salt reduction target and implicitly other targets associated with NCDs.

Application of taxes to the salty foods is another component of the salt reduction strategy that can be applied in the Republic of Moldova. Accumulated money can be redirected towards the health promotion actions, including increasing the availability of fruit and vegetables that are potassium sources with a protective antihypertensive effect. A tax is acting to change the price for consumer, thus reducing the demand and changing consumption at the population level. Examples of unhealthy diets illustrate this concept. Frequent consumption of food high in energy, saturated fat, *trans* fatty acids, sugar and salt is associated with an increased risk of overweight and obesity and NCDs, including hypertension and stroke [14]. As shown by evaluation made in Hungary, the consumption of salty foods is higher among overweight and obese individuals [16]. Increasing in the number of diseases and disability associated with excessive consumption of such food leads to the increasing of medical and social care costs, additionally to the economic productivity losses [1, 40]. As such, the costs for society of unhealthy food consumption (external costs) can be significant but it is not reflected either in the private cost of producing the product or in the price paid by the consumer. This is one example of the 'market failure or collapse' which is an economic justification for the government intervention. In such cases governments can decide to increase the product price by taxation to reduce the demand, as it did Hungary, Portugal and Fiji [16, 34].

The extensive and up-to-date analysis of evidence on salt intake reduction conducted by the academia in countries with strategies to reduce salt intake has been an important tool to stimulate political support needed to launch national strategies to reduce salt intake at population level [14, 29].

The effectiveness of salt reduction strategies is based on its potential to change the food environment. Such an approach involves engagement or imposing the food industry to reformulate foodstuffs as well the government commitment to support coordinated programmes to change the consumer's behaviour. This is also true for the Republic of Moldova, where, unlike the developed countries, a certain proportion originates from the salt added during cooking or when eating. Behaviour change programmes can include food-based guidelines, creating a healthy food environment at the workplace, in educational and other public institutions, in meal proving public and recreation facilities and should be supported by large campaigns of social marketing. Removing of salt shakers from these meals providing public premises can be an important step towards meeting the goal. An intersectoral coalition that includes Ministry of Health, Labour and Social Protection, Ministry of Education, Culture and Research, Ministry of Economy and Infrastructure, National Medical Insurance Company, Nicolae Testemitanu State University of Medicine and Pharmacy and medical colleges, food industry, non-governmental organisations jointly with mass-media, can be the most appropriate for the development and promotion of the national salt reduction strategy.

Iodine deficiency is also a public health problem in the Republic of Moldova. Continuous implementation, starting from 1997, of the salt iodization strategy contributed to the elimination of iodine deficiency disorders in 2012. There are concerns that reducing of discretionary salt intake could jeopardise success achieved through universal salt iodization programmes. World Health Organization recommends continuous surveillance of the iodine status of population, and with decreasing of salt intake the amount of iodine added as fortifier can be increased, based on needs, without risk of excess [7, 43]. Additionally, communication activities on salt intake reduction and elimination of iodine deficiency disorders should be efficiently coordinated to ensure that the potential health benefit of both programmes is maximised.

Another important element to ensure efficiency and sustainability of adopted strategy is the monitoring and evaluation of thereof and responsibility and accountability of both, government and food industry. Models of Great Britain, Ire-

land, Hungary, France, Finland, Turkey and of other countries can be taken into consideration when developing the national salt reduction strategy. Monitoring of infrastructure should allow the assessment of both, the overall impact and the differential impact on different social groups.

Ministry of Health, Labour and Social Protection should lead the process of salt reduction strategy development and implementation within a large intersectoral coalition of all interested stakeholders. National Agency for Public Health with its territorial Centers of Public Health can ensure continuous monitoring of food industry engagements and compliance, and evaluation of targets' meeting. Evaluation of urinary sodium excretion as a measure of sodium intake estimation, should become part of NCDs surveillance system, with periodic evaluations. Consumer knowledge, attitudes and practices should be also monitored.

Conclusions

1. Salt intake is strongly linked with hypertension, one of the main risk factor for CVD and stroke. Present research exemplifies that regardless of income countries can implement efficient strategies to reduce salt intake.

2. The burden of cardiovascular disease is high in the Republic of Moldova. Reducing salt intake at the population level will substantially reduce the public burden of preventable cardiovascular disease and stroke, as well as health care costs and health disparities.

3. The biggest reduction in salt consumption can be achieved by comprehensive multicomponent strategy involving legal measures efficiently enforced, mandatory reformulation, nutritional labelling, taxation, and a good leadership.

4. Scientific community has an essential role in collecting, analysing and disseminating of evidence at the international and national levels on salt intake reduction for public health benefit and social and economic development of the country.

5. Monitoring interventions and assessing their impact is essential to meet the targets and to determine the efficiency and effectiveness of adopted decisions.

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DOI: 10.5281/zenodo.1299012
UDC: 616.995.132-053.2

Monoinvasion with toxocara canis in children

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Abstract

Background: Toxocariasis is a clinical notion that reflects human involvement with the Toxocara larvae in the second stage of development. Its typical syndromes are Larvae Migrans Visceralis, ocular toxocarosis , neurological form and occult form. The most common toxocariasis is asymptomatic, only eosinophilia and the positive serological result being the only manifestations of this helminth. The epidemiological examination and a detailed anamnesis of the patient can be helpful in establishing this diagnosis. Children are part of the risk group, especially because of the childhood habits, their undoubted attraction to puppies, and because of their low immune system.
Material and methods: The elaborated prospective-descriptive study presents the evolution features in a group of 94 children with *T.canis* monoinvasion in which were examined the clinical signs, important laboratory indices, the applied treatment and its influence.
Results: The study revealed the most common clinical signs and paraclinical changes in a group of 94 children with *T.canis* monoinvasion. Was examined the specific treatment by highlighting its influence on laboratory indices representative for this parasitosis.
Conclusions: The most common clinical signs were headache, dry cough and abdominal pain. The specific therapy for vicerial chronic toxocariasis in children was far superior in clinical outcomes, this being reflected by maintaining ALT levels within the normal values demonstrating the direct involvement of larvae and toxocara toxins in the establishment and maintenance of hepatic cytolytic syndrome in children.
Key words: Toxocariasis in children, larva migrans visceralis.

Introduction

Lately, parasitic diseases have returned in force in medical practice putting major problems of diagnosis, treatment and evolution [1,2]. The geographical study of parasitosis as well as the high number of human diseases justify the involvement of substantial resources to prevent and exert effective control of these infections [3,4].

Toxocariasis is a term representing human infection caused by larvae of helminths of the genus Toxocara that parasites in dogs (*Toxocara canis*), cats (*Toxocara cati*) [5], and possible in horned animals (*Toxocara vitulorum*) and swine [6]. Definitive host is shown by the above-mentioned animals, but once they enter the human body it can lead to bad consequences.

Toxocara canis and *cati*, being the most common intestinal parasites have a variety of random hosts including humans, birds, pigs, rodents, monkeys and rabbits. The man is infected by the ingestion of *T. canis* or *T. cati* embryonic eggs. Larvae hatch in the intestine of the intermediate host without the ability to fully mature and remain throughout the infected period at the second stage of development. The degree of the intermediate host damage depends on the amount of larvae circulating through the bloodstream and the affected organs, as well as on the immune response of the host [7]. Of course, it is much more difficult to establish the diagnosis of Toxocariasis in those with whom the disease is asymptomatic or discrete, even if it can evolve for a long time, sometimes up to several decades [8].

Transmission to humans most often occurs through the ingestion of toxocara eggs. *Toxocara canis* has an ability to produce about 200,000 eggs per day, and one gram of puppy feces can content around 100,000 eggs per gram. However, eggs need a few weeks to stay out of the host to mature and become invasive, so recently eliminated eggs are not dangerous [9].

The basic classification of toxocarasis divides this pathology depending on the affected organ. Thus, there are two basic syndromes: Larvas Migrans Visceralis (LMV) where pathology occurs with major organs damage and Migrans Larvas Ocular (OLM) [10] where the disease is limited to damage of the eye and the optic nerve [11]. The asymptomatic and occult form has also been described [12].

Clinical syndrome of LMV may be acute or subacute with splenomegaly, hepatomegaly, gastrointestinal symptoms, skin manifestations, pulmonary involvement, and CNS involvement (this particular form was called Neurological Toxocariasis) [13] and eosinophilia[14].

The occult form occurs as a result of the development of non-specific symptoms due to the action of the parasite antigen in the host's immune system. Asymptomatic form is established when accidentally in patient without signs of typical damage are detected antibodies to Toxocara and eosinophilia [15].

Multiple studies in this area, although most of the time having contradictory results, had a common element: the distribution of *T. canis* and *T. cati* is global, with cases identified in very limited populations. The seroprevalence rate ranges from less than 10% [16,17] to more than 80% [18,19,20], increased seroprevalence being more common in economically underdeveloped countries or countries in

developing cource[14,21]. Besides these well-known types, have also been discovered new varieties of Toxocara as *Toxocara malayasiensis* affecting domestic cats [22] and *T. lyncus* infecting lynx [23]. However, there is no data on the involvement of these new types discovered in human damage.

Children, due to childhood habits (especially geophagia), poor personal hygiene, even if the children are part of families with a high income and good living standard, the tendency to contact with the animals, especially with puppies without parental supervision, leads to an increased risk of receiving toxocariasis[15].

Material and methods

A prospective descriptive study was developed with the inclusion of 94 children aged 4 to 18 years with no other associated parasitosis (ascaridiasis, oxyuriasis, giardiasis, etc.). The patients were examined both clinically, with developing the most common clinical and paraclinical signs, revealing the general blood counts, biochemical examination (ALT and AST), total IgE level, antibody titre to *T. canis*.

Results and discussion

The study involved 94 children with Toxocara canis monoinvasion. Duration of toxocara invasion ranged from 1 year to 9 years, in most children (70.1%) with a duration of 2 to7 years.

Among the studied group the average age was 11.4 ± 0.47 years. Children aged 4-7 were 21 (22.3%), 8-12 years – 36 (38.3%) and 13-18 years – 37 (39.4%).

The bronchopulmonary form prevailed with the involvement of 32 children (34.0%) followed by the neurological form with 30 children (31.9%), the cutaneous form was discovered in 16 children (17.0%), the digestive form – in 10 children (10.5% %), and in 6 cases (7.3%) other clinical forms were described.

The frequency of major signs encountered during the course of the disease in children in the studied group is shown in fig. 1. Clinical manifestations behaved with a variety of different intensity and frequency. The most frequent clinical manifestations in the studied group were headache and long-term cough, both in 33% of cases, followed with a decrease in percentage (from 25.5% to 20.3%) with hepatomegaly, vertigo, abdominal pain, diffuse liver changes on ultrasound exam, skin pruritus, sleep disturbances. The other 6 clinical signs with a decrease in percentage from 20% to 10%: maculo-papular rash, splenomegaly, neuropsychiatric disorders (impulsivity, inability to concentrate, poor memory, chronic apathy, etc.), physical asthenia and weight loss (fig. 1).

Clinical manifestations with an incidence of less than 10% in the course of chronic toxocariasis in children are shown in fig. 2. In 8.1% of cases of monoinvasion with *T. canis* were noted epileptiform seizures, signs of rhinitis and dyspnoea, in 6.8 % of cases is described bronchopneumonia, in 5.4 cases – high level of ALT.

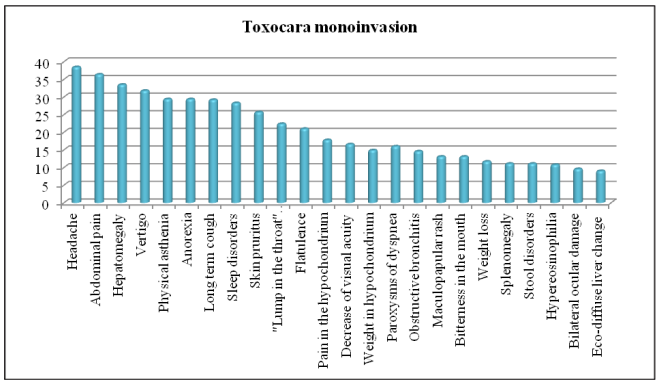

Fig. 1. Percentage distribution of the most common signs in children with chronic visceral toxocariasis, monoinvasion.

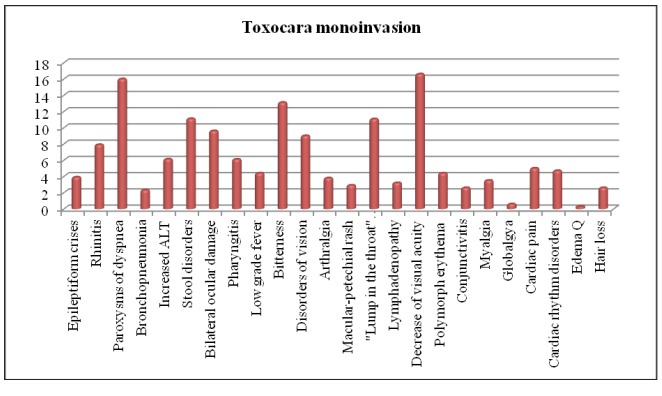

Fig. 2. Percentage distribution of the less common signs in children with chronic visceral toxocariasis, monoinvasion.

The specific treatment indicated in toxocariasis has been shown in most cases with benzimidazole derivatives with a 2-times daily intake of 10 mg / kg / body with duration of 10-14 days.

The number of leukocytes was not deviating from normal mean values, but significantly declined in patients with specific treatment from 6.8 ± 0.47 to 5.5 ± 0.47 (P <0.05). Also, the number of erythrocytes increased compared to the values before the treatment, with statistical significance regardless of the specific treatment applied (P <0.001). The percentages of the lymphocytes had minor decreases compared to the baseline in the patients regardless of the therapy applied; however, they remained above the mean values compared to the healthy ones. The percentage of eosinophils, starting from over-normal values compared to healthy subjects (6.3 ± 0.61 versus 1.8 ± 0.10, P <0.001), decreased regardless of the treatment applied to 3, 7 ± 1.0; in the absence of this treatment – 3.1 ± 0.76 (P <0.05).

In the group of children without specific treatment, ALT levels have increased significantly from baseline values and those seen in healthy individuals. In monoinvasion with *T. canis*, they increased from 23.8 ± 2.4 to 62.8 ± 14.2 (P <0.01). These liver enzyme behavioral findings demonstrate the direct involvement of larvae and toxocara toxins in the hepatic cytolytic syndrome. At the same time, the ALT activity in the group with specific treatment remained close to the mean baseline values, no different from those seen in

healthy individuals, which proove the safety of antilarvaric treatment in children. Activity levels of the AST enzyme did not change during the different treatment regimens, and remained constant within the normal mean values.

Paradoxically, however, regardless of treatment, levels of antibodies to *T. canis* increased, averaging higher than those found up to treatment.

The total IgE level was in the medium of 302.2 ± 41.0. Specific treatment had a beneficial effect on the evolution of total IgE, registering significantly lower values than baseline (145.4 ± 29.2 vs. 302.2 ± 41.0, P <0.01).

In order to identify the capacity of these children to form and maintain the postvaccinal response, randomly in every third child, with an average age of 9.18 ± 0.64 years, timely vaccinated according to the national vaccination schedule with three doses of the vaccine HepB, we tested the presence of anti-HBsAg in the serum. In these patients, the result was the titre of the protection antibodies below the detection limit, 1/3 of who were under the age of 7 years.

Because, as we mentioned, clinical manifestations were varied in intensity and frequency, the changes in the clinical picture during chronic toxocariasis in children were analyzed according to the percentage decrease of each symptom, shown as a percentage of the intensity expressed before the onset of treatment applied. The average percentage reduction in symptoms determined clinical effectiveness. Thus, clinical efficacy was noted in terms of diminishing or negating clinical signs: very pronounced – efficacy noted with a reduction of 75%-100% of the intensity of previous manifestations, pronounced – 50-75%, poorly pronounced – less than 50% and absence. As observed in the study group, more pronounced and poorly pronounced clinical efficacy was found, and the lack of clinical efficacy was found only in 17.0% of the 94 tested children (P <0.01).

Out of the 94 children studied, antilarvaric specific treatment received 56 (59.6%) children and 38 (40.4%) did not receive this treatment. The very pronounced clinical efficacy in children with antilarvaric treatment was recorded in 16.1% of cases, whereas in the non-treated group only in 5.3% (P <0.05). Significant clinical efficacy was found in 48.2% of the 56 treated versus 23.7% of the 38 without treatment (P <0.05). And the percentage of those with specific treatment with poor clinical efficacy was higher – 23.2% versus 15.8%.

Lack of clinical efficacy was seen in 55.2% of children without specific therapy and only in 12.5% in those who received this therapy (P <0.001).

The level of antibodies to *T. canis* in children in the investigated group receiving specific treatment decreased to 31 (55.4%) compared to 18 (47.4%) found in those without specific therapy. However, after the anti-toxocara treatment, it was noted that in approximately one third of the patients the antibody levels increased (39.2%), although most of these children had expressed clinical improvement.

The percentage of eosinophils also had a greater decrease in children with specific treatment – 66.9%. Among the children with specific treatment, the cumulative percentage in increase or maintenance at the same eosinophil values was 30.4%, compared to 71.1% in the non-specific anti-toxocara treated group (P <0.001).

Total IgE concentration also declined more frequently among patients with anti-toxocara therapy – 33.9% of cases, compared with 26.3% in children without anti-toxocara treatment (P <0.001). The percentage of patients who did not experience essential changes was quite high, ranging from 42.3% to 48.2% (P <0.05).

Therefore, clinical manifestations of chronic toxocariasis in children are very numerous (more than 40 clinical signs), varying in intensity and incidence, and the most common of these are headache and dry cough, both of which are recorded in 33.0% of cases. In the investigated group hyper-eosinophilia is recorded in every 6-7th child, with a percentage of 13.5%. Summarizing the correlation of the laboratory indices in chronic toxocariasis in children, there is a decrease in the number of leukocytes, especially with applied specific antilarvar therapy (P <0.05). Parasitic offensive action on laboratory indices was evident, demonstrating high levels of total IgE (302.2 ± 41.0 P <0.05) and eosinophils (6.27 ± 0.61, P> 0, 05). Prolonged invasive parasitic action in children keeps the immune system permanently alert, contributing to the depletion of postvaccinal response formation and maintenance capacity.

Specific treatment significantly reduced the percentage of eosinophils and total IgE (P <0.01). The increase in ALT from 23.8 ± 2.4 to 62.8 ± 14.2 (P <0.01) in the absence of specific treatment, and maintenance of this enzyme within the normal range throughout the duration of the specific therapy demonstrates the direct involvement of the larvae and toxocara toxins in the development of hepatic cytolysis syndrome in children. The antibody levels to *T. canis*, regardless of the treatment applied, tended to increase. The main laboratory indices that directly reflect parasitic activity (the level of antibodies, total IgE and percentage of eosinophils) had a higher positive change frequency in the specific treated group (P <0.05). However, in approximately 1/3 of the children with anti-toxocara treatment the antibody titres increased (to 39.2%) and total IgE remained unchanged in 48.2% of cases, although most of them had apparent clinical improvement.

Conclusions

1. Clinical manifestations during chronic toxocariasis in children are very numerous (over 40 clinical signs); varying in intensity and incidence, the most common of them being headache, dry cough and abdominal pain. Blood hyper-eosinophilia is recorded in every 6-7th child at a rate of 13.5%.

2. The specific therapy in chronic visceral toxocariasis in children had a much higher clinical rate, being reflected by maintaining the ALT level within the limits of normal values demonstrating the direct involvement of larvae and toxocara toxins in the occurrence and maintenance of hepatic cytolysis syndrome in children.

3. Specific treatment significantly reduced the intensity of clinical manifestations, the percentage of eosinophils and

total IgE (P <0.01), much less acting on the level of antibodies against *Toxocara canis*.

4. Toxocariasis in children takes the form of a complex evolutionary chronic process with periods of remissions, relapses and exacerbations, which implies the need for further surveillance of these children, if necessary with the involvement of other specialists (neurologist, ophthalmologist, etc.).

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DOI: 10.5281/zenodo.1299020
UDC: 618.3-06:616.8-009.24:612.015.11

The oxidative stress in preeclampsia

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Abstract

Background: The pathogenesis theories of preeclampsia include inappropriate trophoblast invasion and incomplete remodeling of spiral uterine arteries. This is followed by a reduced utero-placental perfusion; ischemia is the cause of oxidative stress activation. The imbalance between the oxidant and antioxidant components in favor of pro-oxidation, induce endothelium activation and / or dysfunction with the occurrence of clinical and laboratory manifestations.

Material and methods: It was proposed a comparative prospective study including 105 pregnant women with preeclampsia in comparison with 367 pregnancies without preeclampsia. Pro-oxidant and antioxidant status and its correlation with systolic blood pressure values and classical laboratory marchers were analyzed.

Results: In preeclampsia there is an excess accumulation of pro-oxidants comparing to control group p<0.001**.** Malondialdehyde and Advanced Oxidation Protein Products are most implicated in hypertensive syndrome r 0.7, p<0.001. Inflammatory diseases of uro-excretory tract accompanying preeclampsia, demonstrate excess accumulation of Advanced Oxidation Protein Products and AGE verprelizine-like and AGE pentozidine-like r 0.7, p<0.001. Modified ischemic albumin did not get the modified value in the research group.

Conclusions: Excess accumulation of pro-oxidants in preeclampsia plays an important role in pathogenesis pathway, clinical and laboratory manifestation of preeclampsia.

Key words: preeclampsia, oxidative stress, pro-oxidants, hypertension, proteinuria.

Introduction

Preeclampsia (PE) is a heterogenic and multisystem pathologic condition appeared in pregnancy, characterized by hypertension and commonly accompanied with proteinuria after 20 weeks of gestation. It is mediated by endothelial dysfunction, vasospasm and implication of one or more organs inclusively utero-placentar system [1].

One of actual PE etiology theories is poor trophoblast invasion and improper spiral arteries remodeling, preceded by reduced utero-placentar perfusion, ischemia which is the principal cause of oxidative stress imbalance in favor of pro-oxidants [2, 3]. The most important effect of pro- oxidants excess in PE is endothelial damage [4], vasospasm and increased peripheral resistance [5].

Relevant to PE pro-oxidative factors are: Advanced Glycation End Products, Advanced Oxidation Protein Products, Malondyaldehide, and Ischemic Modified Albumin. All of them are known to have a negative endothelial effect.

Advanced Glycation End Products bind to their receptor (RAGE) and alterate the invasion of trophoblast and placenta, activating general inflammatory pathway and local inflammatory response being responsible for the placental dysfunction and IUGR in PE [6,7, 8].

Advanced oxidation protein products are considered a biomarker of oxidative stress known to be in excess in different metabolic syndromes and kidney diseases. It plays an important role in endothelial dysfunctions and proteinuria [9]. Also it's suggested that AOPP are accumulated in placenta and play a negative role in trophoblast invasion and increases apoptosis [10, 11].

Malondyaldehide is a result of lipid peroxidation which results in oxidative stress. Lipid peroxides are considered to be toxic for endothelial cells, increase the synthesis of tromboxane with a decrease of prostacyclin synthesis with resulted vasoconstriction [12]. Excess of Malondyaldehide in PE is the cause of accumulation of reactive oxygen species and free radicals accumulation are involved in endothelial lesions [13]. So, all of pro-oxidants discussed manifesting endothelial injury and vasoconstriction are responsible for clinical and laboratory manifestations of PE as hypertensive syndrome, and proteinuria.

As antioxidant capacity are discussed Total Antioxidant Activity and Cupric Ionic Oxidation Capacity. The results of most studies indicate a decrease of antioxidant factors in PE [14, 15].

Material and methods

It was proposed a comparative prospective study which included sample research in 105 pregnant women with preeclampsia and comparative sample in 367 pregnant women without preeclampsia. Including criteria in research sample were: singleton gestation with 27-41weeks gestation with preeclampsia. Excluding criteria were: multiple pregnancies, diabetes, superimposed preeclampsia, congenital malformations of the fetus and pregnancy with rhesus conflict. Including criteria for comparative sample were: singleton pregnancy without preeclampsia. Excluding criteria were: multiple pregnancy, diabetes, congenital malformations of the fetus and pregnancy with rhesus conflict.

The following pro-oxidant parameters were determined in our research: advanced oxidation protein products (AOPP), advanced glutathione end products verprelizine-

like (AGE-Verpelizine-like) and advanced glutathione end products pentozidine-like (AGE-Pentozidine-like), Malondialdehyde (MDA). Antioxidant effect was studied based on total antioxidant capacity (TAC) and cupric ionic reducing capacity (CUPRAC).

The values of systolic blood pressure were measured 4 times, every 6 hours daily. The results were introduced in data base ACCESS.

Results and discussion

First of all was studied the balance of oxidative stress in preeclampsia. As it is known, PE is a disease associated with an imbalance between pro-oxidant and antioxidant parameters of oxidative stress [15, 16, 17]. Comparative results of these parameters were set out in the table below:

Table 1

Average values of pro-oxidants and antioxidants of oxidative stress in the research and comparative groups (Media, SE, p)

Pro-oxidant/antioxidant factor	Research sample	Control sample	P
Advanced oxidation protein products	173.4±17.1	46.4±7.8	0
AGE verprelizine like	1209.5±201.5	349.9±26.3	0
AGE pentozidine like	2137.1±179.7	529.6±37.1	0
Malondialdehyde	34.5±4.0	18.3±2.2	0
Total antioxidant activity	8.8±16.7	8.1±10.6	0.9
Cupric ionic oxidation capacity	1.9±0.3	3.5±0.4	0.1
Ischemic modified albumine	0.4±0.1	0.4±0.1	0.3

As can be seen in the table, the mean values of pro-oxidant substances in PE are significantly different compared to these values in the control group. But the mean values of antioxidant substances are similar in both groups. Thus, we can deduce that PE is associated with an imbalance of oxidative stress in favor of pro-oxidants at a concentration of antioxidants similar to the control group [15, 18]. According to Burton G.J. and other authors, pro-oxidants play a major role in pathophysiology of different pathologies including PE. Because of superficial invasion of trophoblast and endothelium activation the placental barrier is broken, creating a leakage of pro-oxidants and placental debris in maternal circulation with activation of inflammatory cascade and accumulation of more pro-oxidants. Accumulated pro-oxidants have a negative effect on maternal endothelium being the most important cause of clinical manifestations of PE [19].

Malondialdehyde and Advanced Oxidation Protein Products are most relevant pro oxidants excess responsible for endothelial cell damage. MOA are the final products of lipids peroxidation. Activated endothelium in cerebral, renal and hepatic system decreases the synthesis of endothelium derived vasodilatations such as Nitric Oxide and increases the synthesis of vasoconstrictors like Endotheliin1 and Tromboxane A2 which are responsible for vasoconstriction and hypertensive manifestations of PE [20, 21].

Based on the above, we assumed the existence of correlation between the higher values of the pro-oxidant markers with first mandatory manifestation of preeclampsia- high values of systolic blood pressure which were measured before labor. As a result, we have achieved an intense and true correlation between these parameters and pro-oxidant markers of oxidative stress r 0.7, p <0.001 in the case of association of the maximum blood pressure measured in the morning and r 0.5, p <0.001 to maximum values of systolic blood pressure at night and at midnight, and r 0.6, p <0.001 at nocturnal systolic blood pressure. Furthermore, the highest values of systolic blood pressure were determined in the morning and in the night.

The next step in our study was evaluation of correlation grade of pro-oxidants with biochemical parameters as total protein, urea, creatinine and fibrinogen blood levels which frequently are pathological in PE.

Table 2

Representation of direct and indirect correlation between pro-oxidants parameters of oxidative stress and blood parameters in preeclampsia (correlation coefficient "r", p)

Pro-oxidant parameter	Biochemical parameter	Correlation coefficient "r"	P
AGE pentozidine like	Proteine in blood	-0.4	<0.0001
AGE verprelizine like	Ureia in blood	0.3	<0.0001
AOPP	Fibrinogen	0.5	<0.0001
AOPP	Creatinin in blood	-0.3	<0.0001
MDA	Proteine in blood	0.3	<0.0001

In terms of the results obtained and presented in the table above, there are various correlations between the pro-oxidant parameters and the biochemical parameters, which denote the systemic involvement of the pro-oxidant substances in the pathogenesis mechanisms of PE. As seen in the table above, there is obtained a negative correlation between AGE pentozidine-like marker and total amount of blood protein. With this was demonstrated that low concentration of general blood protein is dependent of high accumulation of AGE pentozidine-like which is known to be implicated in high permeability of endothelium and mediation of inflammation [22]. Another important correlation was found between AOPP and blood concentration of fibrinogen (r 0.5, p<0.0001) which is sugestive for association between inflammation and excessive acumulation of pro-oxidants like AOPP. The next biochemical blood markers as ureia and creatinine demonstrated moderate but sure correlation with AGE verprelizine-like and AOPP which also indicated a moderate implication of pro-oxidants in pathological process of uroexcretor system.

One of frequent diagnostic criteria of PE is proteinuria. The following significant correlations were found between this criteria and pro-oxidants factors: AGE verprelizine-like and AGE pentozidine-like were in moderate correlation,

r 0.4, p <0.0001 and AOPP, MDA and proteinuria with r 0.3, p <0.001. An interesting correlation has been found between the presence of bacteriuria in the urine investigation which was significantly correlated with AGE verprelizine-like, AGE pentozidine-like and AOPP: r 0.7, p <0.0001 [23, 24]. In support of the opinion that pro-oxidative factors are accumulating in excess in different independent pathological conditions accompanying pregnancy with PE such as obesity, cardiovascular pathology, inflammatory disease of uro-excretor and genital system, the following significant correlations have been found. AOPP was mostly increased in pregnancy with PE and obesity, F 8.3, p<0.005 but in case of chronic pyelonephritis the values of AGE vertelizine-like and AGE pentozidine-like were significantly increased in the research group compared to their values in the control group, F 15.6, p<0.0001. [25]. In case of cardiovascular and endocrine pathologies there were not found any significant correlations. Moreover, statistically significant correlations were found between vertelizine-like AGE concentrations in neo-natal outcome with ischemic brain lesions as compared to women in the research group without this result at birth $\chi 2$ 17.6, p< 0.001[26, 27].

The last discussed pro-oxidant is Ischemic Modified Albumin. There is a contradictory opinion about its role and importance in PE. Some of them consider it an important marker of ischemia while others did not find any correlation with PE in their studies [28, 29, 30]. In our study, no correlation of IMA with clinical and/or laboratory parameters of PE was found.

Conclusions

Our study demonstrates the importance of oxidative stress imbalance in PE in favor of pro-oxidants p<0.0001. Most pro-oxidants implicated in PE have endothelial toxicity effect and promote inflammation mechanisms being responsible for vasoconstriction, hypoxia and hypertension. Inflammation of uro-excretory system (p<0.001) and obesity are the most relevant medical conditions in PE with excess accumulation of pro-oxidants p<0.005. Also we found a moderate correlation between AGE verprelizine- like concentrations and fetal hypoxic brain lesions in PE.

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DOI: 10.5281/zenodo.1299024
UDC: 616.24-002.5-036.22(470+571)

Challenging issues of tuberculosis control in the Russian Federation

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Abstract

Background: The comparative assessment of the epidemiological indices and challenging issues of the tuberculosis control in the Russian Federation.
Material and methods: In the study were used the analytical annual reports of the statistical indices registered and the electronic resources of the Federal Research Institute for Health Organization and Informatics of the Russian Ministry of Health.
Results: The tuberculosis epidemiological situation in the Russian Federation is continuously improving. The achieved indices in disease control were 71.3% of the population annually screened and the rate of 51.5% patients with microbiological sputum conversion from positive to negative. During the period from 2000 till 2017, the incidence decreased from 90.4 to 48.3 per 100 000 population, the incidence in children aged between 0 and 14 years old decreased from 19.1 in 2001 to 9.7 in 2017 and in children aged between 15 and 17 reduced from 40.5 (the highest level) in 2005 to 21.6 in 2017 (the lowest level 16.6 in 1992). One of the contributing factors with an important impact on the epidemiological state represents the HIV-infection, the incidence of which went up from 6.5% in 2009 to 20.9% in 2017. In 2017, the effectiveness of treatment based on bacterial conversion was reported in 70.7% of new cases, and closure of cavitary lesions was reported in 62.9% of cases.
Conclusions: Russia made certain progress in tuberculosis control, however, the vast territory, its heterogeneity, including climatic and social economic differences, affect the epidemiological situation. Big efforts were performed for solving the problems associated with the infection control. The epidemiological data determined an increasing rate of the MDR-TB and TB/HIV co-infected patients, which required the development of the new approaches in tuberculosis control.

Key words: tuberculosis, federal programme, incidence, prevalence, mortality, HIV infection, MDR-TB.

Introduction

Tuberculosis (TB) control in the Russian Federation (RF) has a national importance. Since 1993, the Russian Federation is consistently implementing the federal TB control programmes, established by the regulations of the Ministry of Health, according to the requirements of the World Health Organization. Starting from 2015 a common electronic platform for case surveillance was developed and implemented in the RF. It allowed the monitoring of diagnosis and treatment, including MDR-TB and TB/HIV co-infected patients. Actually the electronic platform is used in some regions of the RF and its national implementation depends on the project outcomes. The RF joined the resolution of the 67th session of World Health Assembly, which set up the goal to end tuberculosis globally by 2050. In this way the Russian TB control strategy outlined the following targets: 1. Early detection of TB patients by active screening with a coverage of at least 70% of the population; 2. Chemopreventive treatment of 100% TB/HIV coinfected individuals with a CD4 lymphocytes count below 350 cells/ml; 3. Total supply with the anti-tuberculosis drugs for MDR-TB including XDR-TB in 100% of patients; 4. Ensuring a treatment success rate at least 75% in drug susceptible cases and 55% in MDR-TB cases; 5. Research, including innovative development for improving the patient's care; 6. Extension of the collaboration with the Commonwealth of the Independent States, countries of the Eurasian Economic Community and BRICS countries.

Actually the TB control activities in the RF are considered a part of the state programme "Healthcare Development". The national TB control is reflected in the President's decrees. According to the Decree of 07.05.2012 regarding "The improvement of the state policy in health care" and the state program "Healthcare Development for 2013-2020" the TB mortality should target 11.8 cases per 100 000 population. The value was achieved in advance and in 2017 the disease mortality decreased to 6.4 per 100 000 population. The "Healthcare Development" program provided the reduction of the TB incidence to 35 per 100 000 population. In order to reduce the disease mortality and incidence, the national program provided the assessment of such indicators: the rate of the population covered with the annual active screening (target value was established 70.1%) and the rate of patients with the sputum conversion among microscopic positive patients (target value 45%). The achieved indicators were registered in terms of 71.3% of the population actively screened and 51.5% of the microscopic positive patients converted their microscopic positive state [5].

Material and methods

In the study were used the analytical annual reports of the statistical indices registered in the Russian Federation

and the electronic resources of the Federal Research Institute for Health Organization and Informatics of the Russian Ministry of Health.

Results and discussion

Assessing the epidemiological indicators registered between 2000 and 2017 the incidence of TB went down by 46.6% (from 90.4 to 48.3 per 100 000 population) and by 7.3% in the period 2016-2017 (from 53.3 to 48.3 per 100 000 population). The mortality due to tuberculosis decreased 3.5 times, from 22.1 per 100 000 population in 2005 to 6.4 per 100 000 population in 2017[1, 2, 3]. The indices were reflected in the figure 1.

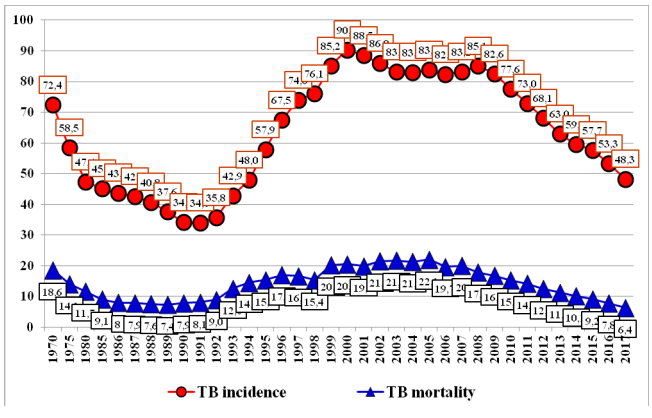

Fig. 1. Tuberculosis incidence and mortality during the period 1970 and 2017.

Tuberculosis affected more men than women in the RF. So, in the young age groups, in the economic and reproductive period (18-44 years old) men were 61.2% of patients. The rate of the women aged between 25 and 34 years during the period 2009-2017 was in the range of 30.5-32.1% from the total number of patients. A decreasing tendency of the TB incidence in both men and women' groups was established. So, the TB incidence in men in the period 2009-2017 decreased from 124.1 per 100 000 in 2009 to 70.7 per 100 000 and in women from 46.9 per 100 000 to 28.9 per 100 000 population.

The TB incidence registered in children aged between 0 and 14 years old decreased by 49.2% in the period 2001-2017 from 19.1 per 100 000 population to 9.7 per 100 000 population. The lowest value was reported in 1992 in terms of 9.4 per 100 000. The TB incidence in children aged between 15 and 17 years old decreased by 46.7% during the period 2005-2017. So, the incidence registered in 2005 declined from 40.5 per 100 000 to 21.6 per 100 000 in 2017. However, the lowest value reported in 1992 of 16.6 per 100 000 was not achieved [1, 2, 3, 5]. Data are shown in the figure 2.

A positive evolution of the TB incidence was registered in both, urban and rural population of the RF. The TB incidence in the rural population was higher than in the urban population and decreased during the period 2008-2017 from 96.4 to 53.5 per 100 000 population in rural residents of the RF and from 80.9 per 100 000 population to 46.5 per

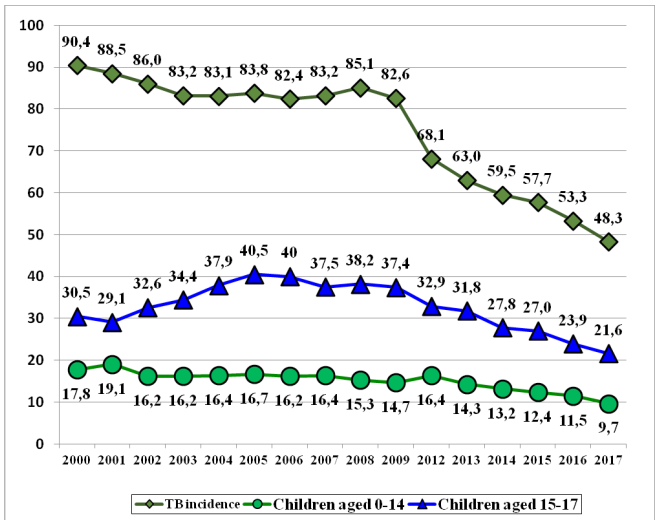

Fig. 2. Distribution of disease incidence in adults, children 0-14 years and 15-17 years old.

100 000 population in urban residents. The proportion of newly diagnosed TB patients in the rural areas is almost one-third from all registered cases (28.4%). Data are shown in the figure 3.

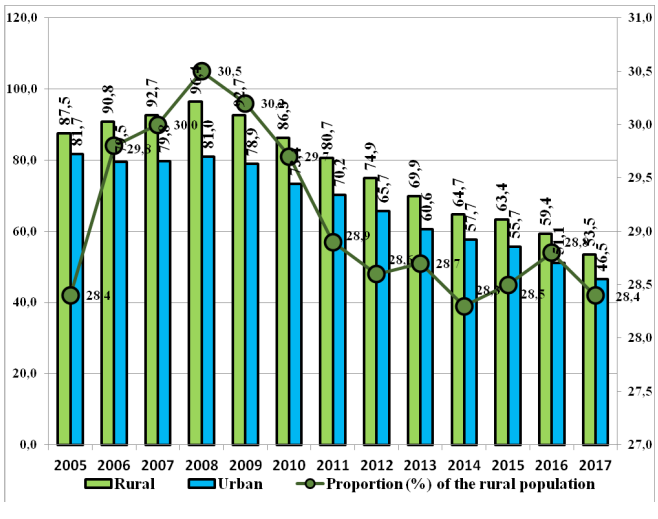

Fig. 3. Distribution of the epidemiological indices according to the demographic area of registered patients.

While distributing patients according to the regions it was established that the severest epidemic state of tuberculosis was reported in the Far East, Siberian and Ural federal districts. The lowest incidence and mortality indices were registered in the Central, North-Western and North-Caucasian federal districts which were approached to the Central European ones[4]. Data are shown in the figure 4.

One of the emergent epidemiological problems with a negative impact on the TB indices in the Russian Federation is the rate TB/HIV co-infection in newly detected patients. According to the executive order of October 2016 the Russian Federation government approved the National Strategy to Combat the Spread of HIV-infection. Over the period from 2009 to 2017 the proportion of newly diagnosed TB patients among HIV-infection increased 3.2 times from

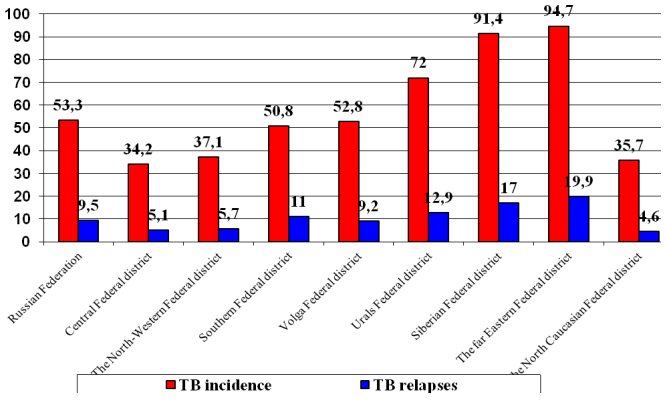

Fig. 4. The distribution of the epidemiological indices: incidence and relapse according to the Districts of the Russian Federation.

6.5% in 2009 to 20.9% in 2017. Among previously treated patients the associated TB/HIV co-infection increased 3.4 times from 5.5% in 2009 to 18.5% in 2017 [6]. Indices were shown in the figure 5.

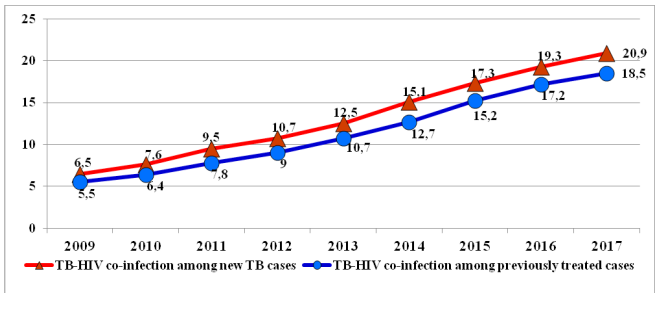

Fig. 5. The rate of TB/HIV coinfection among new and previously treated cases.

Another problem that negatively affects the dynamics of the epidemiological indices in the Russian Federation is the increasing rate of the multidrug-resistant tuberculosis (MDR-TB) among new and previously treated patients. The MDR-TB registration started in the Russian Federation in 1999. So, the rate of the MDR-TB among new microbiological positive cases increased 4 times from 6.7% in 1999 to 27.4% in 2017 and 5 times from 10.5% to 54.0% in previously treated cases. Multiple causes contributed to the rising of the MDR-TB rate in the previously treated cases. The most important were: inadequate treatment without taking into consideration the drug susceptibility, low treatment adherence reflecting the poor patients' supervision, lack of an appropriate variety and quantity of the anti-tuberculosis drugs[5].

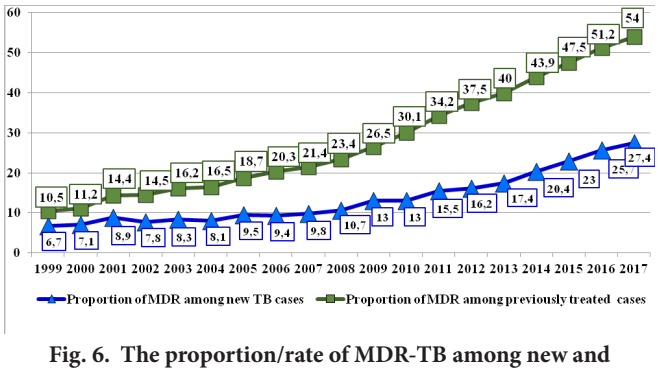

Fig. 6. The proportion/rate of MDR-TB among new and previously treated cases.

During the period from 2002 till 2017 the disease prevalence decreased by 59.8% from 272.8 per 100 000 population to 109.8 per 100 000 population. The rate of the severest form with the highest epidemiological danger fibro-cavernous tuberculosis decreased 2 times from 25.0 to 10.2 per 100 000 population during the period 2002 till 2017. The rate of the microscopic positive patients decreased during the period 2002 till 2017 two times from 88.5 per 100 000 population to 46.0 per 100 000 population. It can deduct an earlier screening of limited forms and increasing the use of the molecular genetic methods in the diagnosis of tuberculosis.

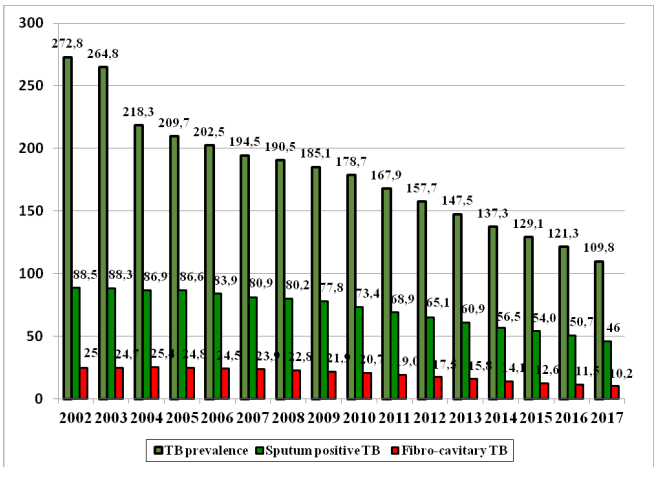

Fig. 7. The distribution of the epidemiological indices: prevalence, the rate of the sputum positive cases and the rate of the fibro-cavitary tuberculosis.

The anti-tuberculosis treatment effectiveness reflects an important impact on the disease prevalence. Using a 12 months regimen the closure of the lung cavities was achieved in 62.9% of new pulmonary cases, compared with 62.8% in 2016 and the microbiological positive state converted into negative in 70.7% of cases in 2017, compared with 70.2% in 2016. The closure of the lung cavities in relapsed cases using a 12 month regimen achieved 40.3% of cases in 2016, compared with 41% in 2015 and 41.9% in 2014. Microbiological conversion of the positive state into negative was reported in 46.6% in 2016 compared with 43.5% of cases in 2009. The rate of the clinical cured patients increased from 28.5% in 2005 to 35.7% of cases in 2015, 35.6% in 2016 and 38.2% in 2017. The rate of the patients with sputum conversion in culture positive patients increased from 30.8% in 2005 to 51.5% of cases in 2017. The increased rate of the microbiologic conversion in the MDR-TB cases from 5.7% in 2005 to 28.7% in 2017 was due to the allocation of funds from the federal budget for the procurement of the expensive anti-tuberculosis drugs[4].

Conclusions

The Russian Federation made certain progress in tuberculosis control, however, the vast territory, its heterogeneity, including climatic and socio-economic differences, affect the epidemiological situation. Big efforts were performed for solving the problems associated with the infection control.

The epidemiological indices went down. More expressed was the reducing of the the mortality rate. The rate of the affected men is maintaining at the same level, demonstrating the persistence of the social and behavioral risk factors in the male groups.

The epidemiological indices in the pediatric population went down, however the lowest level registered in 1992 was not achieved. The rate of tuberculosis in urban and rural population remains in average at the same level.

Big differences of the epidemiological indices were registered in different districts, demonstrating regional disparity in the social economic development and accessibility to the health care services.

The increasing rate of the TB/HIV infection demonstrated the limited resources available in the control of both infections.

The epidemiological data determined several times increase of the MDR-TB rate in primary and previously treated cases, due to multiple causes.

The decreasing rate of the fibro-cavitary tuberculosis and the rate of microscopic positive cases demonstrated the early diagnosis using the annual radiological screening and the molecular genetic method with substantially improved diagnosis sensibility.

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REVIEW ARTICLES

DOI: 10.5281/zenodo.1299028
UDC: 616.346.2-002.1

Acute appendicitis

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Abstract

Background: It has been more than 130 years since Reginald Fitz described for the first time the pathomorphological changes of the acute appendicitis and after the first appendectomy that was performed by H. Hancock, in the case of acute appendicitis, but the problem remains to be actual till now. Despite of the acute appendicitis is the most common abdominal emergency, there still are some compartments of this problem that need to be studied, starting with particularities of etiology of acute appendicitis and ending with particularities of clinical picture, differential diagnosis, and treatment. The article describes anatomy and physiology of the appendix. Authors presented data on history, epidemiology, etiology, pathophysiology, classifications, complications, management, treatment and prognosis of acute appendicitis. Also the article reflects characteristics of acute appendicitis in children, elderly patients and pregnancy and clinical signs depending on anatomical position of appendix.

Conclusions: Earlier diagnostics and an early surgery performance in patients with acute appendicitis are the key to success, and lead to significant reduction in number of postoperative complications. We hope that our article will be useful for medical students, family doctors, and for young surgeons.

Key words: appendix, acute appendicitis.

Appendix, definition

The vermiform appendix is a narrow blind tube structure attached to the cecum and is named after the Latin word "vermiform" which means 'worm-shaped'. The appendix is a true diverticulum of the cecum. In contrast to acquired diverticular disease, which consists of a protuberance of a subset of the enteric wall layers, the appendiceal wall contains all of the layers of the colonic wall.

Anatomy of appendix

It varies essentially in length from 1 to 25 cm; but mostly it varies between 5-10 cm [16]. The histological features of the appendix include the following: serosa, the muscularis layers, the submucosa and mucosa. The three taenia coli converge at the junction of the cecum with the appendix and can be a useful landmark to identify the appendix.

The appendix receives its arterial supply from the appendicular branch of the ileocolic artery. Findings in literature show that the retrocecal appendix position can be found in 26-70% of cases, followed by paracecal and then the other positions of the appendix in different percentage. Retrocecal position – when appendix lies behind the cecum although in majority of cases in an intraperitoneal location. Only in case of a long retrocecal appendix the tip of the appendix remains in the retroperitoneal tissue close to the ureter [24]. Pelvic position of the appendix is the second most common irregular position –25% [24]. Subcecal position of the appendix (2%) – that means position of the appendix behind the cecum. Subhepatic position of the appendix (3%) – it means that the tip of the appendix is towards the liver [24]. Absent appendix is too rare a condition and until this date only 68 cases have been reported in the literature [22]. Similarly duplication of appendix is also a rare anomaly and fewer than 100 cases have been reported [19]. The peculiarity of the musculature of the appendix is that there are a few gaps in the muscular layer called "hiatus muscularis". Through this infection from the submucosal coat directly comes to peritoneum and regional peritonitis occurs [24]. The appendix is fixed retrocecally in 16% of adults and is freely mobile in the remainder [24]. The function of the appendix is not clearly understood, although the presence of lymphatic tissue on it suggests a role in the immune system.

Physiology of the appendix

- Secretion function, the appendix mucosa makes and releases the special fluid that contents mucus and proteolytic enzymes.
- Hemopoetic function – limphopoetic and immune.

Acute appendicitis is one of the more common causes of acute abdominal pain and is the most common emergency surgical presentation requiring operation.

Acute appendicitis is an inflammation of vermiform appendix caused by nonspecific purulent infection [17].

History

Appendix was first depicted in anatomic drawings in 1492 by Leonardo da Vinci. Appendix was described in 1521 by Berengar Carpi, a famous surgeon and anatomist from Bologna and in 1543 was remarked in "The humanus corporis factory" of Vesalius. The first report of an appendectomy came from Amyan, a surgeon of the English army that performed an appendectomy in 1735 without anesthesia to remove a perforated appendix [24]. Reginald Fitz, an anatomopathologist at Harvard, first described acute and chronic appendicitis in 1886 and it has been recognized as one of the most common causes of severe acute abdominal pain worldwide [11]. The greatest contributor to the advancement in the treatment of appendicitis was an American surgeon from York, Charles McBurney. In 1889 he described some symptoms, anatomical point projection of inflamed appendix, and described the classic oblique incision that nowadays is used for appendectomy, and in the same year, he published his landmark paper in the *New York State Medical Journal* describing the indications for early laparotomy for the treatment of acute appendicitis.

Then, at the end of the 19th century, the English surgeon H. Hancock successfully performed the first appendectomy in a patient with acute appendicitis.

Semm is widely credited with performing the first successful laparoscopic appendectomy in 1982 [25].

Epidemiology

Approximately 7% of people in western countries have appendicitis at some time during their lives, and about 200,000 appendectomies for acute appendicitis are performed annually in the United States [14]. About 10% of the population will develop acute appendicitis during their lifetime [23]. Between 15 and 30 years of age there is an increase of 230 cases per 100,000 population/year, and then a decline of cases with aging. The frequency of acute appendicitis is different in each country. The current incidence of appendicitis is about 100 per 100,000 person-years in Europe/America and 220 cases per 100.000 in the Republic of Moldova (2007). In Asian and African countries, the incidence is probably lower because of the dietary habits. The incidence is lower in populations where a high-fiber diet is consumed [4, 12]. Appendicitis occurs more frequently in males than in females, with a male-to-female ratio of 1.7:1 [24] although some authors described the prevalence in women. Appendicitis is a disease of the young, with 40% of cases occurring in patients between the ages of 10 and 29 years. According to Yui-Rwei Y 90% of the cases of appendix infection occur in patients under 60 years old [35]. The lower number was in the age from 70 to 80 years of life. It is unusual under the age of 1 year. Incidence of acute appendicitis decreases with age, because lumen of the appendix enlarges after lymphoid tissue atrophies. Petroianu A. mentioned that the most of patients are whites (74 %), and it is very rare in patients with black skin (5 %) [21]. Some authors have described a seasonal variation in the incidence of appendicitis, a higher incidence occurring at summer time, or at spring and autumn, or even in raining weather [5]. The cost per patient for the surgical treatment of appendicitis in the United States ranges from $11,577 to $13,965.

Etiology

Although it was originally described more than 125 years ago, the etiology of acute appendicitis continues to be debated. Obstruction of the lumen is the dominant etiologic factor in acute appendicitis. Obstruction of the proximal lumen by fibrous bands, submucosal lymphoid hyperplasia, fecaliths, inspissated barium, calculi, vegetable/fruit seeds, worms, tumors of cecum/appendix has long been considered to be the major cause of acute appendicitis, though that theory is doubted by many experts. Fecalith or calculus is found in only 10% of acutely inflamed appendices [10,14]. Fecalith alone causes simple appendicitis in 40%, gangrenous non-perforated appendicitis in 65%, and perforated appendicitis in 90% of cases [6]. Although it is widely accepted that obstruction is the inciting event in most cases of acute appendicitis, it is worth pointing out some observations that are not consistent with this hypothesis [16]. The first observation is that impacted fecaliths have been observed with no accompanying local inflammation or syndrome of appendicitis. In addition, fecalith impaction or functional evidence of obstruction cannot be demonstrated in a substantial number, up to half, of cases. Thus obstruction may be just one of many factors involved in the etiology and pathogenesis of acute appendicitis [16].

Some researchers suggest that the forms of uncomplicated and complicated appendicitis are two distinct diseases, with different etiologies, each of which leads to the final pathway of invasion of the appendiceal wall by intraluminal bacteria.

One of the theories of acute appendicitis is *Aschoff infectious theory.* Advocates of infectious theory consider that the main cause of acute appendicitis is a polymicrobial infection that occurs in a healthy intestine (E. coli, Staphylococcus, Streptococcus).The significant place has had the predisposing factors, that lead to the trauma of the mucous membrane of the appendix, like: foreign bodies, pieces of solid food, intestinal parasites, as well as intestinal atony, changes in the reactivity of the organism, etc. That leads to violations of the barrier function of the epithelium of appendix and penetration of the bacteria inside appendix wall. As in other intra-abdominal infections, such as salpingitis, diverticulitis and enterocolitis, which are often treated only with antibiotics, the infectious etiology of acute appendicitis is advocated by some scholars [26].

Hematogenous theory. Bacteria reach the appendix by blood, from an infection source. This theory is especially true in children after infectious disease. The infection can occur in outbreaks. Nearby situation occurs frequently in women, the inflamed ovaries may be the source of infection. Advocates of *neurovascular theory* consider that the most important moment in acute appendicitis pathogenesis is a reflex abrupt violation of the regional blood flow inside the appendix (vasospasm, ischemia), which leads to the thrombosis of the blood vessels of the appendix and development

of trophic disorders and necrosis inside the appendix wall.

Some researchers attach importance to the *allergic factor* endpoint to significant amount of mucus and Charcot-Leiden crystals in the lumen of the appendix.

Recently, with the advent of *neurogastroenterology*, the concept of neuroimmune appendicitis has evolved [40].

Pathophysiology

The lumen distal to the obstruction starts to fill with mucus and acts as a closed-loop obstruction. This leads to distension and an increase in intraluminal and intramural pressure [6]. As the pressure of the lumen exceeds the venous pressure, the small venules and capillaries become thrombosed, but arterioles remain open, which leads to engorgement and congestion of the appendix. With vascular compromise, epithelial mucosa breaks down and bacterial invasion by bowel flora occurs. The inflammatory process soon involves the serosa of the appendix, hence the parietal peritoneum in the region, which causes classical right lower quadrant pain [6]. Once the small arterioles are thrombosed, the area at the antimesenteric border becomes ischemic, and infarction and perforation ensue. Bacteria leak out through the dying walls and pus forms (suppuration) within and around the appendix. Perforations are usually seen just beyond the obstruction rather than at the tip of the appendix [6].

Kolesov's classification of acute appendicitis (1952)

1. Appendicular colic.
2. Simple superficial appendicitis.
3. Destructive appendicitis:
   a) phlegmonous;
   b) gangrenous;
   c) perforated.
4. Complicated appendicitis:
   a) appendicular mass;
   b) appendicular abscess;
   c) diffuse purulent peritonitis.
5. Other complications of acute appendicitis (pylephlebitis, sepsis, retroperitoneal phlegmon, local abscesses of abdominal cavity) [38, 39].

L. Kovalchuk remarked that [17] four phases are distinguished according to clinical features of acute appendicitis: 1) epigastric; 2) local symptoms; 3) calming down; 4) complications [17].

During the last World Society of Emergency Surgery Congress in Israel (July 6th, 2015), the authors submitted a new comprehensive disease grading system, based on three aspects of the disease (clinical and imaging presentation and laparoscopic findings).

Acute appendicitis grading system

Grade-0 (normal looking) – the patient has a clinical diagnosis of acute appendicitis and laparoscopy shows a macroscopically "normal looking appendix", the histopathological study shows intraluminal inflammation.

Grade-1 (inflamed) – the image shows hyperemia and edema of appendix.

Grade-2A and 2B (necrosis)

Grade 2A – the necrosis was an isolated phenomenon, restricted to the appendix, without or with minimal local exudation.

Grade 2B – presence of necrosis involving the appendicular base, at the level of its insertion on cecal wall.

Grade-3A -3B -3C (perforated – inflammatory tumor)

Grade 3A – the CT scan of abdomen showing an inflammatory tumor in the lower right quadrant. The patient was managed with antibiotics only; non-operative treatment.

Grade 3B – acute appendicitis complicated with inflammatory tumor and an abscess less than 5 cm, managed by laparoscopic approach.

Grade-4 (perforate – diffuse peritonitis)

Clinical diagnostics of acute appendicitis. Complaints

Only half of the patients present the classical clinical diagnosis of appendix infection.

Pain localization

Typically begins as epigastric pain or periumbilical (in 70% of patients) followed by brief nausea, vomiting, and anorexia [23]. After a few hours, the pain shifts to the right lower quadrant (the Kocher's and Kiumeli's symptoms).

Patients may present with localized pain in the right upper quadrant from a long appendix, in the left lower quadrant if malrotation is present, and in the anterior wall of the rectum if the appendix is located in the pelvis [8,42,43]. The most common location of 'atypical' somatic pain is the right flank in patients with a rectrocecal appendix [8].

The typical patient will therefore present with an initial central colicky abdominal pain, which after a few hours progresses into a constant right iliac fossa pain (the pain moves; it does not radiate) [2]. As the response to luminal obstruction evolves to include luminal distension, intramural edema, and ischemia, the pain becomes constant [16]. The disease begins with a sudden pain in the abdomen. It is localized in the right iliac region, has moderate intensity, permanent character and does not irradiate [17, 24].

The second characteristic feature of the pain in appendicitis is its permanence. Pain remains while the inflammatory process is present, may increase and wane, but not completely stopped. The intensity of pain depends from the location and extension of the process of inflammation. For the vast majority of patients moderate, constant aching pain is typical, but sometimes (4-19%) cramping occurs. Pain may be very acute, especially in perforation appendicitis and in the cases of peritonitis, but it does not reach such a force as in patients with perforated gastric ulcer, acute intestinal obstruction, acute pancreatitis or renal colic. Pain intensity does not always correspond to the degree of pathological anatomical changes. Sometimes intense pain before operation has had slight morphological changes inside the appendix, and, conversely, the presence of even gangrenous and perforated appendicitis sometimes do not complain of severe pain [36].

Pain increases with cough and motion that is caused by movement of the inflamed appendix against the peritoneum. Patients usually lie down, flex their hips, and draw their knees up to reduce movements and to avoid worsening their pain.

The duration of symptoms is less than 48 hours in approximately 80% of adults but tends to be longer in elderly persons and in those with perforation.

Anorexia is the most constant symptom of appendicitis. If it is absent, the diagnosis should be questioned [8]. A patient reporting a normal appetite is very uncommon [16]. Vomiting occurs in the first hours of the disease and more often is single. In catarrhal appendicitis vomiting is absent in 90% of all cases. Repeated vomiting is observed in acute appendicitis complicated by peritonitis. Vomiting does not bring relief to the patient. With involvement of the peritoneum inflammation some patients have constipation due to intestinal paresis.

Temperature elevation is rarely >1°C. Fever above 100°F or 38.2°C rarely occurs early in the appendicitis syndrome and usually appears after the time when local tenderness appears [16].

Less common symptoms include diarrhea, which may occur early or late in the course of appendicitis. Early in the course, patients may have one or two loose bowel movements, or they may have an episode of massive evacuation of normal stool. This sequence represents a response to visceral pain and is usually limited to one or two episodes, rather than the persistent diarrhea caused by viral or bacterial infection. Later in the course of appendicitis, diarrhea may return because of irritation of the rectum by an inflamed pelvic appendix [8].

Diarrhea, urinary frequency, pyuria, or microscopic hematuria may suggest a retrocecal appendix, causing irritation of adjacent structures [23]. Mild fever and tachycardia are common in appendicitis [23].

Clinical examination

The general condition of patients is usually normal and gets worse only in case of growth of destructive changes in appendix [24]. Pulse rate is normal or slightly elevated.

Do not administer analgesics and antipyretics to patients with suspected appendicitis who have not been evaluated by the surgeon.

Painfulness is the basic and decisive sign of acute appendicitis during the examination by palpation in a right iliac region. Tension of muscle of abdominal wall is a positive symptom of peritoneum irritation [9, 24]. Tenderness over the site of the appendix is the hallmark of appendicitis. However, tenderness may be absent early in the course of the illness or unelicitable in obese individuals. Patients with a retrocecal appendix may experience some mild right-sided or right-flank tenderness [9]. Classically, the area of maximal tenderness will be one-third of the way from the anterior superior iliac spine to the umbilicus, but in fact it will be wherever the appendix is in the individual patient [8]. Cutaneous hyperesthesia often overlies the region of maximal tenderness [23].

Dieulafoy triade – cutaneous hyperesthesia often overlies the region of maximal tenderness and pain.

Kocher (Kosher)'s sign. The classic history – the appearance of pain in the epigastric region and migrating or subsequent shift to the right iliac region occurs in only 50 percent of patients.

Kiummel sign. The appearance of pain in the umbilical region and migrating or subsequent shift to the right iliac region.

The Rovsing's sign. Continuous deep palpation starting from the left iliac fossa upwards (anti-clockwise along the colon) may cause pain in the right iliac fossa, by pushing bowel contents towards the ileocecal valve and thus increasing pressure around the appendix.

Psoas sign or Coupe 1 sign. With the patient lying on the left side, slow extension of the right hip causes local irritation and pain**.** This indicates presence of irritative inflamed appendix in close proximity to the psoas muscle. This is possible in retrocecal appendicitis [23, 24].

Obturator sign or Coupe 2 sign. With the patient supine, passive internal rotation of the flexed right hip causes hypogastric pain [23].

Shake test. Another useful sign in establishing the presence of local peritonitis. Most surgeons perform this by grasping the iliac wings and shaking the pelvis from side to side. The patient complains of pain at the site of the appendix if local peritonitis is present [8].

Blumberg's sign. After gradual pressing by fingers on a front abdominal wall from the place of pain quickly, but not acutely, the hand is taken away. Strengthening of pain is considered as a positive symptom in that place. Tension of muscles of front abdominal wall is obligatory here.

Voskresensky's sign. By left hand the shirt of patient is drawn downward and fixed on pubis. By the taps of 2–4 fingers of right hand epigastric region is pressed, the hand slides in the direction of right iliac region, without taking the hand away. Thus there is an acute strengthening of pain.

Sitkovsky's sign. A patient, that lies on the left, feels the pain which arises or increases in the right iliac region. The mechanism of intensification of pain is explained by displacement of blind gut to the left, by drawing of mesentery of the inflamed appendix.

The Obrazcov's sign. With the position of patient on the back by index and middle fingers the right iliac region of most painful place is pressed and the patient is asked to heave up the straightened right leg. At appendicitis pain increases acutely.

Bartomier's sign. The increase of pain intensity during the palpation in right iliac region of patient in position on the left side. At such position an omentum and loops of small intestine are displaced to the left, and appendix becomes accessible for palpation.

Rosdolsky's sign. At percussion of the anterior abdominal wall the pain increases in a right iliac region.

Krimov symptom. Pain during peritoneum palpation throws right external inguinal orifice.

Iaure–Rozanov sign. Palpation in the projection of Pti triangle is painful.

Laboratory evaluation

Although the diagnosis is clinical, the complementary tests may be useful in doubt. What are the main alterations of laboratory tests in patients with acute appendicitis?

Leukocyte count greater than 11,000 cells/ul with polymorphonuclear cell predominance is common in children and young adults [23]. An elevated white blood cell count cannot be used alone as a sign of appendicitis [24]. A leukocytosis of over 20 000 mm^3 suggests perforation of the appendix or another diagnosis [8]. Neutrophilia with deviation to the left is frequently associated to lymphopenia and can be presented along with monocytosis. They are predictive for the diagnosis of acute appendicitis, whereas elevated levels of fibrinogen, high lymphocyte and PLT count are predictive for non-appendiceal pathology with low diagnostic accuracies [41]. In 20-30% of acute appendicitis, the number of white blood cells is normal or only slightly increased. Some studies mention that the ratio neutrophils / lymphocytes of greater value than 3.5 would indicate for diagnosis of acute appendicitis and would be more sensitive diagnostic test if compared to the WBC account [15].

The advanced stages related to the initial ones, show lower quantities of lymphocytes (9,3% e 14,8%, respectively), with value of $p<0,05$ [3]. C reactive protein levels are also related to the evolution stage of appendicitis, according to literature. Values above 50 mg/dl are related to appendix necrosis and perforation.

Urine analysis is abnormal in 25% of patients with appendicitis. Pyuria, albuminuria, and hematuria are common. Bacteria suggest urinary tract infection. Hematuria suggests urolithiasis [23]. The urinalysis also may be abnormal with appendicitis because the appendix lies near the ureter and bladder. Most patients with appendicitis, however, have a normal urinalysis [24].

Serum pregnancy test should be performed in women of childbearing age. A positive test suggests an ectopic pregnancy [23].

Abdominal X-rays. Plain radiography is infrequently able to give the diagnosis, however, it is useful for identifying free gas, and may show an appendicolith in 7-15% of cases [8].

An appendicolith can be seen in only one-third of children and one-fifth of adults with appendicitis [23].

Plain abdominal films may reveal localized ileus and soft-tissue density in the right lower quadrant, or free intraperitoneal air (0-7%). These are so non-specific as to be of no value in the diagnosis of appendicitis [8].

Ultrasonography showed the highest diagnostic accuracy (92.9%). Sonographically, the appendix is identified as a blind-ending, nonperistaltic bowel loop originating from the cecum. Findings associated with appendicitis include wall thickening, luminal distention, lack of compressibility, abscess formation, and free intraperitoneal fluid [32]. Nevertheless, during appendicitis, the appendix can be seen in only 50% of patients. Therefore, not seeing the appendix during an Ultrasonic test does not exclude appendicitis [24]. Ultrasonographic criteria for the diagnosis of appendicitis are: a threshold 6-mm diameter of the appendix under compression surrounded by a hypoechoic, thickened wall more than 2 mm in diameter [8]. Remark that a sensitivity of only 75 per cent is too low to be acceptable in a diagnostic test.

CT scan of the appendix region is useful in acute appendicitis and periappendicular abscesses diagnosis as well as in excluding other diseases inside the abdomen and pelvis that can mimic appendicitis [24]. CT is highly sensitive (94-98%) and specific (up to 97%) for the diagnosis of acute appendicitis and allows for alternative causes of abdominal pain also to be diagnosed.

CT findings include: dilated appendix with distended lumen (>6 mm diameter), thickening of the cecal apex (up to 80%): pericecal adenopathy, including stranding of the adjacent fat and thickening of the lateroconal fascia or mesoappendix, pericecal collections of fluid, inflammatory phlegmon, abscess formation, appendicolith may also be identified [8].

Laparoscopy can be used in the diagnosis and treatment of patients with suspected acute abdomen that imitates acute appendicitis and cannot be differentiated with physical examination and laboratory methods. Delays in diagnosis and unnecessary appendectomy will be prevented in this way.

Scoring Systems

The diagnosis of acute appendicitis can be difficult and any delay in definitive treatment with surgery can lead to an increase in mortality and morbidity as the disease progresses to appendiceal perforation.

The most widely cited score in the diagnosis of adults with acute appendicitis is the Alvarado score (Alvarado, 1986) [1]. Whereas, in children the pediatric appendicitis score or Samuel score is most widely used (Samuel, 2002) [23].

Alvarado score resulted in the formation of a simple score, from 0 to 10, consisting of three symptoms (Migration of pain to right iliac fossa – 1 point, anorexia or acetone in urine – 1 point, nausea-vomiting – 1 point, three signs (tenderness over right iliac fossa – 2 points, rebound tenderness over right iliac fossa – 1 point, elevation of temperature 37.3C – 1 point,) and two laboratory markers of inflammation (leukocytosis more than 10000 – 2 points, shift to the left more than 75% – 1point).

The maximum total score achievable is, therefore, 10. Group-I with total score 1-4 diagnosis of acute appendicitis unlikely. A score of 5 or 6 is compatible with a diagnosis of possible acute appendicitis. Score of 7 or 8 indicating probable appendicitis and a score of 9 or 10 indicating a very probable acute appendicitis. It has been suggested that score can be used as a guide to determine which patients require further observation and which patients require surgery. Those with a score of 5 or 6 required observation while those with a score of 7 or above needed to proceed to surgery as it was likely that they had appendicitis.

Differential diagnosis. Acute surgical diseases

Acute cholecystitis. The attack of pain can arise after the reception of spicy food and, is accompanied by nausea and frequent vomiting by bile. In anamnesis patients often have information about a gallstone disease. During examination intensive painfulness is observed in right hypochondrium,

increased gall-bladder and positive symptoms Murphy's and Ortner's.

Perforated peptic ulcer disease. The pain in the right ileac area is caused by gastric and duodenal fluids that tend to settle in the right paracolic gutter causing peritonitis and RLQ pain. The syndrome carries its name after Rudolph Valentino, a famous American film actor, who died in 1926 due to complication related to the perforated peptic ulcer. He presented with pain in right lower abdomen and was diagnosed and treated as a case of acute appendicitis and later on autopsy, he was found to have perforated peptic ulcer [34].

The pain in right ileac area usually is moderate in acute appendicitis and is usually dramatic, sudden, severe, midepigastric pain, like a knife stab, which spreads rapidly to involve the entire abdomen. Both pathologies need an emergency surgery. At surgery, the appendix was found to be normal but with surrounding turbid fluid in the right paracolic gutter and subhepatic space.

An strangulated right inguinal or femoral hernia may present with right ileo-inghinal pain. There will be tenderness and an irreducible swelling over the hernial orifice, and symptoms and signs of bowel obstruction. Cough impulse is lost if hernia is strangulated.

Acute intestinal obstruction

In acute intestinal obstruction the patient has the cramp abdominal pain, distended abdomen; constipation progressing to obstipation, vomiting. In acute appendicitis the patient has permanent, moderate pain usually in the right ileac area, non-distending abdomen, rare vomiting.

Gastrointestinal diseases

Gastroenteritis is characterized by nausea, emesis prior to the onset of abdominal pain, malaise, fever, and poorly localized abdominal pain and tenderness. The WBC count is less frequently elevated. Salmonella typhimurium infection causes mesenteric adenitis and paralytic ileus with symptoms similar to those of appendicitis. The diagnosis can be established by serologic testing. *Campylobacter jejuni* causes diarrhea and pain that mimics that of appendicitis. The organism can be cultured from stool.

Meckel's diverticulitis may mimic appendicitis, and practically is impossible to put correct diagnosis before surgery, because clinical signs are the same.

Meckel's diverticulitis is associated with the same complications as appendicitis and requires the same treatment – prompt surgical intervention. Resection of the diverticulum or resection of the segment of ileum bearing the diverticulum with end-to-end anastomosis can nearly always be done through a McBurney incision, extended if necessary, or laparoscopically.

Mesenteric lymphadenitis is characterized by alarming discrepancy between general symptoms (headache, cough, fever, diffuse muscle aches) and slight pain in the right iliac area; white blood cell count is normal or even low, and a relative lymphocytosis will be present. During surgery the surgeon finds a normal appendix and mesenteric lymph, increased in size. Acute mesenteric adenitis is the disease most often confused with acute appendicitis in children. The pain usually is diffuse, and tenderness is not as sharply localized as in appendicitis. Voluntary guarding is sometimes present, but true rigidity is rare. Generalized lymphadenopathy may be noted.

Crohn's disease. The manifestations of acute regional enteritis – fever, right lower quadrant pain and tenderness, and leukocytosis – often simulate acute appendicitis. The presence of diarrhea and the absence of anorexia, nausea, and vomiting favor a diagnosis of enteritis, but this is not sufficient to exclude acute appendicitis. In an appreciable percentage of patients with chronic regional enteritis, the diagnosis is first made at the time of surgery for presumed acute appendicitis. In cases of an acutely inflamed distal ileum with no cecal involvement and a normal appendix, appendectomy is indicated. Progression to chronic Crohn's ileitis is uncommon.

Perforating carcinoma of the cecum, or of that portion of the sigmoid that lies in the right side, may be impossible to distinguish from appendicitis. These entities should be considered in older patients. CT scanning is often helpful in making a diagnosis in older patients with right lower quadrant pain and atypical clinical presentations.

Urologic diseases

Pyelonephritis is associated with high fever, rigors, and costovertebral pain and tenderness. Diagnosis is confirmed by urine analysis.

Ureteral colic. Intensity of pain in kidney colic is one of the basic differences from acute appendicitis. Pain at first appears in right lumbar region and irradiates downward after passing of ureter in genital organs. Tenderness is usually minimal and hematuria is present. The excretory urography will be very useful to exclude the ureteral stone. Absence of function of right kidney allows to eliminate the diagnosis of acute appendicitis.

Gynecologic diseases

Pelvic inflammatory disease. In pelvic inflammatory disease the infection usually is bilateral but, if confined to the right tube, may mimic acute appendicitis. Nausea and vomiting are present in patients with appendicitis, but in only approximately 50% of those with pelvic inflammatory disease. Pain and tenderness are usually lower, and motion of the cervix is exquisitely painful. Intracellular diplococci may be demonstrable on smear of the purulent vaginal discharge.

Ultrasound can help distinguish PID from appendicitis.

Ectopic pregnancy. A pregnancy test should be performed in all female patients of childbearing age presenting with abdominal pain. Ultrasonography is diagnostic.

Ovarian cysts can cause sudden pain by enlarging or rupturing. The cysts are detected by transvaginal ultrasonography.

Ovarian torsion. The ischemic ovary often can be palpated on bimanual pelvic examination. The diagnosis is confirmed by ultrasonography.

The apoplexy of ovary a more frequent is with young women and, as a rule, on 10-14 day after menstruation. Pain appears suddenly and irradiates in the thigh and perineum. At the beginning of disease there can be a collapse. However, the general condition of patients suffers insignificantly. When not enough blood was passed in the abdominal cav-

ity, all signs of pathology of abdominal cavity organs calm down after some time. Signs, which are characteristic of acute anemia, appear at considerable hemorrhage.

Appendicular mass is necessary to be distinguished with cecum or colon neoplasms, from ileo-cecal tuberculosis, terminal ileitis and from ileal and colonic locations of Crohn's disease, from ileocecal intussusceptions, ovarian cysts torsion, retroperitoneal tumors, etc.

Some rare medical conditions, like Schonlein-Henoch abdominal purple, abdominal saturnine colic, porphyrinuria, anaphylactic abdominal pain can cause clinical pictures of fake acute abdomen and fake clinical sign of acute appendicitis [10].

Treatment

The treatment of acute appendicitis is appendectomy. Appendectomy is the most common nonelective surgery performed by general surgeons. Several methods of performing an appendectomy include open incision method and laparoscopic approach.

The surgery may be performed through a standard small incision in the right lower part of the abdomen, or it may be performed using a laparoscopy, which requires three to four smaller incisions [24].

Management of acute appendicitis

Alvarado score [1] is found to be helpful in the diagnosis and management of acute appendicitis. Diagnosis of acute appendicitis is virtually confirmed with a score of 7-10 especially in males and they should undergo appendectomy. Diagnostic laparoscopy is advised to minimize the unacceptable high false negative rate in women. Patients with score 5-6 must be admitted and scored frequently. Score 1-4 can be discharged unless otherwise indicated.

It should be noted that excessive involvement in the surgery, sometimes a frivolous approach to surveys of acute appendicitis leads to the fact that appendectomy is performed in those cases when it is not clinically justified. In more than 70% of patients suffering from an adhesion disease, the first operation was the removal of the unchanged vermiform appendage [37].

Open appendectomy is indicated when the surgeon or patient prefers an open procedure to a laparoscopic procedure, or when the laparoscopic approach is contraindicated.

An incision is made in the right iliac fossa. The position of the incision is based upon the location of the McBurney point, which is a point one third of the distance from the anterior superior iliac spine to the umbilicus [44]. Place the incision between the first third and the second third of the distance from the anterior superior iliac spine to the umbilicus. An incision is then made down through skin and subcutaneous tissues until the muscle layers are reached. External oblique muscle is split bluntly by using Roux retractors. The external oblique, internal oblique and transverse abdominus muscles are then opened. This is done by a muscle-splitting incision along the lines of the fibers with no fibers actually being cut. The peritoneum is open and the abdominal cavity can be entered. Once the peritoneal cavity is opened, any fluid encountered should be sent for Gram stain and culture. The appendix and cecum are then identified and pulled up through the incision. The appendix can be removed through either an antegrade or a retrograde technique. After exteriorization of the appendix, the mesoappendix is held between clamps, divided, and ligated. The appendix is clamped proximally and is cut above the clamp with a scalpel [24]. Many surgeons then bury the stump of the appendix with a purse-string and z-stitch around the cecum. The cecum is then returned to the abdomen. Any fluid or pus is carefully sucked and swabbed out. If there is severe contamination, a drain may be left [2]. In cases of perforated appendicitis, some surgeons leave the wound open, allowing for secondary closure or a delayed primary closure until the fourth or fifth day after operation. Other surgeons prefer immediate closure in these cases [24]. Although most authors recommend leaving the incisions open when there is gross contamination by pus and fecal material, there is increasing evidence that this may be no more unsafe and less cost-effective than closing all wounds (where it is feasible) and later treating any wound infections that result. This decision should be individualized to each patient [16].

Laparoscopic approaches

Based on the most recent information available, it seems clear that in uncomplicated cases the diagnosis is secure, the laparoscopic approaches may offer a small reduction in pain scores, a mild reduction in hospital stay, and possibly a reduction in wound infection rates. Return to work may also occur earlier [16].

Contraindications to the laparoscopic approach include the lack of surgical expertise and necessary equipment, severe pulmonary disorders, a bleeding diathesis, severe heart failure, portal hypertension, intolerance of Trendelenburg positioning, poor visualization, and severe adhesive disease from previous abdominal surgical procedures.

The operation should be covered with prophylactic antibiotics, usually Metronidazole, given intravenously at induction of the anesthetic [2]. We could sometimes meet the information about the fact that spontaneous resolution of early acute appendicitis can occur. Several authors suggest that antibiotics may be used as primary treatment for selected patients with suspected uncomplicated appendicitis. However, due to the small number of patients included and the lack of the characteristics of the patients, it is very difficult to analyze these studies. I must remark that it is a very dangerous treatment way for acute appendicitis, but sometimes it is possible to try to do it,if facilities for appendectomy are not available. Conservative or unreasonable long-termdynamic observation can lead to a time lost for an optimal surgical treatment of acute appendicitis that will certainly increase the number of postoperative complications and mortality [29].

Acute appendicitis in children

The mean age in the pediatric population is 6-10 years, we can meet only 2% appendicitis in children under 2 years. It is common after age of two years. In infants acute appendicitis can be seen infrequently, but, quite often carries atypical character. Historically, the lack of classic symptoms and delay in presentation make diagnosing acute appendi-

citis more difficult in children, resulting in a higher perforation rate. All this is characterized, mainly, by the features of anatomy of appendix, insufficient of plastic properties of the peritoneum, short omentum and high reactivity of child's organism [17]. The temperature reaction is also expressed considerably acuter. In the blood test there is high leukocytosis [24]. Very often the children do not report pain, because they are afraid, in this situation are very important patient's mimics ("what the word hides, the face betrays").

Acute appendicitis in elderly patients

Appendicitis is known to be the disease of the younger age groups with only 5-10% of cases occurring in the elderly population. It is widely recognized that elderly patients with appendicitis present with less acute symptoms, less impressive clinical signs, and leukocytosis [33]. The triad of right lower abdominal pain and tenderness, fever and leukocytosis is reported to be present in not more than 26% of patients above 60 years [27]. Up to 30% of elderly patients present more than 48 h into the illness, and between 50% and 70% have a perforation at the time of surgery [16]. The reasons behind this high rate were postulated to be due to the late and atypical presentation, delay in diagnosis and surgical intervention, presence of comorbid diseases and to the age-specific physiological changes [13]. The early use of CT scan can cut short the way to the appropriate treatment. Elderly patients have a higher risk for both mortality and morbidity following appendectomy. It was estimated to be around 70% as compared to 1% in the general population [30].

Acute appendicitis in pregnancy

Acute appendicitis can occur at any time during pregnancy, although it occurs most often during the second trimester (45%) and 30% during the first trimester and the remaining 25% in the third trimester. The overall incidence being 0.15 to 2.10 per 1000 pregnancies [7]. In pregnancy, the percentage of perforated appendix can be as high as 43%, compared to 19% in the general population [31]. Appendicitis is associated with a fetal loss risk of 1.5% to 9%, however the risk increases up to 35% after perforation.

The diagnosis of acute appendicitis during pregnancy is one of the most challenging of all clinical problems. Appendicitis occurs at the same rate in pregnant and nonpregnant women, but pregnant women have a higher rate of perforation.

Pregnancy itself, especially in the early stages, is associated with nausea, vomiting, and pain. In the first and early second trimester, the evolution of symptoms and signs is not different from that in nonpregnant women. During pregnancy the appendix is superiorly and laterally displaced by the enlarging uterus, thus, pushing it away from Mc Burney's point. After the fifth month, the cecum and appendix are shifted upward by the expanding uterus. In the last trimester, localized tenderness from the appendix may be found in the upper flank and right upper quadrant of the abdomen. In addition to this, rebound tenderness and guarding of the abdominal wall are less commonly elicited during examination due to the laxity of the abdominal wall muscles during pregnancy. Fever, hypotension, and tachycardia are also unreliable and may not be present during pregnancy [10,15,18]. Leukocytosis is a normal physiological response of pregnancy (up to 12 500 leukocytes/mm^3) and cannot be relied upon to help confirm the diagnosis of appendicitis and bandemia (immature WBC) are normal physiological alterations during pregnancy. Not all pregnant women with appendicitis have leukocytosis [20]. The diagnosis of appendicitis in pregnancy is mainly clinical but ultrasound is very helpful in this setting, as it may provide images of the appendix, gallbladder, uterus, and other pelvic organs [16].

Complication of acute appendicitis

The more serious complications of acute appendicitis usually include: local and genera1 peritonitis, appendicular or periappendicular mass or abscesses, pylephlebitis, liver abscess.

General peritonitis. The diagnosis of acute appendicitis with genera1 peritonitis is not difficult. The patient complains of severe general abdomina1 pain, with nausea and vomiting. The abdomen becomes distended, tense and rigid. There is tenderness throughout, although it remains more marked in the right lower quadrant. Auscultation reveals a silent abdomen. The temperature is considerably higher (39 - 40 C.) than in uncomplicated acute appendicitis, and the pulse rate is increased. The leukocytes count is also high, more than 20,000.

The patient requires an emergency surgery with preoperative preparation during 1 or 2 hours. The meaning of surgery – large laparotomy, appendectomy, toilet and drainage of the peritoneal cavity.

An appendiceal mass is as an inflammatory mass consisting of an inflamed appendix which has become adherent to the omentum and surrounding viscera. This is an attempt of the nature to prevent general peritonitis even if rupture of the appendix occurs [24]. Appendicular mass is a common surgical clinical entity encountered in 2-6% of the patients presenting with acute appendicitis.

It develops, certainly, on 3-5th day from the beginning of disease. Acute pain in the stomach decreases, the general condition of the patient gets better. Dense, not mobile, painful, with unclear contours, mass is palpated in the right iliac area [24].

At reverse development of the mass (when resorption comes) the general condition of the patient gets better, sleep and appetite improve, activity grows, the body temperature and WBC account become normal, pain in the right iliac area decreases, the mass diminishes in size.

Classical management involves initial conservative treatment with broad-spectrum antibiotics and intravenous fluid until the inflammatory mass resolves. Surgery at this stage is difficult and dangerous as it is difficult to find appendix due to adhesions and ultimately faecal fistula may form [28]. In the majority of cases the mass will resolve and an interval appendectomy (usually after 2 months) can be performed.

If the general and local conditions of the patient become worse: increase in general amount of WBC, pulse rate and body temperature, increase of the abdominal pain, and the mass becomes larger in size, that indicates the abscess formation inside the appendicular mass. In this situation the

patient need an emergency surgery – open and drainage of the appendicular abscesses without appendectomy. Enforce appendectomy, in this situation, can lead to possible damage of the inflammated cecum wall with fistula formation or peritonitis.

Patients with an **appendix abscess** present with a swinging pyrexia associated with a tender mass in the right lower quadrant and a leukocytosis. The diagnosis is often confirmed by ultrasound or using computed tomography. The abscess can be drained percutaneously but open drainage allows appendectomy to be performed. In untreated cases lethal form of peritonitis is produced by secondary rupture of appendicular abscess.

Treatment. Immediate drainage under antibiotic cover is the treatment of choice. Appendectomy is not performed.

Pylephlebitis. Pylephlebitis is defined as septic thrombophlebitis of the portal vein, a precursor of liver abscess, is an extremely rare and frequently fatal complication of diverticulitis and rare nowadays in acute appendicitis. Septic clots from involved mesenteric veins produce multiple pyogenic abscesses in the liver. It is more frequent in patients with acute retrocecal appendicitis. It is heralded by chills, hectic fever, right upper quadrant pain and jaundice. In case with rapid passing of disease the icterus appears, the liver is increased, kidney-hepatic failure progresses, and patients die in 7–10 days from the beginning of disease. At gradual subacute development of pathology the liver and spleen are increased in size, and after the septic state ascites arises. The ideal duration of antibiotic therapy for pylephlebitis is unclear [22]. Given frequency of liver abscess as a complication of pylephlebitis, however, a minimum 4 weeks of therapy seems prudent since developing abscesses may not be visualized on CT scans. Patients with demonstrated macroscopic liver abscess complicating pylephlebitis should probably receive at least 6 weeks of antibiotic therapy, with or without drainage. Surgical intervention for pylephlebitis typically involves opening and drainage of the focus of infection.

Postoperative complications after appendectomy are: intestinal obstruction, major wound sepsis, pneumonia, empyema, pulmonary embolism, postoperative hemorrhage and thrombophlebitis, fecal fistula.

Wound infection

The rate of postoperative wound infection is determined by the intraoperative wound contamination. Rates of infection vary from < 5% in simple appendicitis to 20% in cases with perforation and gangrene. The use of perioperative antibiotics has been shown to decrease the rates of postoperative wound infections.

Intra-abdominal abscess

Intra-abdominal or pelvic abscesses may form in the postoperative period after gross contamination of the peritoneal cavity. The patient presents with a swinging pyrexia, and the diagnosis can be confirmed by ultrasonography or computed tomography scanning.Abscesses can be treated radiologically with a pigtail drain, although open or per rectal drainage may be needed for a pelvic abscess. The use of perioperative antibiotics has been shown to decrease the incidence of abscesses.

Intraperitoneal bleeding after appendectomy is rare, but dangerous complication. At the Sklifosovsky Emergency Institute in Moscow, Russia, this complication was observed in 3 patients over a period of 20 years [37].

Mesentery vessels or the adhesions vessels may be the source of bleeding into the abdominal cavity. Causes of bleeding may be diverse: not complete ligation of the mesentery vessels or slugging of ligature after the decrease in mesentery swelling; insufficiently reliable hemostasis from adhesions; increased bleeding due to common diseases (hemophilia, leukemia, scurvy).

Bleeding inside the peritoneal cavity after appendectomy requires relaparotomy. Often during relaparotomy the source of bleeding is not detected and the operation is ended with the removal of blood and clots, tampon application and drainage of the place of bleeding.

The rate of **acute intestinal postoperative obstruction** fluctuates in the limits of 0.1-2.5%. Diagnosis of early acute intestinal obstruction often is difficult, deterioration of patient's condition is explained by postoperative paresis, continuing peritonitis, that lead to time missing. **Fecal fistula** is an uncommon complication of operation for acute appendicitis, either with or without an associated peritonitis. The incidence of fistulas for the total number of operations for acute appendicitis with and without perforation is only 0.8%. The diagnosis of an intestinal fistula ordinarily is not difficult. In the case of most ceca1 fistulas, the discharge is semisolid and is not especially irritating to the skin. The fistulas higher in the small intestine are associated with an irritating discharge and, due to the fluid character of the contents, considerable quantities of fluid, electrolytes and food substances may be lost. The fistulas associated with appendicitis tend to heal spontaneously. This is particularly true of the cecal fistulas. When healing fails to occur in these cases there is usually an outgrowth of the cecal mucous membrane so that it becomes continuous with the skin, producing the direct or lip type of fistula. In such cases, surgical closure is necessary. The ileal fistulas are more apt to be complete and are less likely to heal spontaneously than are those in the cecum.

After hospital discharge, patients must have a light diet and limit their physical activity for a period of 2–6 weeks based on the surgical approach (i. e., laparoscopic or open appendectomy) [24].

Prognosis

The mortality from appendicitis in the United States has steadily decreased from a rate of 9.9 per 100,000 in 1939 to 0.2 per 100,000 today. Among the factors responsible are advances in anesthesia, antibiotics, IV fluids, and blood products. Principal factors influencing mortality are whether rupture occurred before surgical treatment and the age of the patient. The overall mortality rate in acute appendicitis with rupture is approximately 1%. The mortality rate of appendicitis with rupture in the elderly is approximately 5% – a fivefold increase from the overall rate. Death is usually attributable to uncontrolled sepsis peritonitis, intra-abdominal abscesses, or gram-negative septicemia. Pulmonary embolism continues to account for some deaths.

Conclusions

1. The diagnosis of acute appendicitis is determined by the combination of clinical and instrumental methods.

2. When additional methods of research have exhausted themselves and the diagnosis is not yet clear, the preference will go to the clinical method of examination.

3. Instrumental methods of research play a great and sometimes decisive role in the differential diagnosis of acute appendicitis.

4. The method of treatment of the established diagnosis of acute appendicitis is appendectomy by an open or laparoscopic method.

5. The best method of surgical treatment (open or laparoscopic) is the one that is optimal for this patient.

6. Conservative treatment of acute appendicitis only with antibiotics is dangerous, and cannot be taken as a basis for treatment.

7. The earlier diagnosing and an early surgery performance in patients with acute appendicitis are the key to success, and lead to significant reduction in number of postoperative complications.

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DOI: 10.5281/zenodo.1299030
UDC: 616.993.162

Cutaneous leishmaniasis

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Abstract

Background: Leishmaniasis is a disease caused by parasites of the *Leishmania* type. Cutaneous leishmaniasis is a neglected worldwide, zoonotic, vector-borne, tropical disease. The clinical spectrum of leishmaniasis ranges from a self-resolving cutaneous ulcer to a mutilating mucocutaneous disease and even to a lethal systemic illness. People who recover from cutaneous leishmaniasis are protected against future infections. The risk of infection is for people of all ages if they live or travel where leishmaniasis is found. Leishmaniasis usually is more common in rural than in urban areas, but it is found in the outskirts of some cities. The transmission risk is highest from dusk to dawn because this is when sand flies generally are the most active. Cutaneous leishmaniasis causes skin lesions, which can persist for months, sometimes years. The skin lesions usually develop within several weeks or months after the exposure but occasionally first appear years later. Presented here is a clinical case of leishmaniasis of the cutaneous form, diagnosed by the microscopic method. The patient was diagnosed, monitored and treated in Clinical Hospital of Infectious Diseases "Toma Ciorbă" from 10.01.2018-09.02.2018. The progression of the disease was favorable following the etiotropic treatment with antimony meglumine (Glucantime), requiring careful monitoring due to adverse reactions.

Conclusions: Clinical symptomatology was characteristic for cutaneous leishmaniasis: skin lesions of various pink-cherry sizes, some with ulcers on the body. The first etiotropic treatment with antimony meglumine was effective. Antimonate Meglumine treatment at a dose of 15 ml resulted in adverse reactions: asthenia, fever, myalgia and arthralgia.

Key words: cutaneous leishmaniasis, diagnosis, treatment, adverse reactions.

Introduction

Leishmaniasis owes its name to Sir William Leishman, a British army medical officer, who discovered the disease in 1901 and published his findings in 1903 [28]. Leishmaniasis is a polymorphic disease produced by several protozoa species of Leishmania genus that can affect the skin, mucous or internal organelle. Infections in humans are caused by more than 20 species of *Leishmania* [27, 28]. Cutaneous leishmaniasis can be caused by several *Leishmania* spp and is transmitted to human beings and animals by sandflies [19, 20, 24, 27]. Other modes of transmission of leishmaniasis to humans, though less common, include blood transfusion, sharing of contaminated needles, and mother-to-child transmission during pregnancy [16, 17]. Risk factors include poverty, malnutrition, deforestation, and urbanization [3, 27]. Some people have a silent infection, without any symptoms or signs. People who develop clinical evidence of infection have one or more sores on their skin. The sores can change in size and appearance over time [9]. Leishmaniasis has three principal clinical forms: cutaneous (CL), mucocutaneous (ML) and visceral (VL). The distributions are: ≈1.5 million cases of CL and ≈50 000 cases of VL occur annually [11]. Cutaneous leishmaniasis (CL) is a neglected worldwide, zoonotic, vector-borne, tropical disease that is a threat to public health. This threat may spread from endemic to non-endemic areas, is endemic in the tropics and neotropics [13, 20]. The World Health Organisation (WHO) estimates that 350 million people are at risk of contracting leishmaniasis and an estimated 1.6 million new cases occur annually [23]. The morbidity associated with human CL is 0.7-1.2 million cases distributed worldwide resulting in extensive integumentary lesions [1]. Since 2002, there has been a dramatic increase in the cases of cutaneous leishmaniasis: from 1 case per 100,000 in 2002 to 4.5 cases per 100,000 in 2012. Since 2012, the incidence rates have decreased (2.7 cases per 100,000 in 2015) [6].

There are two groups of CL, New World and Old World leishmaniasis, with only the latter group identified in the Middle East and it includes three main species; *L. major*, *L. tropica* and *L. infantum* [22]. The majority of cutaneous Leishmaniasis cases occur in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia and the Syrian Arab Republic [1, 28]. Recent studies showed a high prevalence of CL in Iran [7, 8], Turkey and Syria [12]. Although Iraq shares long borders with these countries and leishmaniasis is endemic, the World Health Organization has not classified it as a country with a high burden profile [26]. CL has long been endemic in Israel. More recently, illness caused by *L. tropica* parasites

has been reported in several semi-arid hilly areas in Israel's more densely populated, and less dispersed, central and northern population centers [10, 21].

It is often referred to as a group of diseases because of the varied spectrum of clinical manifestations, which range from small cutaneous nodules to gross mucosal tissue destruction. Cutaneous leishmaniasis is the most common form of the disease. It usually produces ulcers on the exposed parts of the body, such as the face, arms and legs [2, 28]. The lesions typically evolve from papules to nodular plaques to ulcerative lesions, with a raised border and central depression, which can be covered by scab or crust; some lesions persist as nodules. The lesions usually are painless but can be painful, especially if ulcerative lesions become infected with bacteria or if the lesions are near a joint. The healing process typically results in atrophic scarring [9]. The gold standard for confirmation of Leishmania infection is visualisation of parasites by microscopy and scrapings or fluid from cutaneous sores in the case of CL [23]. In cutaneous and mucocutaneous leishmaniasis, clinical manifestations with parasitological tests confirm the diagnosis but serological tests have limited value [28]. The risk factors: poverty and malnutrition play a major role in the increased susceptibility to leishmaniasis. Another risk factor is the movement of susceptible populations into endemic areas, including large-scale migration of populations for economic reasons [3].

Leishmania may live quietly for years in the body and then begin to multiply (reactivate) if the person's immune system becomes suppressed. Thus, people who were born in a country with leishmaniasis and those who have had travel-related exposure are at risk if they become immunosuppressed by conditions such as chemotherapy, use of steroids, or infection with HIV. Patients who have previously had cutaneous leishmaniasis acquired in certain parts of the New World are at risk for mucocutaneous leishmaniasis [14]. Meglumine antimoniate (Glucantime) is a pentavalent antimony (Sb^V) recommended by the World Health Organization as the first-choice drug for the treatment of all types of leishmaniasis; the maximum dose recommended is 20 mg/kg of body weight/day via the intramuscular route [4, 5, 15, 25]. Some of the side-effects may be rare but serious: fever, irregular heartbeat, nausea, back pain, upper abdominal pain, vomiting, chills, cough, skin rash, drowsiness [18,].

Results and discussion

Patient U.V., aged 44 years, from Chisinau, was hospitalized in the IMSP Clinical Hospital of Infectious Diseases "Toma Ciorba" on 10.01.2018. At the time of admission there were the following complaints: physical asthenia, rash all over the body, skin pruritus - more pronounced in the eruption region. The patient is considered ill from mid-September 2017, when moderate skin pruritus appeared in the large joints, and then on the entire body surface, increasing in intensity until it became unbearable. On October 9-10, 2017, there appeared skin rashes (papules) in the joints region, which then spread throughout the body. In November, he contacted a dermatologist who prescribed treatment with Betaden, Erolin, Flosteron, Ung. Dermovate, Maxitrol, Central -B. The indicated treatment improved the pruritus, but the rash continued to progress to ulceration.

On 27.12.17, the patient came to the Diagnostic Advisory Center of Parasitic and Tropical Diseases of the Toma Ciorbă Infectious Diseases Clinical Hospital, and was suspected to have Leishmanioza, of skin form, which was later confirmed parasitologically, by detecting leishmanias in the smear taken from the ulcer of the left leg region (fig. 1) [23].

From the epidemiological history, it was found that during May-June 2017 the patient was in Denmark, where he was bitten by mosquitoes, and from August to October 2017 (45 days) he was in Israel, where he worked in construction, and had satisfactory living conditions in the first three weeks; however, afterwards, he lived in precarious conditions, where there were many vagabond cats. In his spare time he sunbathed and swam in the sea. The patient was often pricked by insects, but without consequences. He also worked in Israel in the years 2013-2014, when he had an episode of hives that ceased after treatment.

At the first examination, the general condition of the patient was of average severity. He had usual skin color and several moist skin lesions of various sizes (1-8 cm) of pink-burgundy color, some of which were covered with ulcerations, while others were covered with crusts surrounded by an indented, pointed, and well-defined by a wave of edema and hyperemia, edge. There were also rashes characteristic of the first clinical manifestations of leishmaniasis and 4 papules located on the chest and abdomen. The largest lesions were found in the forearm and right leg 5 × 7-8 cm, on the face and head – rashes absented [2, 28]. In total there were 24 eruptive outbreaks, and 3 more appeared in the first days of staying.

The exam on the systems did not show any special changes.

On 11.01.18, treatment with Meglumine antimonate (Glucantime) 1.5 g/5 ml – 60 mg/kg /day was initiated according to the scheme: 1st day – 1/3 of the total dose / day (5 ml); day 2 – ½ of total dose / day (7.5 ml); 3rd day – ¾ – of total dose / day (12.5ml); 4th day total dose (15.0 ml). Sol. Clemastin 2.0 ml i / m. Sol.Dexamethasone 4mg i / m (for 3 days after initiation of treatment), group B vitamins.

Beginning from 21.01.18 (on the 11th day of the treatment), the patient started having fever, up to 38.5 ° C, that became pronounced in the second half of the day. Initially, the fever did not significantly affect the patient's general condition, but, after one week, the condition gradually worsened: pronounced asthenia, muscle and bones pain, suffocation, pronounced palpitations. Data from additional investigations have excluded the association with other pathological conditions, including sepsis, pneumonia, urinary infections, endocarditis, etc. On 29.01.18 (on the 19th day of the treatment), the general condition of the patient continued to worsen, as the signs of general intoxication have intensified, manifesting themselves through pronounced physical

asthenia, muscle pain, arthralgia, fever over 39.0 ° C, and insomnia. These symptoms were considered to be caused by the adverse reaction to treatment with Meglumine antimonate [18, 25]. Following the occurrence of these side effects, the dose of antimony meglumine was reduced to 5 ml/day. On 30.01.18 the condition of the patient improved, the fever decreased, the signs of intoxication disappeared, and physical asthenia was decreasing. The patient continued treatment with Meglumine antimonate 5 ml / day until 06.02.18. He was discharged on 09.02.18 in satisfactory condition. 1 month after the discharge, the general condition was satisfactory, and no serious consequences surfaced. Some crusts of very small size were present.

In fig. 2, tab. 1 and tab. 2 we present the evolution of cutaneous lesions and laboratory investigations during the treatment period:

Specific clinical data

The smear of the eruptive element from the right leg region (27.12.17) - gen. Leishmania was found.

The smear of the eruptive element from the right forearm region (15.01.18) - gen. Leishmania was not found.

The smear of the eruptive element from the right lateral abdominal region (18.01.18) - gen. Leishmania was not found.

Non-specific clinical data

11.01.2018 – FCC - 86 b / min; AEC – normal; sinusoidal rhythm.

29.01.2018 – FCC – 110 b / min. Sinusoidal rhythm, tachycardia. Moderate deregulations of the ventricular repolarization processes in the anterolateral, apical VS region. Signs of overloading VS.

USG abdominal organs (11.01.2018): Liver: LD – 153 mm; LS – 73 mm; V. Portae – 10 mm;

V. splenic – 6 mm; Gallbladder – deformed; walls – 2 mm; calculus – absent. Pancreas – Regular contour; Structure – poorly homogeneous. Splint – 116x41mm; Ecogeneity – slightly increased. Kidney: Nephroptoza of grade I-II on the right. In the calcification system – micro-calculus with d = 1- 3 mm.

Conclusion USG: Hepatomegaly. The slightly increased ecogeneity of the liver and spleen. Deformed collector. Chronic pancreatitis. Renal microlithiasis, more pronounced on the left, uric diathesis. Nephroptotic grade I-II on the right.

Flora hemoculture at sample I (31.01.18) – Aerobic flora – negative; Anaerobic flora – negative.

Flora Hemoculture on Sample II (05.02.18) – Aerobic Flora – Negative; Anaerobic flora – negative.

Table 1

Hemoleucogram in dynamics

Indicators / Data	**11.01.18**	**15.01.18**	**22.01.18**	**27.01.18**	**29.01.18**	**30.01.18**	**07.02.18**
Hemoglobin (g / l)	148	157	-	129	126	-	-
Erythrocytes (10^{12}/l)	4,9	5,2↑	-	4,2	4,2	-	-
Color Index	0,9	0,9	-	0,92	0,9	-	-
Platelet count (10^9/l)	248,0	288,0	-	264,0	-	-	-
Leukocytes (10^9 / l)	6,1	12,3↑	10,8	8,6	12,0↑	9,8	9,8
Unsegmented (%)	15↑	11↑	21↑	22↑	7	7	8
Segmented (%)	41↓	63	52	47	56	72	49
Eosinophils (%)	5	-	4	3	2	2	9
Lymphocytes (%)	29	19	20	25	27	15	23
Monocytes (%)	10	7	3	3	8	4	11
RSH (mm / h)	13↑	8	-	47↑	61↑	49↑	22↑

Table 2

Biochemical analysis of blood in dynamics

Indicators / Data	**11.01.18**	**15.01.18**	**16.01.18**	**27.01.18**	**30.01.18**
Bilirubin mcmol / l	10,8	-	-	7,6	-
ALT U/l	22,1	-	-	50,7↑	39,4
AST U/l	17,5	-	-	31,6	-
Total cholesterol mmol/l	6,6	-	-	-	-
Urea mmol/l	4,0	5,2	-	5,7	5,9
Creatinine mcmol/l	88,0	84,0	-	72,0	82,0
Glucose mmol/l	-	-	4,8	4,8	-
GGT U/l	-	38,5	-	77,5	74,8
Amylase U/l	28,0	-	-	58,0	-
Total protein g/l	68,9	-	-	-	-
Prothrombin Index %	90,0	-	-	-	-
Alkaline phosphatase U/l	163,0	-	-	-	-

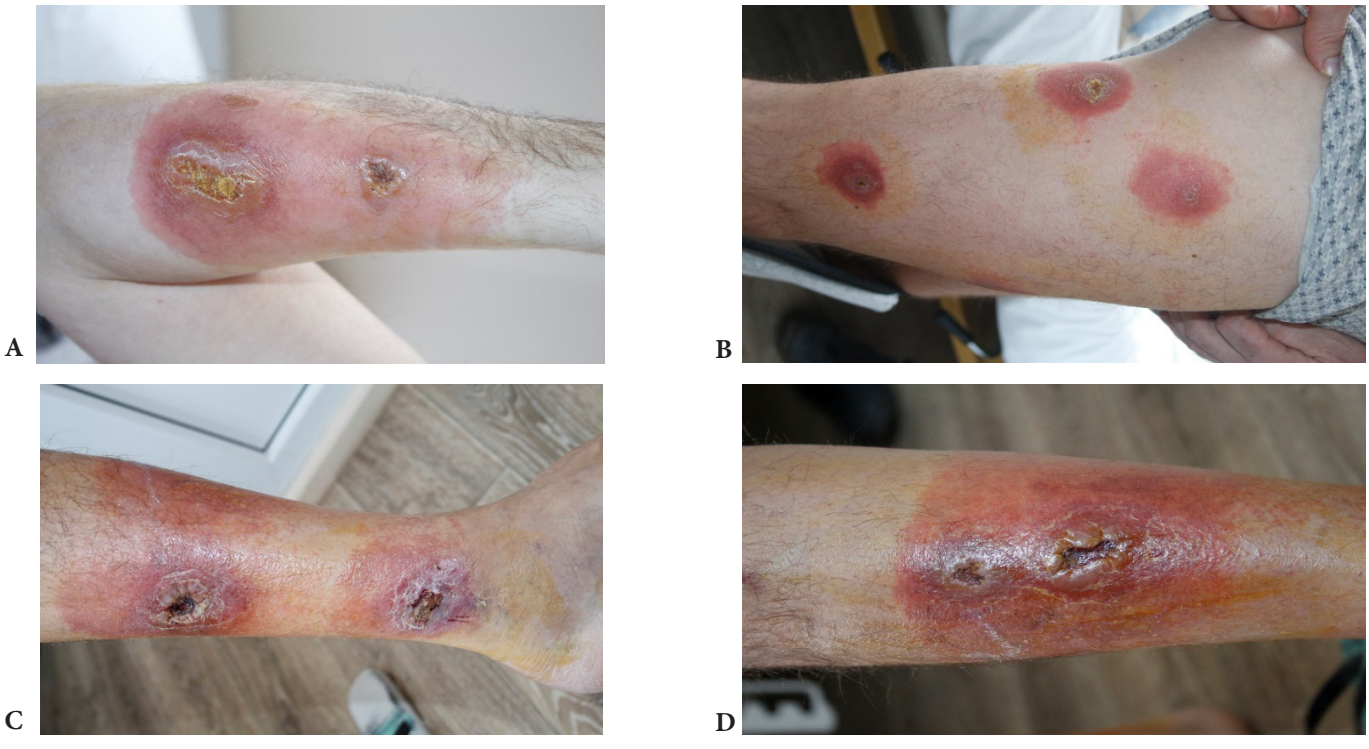

Fig. 1. Skin lesions before admission on the 08.01.2018.

A – Right forearm, **B** – Right leg anterior surface, **C** – Left leg, **D** – Right leg posterior surface.

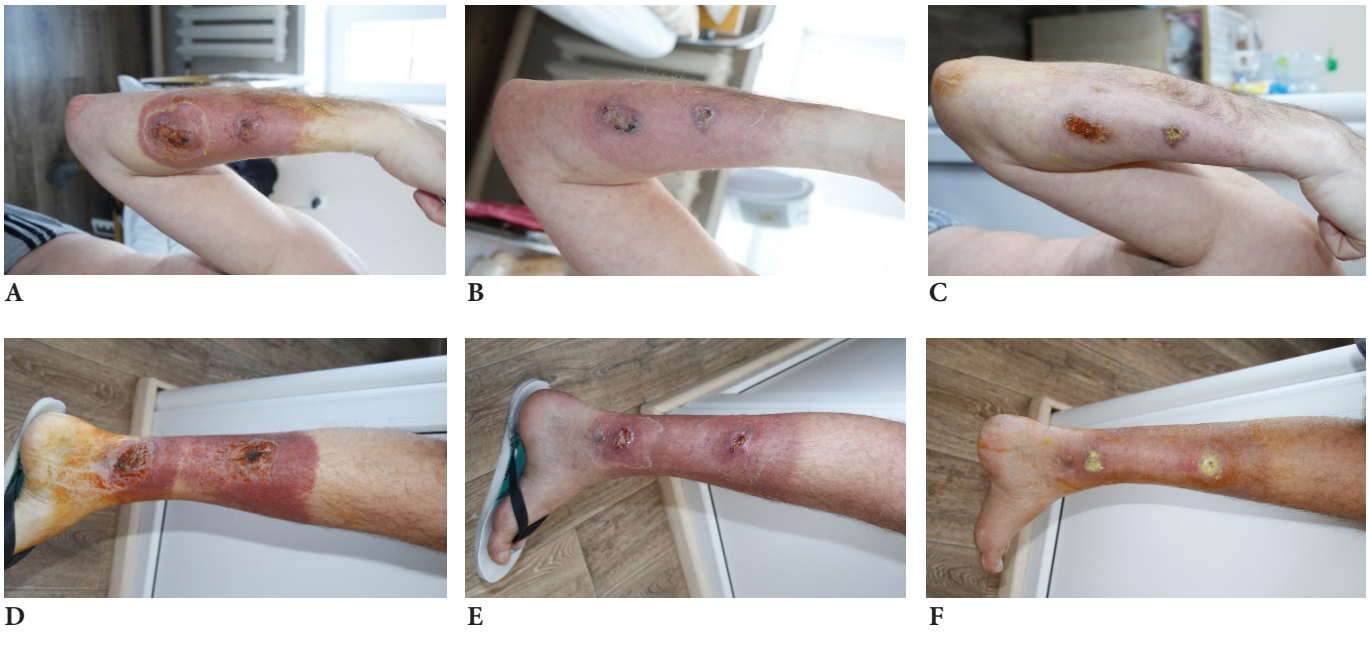

Fig. 2. Evolution of cutaneous lesions during the treatment period.

The right forearm region on the: **A** – 5th day of treatment, **B** – 12th day of treatment, **C** – 23rd day of treatment.

The left leg region on the: **D** – 5th day of treatment, **E** – 12th day of treatment, **F** – 23rd day of treatment.

Conclusions

1. This case of Cutaneous Leishmaniasis is the first of this form registered with a national citizen returned from a region where the morbidity through this parasitosis varies from 2.7-4.5 cases in 100.000 according to literature data.

2. It is necessary to raise the level of knowledge among practitioners, because, due to global warming, these infections may spread through new areas linked by the presence of transmission vectors.

3. A thorough management is needed to highlight the adverse events caused by etiotropic treatment with those of other clinical syndromes of other origins.

4. The emergence of similar cases requires the familiarization of the local medical community with the modern methods of diagnosis and treatment of this disease through continuous medical education.

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DOI: 10.5281/zenodo.1299033
UDC: 616.216-002.828

Fungal rhinosinusitis: pathophysiology, diagnosis and treatment

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Abstract

Background: Fungal rhinosinusitis is a major clinical problem which should be considered in all patients and immunocompromised patients with chronic rhinosinusitis. It may be non-invasive or invasive with five main subtypes. Acute invasive fungal rhinosinusitis affects immunocompromised patients, usually with poorly controlled diabetes. Orbital and intracranial invasions are common, and mortality is high, except in cases of early detection and aggressive treatment. Chronic invasive fungal rhinosinusitis and chronic granulomatous fungal rhinosinusitis are characterized by a prolonged clinical course with slow progression of the disease, frequent invasion of the orbit and skull. Allergic fungal rhinosinusitis is a disease of young atopic individuals. There are usually involved all the sinuses with mucosa thinning and specific secretions. Fungus ball appears in one sinus, most frequently in the maxillary sinus, and affected individuals are not usually atopic. Anatomical and physiological interactions of the nose and paranasal sinuses form a dynamic system. Mucus is the first line of defense against inhaled irritants and pathogens. The hygiene of a normal airway is maintained by the mucociliary clearance. The immune system includes nasal epithelial surface properties, or non-specific innate immunity and specific acquired immunity.
Conclusions: The detailed knowledge of anatomical, histological and immunological properties of the nasal and sinus mucosa is essential for understanding the pathophysiology of sinus diseases, treatment planning and surgical approach in order to obtain a favorable result.
Key words: fungal rhinosinusitis, mycological examination, nasal mucociliary epithelium, immunology.

Introduction

Fungal rhinosinusitis (FRS) is a potentially dangerous condition, depending on the type developed and the opportunity for diagnosis. It is a highly controversial subject in today's medical world for the different research directions it offers [1, 2, 3].

In an attempt to elucidate the etiopathogenesis of rhinosinusitis (RS), several controversial hypotheses have been launched. A true "storm" among otorhinolaryngologists occurred in 1999 when Ponikau and Kern (Mayo Clinic, USA) launched the hypothesis that chronic rhinosinusitis (CRS) without nasal polyps has predominantly (in 93% of cases) a fungal etiology [1]. Despite the fact that until recently, bacteria have been considered responsible for CRS pathogenesis, the role of fungi is now recognized in the occurrence of certain forms of CRS. Fungal spores, by their ubiquitous nature, are always inhaled and stored in the respiratory tract mucosa. Although in healthy individuals in general, fungi have a saprophytic behavior, in some patients, under certain conditions, especially related to host immunity, fungi can induce diseases. FRS may include a wide variety of fungal infections that may vary in intensity, sometimes being lethal [2, 26].

Despite the recognition of FRS as a serious entity for more than two centuries and due to all studies carried out in recent years, the condition remains a controversial disease with evasive pathophysiology, incomplete knowledge about epidemiology and medical mycology. Further research is needed to elucidate the exact etiological and pathogenic role of fungal species in CRS, to improve FRS diagnosis and treatment in order to determine a better prognosis [1].

In recent years, the incidence of FRS has considerably increased due to patient's survival, contemporary diagnostic equipment and high frequency of conditions favoring fungal infections (diabetes mellitus, long-term pharmacological treatment with antibiotics, corticosteroids and immunosuppressants, radiotherapy, chemotherapy, immunodeficiency disorders). Fungal rhinosinusitis is classified into two main categories based on histopathological findings: invasive and non-invasive [1, 2, 3].

Epidemiology. Among patients with CRS, from 6% to 12% are found to have fungi in culture or at histopathological examination [40]. According to another study, 5-15% of all cases of CRS are considered RS of fungal etiology [41]. The incidence of fungal infections among patients operated for inflammatory rhinosinusitis accounts for 4.3% [43]. Globally, the FRS prevalence is about 900 million cases or 15% of the world's population [42].

In prospective studies, on large cohorts of patients with CRS (349-450 patients), FRS was diagnosed in 19.3-25.8% of cases. Approximately 10.3% of patients had allergic FRS (AFRS), 15.2% of patients - chronic FRS and 0.3% patients – fungus ball. Probably, the prevalence figures of different forms of FRS are underestimated because some patients with fungal diseases did not have clinical characteristics. This raised suspicions of FRS and therefore did not collect and send specimens for fungal analysis. In this case, the prevalence of non-invasive FRS may be higher [4, 5].

Etiology. The most common pathogens in FRS are Aspergillus (Fumigatus, Flavus and Niger), which represent 45% of all positive cultures, and *Mucormycosis,* but many other fungal species (Alternaria, Curvularia, Bipolaris, Candida etc.) are also reported. These fungal spores are omni-

present in the environment and can cause invasive and non-invasive conditions [2, 4, 5, 6, 26].

FRS pathophysiology remains unknown. In order to develop, fungal hyphae and spores must penetrate a paranasal sinus, and the conditions should contribute to the fungal growth. These conditions develop when some disorders disrupt normal mucociliary clearance (MCC) and / or obstruct sinus ostium [7]. The epithelial events, including specific and non-specific immunity, require a broader description because they allow understanding the physio-pathological mechanisms of FRS and are potential therapeutic targets [8]. In this context, we briefly recall the fundamental elements of rhinosinusal histology, immunology and physiology.

Notions of rhinosinusal histology. The internal plan of nasal pyramids consists of the nasal mucosa and the following types of epithelium:

1. Pseudostratified columnar epithelium (respiratory epithelium) composed of five major cell types: ciliary cells (columnar), non-ciliary cells (columnar), caliciform cells, basal cells (small polygonal stem cells – progenitor cells of other cell types); and small granular cells. This epithelium is found in the two posterior thirds of the nasal cavity.

2. Squamous and transitional epithelium (stratified epithelium containing cuboidal cells with microvilli) is found in the first third of the nasal cavity.

3. Epithelium of paranasal sinuses is a simple ciliary columnar type, with some caliciform cells and glands.

4. Olfactory epithelium in the olfactory area is a pseudostratified epithelium containing olfactory cells, basal cells and Bowman glands (small serous alveolar glands) [9, 10, 11].

The main glandular components of the lamina propria consist of serum glands, seromucous glands or Bowman glands. Seromucous glands and caliciform cells secrete acid glycoproteins (sialomucins and sulfomucins), while serum cells secrete neutral glycoproteins (fucomucin), enzymes (lysozyme, lactoferrin) and immunoglobulins [12].

The epithelial cells protect the upper and lower airways directly through MCC. The apical part of the ciliated cells, which accounts for about 80% of all epithelial cells, is covered by cilia (over 200 cilia per cell) whose apexes are located in the periciliary layer. The frequency of ciliary beats, a determining factor in the mucociliary transport rate, is between 10 and 20 Hz (800-1000 beats per minute) at normal body temperature, and the ciliary rhythm/beat consists of three phases: fast forward rhythm/beat (effective movement), during which the cilia expand to the maximum and are perpendicular to the cell surface, the tip being in contact with the mucus; the rest phase, in which the cilia are parallel to the cell surface and a slow return rhythm/beat (recovery rhythm/beat) [9, 10, 12, 13, 14].

The caliciform cells or mucus secreting cells produce an acid mucin in the amount of 0.1-0.3 mg/kg /day or 20-40 ml of mucus. The sufficient production of viscous, elastic and adhesive mucus is important in maintaining normal MCC. To prevent infections, mucus is weakly acidic, with a physiological pH value of 5.5-6.5 and has the capacity of a small chemical buffer [9, 10, 11, 12, 14].

An outpatient study of monitoring nasal pH for 24-hours showed neither a diurnal pH variation nor significant fluctuations in daily activities (ingestion of food and fluids, rest, sleep). The mean pH varied within the range of 5.97-7.85, while in the anterior part of the lower meatus the pH was higher than in the posterior part (7.1 versus 6.6) [15]. According to another study, the mean pH value in the nasal cavity was 6.3, while in the anterior part of the nasal cavity - 6.40 (from 5.17 to 8.13) and in the posterior part of the nasal cavity - 6.27 (from 5.20 to 8.00) [16]. In patients with CRS, the pH in the middle meatus is alkaline and is on average 7.81 ± 0.83, statistically significantly higher, compared to practically healthy subjects (7.35 ± 0.82, p = 0.00011) [17].

Both edema with the inflammation of the nasal mucosa and obstruction of sinus ostia may occur in the case of acid or alkaline nasal pH, resulting in ciliostasis – a cause known to develop CRS [18].

Therefore, the detailed knowledge of the mucosal histology of each nasal anatomical portion is essential to understand the pathophysiological mechanisms of nasal disorders and to plan the medical treatment and appropriate surgical intervention in order to obtain a favorable outcome.

Aspects of rhinosinusal physiology. The normal functioning of paranasal sinuses depends on three essential components that ensure a continuous secretion clearance: normal secretion, ciliary function integrity, and ostial patency [9, 10, 11, 13, 14].

There are two mechanisms that protect the respiratory system against several irritants, microorganisms and inhaled allergens – the nonspecific system (filtering function of the nose, nasal mucus with MCC and inflammatory reaction) and specific system (humoral and cellular immune responses) [11].

Assessment of MCC along with the use of rhino-scintigraphy and other objective and subjective methods in patients with CRS, with or without nasal polyps, treated medically and/or surgically, allows understanding of ciliary function and its role in CRS pathogenesis [19]. The ciliary function plays an important role in sinus clearance and prevention of chronic inflammation. Although knowledge of CRS has considerably increased, there are very limited data on predisposing factors for these conditions. The mucociliary transport speed is considered to be an important index of MCC function of the upper respiratory tract, an important mechanism for the protection of the respiratory ciliary epithelium [8].

The average speed of mucus flow and particle transport in healthy adults and in normal conditions is about 5-6 mm/min, ranging from 3 to 25 mm/min [9, 10]. Different factors may affect the ciliary function of epithelial cells. The MCC is reduced with age, being affected in the congenital abnormalities of the ciliary structure constitution (Kartagener triad, primary ciliary dyskinesia). The nasal mucosa dryness significantly affects the ciliary activity. At 50% relative humidity of inspired air, the ciliary motion stops after

8-10 minutes and at 30% relative humidity of inspired air – it stops after 3-5 minutes. The ciliary activity is optimal at 32-40°C. At 19-32°C temperature, the frequency of ciliary beat increases, at temperature above 40°C it decreases, and at temperature 7-12°C and above 45°C the ciliary activity ceases. Other factors, such as locally applied drugs, inhaled gases, exposure to large amounts of wood dust and chromium vapor, tobacco smoke, infections (viral, bacterial, fungal), chronic rhinosinusitis (allergic rhinitis, CRS, nasal polyps) can severely affect the ciliary function [9, 10, 12, 13, 14].

CRS causes significant changes in nasal mucosa, including secondary ciliary dysmorphology. These secondary changes may be reversible, but the time required to return to normal morphology depends on the severity of disorders, the persistence of the infection and other predisposing factors. Secondary ciliary dyskinesia and cytopathic epithelial changes play an important role in CRS pathophysiology [20].

Therefore, the anatomical and physiological interactions of the nose and paranasal sinuses form a dynamic system. Mucus forms a protective barrier to the airway epithelium; it is the first line of defense against the irritants and inhaled pathogens. The normal airway hygiene is maintained by MCC, the efficiency of which depends on the structure, number, movement, strength and coordination of cilia, quantity, composition and rheological properties of the periciliary layer and mucus layer, temperature. The unique rheological properties of mucus (viscosity, elasticity and adhesion) are significant determinants of these two protective mechanisms.

Rhinosinusal immunology. The nasal immune system includes:

1. Superficial properties (mechanical, epithelial, physical characteristics of the mucus layer, mucociliary transport).

2. Inborn or non-specific immunity (bactericidal activity of mucus, proteins – lactoferrin, lysozyme, α2-macroglobulin, C-reactive protein, complement system, cellular – polymorphic cells and activated phagocytes, including neutrophils, monocytes and macrophages).

3. Acquired or specific immunity (immunoglobulins – IgA, IgM, IgE and superficial IgG, informed macrophages, submucosal macrophages, IgM, IgG, T and B lymphocytes, mucosa-associated lymphoid tissue and remotely located (adenoids, lymph nodes and spleen) 11, 21, 22].

The 4 subclasses of IgG represent 75% of immunoglobulins found in the serum, with a concentration of about 10 mg/ml in healthy individuals. The least abundant immunoglobulin in serum is IgE, with a normal concentration of approximately 150 ng/ml [23].

The defense mechanisms of the innate immunity are MCC, antimicrobial secretions and cells of the innate immune system. The innate immunity involves a set of resistance mechanisms, such as phagocytosis, which is not specific to a particular pathogen, while adaptive immunity has a high degree of specificity, such as the remarkable "memory" property. In spite of these differences, the innate and adaptive immune responses are linked and interact with each other, and both are necessary for an effective immune protection [21, 22].

The immune system cells responsible for the reaction and release of soluble molecules are lymphocytes (B and T), phagocytic cells (dendritic cells, macrophages, neutrophils and eosinophils) and auxiliary cells (basophils and mast cells). The molecules released by these cells are antibodies, cytokines (interleukins - IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α and interferons), chemokines, complement and various inflammatory mediators. There is evidence that IL-13 is a central mediator that independently promotes eosinophilic inflammation [21, 22].

IgA is a primary mucosa induced immunoglobulin; it is produced in humans more than any other class of immunoglobulins, and its major role lies in mucosal immunity. IgA can trigger cellular functions, such as degranulation and respiratory activation. Most people with IgA deficiency are not ill, IgA deficiency being associated with a large number of specific disorders: sinopulmonary, gastrointestinal, autoimmune and allergic diseases [21, 24].

The nose has two types of acquired cellular reactions as the first line of defense: IgA production, which forms insoluble complexes in mucus, and informed activated superficial immunological cells which are capable of phagocytosis. IgA is found in considerable amounts in nasal secretions [21, 24].

IgE is an immunoglobulin that causes allergic reactions and is mainly produced by lymphoid structures (tonsils and adenoids) and submucosa. IgE mediates immediate hypersensitivity reactions and has a hypersensitivity impact on MCC function [21, 24].

Thus, the nasal immune system includes superficial properties (mechanical, epithelial, physical characteristics of the mucus layer, mucociliary transport), innate or non-specific immunity (bactericidal activity of mucus, proteins, complement system, cellular immunity) and acquired or specific immunity (immunoglobulins, macrophages, T and B lymphocytes, lymphoid tissue).

Several predisposing factors for FRS (poor nutrition, low immunity, diabetes, long-term anti-TB treatment or antibiotics [25]) have been described, there are several possible pathophysiological pathways involving fungi in CRS and they can also act simultaneously or independently in a particular patient:

- Systemic or local IgE mediated reaction to fungi,
- Fulminant invasive infections – acute IFRS,
- Chronic invasive infections – chronic IFRS, chronic granulomatous IFRS,
- Epithelial lesions of superficial mucosa caused by eosinophilic proteases (major basic protein),
- Impairment of epithelial barrier with subsequent immunological reaction,
- Biofilms containing fungi [26, 27, 28, 29].

The mucin in patients with CRS contains heterogeneous eosinophilic clusters with high level of eosinophilic granules of major basic protein, a toxic cationic protein for extracellular microorganisms but also for the respiratory mucosa,

predisposing patients with CRS to secondary bacterial infections. Eosinophils can migrate into the respiratory mucosa by IL-13 expression, induced by adhesion molecules in the microvasculature, with subsequent migration from vessels to tissues. Another cytokine – IL-5, by inhibiting apoptosis, promotes the differentiation, activation and survival of eosinophils in tissues [26, 30].

Exposure of peripheral blood mononuclear cells to fungal antigens in vitro contributes to increased IL-5 and IL-13 production in 89% of patients with CRS. The increase in humoral response (serum IgG) correlates strongly with the increase in cellular response (IL-5 production). Less than 30% of patients with CRS have specific IgE antibodies to fungi [26, 30, 31].

These findings have led to a hypothesis in which fungi on the sinus mucosa surface could activate the immune system in sensitized patients and induce cytokine production, which promotes the migration of eosinophils through the epithelium to mucin. Eosinophils reach the mucin containing fungi and release cationic proteins to destroy fungi, thus they perpetuate and potentially aggravate the inflammation of the mucous membranes observed in CRS. Therefore, reducing the fungi in nasal and sinus cavities by antifungal treatment could reduce the immune and inflammatory responses in these clinically beneficial organisms of patients with CRS [26, 30].

Clinical trials with antifungal therapy for CRS, including CRS with nasal polyps, contributed to a symptomatic improvement, but did not demonstrate a substantial clinical effect [26, 32]. Some studies have found that antifungal treatment is safe and effective, reducing fungal antigenic load in nasal and paranasal cavities and then lowering the eosinophilic response. However, in order to determine the role of intranasal antifungal drugs in CRS treatment, controlled and blind studies are required [30, 32].

Thus, fungi are more frequently involved as an important pathogen in CRS etiology - they may play a minor role in CRS as part of a more complex multi-factor interaction, and conversely may be the main factor in some forms of CRS, however, fungi are not a universal etiological factor. However, the incidence and prevalence of various forms of FRS have not been accurately documented in prospective studies. It is crucial to estimate the exact physio-pathological mechanism in order to determine whether any changes in the treatment of CRS are needed and, if so, how to address them. In order to establish adequate and effective therapeutic strategies, and to minimize side effects, it is necessary to elucidate the pathophysiological mechanisms by which fungi initiate or perpetuate the inflammation, the nature of fungal interactions with the mucosal surface (e.g. as part of a biofilm or as a non-specific invader of disrupted epithelial barriers), optimal drug delivery methods [1, 4].The presence of fungi in the sinus mucosa does not explain the chronic inflammation in patients with CRS because they are only in the mucus and histologically do not invade the tissues. Since IgE antibodies to fungi have been detected in less than 50% of patients, type I hypersensitivity reaction in fungi does not fully explain the pathological process. Therefore, CRS could be caused by an immunological response to fungi in nasal and sinus cavities of patients with CRS, but not necessarily type I hypersensitivity reaction [32].

Fungal infections of paranasal sinuses can manifest as two distinct entities. The most severe (invasive) infections occur in patients with a compromised immunity (malignancies, autoimmune diseases, malnutrition, HIV infection, diabetes mellitus or immunosuppressive therapy) and are relatively easy to recognize by symptomatology and fulminant progression. The mortality rate is quite high in IFRS, early diagnosis and appropriate treatments are vital [33].

Non-invasive infections are chronic and, unfortunately, are often confused and treated as bacterial CRS for long periods of time, until the disease is exactly diagnosed [1, 33].

Clinical picture. FRS can often be difficult to diagnose, since its symptoms may be easily confused with the symptoms of bacterial CRS. The most common symptoms are pressure and /or numbness in the face area, frequent nasal congestion, inflammation of sinuses, nasal polyps, frequent sneezing, cough, headache, facial pain [6].

The clinical picture of invasive infections involves the presence of a viscous dark secretion in sinus cavities, spreading into adjacent tissues – orbit and intracranial structures [6].

FRS classification. To be able to predict patient's prognosis and response to treatment, a FRS classification is needed. It is important to make a distinction between invasive and non-invasive forms of FRS. All attempts to systematize FRS confirm that there is no single opinion concerning FRS so far, but fungi are definitely involved in its etiopathogenesis, and its incidence and prevalence are much higher than previously thought [1, 26].

Currently, most rhinologists acknowledge the following clinical and pathological forms of FRS:

1. *Non-invasive FRS (absence of mucosal layer invasion):*
- Local colonization with saprophytic fungi
- Fungus ball
- FRS caused by eosinophils (AFRS, eosinophilic FRS, eosinophilic mucin RS).

2. IFRS (*with mucous layer invasion*):
- Acute (fulminant) IFRS,
- Chronic IFRS,
- Chronic granulomatous (indolent) IFRS [2, 33, 34, 35].

Therefore, there is invasive and non-invasive FRS. Acute FRS includes acute IFRS (fulminant), and chronic FRS - chronic IFRS, chronic granulomatous IFRS, fungus ball, AFRS. FRS forms are distinct entities with different clinical, laboratory and radiological characteristics. Each FRS form can be differentiated and has different treatment and prognosis approaches.

General criteria for diagnosing different types of FRS. The most important step in FRS management is correct diagnosis, based on solid criteria, which will lead to a better prognosis of this condition. Due to potential invasiveness, especially in patients at risk, a correct and rapid diagnosis of FRS is essential in order to initiate the treatment as early as

possible and to ensure a favorable prognosis. FRS diagnosis should be based on clinical examination and paraclinical investigations, the most important of which is histopathological evidence of fungi presence [1].

Two essential conditions are required for FRS diagnosis: RS diagnosis (the ubiquitous nature of fungi should not be forgotten) and evidence of fungal infection. The latter can be confirmed by histopathological and/or mycological examination. Histopathology, according to some literature data, is still the standard method ensuring the best sensitivity in the detection of rhinosinusal fungal infection. Mycological examination is a useful tool and has a certain value, but it involves special conditions for harvesting, transporting and processing in order to obtain positive results. Correct sample harvesting and transporting are essential for the precise identification of fungi [1].

The diagnosis of FRS begins with a detailed anamnesis. Often, patients have a history of rhinosinusitis for prolonged periods of time or rhinosinusitis refractory to medical or surgical treatment for bacterial CRS [1, 25].

Histopathological (anatomopathological) examination is a quick and relatively inexpensive technique that often confirms the positive diagnosis or, at least, induces a suspicion of diagnosis. It detects the presence of fungi and confirms the tissue invasion. In addition, some histopathological parameters in CRS are predictive of the favorable response to functional endoscopic sinus surgery [1, 36, 37]. The histopathological examination for the detection of fungi reveals inflammatory cells in tissues and mucus, as well as the existence of specific reactions (Charcot-Leyden crystals). Staining can be done using hematoxylin-eosin, periodic Schiff acid or Grocott-Gomori silver hexamine impregnation, the latter can also identify fungal morphology [1]. However, some fungi (Aspergillus and Mucorales) have a similar morphology, making it difficult to distinguish between them by Grocott-Gomori silver hexamine stain. Immunohistochemical staining MUC5B is a much more sensitive method for the detection and identification of fungi in FRS, especially differentiating Aspergillus species from Mucorales species [44].

Mycological examination is also an essential step in the analysis and can be done with or without staining. The poor sensitivity of fungal sinus culture techniques with significant false-negative rates makes difficult the determination of the exact FRS incidence and prevalence [4]. The fungal detection rates using the culture method vary greatly – from 6% to 93% [43, 45]. Since fungi cultures are frequently negative, Bent and Kuhn have accepted that if all the other diagnostic criteria exist, including the positive histopathological examination of the sinus mucin, positive fungal culture is not required to confirm the diagnosis [38].

The usefulness of immunofluorescence techniques in the diagnosis of fungal infections has been confirmed in many studies. They can be used to early detect and identify fungi on different cultures or almost any biological product (blood, urine, cerebrospinal fluid, etc.) [1].

To diagnose fungal infections, there are some other techniques using the immunoassay (ELISA) to determine active antigens or genomic amplification by molecular biology techniques (polymerase chain reaction – PCR) [1].

The serological test aims at identifying specific immunoglobulins that are a marker of early or present fungal infection. It is noteworthy that two essential conditions are required to determine specific serum IgG: long enough contact of the fungal antigen with the host immune system and competent host immune system. This explains why the serological test is negative in localized fungal infection of fungus ball and in immunosuppressed patients (AIDS, leukemia, etc.) [1].

Skin tests are very important diagnostic tools in the case of allergic fungal disease. Lately, skin prick tests have become a norm, standardizing fungal extracts for classical intradermal tests [1].

Unfortunately, we have no standard criteria for imaging diagnosis of FRS. CT scan is the most useful imaging method, due to an increased sensitivity and ability to identify signs at early stages, but with reduced specificity for this condition. CT is performed at a 3 mm interval in the axial and coronal planes, using both bone and tissue windows. Magnetic Resonance Imaging (MRI) has a limited value for RSF diagnosis, being a starting point for the diagnosis of these clinical entities. It is often required in order to double the CT examination [1].

Several suspicions about FRS diagnosis are described:

- Isolated damage to a paranasal sinus (maxillary, sphenoid) or asymmetric disorders (significant percentage of unilateral damage) with opacification and calcification inside and / or different density on CT scans, hyposignal in secretion and hypersignal in the mucosa injured on T2 sections of MRI.
- Exacerbated facial pain, signs and symptoms non-specific to RS (nasal congestion, headache, rhinorrhea, etc.), nasal and facial edema.
- Severe thick brownish (mucin) and / or caseous secretion during endoscopic diagnosis or during surgery.
- Ischemic or necrotic areas on endoscopic or surgical examination.
- Direct examination of secretions with degranulated and / or necrotic eosinophils (Charcot-Leyden crystals).
- Direct identification of hyphae, if positive culture is negative, if both are negative – positive PCR (considering the clinical and radiological data described above).
- Mucosa with non-specific inflammation, if the fungus is present in the epithelium, submucosa and / or bone - invasive presentation (correlates with the clinical characteristics, findings and patient`s immune status).
- Clinical and radiological presentation similar to eosinophilic mucin; there are no available methods for positive fungal identification, which may indicate non-fungal eosinophilic CRS (mucinic, atopic or non-atopic).
- The presence of fungi on direct examination by cul-

ture harvesting and / or PCR can also be determined in normal subjects [35].

The diagnosis of FRS is primarily histological. The distinction between IFRS and non-invasive FRS is based on histopathological evidence of fungal invasion of the sinus and bone mucosa and, eventually, spread to the adjacent structures and tissues (orbit, anterior skull base and pterygopalatine fossa). In non-invasive FRS, fungal infection is limited to the sinus cavity without fungal invasion of the mucous membrane and bones [6].

Non-invasive FRS includes fungus ball and AFRS, which generally do not invade bone or tissues and, more commonly, are a result of skin hypersensitivity reactions. But a long-term development of the disorder may eventually erode the bone (osteitis, osteomyelitis) that may cause an intracranial or intraorbital complication. Complications may occur in an immunocompetent patient and are characterized by the presence of allergic mucin, Charcot-Leyden crystals, eosinophils and other inflammatory cells [6, 25, 34].

The diagnosis of fungus ball is often delayed because the symptoms are generally similar to those of bacterial CRS, the course of the disease is slow, oligosymptomatic and non-invasive. At the same time, fungus ball tends to appear in a single sinus, unilaterally, most often in the maxillary sinus, usually the affected individuals being non-atopic. The sinus contains hyper attenuated material and there may be evidence of chronic sinus disease or smooth bone erosion. Surgical removal is the basic treatment, recurrences being unusual [34].

AFRS is more common in young atopic people. Pansinusitis is usually found with expansion and thinning of the affected sinuses. The disease is characterized by the presence of allergic mucin, Charcot-Leyden crystals and eosinophils [6, 25, 34, 46]. The sinus content is hyper attenuated with high signal intensity on MRI images T1 and low signal intensity on MRI images T2. Surgical extirpation and antiallergic remedies are the basis of the treatment, the systemic or local antifungal toxic therapy not being necessary [34].

IFRS is a more fatal condition, defined by the presence of fungal hyphae in the mucosa, submucosa, bones or blood vessels of paranasal sinuses with orbital and intracranial extension. These subtypes are distinct entities with different clinical and radiological characteristics, with different treatment and prognosis strategies, which predominantly occur in immunocompromised patients in about 50% of cases. According to some studies, IFRS incidence is 0.5-4% of patients with bone marrow transplant. The imaging features are often subtle at the initial stages, and sinus evaluation in these patients is performed to determine early invasion signs. The mortality rate tends to be high, except the cases of early detection and aggressive treatment. In patients with untreated IFRS the mortality is significant and may reach 85-100% [3, 25, 34, 35, 39].

The risk factors for RSFI development include the use of antibiotics for prolonged periods of time, permanent sinonasal catheter, prolonged nasal intubation, immunosuppressive medications, metabolic or steroid abnormalities, poorly controlled diabetes, long neutropenia, and sinus disease [3].

The acute forms of IFRS progress rapidly within hours or days to fulminant intracranial infections. The chronic forms show slow growth and cause slow tissue destruction with subsequent invasion. Disease duration of less than 4 weeks differentiates acute form from chronic one. The term "subacute IFRS" can be used in rare situations – in patients with disease duration within 1-3 months [25, 34, 39, 42].

Chronic IFRS and chronic granulomatous IFRS are characterized by a long-lasting clinical development (more than 3 months) with slow progression of the disease, orbital complications and intracranial complications. Imaging manifestations can mimic agressive neoplastic lesions [34, 42].

FRS complications range from relatively benign to potentially lethal and are divided into three categories:

- Local - mucocele of paranasal sinuses, frontal bone osteomyelitis, subperiosteal abscess of the frontal bone,
- Orbital - inflammatory edema, orbital (post septal) cellulitis, subperiosteal abscess, orbital abscess, cavernous sinus thrombosis,
- Intracranial - meningitis, epidural abscess, subdural abscess, intracerebral abscess, cavernous sinus thrombosis, upper sagittal sinus thrombosis [25].

The intracranial, insidious and rapid extension is the most feared complication of IFRS with high mortality rates. The following forms of extension are known: 1) direct – the most common, 2) haematogenic – dangerous and asymptomatic with emboli and mycotic thrombus formation, 3) perineural with the cranial nerves paralysis and extension to the base of the anterior skull, 4) through the cribriform plate of the ethmoid bone at the base of the anterior skull, 5) very rarely by surgery or blood transfusion [25].

Therefore, the most appropriate approach is early diagnosis and intervention. With the current available diagnostic means (CT, MRI, microscopic examination, cultures and nasal endoscopy), diagnosis is much simpler, however, a high degree of clinical suspicion is required. The treatment results have greatly improved with advances in medical and surgical technology. New antifungal agents and other remedies have greatly contributed to better results through increased efficacy and minimized toxic side effects of traditional medicines [25].

FRS management is divided into two main directions: surgical treatment - which is aimed at eliminating fungal antigen and is most commonly the main treatment and conservative treatment – which seeks to prevent relapses, but has not been standardized so far and there is no clear evidence of the efficacy of any of the therapeutic agents used. Functional endoscopic sinus surgery is used together with long-term conservative treatment, oral and intranasal glucocorticosteroids, immunotherapy, antifungal medication and antimicrobial agents [25].

Functional endoscopic sinus surgery is the main option. There are various surgical methods in FRS. In the case of non-invasive and invasive disease, which is limited to sinuses without obvious dural involvement or osteomyelitis,

endoscopic elimination is the method of choice. If there is FRS extension, particularly intracranial extension, non-endoscopic approaches with the involvement of neurosurgeons team are considered [25].

In fungus ball, surgery always resolves the disease without the need for further pharmacological treatment. In AFRS, surgical treatment improves the symptoms of nasal respiratory obstruction, but local drug therapy is necessary. With the help of medical treatment, surgery resolves chronic IFRS and prevents the intracranial spread of complications. In patients with acute (fulminant) IFRS, timely surgical treatment prevents the onset of intracranial complications and makes the antimycotic polychemotherapeutic treatment possible in order to control the disease [43, 47].

The main pillar of the medical treatment of FRS is the administration of antifungal preparations: Amphotericin B at a maximum dose of 2-4 g/day, Lipozomal Ampho B at a dose of 4 mg / kg / day and can be increased to 10-15 mg / kg / day, fluconazole or itraconazole 400 mg twice daily [25].

Postoperative treatment includes regular endoscopic examination and follow-up, nasal lavage twice daily with Amphotericin B. Antifungal preparations are continued for approximately 4 weeks or until complete recovery confirmed by endoscopic examination [25].

Conclusions

FRS is one of the most challenging diseases for otorhinolaryngologists, primarily in terms of diagnosis and treatment. Because of the lack of standard diagnostic criteria and the potential FRS invasiveness, especially in at-risk patients, it is essential to have a correct and rapid diagnosis in order to initiate treatment as quickly as possible to get a favorable prognosis. The only way to establish a reliable diagnosis is to perform a detailed clinical examination and biopsy sampling [1].

FRS is an important clinical issue with various manifestations that should be considered in all immunocompromised patients and in all patients with CRS. It may be invasive or non-invasive, with five main subtypes [34].

Acute IFRS affects immunocompromised patients and patients with poorly controlled diabetes. The orbital and intracranial invasion is common, and mortality is high, except in cases of early detection and aggressive treatment. The imaging features are subtle at the initial stages and require attention to detect early signs of invasion [34].

Chronic IFRS and chronic granulomatous IFRS are characterized by a prolonged clinical development, slow progression of the disease, orbital and cranial invasion. Imaging manifestations can mimic aggressive neoplastic lesions [34].

AFRS is a disease of young atopic individuals. There is usually pansinusitis expansion and thinning of the affected sinuses. The contents of sinuses are hyper attenuated, with an increased signal intensity of T1 and a low signal intensity of T2 on MRI images. Surgical removal and antiallergic treatment are the main management methods, systemic or local antifungal toxic treatment not being necessary [34].

Fungus ball occurs in a single, unilateral sinus, most commonly in the maxillary sinus, and the affected individuals are usually not atopic. Sinuses contain hyper attenuated material and there may be evidence of chronic rhinosinusitis or smooth bone erosion. Surgical removal is the method of choice, the recurrence being unusual [34].

Therefore, understanding the different types of FRS and their special radiological characteristics allows diagnosing and initiation of the early treatment to avoid a delayed outcome, complications or fatal outcomes.

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DOI: 10.5281/zenodo.1299035
UDC: 616.248-053.2

Modern approach to pediatric asthma

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Abstract

Background: The article reviews aims to promote the communication between scientists engaged in basic research and clinicians working with children, in order to better understand the mechanism and work together towards an optimal management of this prominent pediatric condition. We reviewed the recent advancements in regards to pediatric asthma related to pathogenesis, diagnostic criteria and differential diagnosis, and peer-reviewed evidence for efficiency of pharmacological and non- pharmacological/alternative/adjuvant treatment; we also emphasize the importance of education of all parties involved in care, outpatient management, age-appropriate involvement of children in their own care, and asthma prevention measures in children. Clinically asthma is characterized by recurrent episodes of wheezing, dyspnea, and cough; at the pathophysiological level the airway wall thickness is increased and involves both smooth muscle and collagen tissue, the mucous glands and mucus production are increased, and the vascularity of the airways is increased, all leading to chronic inflammatory remodeling of the airways and resulting in reduction of lung function.

Conclusions: Asthma among children has been on the rise for decades. With an estimated prevalence of 10-30%, asthma is the most prevalent chronic disease of pediatric population worldwide and one with a large health care burden. The approach to diagnosis and treatment of asthma in children is distinct from adults; so is the management, prevention and the education of parties involved in care. Recently there has been a significant progress in mechanistic understanding of asthma; further, asthma management in children is becoming a top priority.

Key words: asthma, recommendations, pediatric, children, pathogenesis, therapy, management.

Introduction

Asthma is known to humanity since ancient times. The name asthma is derived from ancient Greek meaning gasping or panting. The description of asthma dates back to Hippocrates (460 - 357 BC) who first described asthma spasms, and to Galen (201-130 BC) who defined asthma symptoms and established that asthma was caused by bronchial obstructions. Despite long history, it was believed that asthma is a disease of adults; only with industrialization was acknowledged that asthma also affects children.

Childhood asthma: how big the problem really is?

Based on the largest cross-sectional study from ISAAC Phase Three study, which included 193,404 children aged 6-7 years from 66 centers in 37 countries, and 304,679 children aged 13-14 years from 106 centers in 56 countries, asthma has a prevalence of 10% to 30% in children worldwide (1). With such magnitude, asthma is the forerunner chronic medical condition in childhood worldwide. In US alone, the prevalence of asthma among children more than doubled in less than 2 decades (1980-1996) and has reached an alarming 9.4% in 2008 (2), up from 8.9% in 2005 (2).

Morbidity and mortality from childhood asthma: estimated and hard-to-estimate costs

Health care use for asthma in all ages includes outpatient visits to doctors' offices and hospital outpatient departments, visits to hospital emergency departments (EDs), and hospitalizations (1-3); the pediatric population adds the financial burden from the missed school days, estimated 12.8 million in children 5-17 old, and from days that their parents miss from work while taking care of their children (3). In 2005 in US, children had 7.0 million visits and an outpatient visit rate of 958 per 10,000 pediatric population. Children also had over 754,000 ED visits, at a rate of 103 per 10,000. The ED visit rate was highest among children aged 0-4 years at 168 per 10,000. Among children 0-17 years, there were 198,000 hospitalizations (27 per 10,000). Hospitalizations were highest among children 0-4 years, 60 hospitalizations per 10,000. Among children, asthma deaths are rare (2.3). In contrast, asthma causes significant morbidity and is the most frequent reason for preventable childhood hospitalizations (4). In addition to the sizable costs, there are largely underestimated costs from the psychological burden of asthma on children due to impaired quality of life, long-term health outcomes of childhood asthma during adulthood, and impaired quality of life in caregivers (5).

The face of childhood asthma: age, gender, race, genetic background, socio-economic status, access to and quality of child care and more

Age. Asthma often appears in early childhood, is diagnosed by 3.5 years of age in most of children who will develop childhood asthma and the rate keeps at steady frequency by 5.5 years (1-3). While it is difficult to predict the prospective identification of asthma phenotype and its clinical course, it is currently estimated that 3 out of 4 school-aged children with asthma will have outgrown their disease by mid-adulthood. The risk of persistence of childhood asthma

into adulthood increases with severity, sensitization, exposure to environmental offending agents including smoke, and female gender (7).

Gender. As of 2005, in US asthma prevalence for boys (10%) was 30% higher than for girls (7.8%) (3); it has been reported that hormonal status is important for asthma development, however it remains to be determined which factors contribute to the fact that more adult women are diagnosed with asthma than adult men (8).

Race and population/ethnicity. According to Akinbami LJ et al, at least in US, there are no significant differences in asthma prevalence between race groups (9). In contrast, asthma-related morbidity is higher among black children. Among racial categories, black children are most likely to have activity limitations due to asthma but only if they are economically disadvantaged: 49% of those who were also and poor were limited compared with about 20% of black non-poor, white poor, and white non-poor children (9). Even when access to healthcare is accounted for, as described in a study by Stewart KA et al involving children whose parents were in the military and who therefore had access to health insurance and treatment, black children were more likely to be diagnosed with asthma than either Hispanic or white children (9.6% versus 8.0% and 6.3%, respectively) (10). Black children with an asthma diagnosis also were more likely than Hispanic or white children to have a potentially avoidable hospitalization for asthma (2.6% versus 2.0% and 1.3%, respectively) or for any other medical reason (2.9% versus 2.2% and 1.6%, respectively) (10). Finally, black poor children had the lowest level of ambulatory care use for asthma after accounting for disease severity (9). Besides being linked to race, asthma prevalence in the US varies drastically between diverse US populations/ethnicities (11). In US, asthma prevalence is highest in minorities, including Puerto Ricans, African Americans, Filipinos, Irish Americans, and Native Hawaiians, and lowest in Mexicans and Koreans (12-14). Mortality rates from asthma-related morbidity follow similar trends in all races and ethnicities; response to medication, including Salbutamol, is lower in descendants of Indian and Puerto Rican origin than in African Americans or Mexicans (15,16). Asthma prevalence also differs between populations of the same ethnicity who are born and live in different places (17). US-born Mexican populations, for example, have higher asthma rates than non-US born Mexican populations that are living in the US (18).

Genetic background. There are compiling data suggesting that the genetic background may be implicated in susceptibility, and severity, of asthma. Studies from Thomsen SF et al showed that the risk of asthma in the co-twin of an affected twin was higher in mono-zygotic than in di-zygotic twins (19). The risk of asthma in the co-twin decreased with increasing age at onset of asthma in the index twin; the effect was attenuated in di-zygotic twins relative to mono-zygotic twins. According to these studies, genetic factors explain ~34% of the variation in the age at onset of asthma, and environmental factors account for ~66% (19).

Current literature indicates that asthma signs and symptoms are more common in those with alpha-1 antitrypsin deficiency (AATD) (20), or mutations in *DENND1B*, a protein that interacts with the tumor necrosis factor (TNF) α receptor and represses inflammatory-cell TNF-receptor signaling (21), ADAM33 (ADAM metallopeptidase domain 33, a disintegrin and metalloproteinase domain 33) (22) or in sphingosine-1-phosphate receptor (S1PR1), a regulator of vascular permeability and an essential participant regulating lung vascular integrity and responses to lung inflammation (23). Children with CHIT1 mutations are at higher risk for severe asthma with fungal sensitization (24). The genome-wide association studies (GWASs) revealed an asthma-predisposing SNP signature in interleukin 1 receptor- like 1/ interleukin 18 receptor 1 (IL1RL1/IL18R1), dipeptidyl-peptidase 10 (DPP10), phosphodiesterase 4D (PDE4D), V-myb myeloblastosis viral oncogene homolog (MYB), PDE10A, IL33, and especially protein tyrosine phosphatase, receptor type D (PTPRD) genes. In addition, rs10938397 near glucosamine-6- phosphate deaminase 2 (GNPDA2) and protein kinase C alpha (PRKCA) were top gene SNPs common among both increased BMI (obesity) and asthma (25). In contrast, TLR2/rs7656411 TT variant homozygote had a significantly reduced risk of asthma when compared with those with the GG wild- type homozygote. Furthermore, a positive association was observed between the T allele of rs2381289 in TLR6 and allergic rhinitis in asthma, while the A allele of rs11466651 in TLR was negatively associated with allergic rhinitis (26). MHC haplotype has significant effect on CD8 cell response and is an important determinant of the outcome of neonatal RSV infection; it remains to be determined if these factors are important for virus-dependent sensitization and development of asthma (27). Collectively these data suggest a role for innate immunity and inflammation in asthma.

Socio-economic status. The incidence of asthma is highest among low-income populations; such disparity is observed in US and worldwide (9, 28). Asthma deaths are most common in countries with low income and in neighborhoods with limited income and increased frequency of ethnic minorities. Given the fact that asthma has been strongly associated with the presence of household insects, including cockroaches; and the most prevalent presence of these insects in low-income neighborhoods, it is also indicative of association between low socio-economic status and asthma. It remains to be determined if limited resources and/or limited access to medical care can account for the low incidence of and treatment quality for asthma that varies significantly among different racial groups (29). For example, poor African Americans are less likely to receive outpatient treatment for asthma, more likely to require an emergency room visit or hospitalization for their asthma symptoms and have higher likelihood as a race of dying from an asthma attack compared to whites. The prevalence of "severe persistent" asthma is also greater in low-income communities than those with higher income (29, 30).

Access to and quality of child care. A study by Gurka et al

shows that the number of children in the child-care environment when the child was a toddler is significantly associated with odds of asthma, even after adjusting for respiratory illnesses and other risk factors (31). The fewer the children are exposed to triggering agents as toddlers, the higher the probability of persistent or late-onset asthma by age 15 (31).

Epigenetic. It is well established that maternal, and even paternal, health status, and their habits, such as consumption of alcohol or tobacco, may influence the health of the off-spring; such connections were closely established for low birth weight infants, immunity, and obesity. More recently, Folic acid taken in supplement form in late pregnancy was associated with an increased risk of childhood asthma at 3.5 years and with persistent asthma (32).

Pathophysiology of asthma

Asthma is characterized by inflammation and hyper-reactivity of airway in response to triggering agents that include allergens such as pollen or mold, viral respiratory infections, or other stimuli/irritants, such as tobacco smoke, cold and exercise. How the irritants trigger asthma is less well understood; in case of allergens and viruses, they are inhaled and taken up by the epithelial and immune cells in the upper airways and in the lungs. Upon exposure to triggers, a complex immune cascade is initiated in both innate and adaptive immune cells (33-37). Innate immune cells, including macrophages and dendritic cells, are activated and attempt to eliminate the trigger by degradation while producing pro-inflammatory signals, including cytokines and chemokines. In addition, innate immune cells process the triggering molecules and present them to adaptive immune cells, including T and B lymphocytes (34). Lymphocytes attack the infected/damaged cells in the airways, by cytokines/chemokines, and by secreted antibodies. It is believed that Th2 is predominant in asthma with production of IL-4, IL-5, IL-9, and IL-13. IgE production by B cells is most detrimental in asthma. Altogether, these complex immune reactions promote inflammation, which further activate the release of pro-inflammatory mediators from and recruitment of mast cells, macrophages, eosinophils and other immune cells in the airways (35). It is this complex response that collectively causes injury of the airway epithelium, prolonged contraction of smooth muscle, edema and glandular hypertrophy followed by exaggerated secretion of mucus; inflamed airways become more narrow and obstructed and are further hyper-responsive to subsequent stimuli (35,36). Airway obstruction can develop suddenly or gradually and accounts for asthma symptoms. In the long run, if the inflammation and hyper-responsiveness persist, a remodeling of the airway occurs. Airway thickening of all components of the airway wall (inner, outer, and total) occurs in long-term asthma from the epithelial hypertrophy, mucus metaplasia, increased muscle mass from myofibroblast hyperplasia, and connective tissue deposition followed by sub-epithelial fibrosis (33). All these events contribute to progressive decline in lung function and continuous sensitivity to trigge-ring agents in asthma (33-37) (fig. 1). However, it is wise to acknowledge that our understanding of the mechanisms of asthma is yet minimal, even with the fast pace of recent discoveries in the areas of environmental biology, immunology, omics (genomics, proteomics, inter-

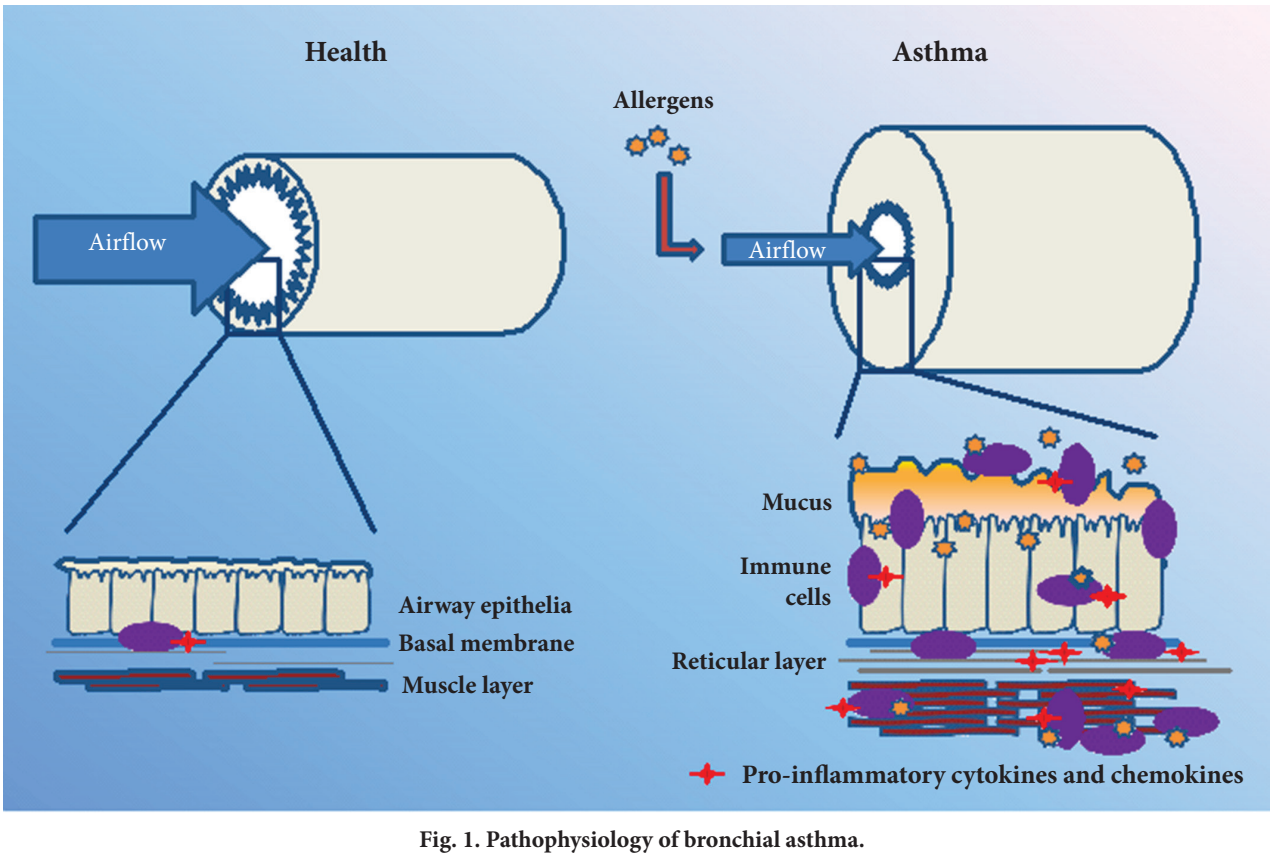

Fig. 1. Pathophysiology of bronchial asthma.

actomics) and our symbiosis with airway, skin and gut microbiota. While we managed to identify individual players in many of the processes involved in asthma, we still lag behind in our ability to connect all the discoveries in a bigger picture of asthma. In these conditions, extensive research is needed, more than ever, to fully understand the pathogenesis of asthma, with special focus on early pediatric age.

Clinical manifestations of asthma in childhood

Clinically asthma is characterized by recurrent episodes of wheezing, shortness of breath, cough and chest tightness. Symptoms are often worse at night or in the early morning, and often occur in response to exercise, cold air or triggering allergens.

The diagnostic process of asthma

Accurate diagnosis is critical in pediatric asthma; unfortunately in pediatric population additional difficulties are often encountered in the diagnosis process, including the intermittent and non-specific clinical presentation, limited ability of the patient caregiver to present a detailed history of the disease in the proband and health status in related family, etc. Nevertheless, several clues are available to aid the diagnosis of asthma in children. First, asking the right questions in a case when asthma is suspected helps: history of cough occurring with or without colds which are aggravated at night, recurrent wheezing, dyspnea and chest tightness, some or all of which are precipitated or aggravated by physical activity, exposure to house dust, smoke, animals with fur, nature (grass, pollens) or alternating emotions are suggestive of asthma. Second, collection of disease history from the proband can be employed in children as early ages of ~5 years old; it is important to note what their individual perception of symptoms is and how they define the effects of their disease on their quality of life. Third, family history is of great importance: asthma, eczema, eosinophilia, allergies, smoking may suggest of origins of asthma. Fourth, a focused physical exam of a child is warranted when asthma is suspected: include respiratory tract, chest, and skin, but do not overlook the general health (nourishment status, neurological and musculo-skeletal status, intellectual status, age-specific milestones).

Diagnostic criteria of asthma in childhood

Currently there is no precise diagnostic test for asthma. Similar to good old days, when medicine was solely based on the ability of the physician to recognize a disease by a combination of clinical symptoms, asthma is largely a symptom-based diagnosis today. The Expert Panel from Asthma Education and Prevention Program (NAEPP) of the National Heart, Lung, and Blood National Institute has issued the G3 report in 2007 outlining the Guidelines for the Diagnosis and Management of Asthma (38). In this report the experts recommended that essential elements in asthma evaluation include the history, symptoms, physical examination, and assessment of quality of life, as well as therapeutical trial with medications are key in the diagnosis of asthma in 0–4 years of age group, while 5–11 years of age group is to be analyzed as adults, based on detailed medical history, physical exam focusing on the upper respiratory tract, chest, and skin (including identification of episodic symptoms of airflow obstruction or airway hyperresponsiveness, airflow obstruction), and exclusion of alternative diagnoses. Spirometry to demonstrate obstruction and assess reversibility was recommended in children 5 years of age or older; reversibility was determined in these guidelines either by an increase in FEV1 of ≥12 percent from baseline or by an increase ≥10 percent of predicted FEV1 after inhalation of a short-acting bronchodilator. It is thus clear that the NAEPP recommendations, listed in table 1, do not fully distinguish between adults and children older than 5 years of age. NAEPP specifies that spirometry is needed to establish a diagnosis of asthma; further, spirometry is recommended at the time of initial diagnosis, after treatment is initiated and symptoms are stabilized, whenever control of symptoms deteriorates, and every 1 or 2 years on a regular basis.

Table 1

The NAEPP recommendations for diagnostic criteria of asthma

- Wheezing-high-pitched whistling sounds when breathing out. Lack of wheezing and a normal chest examination do not exclude asthma.
- History of any of the following:
  - o Cough, worse particularly at night
  - o Recurrent wheeze
  - o Recurrent difficulty in breathing
  - o Recurrent chest tightness
- Symptoms occur or worsen in the presence of:
  - o Exercise
  - o Viral infection
  - o Animals with fur or hair
  - o House-dust mites (in mattresses, pillows, upholstered furniture, carpets)
  - o Mold
  - o Smoke (tobacco, wood)
  - o Pollen
  - o Changes in weather
  - o Strong emotional expression (laughing or crying hard)
  - o Airborne chemicals or dusts
  - o Menstrual cycles
- Symptoms occur or worsen at night, awakening the patient

Table 2

Diagnostic criteria of asthma used by the British Thoracic Society

BTS recommendations are based on the 'response to therapy' approach: a positive response to treatment, assessed as reversibility of airway obstruction, is considered diagnostic of asthma.
BTS recommends that airflow measurement is diagnostic when:

- ≥20% difference on at least three days in a week for at least two weeks;
- ≥20% improvement of peak flow following treatment, for example:
  - o 10 minutes of inhaled β-agonist (e.g., salbutamol);
  - o six weeks of inhaled corticosteroid (e.g., beclometasone);
  - o 14 days of 30 mg prednisolone.
- ≥20% decrease in peak flow following exposure to a trigger (e.g., exercise).

The British Thoracic Society (BTS) recommends the 'response to therapy' approach for asthma diagnosis (39). A positive response to treatment, assessed as reversibility of airway obstruction, is considered diagnostic of asthma. Further, BTS recommends airflow measurement with a peak flow meter or spirometer. The BTS diagnostic criteria for asthma are listed in table 2; similar to NAEPP recommendations (tab. 4), the BTS recommendations do not fully distinguish between adults and children.

Spirometry

Spirometry is necessary to confirm the diagnosis of asthma, but can be also useful in assessing the degree of congestion. Two conditions are to be assessed: baseline and post-inhalation of a short- acting beta agonist (SABA); two parameters are to be measured during spirometry, including forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1), and the FEV1/FVC ratio is to be calculated. Due to the great need for cooperation from the patient during spirometry, this procedure is recommended only for children older than 5 years old. Disproportionate limitation of FEV1 compared to FVC, indicated by decreased FEV1/FVC ratio, is diagnostic of obstructive disease; the response to SABA is indicative of the reversibility of airway obstruction. If the post-SABA FEV1 rises with more than 200ml or +12% compared to the baseline, the airway obstruction is considered reversible. If the spirometry results are not conclusive and the diagnosis of asthma is still suspected, a bronchial provocation with histamine or methacholine may be employed. This test is limited to those with >65% preserved FEV1; a -20% FEV1 post-stimulation is indicative of asthma.

Differential diagnosis of asthma

There are several diseases that are often misdiagnosed as asthma; among the first criteria to use for a differential diagnosis of asthma in childhood is the child's age. In infants the differential diagnosis task is relatively easy, because asthma is seen relatively rarely in this age group and it is often considered as a differential rather than the primary diagnosis. In older children, differential diagnosis of asthma often requires thorough investigation. Nevertheless, excellent diagnostic skills are required from the physician in order to accurately diagnose asthma at all ages.

In *an infant*:

1. Transient infant wheezing occurs early in life; however, it is without clear ties to atopy but with clear relationship to maternal smoking.

2. Milk aspiration, due to neurological problems or anatomical defects, is usually associated with feeding, and if left untreated, is associated with lung infections and developmental delays.

3. Structural abnormalities of the bronchial tree (tracheo-, broncho-malacia) are often manifested shortly after birth and only pose difficulty in differentiation when are manifested later in life.

4. Cardiac failure associated with congenital or acquired heart diseases is rarely a problem for asthma differentiation at any age.

5. Inhalation of foreign body can occur in any age and is often presented with an acute onset and shows differential air entry in addition to wheezing; when not removed promptly it may be mistaken with asthma and needs a detailed history, thorough examination and imaging.

6. Vascular rings/laryngeal webs. Vascular rings (VR) are caused by the abnormal persistence of embryonic tissue that comprises the aortic arch because of double aortic arch (50-60%), right aortic arch with an aberrant left subclavian (12-25%), or pulmonary artery sling. VRs often present with a clinical picture of tracheo-esophageal compression, wheezing and stridor or sudden apneic and cyanotic spells; they are often diagnosed as recurrent bronchiolitis. *Laryngeal webs* are caused by failure of normal embryonic tissue regression; depending on the severity of anatomical defect, the LW often present with varying degress of respiratory distress ranging from severe subglottic stenosis diagnosed at birth with respiratory failure to identification in older children with weak cry, horseness and recurrent croup. Instrumental investigation is useful for the differential diagnosis in these cases: laryngoscopy and lateral neck films are useful for LW, while barium swallow and MRI are often used for VR.

In *older children*, the differential diagnosis of asthma requires a more extensive investigation as candidate diseases are more abundant.

1. *Genetic disorders*, such as cystic fibrosis (CF) and primary ciliary dyskinesia (PCD) should be always on the watch. *CF* may be suspected if the recurrent wheezing is associated with failure to thrive. *PCD* may be suspected in infancy, when supplemental oxygen is needed early postnatally, or later in life when present with wheezing and cough; however it stands apart from asthma due to its association with recurrent otitis, sinusitis and situs inversus.
2. *Viral infections* accompanied with bronchiolitis may result in a lingering wheezing and dyspnea.
3. *Eosinophilic lung disorders (ELD)*, including allergic bronchopulmonary aspergilosis, can be often mistaken with asthma, however, in ELD the serum IgE is often elevated, along with positive imaging picture (chest infiltrates) and positive skin prick test to Aspergillus antigen.
4. *Mental health problems*, such as anxiety-associated hyperventilation, depression and panic disorders, are often seen on older children and the triggering factor is clearly not of allergic nature; little or no wheezing is present and the spirometry parameters lack FEV abnormality.
5. *Vocal cords dysfunction* (VCD) is rare in children however, it may present with episodic, abruptly starting and stopping wheezing, cough and severe difficulty breathing; unlike asthma, where inspiratory flow is obstructed, the VCD is associated with a preserved expiratory loop and flow and flattened expiration.
6. *Churg-Strauss vasculitis (CSV)* may be mistaken with asthma, especially at the early disease stages. However, unlike asthma, CSV is accompanied by anemia, persistent

eosinophilia, raised erythrocyte sedimentation rate and positive anti-neutrophil cytoplasmic antibody in 30-50% of patients; the definitive diagnosis of CSV requires biopsy of the lungs and other affected organs.

7. *Enlarged lymph nodes or tumors (head and neck, mediastinal)* can press the respiratory pathways to mimic asthma; targeted evaluation of these conditions should be performed in children when both inspiratory and expiratory flows are affected, even if no symptoms of infection/inflammation or cancer are observed.
8. *Exertional dyspnea (ED)* may be the most challenging to distinguish from asthma, however in ED symptoms are present exclusively during exercise. A particular challenge in children is the differential diagnosis of exertion asthma (EA).
9. *Pulmonary embolism (PE)* is seen in ever younger adults and may occur in teens. While difficulty breathing in PE may be mistaken with asthma, a thoroughly collected anamnesis, a rapidly progressing clinical course with acute onset of symptoms and rapid deterioration of respiratory function, aided by indicative imaging investigations are key for differential diagnosis.

Several co-morbidities and triggering factors that are often difficult to separate from asthma; these are usually present in older children.

1. *Diseases of upper airways*, such as chronic rhinosinusitis and nasal polyps, are frequently associated with severe asthma. Nasal congestion/obstruction, purulent nasal and/or retropharyngeal discharge, facial pain or pressure, hypo- or an-osmia, ear pressure/fullness and halitosis despite adequate dental hygiene, are suggestive, while nasal endoscopy and imaging of the nasal cavity and sinuses are confirmative of rhinosinusitis and nasal polyps (40). Medical or surgical treatment of upper airways diseases have been proven useful in managing asthma in adults. Conversely, patients with severe asthma should be systematically evaluated for upper airways diseases.
2. *Gastrointestinal reflux (GERD)* is common in patients with asthma however rarely causes severe asthma symptoms. Regurgitation, dysphagia, heartburn, odynophagia, excessive salivation, nausea, chest/upper abdominal pain, chronic cough, laryngitis, and erosion of dental enamel are suggestive of GERD. Although a 24hrs pH monitoring is needed to confirm the GERD, most physicians prefer an empiric therapy trial with proton pump inhibitors.
3. *Obesity* is a co-morbidity factor for childhood asthma. According to Sulit et al, childhood obesity raised the risk for asthma by 1.8 times and the risk of wheeze 1.6 times (41). It is yet to be identified if the association between asthma and obesity is causal. It is interesting, however, that calorie restriction improves clinical findings and reduces airway inflammation in overweight adults with moderate asthma and has long-term health benefits, including improvement of asthma symptoms (42,43); such events are expected but yet to be defined in pediatric population.

Management of childhood asthma: severity assessment

The assessment of severity is among the most difficult tasks in managing pediatric asthma. The criteria for distinguishing the severity stages in acute and interval asthma are listed in tables 3 and 4, respectively. The golden rule while assessing the severity of asthma in children is to observe often and act promptly; ultimately preventing progression of severity is highly-desired an achievement.

Management of childhood asthma: pharmacological treatment

Asthma is both an easy and a difficult disease: in the former scenario the response to therapy is fast and complete;

Table 3

Severity assessment criteria for acute asthma episodes

Manifestation/Symptom		Severity		
		Severe-to-life threatening	Moderate	Mild
Observational /Subjective	Ability to talk	Unable to speak or separate words	Phrases	Sentences
	Consciousness	Agitated, confused/drowsy or Absent	Normal	Normal
	Central cyanosis	Present	Possible	Absent
	Wheezing	Quiet	Moderate to loud	Variable
Objective	Oxymetry (SaO2)	<90%	90-95%	Above 95%
	Pulse rate	>200/min	100-200/min	<100/min
	FEV1	Unable to perform spirometry or <40% predicted value	40-60% predicted value or previous baseline value	>60% predicted value or previous baseline value
	PEF	Unable to perform or <40% predicted value	40-60% predicted value or previous baseline value	>60% predicted value or previous baseline value

Table 4

Severity assessment criteria for interval asthma

Severity	**Intermittent**		**Persistent**		
	Infrequent	Frequent	Mild	Moderate	Severe
Day-time symptoms between exacerbations	None	None	>1/week but not every day	Daily	Continual
Night-time symptoms between exacerbations	None	None	<2/month and not every week	>1/week	frequent
Exacerbation	Brief, mild, occurs every 4-6 weeks	>2/month	<2/week Affects activity and/ or sleep	>2/week Restricts activity and/or affects sleep	Frequent Restricts activity and/or affects sleep
FEV_1 or PEF	>80% predicted value or previous baseline value	At least 80% predicted value or previous baseline value	At least 80% predicted value or previous baseline value	60-80% predicted value or previous baseline value	<60% predicted value or previous baseline value
PEF variability	<20%	<20%	20-30%	<30%	<30%

however, when the response to therapy is poor, asthma becomes a difficult disease to treat and even more difficult one to manage long-term. Management of asthma depends on the severity and the disease stage at which the medical professional encounters the child.

In *acute asthma*, the assessment of severity (tab. 3) will precede and largely determine the treatment; these children should be assessed promptly and in a calm/comfortable atmosphere, in order to prevent agitation and thus worsening of the respiratory insufficiency. The pharmacological treatment, described in detail in table 5, is aimed at reversing/ managing the bronchial constriction and airway inflammation and limiting mucus production. The children with acute asthma should be accommodated in the best-possible psychologically-comforting and temperature-regulated environment and should be monitored often. Inhaled oxygen, b2-agonists, and anticolinergics, and systemic (per os) corticosteroids are used in mild and moderate asthma attacks, their doses are dependent on age/body weight. More severe asthma attacks require more energetic measures towards decreasing the airflow resistance and enhancing airway penetration achieved with heliox and i/v bronchdialators, including salbutamol, aminophilline and magnesium sulphate, and aided by leukotriene receptor antagonists. The therapy is considered efficient when the patients oxygenation is maintained >95%, the symptoms subside and the respiratory parameters are restored. Alternative and adjuvant therapies and psychotherapy are rarely considered during the management of acute episode of asthma in a child.

In *interval asthma*, the management is aimed to maintenance of good control/ remission (defined as lack of symptoms, lack of limitations to activities and sleep, early morning symptoms, exercise induced cough or wheeze, low frequency of bronchodilator use, preservation of lung functions) and prevention/limitation of recurrences/exacerbations (by desensitization to allergens, prevention of inflammation/remodeling, limitation of therapies and their side effects). The frequency of acute episodes and any chronic symptoms should be reviewed often and action should be initiated promptly. In terms of pharmacotherapy, the first choice should be given to inhaled corticosteroids; spacer devices should be used at all ages, even in younger children in whom use of low volume spacer device with a well-sealing face mask may be needed. In addition, allergen immunotherapy, alternative anti- inflammatory agents (macrolides, xantines, ketotifen) should be considered, when indicated. A key component of interval asthma management is the non-pharmacological intervention, including diet, managed physical activity, and healthy life style. Preventive treatment should be initiated if there are frequent acute episodes or chronic symptoms (more than one disturbed night per week, difficulty participating in physical activities, or bronchodilator use on more than one day per week).

In a nut-shell, the approach to asthma therapy in childhood is on step-by-step nature, as detailed in figure 2. The therapy is administered on sliding scale basis depending on the severity of symptoms, should be tapered as soon as the symptoms subside, and should aim to a symptom-free period during which asthma is considered as well controlled. As with many chronic diseases, in which cures are not yet available, a sustained remission is the target of asthma management.

Management of childhood asthma: management plan

Children with asthma usually see a healthcare provider every one to six months to monitor the child's symptom severity and frequency and response to treatment. If asthma control has been adequate for at least three months, the asthma medication dose may be decreased. If control is not adequate, the medication schedule, delivery technique, and trigger avoidance will be reviewed, and the medication

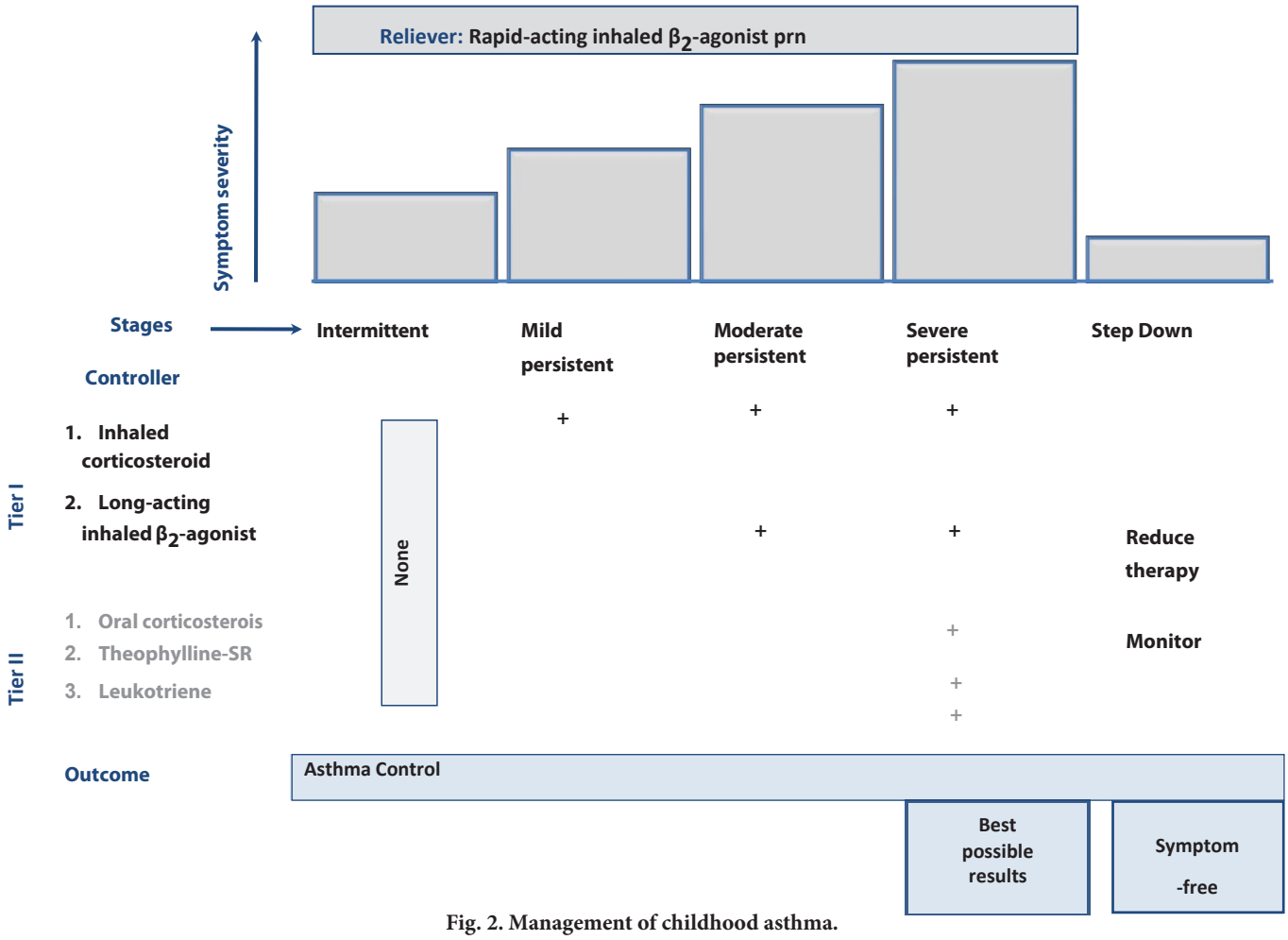

Fig. 2. Management of childhood asthma.

dose may need to be increased. Asthma management goals in children are: symptom free with near normal PFTs; sleep and exercise without asthma symptoms, prevent exacerbations, and minimize medication side effects; none of these is possible without an adequate compliance with the management plan.

National Asthma Education and Prevention Program Guidelines recommend a multimodal, chronic care approach to asthma (38). In this context, the most important component of the management of children with asthma is a specific, customized plan aimed to proactively monitor, identify symptoms in a timely fashion and manage symptoms that involves the caregiver, the child, if sufficiently old, and the medical professional. The treatment plan should be discussed, understood, and agreed upon by all involved parties; the plan should be written down, available at all time for review and adjusted according to changes in symptoms in a timely fashion (48). The healthcare provider may also recommend keeping a daily asthma diary when symptoms are not well controlled or when starting a new treatment; a periodic diary may be recommended for children who have stable symptoms and whose medications have not changed recently.

Management of childhood asthma: challenges for all involved parties

There are challenges for all the involved parties as it relates to following the management plan of a child with asthma; these are related to the age and individual characteristics of the child with asthma, availability and quality of the caregiver, access to medical care, environmental conditions.

The caregiver has to be someone who understands the importance of reducing the child's exposure to allergens, capable of pro-active monitoring the early appearance of the symptoms, and diligent with administering/supervising the therapy of asthma in an outpatient setting. The most often encountered challenges for caregivers are poor socioeconomic status and restricted financial means, leading to inability to protect the children from exposure to allergens, and thus dealing with a never- ending vicious circle of disease progression. In acute asthma exacerbations, the ability of the caregiver to recognize the symptoms and their ability to make arrangements for the delivery of the child to medical care facility in a timely fashion is critical. In interval asthma management, the most challenging task of the caregiver is to prolong an exacerbation-free period along with a healthy life style with age- appropriate activities. The most challenging of all tasks for caregivers is to cope with the specific life style that an asthmatic child brings into the family, all in an efficient yet gracious, cheerful and loving manner.

The children should be encouraged to participate in their own care, as soon as they are sufficiently old to understand the goals and the management plan. Younger children need

to be protected from the symptom-triggering environmental exposure and educated to keep calm upon exacerbations, as stress exacerbates asthma attacks (49). Older children can monitor and identify their symptoms in a timely fashion and even responsibly manage their symptoms with prescribed pharmacological agents. Older children are often tempted by outdoor activities where they can be exposed to cold, athletics with strenuous physical activity, interactions with their peers in crowded, enclosed and thus germ-spreading environments, all of which precipitate asthma symptoms; thus, they need close coaching of their daily lives in order to successfully manage their asthma.

The Medical Professionals are often challenged with difficult differential diagnosis of asthma, especially at extreme childhood ages, rapid progression of the severity of asthma exacerbations, poor management of the diseases requiring ever in need for adjustment pharmacological therapy and insufficient time for outpatient management/follow-up. It is important to stress the need for a follow- up in this particular disease at any stage of care: scheduling an appointment after an emergency department visit increases the likelihood that urban children with asthma would follow up with a PCP (50). Fifield J. et al reported in a recent paper that redesigned medical support is the key to asthma management in children (51). By providing quarterly well-asthma visits using structured visit forms, community health workers for outreach and follow-up, a Web-based disease registry for tracking and scheduling, and a provider education package, the medical professionals have a great potential to truly follow the multimodal, chronic care approach recommended by the National Asthma Education and Prevention Program Guidelines (38,51). Indeed, when intervention sites were given an additional Web- based, computerized patient-specific provider feedback system that produced a guideline-driven medication assessment prompt, the asthma control improved significantly for each of the following visits, suggesting that practice redesign can improve provider adherence to treatment guidelines as well as patients' asthma control (51).

Management of physical exercise in childhood asthma

The physical activity is not to be avoided in children with asthma; moreover, it is to be encouraged, and aim to be of age-appropriate strenuous and symptom-free. In preventing asthma exacerbation upon physical exercise, it is recommended to consult a medical professional before start for comprehensive assessment and thorough planning, start any regimen slowly, keep continuity or warm up gradually at the beginning of each session, avoid cold weather, protect the airway by warming the inspired air. In those who cannot fully comply with these recommendations due to their geographical location or nature of exercise, administer 1-2 puffs of quick and short acting beta agonist (SABA) 5 minutes before starting to exercise.

Management of childhood asthma: adjuvant and alternative treatment

Adjuvant therapy aims to provide support to asthma management in addition to the basic therapeutic regimen. A relatively new approach to long-term care for patients with asthma is the blocking of IgE with monoclonal antibodies (52). Detailed studies of efficiency of such approach in pediatric populations are underway; however, this is not a first-line therapy that will only work in severely atopic patients. In the newer vision of asthma therapy, the inhaled steroids are now recommended in children older than 5 years with persistent asthma; leukotriene modifiers have shown only marginal benefit and are not recommended as first-line therapy or add-on therapy (53).

Alternative medicine (AM) ("holistic", "unconventional", "complementary") includes a broad range of treatments and practices that have not gained wide acceptance in the traditional medical community and so are not considered standard medical treatment. While used in as many is ¼ of adults with asthma (54) and suggested to be useful for asthma control, the alternative medicine use in children is limited. A study by Torres-Llenza et al revealed that alternative medicine use among Quebec children with asthma remained modest (13%), with vitamins, homeopathy and acupuncture being the most popular modalities (55). AM use was associated with preschool age, was highly correlated with ethnicity and episodic asthma. More importantly, AM was also associated with poor asthma control (55). The efficiency of AM in childhood asthma remains to be identified.

Differences between pediatric and adult asthma

For the general practitioners who do not deal with pediatric asthma on daily basis, it is important to summarize the differences between the pediatric and adult asthma. More recently Bush and Menzies- Gow (56) pointed to the age-related variation of asthma phenotypes and classified these age-specific differences based on:

a) Phenotypes of wheezing: often encountered during the first year of life, many remit in the second year of life, and the children who remit do not have later-onset wheeze.

b) Gender/atopy bias: children with severe asthma have no gender bias and are highly atopic, in contrast to the female-preponderant, non-atopic bias seen in adults.

c) Lung function: unlike adults, children with severe asthma have relatively well-preserved lung function.

In terms of pharmacological treatment strategy, children younger than 5 years of age are more difficult to diagnose, treat and manage and require specific approaches; children older than 5 years of age are treated similar to adults, with planned management according to diseases severity and with medication doses corresponding to body weight.

Recent discoveries in asthma research field that promise translational advancement

While avoidance of allergens is often recommended as a primary prevention measure, this is often not possible, not feasible or not effective especially in children. Thus, new preventive measures are needed; in this context measures that aim at naturally occurring mechanisms that might normally limit the development of immune reactions, inflammation and tissue remodeling are most desirable.

Most recently there are several research discoveries that

Table 5

Pharmacotherapy of acute asthma episode

Action	Time-line	Mild episode		Moderate episode		Severe		Life-threatening episode (critical)
Hospitalization	Immediately	Usually not needed		May be needed		Usually needed; may need intensive care		Needed, intensive care
Observation	Immediately	Continuous during the 1st hour, assess after 20 min post- treatment		Continuous during the 1st hour, assess after 60 min post- treatment		Continuous, assess often as needed		Continuous, assess often as needed
Oxygen	Immediately	As needed, monitor SaO2		As needed, monitor SaO2		Required, monitor SaO2		Required, monitor SaO2
Heliox (helium/ oxygen mixture)	Immediately	No		No		May be considered		
SABA (short- acting B2 agonist) Salbutamol (100ug/ puff)	Immediately	Age <6yo	Age >6yo	Age <6yo	Age >6yo	Age <6yo	Age >6yo	All ages
		4-6 puffs	8-12 puffs	6 puffs	12 puffs	6 puffs, 3 times during the 1st hour	12 puffs, 3 times during the 1st hour	Nebulizer
	Review in 20 minutes	Give every 1-4 hrs, as needed		If inadequate response: -repeat 2 more times at 20 min intervals -give every 1-4 hrs thereafter		If no effect , bolus IV salbutamol (15um/kg) over 10 minutes, then 1ug/kg/ min thereafter		If no effect in 20 min, bolus IV salbutamol (15um/kg) over 10 minutes, then 1ug/ kg/min thereafter
Ipratropim (20ug/ puff)	Immediately	Not necessary		Optional		Age <6yo	Age >6yo	All ages
						2 puffs	4 puffs	Nebulizer
						Every 20 minutes, 3 doses during the 1st hour or use in nebulizer		Continuous
Corticosteroids	Immediately	Usually not necessarily. Consider depending on the clinical dynamics		Oral prednisone (1mg/ kg) daily for up to 3 days		Oral prednisone (1mg/kg) daily for up to 5 days. Consider IV methylprednisone.		Methylprednisone IV (1mg/kg) every 6 hours on 1st day, every 12 hrs on 2nd day, and daily thereafter.
Magnesium	During the first hour	No		No		Magnesium sulfate 50% 0.1 ml/kg (50mg/kg) over 20 min, than 0.06 ml/kg (0.06mg/ kg) to achieve target serum 1.5-2.5 mM/L		
Aminophylline	Usually after the first hour assessment	No		No		Only in intensive care: loading dose 10 mg/kg; maintenance dose: 1.1 mg/kg/hr if <9yo, and 0.7 mg/kg/hrs if >9yo.		

look promising in terms of taking advantage of the innate immune responses as means to manage asthma. To date, most researchers in asthma field agree with the "hygiene hypothesis" of asthma and attempt to employ innate immunity- mediated approaches to modulation of adaptive immunity as means to manage asthma. Innate immunity (macrophages, dendritic cells) recognizes pathogen-derived molecules by sensing specific patterns in their structure via Pattern Recognition Receptors (PRR) (34-37). The microbe-specific molecules that are recognized by a given PRR are called pathogen-associated molecular patterns (PAMPs), they alert the innate immune cells to bacterial carbohydrates (e.g. lipopolysaccharide or LPS, mannose), nucleic acids (e.g. bacterial or viral DNA or RNA), bacerial peptides (flagellin), peptidoglycans and lipotechoic acids (from Gram positive bacteria), *N*-formylmethionine, lipoproteins and fungal glucans. PRRs that sense endogenous stress signals are called danger-associated molecular patterns (DAMPs); they sense substances released by stressed or dying cells, including uric acid, heat shock proteins etc.

All these receptors trigger production of pro-inflammatory cytokines and initiate, maintain, and participate in resolution of inflammation. At experimental level, it is possible to employ innate immune tolerance (57-59) or adaptive immune responses (60-63) to control asthma mechanisms; it remains to be determined if these findings have translational value. Even with the promising progress of research, the question remains: can manipulation of immune system aid asthma management?

Unequivocally yes, because inflammation is key to pathogenesis of asthma; however, it is to be noted that manipulation of immune system, along with identification of genetic conditions, and modulation of environmental factors will be needed in order to provide personalized therapy of asthma to children.

Future directions

Several research directions are emergent, in the light of ever-growing number of asthma- affected children:

1. Detailed understanding of the processes of sensitization, inflammation, airway reactivity and remodeling is emerging in order to arrive to a sound prevention and much needed pathogenesis-based treatment asthma. In this context, we foresee that the need for research of allergens, developmental immunology, airway microbiota and omics (genomics, proteomics, interactomics). While this type of research is expected to yield data useful for asthma in general, age-specific characteristics and immune response in the airways are yet to be fully understood.

2. Discoveries of new pharmacotherapy for asthma are also awaited: we are in need of new targets with high specificity, low toxicity, convenient delivery and limited side-effects.

3. A better understanding of the immediate and long-term effects of existing combination asthma therapies, along with development of more efficient assessment, treatment and follow-up protocols, are among the clinical priorities.

4. We need better social support for the asthmatic children and their families in terms of safe housing conditions preventing/minimizing exposure to allergens, access to consistent and quality medical care, availability and adequate conditions for physical activity. Finally, education of all parties involved in care for asthmatic children requires expansion: we need dedicated and excellently-trained medical personnel at all levels, access for caregivers to relevant literature about the latest discoveries in areas of asthma research, diseases prevention and management, and last, but not least, we need a step-by-step action plan on how to educate affected children to efficiently deal with asthma on daily bases and yet have a productive and enjoyable life.

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DOI: 10.5281/zenodo.1299039
UDC: 616.72-007.248-085.276

Osteoarthrosis: possibilities of complex use of the medication Zeel T within the framework of bioregulatory approach

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Abstract

Background: In the article it is considered the essence and possibilities of the bioregulatory approach in the therapy of osteoarthrosis/osteoarthritis (OA) that is realized with the help of the use of complex bioregulatory medications (CBM) of the German company Biologische Heilmittel Heel GmbH. It is described in detail the composition and characteristics of the CBM Zeel T – the chondroprotective compound with a bioregulatory action. Zeel T is registered in the Republic of Moldova as a solution for injections, sublingual tablets and cream. Zeel T contributes to the restoration of the disturbed metabolic and reparative processes in the articular cartilage. There are considered important distinguishable moments of the mechanism of Zeel T action from the traditional chondroprotective compounds and its advantages. It should be noted that Zeel T has more than 40 years research history and it is widely used in more than 30 countries. Every year almost one million patients worldwide are treated with Zeel T. In the article it is presented the overview of clinical studies on the use of Zeel T at OA that demonstrate that Zeel T has comparable clinical efficacy with various traditional medicinal products (MP).
Conclusions: It has also a good tolerability, does not cause characteristic for NSAIDs and corticosteroids (CS) side effects, and therefore can be used for a long time. The medicine is well combined with other medicinal products that increases the effectiveness and reduces the duration of the treatment course of OA.

Key words: Zeel T, bioregulatory approach.

Relevance of the bioregulatory approach

At the present moment, medicine considers the osteoarthrosis/osteoarthritis (OA) as a degenerative-dystrophic process in the articular cartilage, at which there are noted pathologically altered areas of the cartilage and adjacent bone, in which it is disturbed the equilibrium between the degradation and synthesis processes that leads to destruction of the cartilage and a characteristic rearrangement of the subchondral bone plate [3, 15].

At the therapy of the OA are most widely used in the world chondroprotective compounds, NSAIDs, CS, less often – antioxidants, vitamins, minerals, osteotropic medical preparations (MP) and improving intraosseous and regional blood circulation, etc. [2,15]. However, at a long-term use, a number of MP cause various side effects – gastropathies, colitis, cardiomyopathy, influence on arterial tension indices, decrease of the filtration function of kidneys, toxic hepatitis, secondary osteoporosis, etc. [2,3,15]. The same happened also with the COX-2 inhibitors (selective NSAIDs). It turned out that in case of the decrease of analgesic effect, patients increase independently the single and, correspondingly, the daily therapeutic dose that leads to a sharp increase of side effects, including with a lethal outcome. Foreign experts note that this process has an uncontrollable character. The treatment of the OA is complicated also by age restrictions and inability of the use of traditional MP in this or that concomitant pathology. Development of the majority of undesirable side effects, the presence of limitations and contraindications is related to pharmacological paradigm of the accumulation of a certain concentration of the active substance in order to provide an impact on a certain "target" (for example, blocking of COX or certain receptors, occurrence of the excess of the administered substrate at the use of average therapeutic dose, without establishment of individual dose). The development of habituation and decrease of the effectiveness of MP is associated also with the paradigm of the use of large doses of active substance, the task of which is by the "force" of high concentration to keep control over the symptoms in the needed parameters.

In connection with this, there remains an urgent need to optimize the treatment regimens of the OA with the help of MP that have other mechanisms of action and allow ensuring simultaneously high efficacy and safety of treatment. Also, taking into consideration a high risk of the appearance of side effects and poor tolerability of MP at a long-term admission, it is also important the object of the reduction of the duration of their use [15].

As the world clinical experience shows, the above-mentioned tasks are solved successfully by the inclusion in the OA therapy regimens of complex bioregulatory medications (CBM) that were previously called in the literature as antihomotoxic medications (AHTM). The bioregulatory effect is exerted by ultra-small doses (USD) of the active substances of the CBM that are obtained with the help of homeopathic technology. This "pharmacology of ultra-small doses", thanks to a different mechanism of action, introduces fundamentally new possibilities in the treatment regimens of the "pharmacology of large doses" (traditional MP), that at a correct and timely administration, makes it possible to improve significantly the effectiveness of standard therapy regimens. At the same time, the acceleration and quality of recovery is caused by the activation of the processes of self-healing/reparation, due to the launch of USD recovery of

Table 1

Use of the dosage forms of CBM Zeel T depending on the period of OA

Dosage form	Acute period, exacerbation	Continuation of treatment course
Solution for injections* (ampoules of 2,0 ml each)	1 amp. every other day №5	1 amp. 2 times/week - №5-10
Tablets (50 tabl. in container)	1 tab. sublingually every 30 minutes – 1 hour (15 minutes before meals or 1 hour after), up to 12 times per day, the first 2-3 days	1 tabl. 3 times/day
Cream (50 g in tube)	4-5 cm of cream to apply to the skin in the area of the affected joint and to rub in easily: up to 4 times/day in the first 2-3 days	To rub in 2-3 times/day (administration can be combined with phonophoresis, amplipulse)

*solution for injections is injected: intramuscularly, intraarticularly, subcutaneously and intradermally paraarticularly (circumarticularly) and at spondyloarthrosis – paravertebrally.

self-regulation processes in the body [4-6,9,15]. Also, USD are not metabolized in the body and are not energy-consuming for it, and therefore do not have an additional pharmacological load on the body.

Characteristics of Zeel T

For treatment of the OA, in the capacity of basic CBM it is used the Zeel T. In the Republic of Moldova, the medication is presented in three dosage forms: injection solution, sublingual tablets and cream (during the last re-registration by the manufacturer there was specified the basis of the dosage form "ointment" and changed to "cream"). What is the difference between Zeel T and traditional chondroprotective compounds? It does not introduce various components of the connective tissue into the body but has a chondroprotective effect due to bioregulatory effect on the metabolism of chondrocytes and fibroblasts. It restores the efficiency of energetic and metabolic processes in them that improves the synthesis of the missing components of the articular cartilage.

At the same time Zeel T has an anti-inflammatory and analgesic effect. The inclusion of the given CBM in the standard therapy regimens improves the efficiency of the assimilation of connective tissue components that come with classical chondroprotective compounds [7,8,9,15].

Clinical efficiency of Zeel T was confirmed by numerous clinical studies: observational, cohort, prospective, multicentre, randomized, placebo-controlled, double-blind, in vitro [15].

The dosage and mode of administration of Zeel T are presented in the table 1.

Clinical researches

Italian scholars conducted a clinical study "Research of therapeutic efficiency and tolerability of antihomotoxic medication "Zeel T", in which with the help of the method of diffractometric and polarising-microscopic investigation, before and after the course of intra-articular injections Zeel T, there were studied the samples of patients' cartilages. After therapy, the structure of the cartilaginous tissue was significantly improved and no cases of poor tolerance were recorded. This confirms the pronounced chondroprotective effect of Zeel T [10].

The use of Zeel T in the form of ointment at degenerative joint diseases was described by the German specialists in the study "Therapy of Degenerative Joint Diseases by the medication Zeel T – the results of a multicentre investigation of 498 patients". At the evaluation of the tolerability of the ointment Zeel T in 95% of the cases there were registered the results "very good" and "good". At the same time in 75.1% of cases, the effectiveness of therapy with the help of the given CBM was assessed as "good" and "very good", in 17.5% – "satisfactory", i.e. therapeutic effect was observed in 92,6% of cases. Whereby the highest score received the therapy of monoarthrosis with the help of the ointment Zeel T – 76.1% of good and excellent assessments [7,15].

The efficiency of the medication Zeel T (tablets) in comparison with diclofenac was studied in 121 patients with gonarthrosis within the framework of a double-blind randomized study conducted by the German doctors. At the end of the 10-week course of therapy, there was noted a veracious clinical improvement of the mobility and functionality of the affected knee joint in both groups of patients. While the effect of the investigated CBM appeared to be equivalent to the efficiency of diclofenac, in case of the use of the latter there were noted repeatedly side effects (primarily – on the part of gastrointestinal tract). The fact that was not observed at the use of the medication Zeel T. It was concluded that the therapy of light and medium forms of gonarthrosis with the help of the medication Zeel T leads to a significant improvement of the mobility, reduction of pain sensations that improves the quality of life of patients [12].

The data of comparative study showed that to its effectiveness the periarticular injections Zeel T are comparable with intraarticular injections. The results of the therapy by periarticular injections were evaluated as good, the tolerance of the medication was assessed as excellent. It should be noted that at the execution of periarticular injections there is no risk of the contamination of joint. Thus, the injections Zeel T should be administered subcutaneously or intradermally, as close as possible to the affected joint [13].

By Lviv specialists in the work "Osteoarthritis of knee joints in patients with somatic pathology: experience of complex conservative treatment with the inclusion of AHTM" there was studied the possibility of the use of CBM (Zeel T and other CBM) in the therapy of the patients with the OA of knee joints with severe progressive chronic somatic diseases that limited the use of pharmacological allopathic

MP. The results indicate high efficiency, good tolerability and sufficient level of safety of the therapy by Zeel T and other CBM in patients with gonarthrosis of radiologic stages II-III. Also, the authors noted that the treatment with the help of CBM contributes to positive dynamics of clinical signs of acute inflammatory articular syndrome, reducing the pain and movement limitation, improving the functional capacity of joints and improving the quality of life of patients [14].

In the research thesis "Influence of glucocorticoids and antihomotoxic medications on articular cartilage at its mechanical damage in athletes" it was shown that intra-articular injection of the medication Zeel T in the early stages of the damage of articular cartilage of the knee joint causes the suppression of the development of degenerative-dystrophic process and positively influences the formation of chondroid tissue in the defect of articular cartilage [17].

In the research thesis "Treatment of gonarthrosis by homeopathic medication Zeel T in the conditions of polyclinic" there are presented comparative data of two schemes of the 4-week therapy of gonarthrosis in 80 patients. The 1st scheme: 40 people received NSAIDs, vascular medications, physiotherapy. The 2nd scheme: to 40 patients was additionally prescribed the medication Zeel T (tablets). The control of the results of the investigation was carried out according to clinical and ultrasound study of knee joints for 1 year. It is objectively proved that combination of allopathic therapy with the medication Zeel T gives a more pronounced and persistent therapeutic effect, manifested by the reduction of pain and improvement of the function of knee joints. The combination of traditional treatment with Zeel T is recommended in case of periarthritis, tendinitis, mildly/moderately pronounced forms of synovitis and at their combination. The given thesis was awarded the International Prize of G.G. Reckeweg for 2001 [18, 19].

German scientists conducted a comparative study on the use of the medication Zeel T and hyaluronic acid for the therapy of gonarthrosis [21]. Both medications demonstrated similar therapeutic efficiency with a good analgesia, while side effects at the treatment with the help of Zeel T were 2 times less frequent (5,5%) than at the use of hyaluronic acid (11%). Also, the total cost of treatment course with the help of Zeel T was 2 times lower. In another study in Germany in the course of multicentre randomized blind controlled investigation there was carried out the comparison of the efficiency and tolerability of the medications Zeel T and hyaluronic acid in the treatment of the OA of knee joint. Within 5 weeks, patients received 10 injections of Zeel T or 5-of hyaluronic acid (2 groups of 57 patients in each). The therapeutic efficiency of Zeel T was recognized as equivalent to hyaluronic acid, not only in patients suffering of medium intensity pain, but also in patients with severe pain. At the same time, the use of Zeel T twice as less led to undesirable effects. The results of the given investigation confirm the favourable experience of the use of Zeel T, collected for many years [20].

At the department of traumatology and orthopaedics of the Lviv National Medical University there were elaborated methodical recommendations of the Ministry of Health of Ukraine on the application of CBM in the pathology of the joints and vertebral column: "Pathogenetically directed medicinal therapy of destructive-dystrophic diseases of the vertebral column and joints". It is noted that Zeel T contributes to the transfer of arthrogenic disease during the period of persistent remission, and the inclusion of CBM in the therapy schemes contributes to significant improvement of the results of treatment, makes it possible to reduce the dosage of allopathic MP, and in some cases, to cancel them [11].

Table 2

Combination of Zeel T dosage forms and dosage at different stages of OA

Stage of OA	Dosage form	Acute period, exacerbation	Continuation of treatment course	Repeated treatment courses (prevention of exacerbations)
Stage I	**Tablets**	To start with the initiation mode: 1 tab. every 30 minutes – 1 hour, up to 12 times a day, the first 2-3 days	Further to take 1 tab. 3 times/day, 5-7 weeks	- During 5-7 weeks - 2 times/year
	Cream	Up to 4 times/day in the first 2-3 days	Further 2-3 times/day	
Stage I-II	**Solution for injections ***	To start with the initiation mode: 1 amp. every other day, №5	–	- During 7-9 weeks - 2-3 times/year
	Tablets	–	1 tab. 3 times/day During 5-7 weeks	
	Cream	Up to 4 times/day in the first 2-3 days	Further 2-3 times/day	
Stage II	**Solution for injections ***	To start with the initiation mode: 1 amp. every other day, №5	2 times/week №5-10. Total for course: №10-15	- During 10-12 weeks - 2-3 times/year
	Tablets	–	After the course of injections: 1 tab. 3 times/day, 5-7 weeks	
	Cream		2-3 times/day	
Stage III	**Solution for injections ***	To start with the initiation mode: 1 amp. every other day, №5	2 times/week №10-15 Total for course: №15-20	- During ≈ 3 months - 3 times/year
	Tablets	–	After the course of injections: 1 tab. 3 times/day, 5 weeks	
	Cream	Up to 4 times/day in the first 2-3 days	2-3 times/day	

*solution for injections is injected: intramuscularly, intraarticularly, subcutaneously and intradermally – paraarticularly (circumarticularly) and in case of spondyloarthrosis – paravertebrally.

Practical recommendations

A faster and more pronounced clinical effect in the treatment of the OA is achieved by the combination of systemic and local dosage forms of the medication Zeel T: injections with cream and tablets with cream (recommendations in the table 2) [2,8,11].

Conclusions

On the basis of the properties of Zeel T and clinical studies [1-3, 7-15, 19-21], the following conclusions can be made:

1. Zeel T is a complex chondroprotective compound, which, unlike traditional chondroprotective compounds, has a different bioregulatory mechanism of action.

2. Zeel T has similar therapeutic efficiency with traditional MP.

3. Zeel T has a good tolerability, without causing herewith the side effects characteristic for NSAIDs and other MP.

4. The presence of three dosage forms of the medication Zeel T allows to choose for each patient the most optimal or to use the combination of them.

5. Zeel T can be used as a monotherapy or in combination with other MP that contributes to the reduction of the duration of treatment course and increase of its effectiveness.

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Moldovan Medical Journal, June 2018;61(2)

The monograph "The epidemiology of erectile dysfunction in the Republic of Moldova"

Publishing House "Sofiart Studio", Chisinau, 2017, 203 p.

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The monograph "Epidemiology of erectile dysfunction in the Republic of Moldova" is exposed on 203 pages, it contains six chapters. The content of the monograph is based on a thorough analysis of 232 bibliographic sources, mainly from abroad, more than 30% of the references being published in the last 5 years. The paper contains 53 tables and 44 figures.

Chapter I of the monograph describes the history of epidemiological studies of erectile dysfunction, the questionnaires specifically developed for this purpose and the most important investigators who studied the problem. The author presents data on the prevalence of pathology worldwide, starting with the first scientific study that remains a reference, Massachusetts Male Aging Study (MMAS), published in the US in 1994. Subsequent studies conducted over 2 decades in over 60 countries in the world have shown a prevalence of erectile dysfunction from 11 to 57%, being dependent on the socioeconomic state of the investigated region. The risk factors estimated and studied in these works were diabetes mellitus, cardiovascular diseases, depression, some medications and smoking.

Chapter II of the paper describes the methodology of own research and presents the characteristics of the study group. The transversal descriptive study was conducted on a representative sample of 1,186 men aged 18-80. A questionnaire based on similar research and the requirements of scientific societies in the field of urology and sexual medicine has been used, which included questions from 4 scales and questionnaires: BSSCMV – Brief Sexual Symptom checklist: men's version, IIEF -5 International Index of Erectile Function, MSHQ – Male Sexual Health Questionnaire, IPSS-International Prostate Symptom Score. Respondents were assigned according to their living environment, age, family status, schooling level, etc.

Chapter III shows the prevalence of erectile dysfunction in the Republic of Moldova according to self-assessment and validated questionnaires depending on age, living environment, family status, sexual relations, etc. The author shows the prevalence of erectile dysfunction of 19.4% followed by self-assessment and 47.1% following the completion of the IIFE-5 questionnaire, including 12.7% with severe form, 12.4% moderate and 22% of the mild form. The presented data place the Republic of Moldova close to the top of the countries with the obvious predominance of pathology. The author explains the difference between the two questionnaires by the fact that in the case of self-assessment, only the severe and moderate forms of erectile dysfunction were reported, the mild ones not being considered a problem. Another conclusion of the monograph should be noted, the prevalence of erectile dysfunction is about 25% higher in rural areas. At first sight, it seems a paradox considering that the rural environment according to the general opinion is considered healthier.

Chapter IV has 10 subchapters, where the author describes in detail the mechanism of erection and the influence of the principal risk factors on it. Older age is one of the most important non-modifiable risk factors for erectile dysfunction. The author assessed the erectile function of the respondents according to the IIFE-5 index, where a score greater than 21 equals a normal erectile function and less than 8 indicates a severe erectile dysfunction. The erectile function score is presented in age-dependent. Although the tendency to diminish erection is logical with age, it is worrying that in the Republic of Moldova, only 48% did not report problems with erection, men after the age of 40, and after the age of 65 the prevalence of erectile dysfunction was greater than 80%.The author has thoroughly analyzed the literature data regarding the main risk factors blamed for erectile dysfunction, especially cardiovascular and endocrine ones, which have similar mechanisms of action on vascular endothelium. The conclusion of the studies published by this time, including the author of this monograph, is that erectile function reflects men's health, and erectile dysfunction may be an early marker of other illnesses, such as cardiovascular, endocrine or urogenital diseases in apparently asymptomatic patients. Some authors quoted in the paper believe that a man over his 40s diagnosed with erectile dysfunction needs to be interpreted as a potential cardiac patient until proof of the opposite is demonstrated. In the author's paper, about 75% of patients with cardiovascular pathology and 79% of patients with endocrine pathology suffer simultaneously of erectile dysfunction, and in about 50% of them, erectile dysfunction started more than 1 year before other clinical manifestations occurred.

In Chapter V, the author analyzes erectile function depending on the presence of modifiable risk factors. Thus, over 60% of people with obesity and reduced physical activity developed an erectile dysfunction, compared to 19-30% of the control group, in the absence of suspected risk factors ($p < 0.05$). The attitude of respondents to smoking and the influence of smoking on erections are analyzed. It is noted that the risk of erectile dysfunction in smokers is 1.85 times higher than in non-smokers. The author continues to demonstrate that the risk of erectile dysfunction depends on the number of cigarettes smoked daily. Alcohol consumption may influence erection, a dose-effect relationship was established. No significant differences were found in the prevalence of erectile dysfunction in people who do not consume alcohol compared to people who occasionally consume. Instead, daily alcohol consumption leads to a prevalence of erectile problems in about 75% of men, and the probability of erectile dysfunction occurring is 3.69 times higher in people with daily alcohol consumption.

The last chapter, VI, is dedicated to addressability of patients with erectile dysfunction. The author mentions that, as a relatively delicate subject, in most countries, few erectile patients address medical workers. Among respondents in the Republic of Moldova, only 17% of men with erectile dysfunction performed a specialized consultation, and only 15.4% followed treatment. The fact that men with sexual problems remain alone with their problems raises some questions not only because of the social consequences but primarily of the medical ones, making it often impossible to detect early other more serious comorbidities, first of all cardiovascular.

In conclusion, the monograph "Epidemiology of erectile dysfunction in the Republic of Moldova" by Ion Dumbraveanu is a pioneering scientific work for the Republic of Moldova, reflecting the first such study conducted in our country. It is an original work with a certain scientific value and practical utility, which addresses the problems that need to be solved now and in perspective and draws new ways of development of andrology in the Republic of Moldova.

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