ISSN 1857-0666

Curierul medical

SCIENTIFIC MEDICAL JOURNAL

Ministry of Health of the Republic of Moldova
Nicolae Testemitsanu State University of Medicine and Pharmacy

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al Republicii Moldova.
Universitatea de Stat de Medicină și
Farmacie „Nicolae Testemițanu”

Министерство здравоохранения
Республики Молдова.
Государственный университет медицины и
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Issued bimonthly

Vol. 59, No 4
August, 2016

ISSN 1857-0666

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Printing House "Tipografia Sirius"
2, A. Lapusneanu str., Chisinau, MD-2004
the Republic of Moldova
www. sirius.md
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Phone: (+37322) 244751, (+37322) 205209
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RESEARCH STUDIES

Molecular–genetic assay results and clinical, radiological features in patients with pulmonary tuberculosis

Alina MALIC

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Abstract

Background: Early diagnosis of smear-negative and multidrug-resistant tuberculosis represents a national priority, considering the fact that the Republic of Moldova ranks in the list of 30 high burden multidrug-resistance tuberculosis countries, with 26% rate of primary drug-resistance and 64% acquired drug-resistance. In April 2012 it was implemented the real-time fully automated diagnostic molecular test in 15 Moldovan districts and penitentiary institutions. The new assay leads to enhance the tuberculosis early diagnosis, especially multidrug resistant tuberculosis.

Material and methods: A retrospective, selective, descriptive and case-control study was performed using 361 new pulmonary tuberculosis patients, diagnosed and hospitalized in the Municipal Clinical Hospital of Phthysiopneumology of Chisinau city in the period of 01.01.2014-01.01.2015. The total sample was distributed in 2 groups: first group included 174 patients with pulmonary tuberculosis assessed by positive Xpert MTB/RIF assay and the second group included 187 patients with pulmonary tuberculosis assessed having negative Xpert MTB/RIF assay. Investigations were performed according to the National Clinical Protocols.

Results: The biggest rate of patients included in the research was detected by passive way, in the frame of examination of the symtomatic case: 111 (63.8%) patients of the 1st Group comparing with 87 (46.5%) in the 2nd Group, $p<0.001$.

Conclusions: The use of Xpert MTB/RIF technique must be improved in municipality Chisinau for achieving the international quality standards.

Key words: pulmonary tuberculosis, genetic assay results, Xpert MTB/RIF.

Introduction

Tuberculosis is the most important public health threat in the Republic of Moldova, the country ranking among 30 countries with the biggest burden of multidrug-resistance tuberculosis (MDR-TB) [1]. In overall, the primary MDR-TB increased four times in the Republic of Moldova in the period 2003-2011 (6.0% – 2003, 9.9% – 2004, 13.4% – 2005, 19.4% – 2006, 17.8% – 2007, 24.0% – 2008, 22.2% – 2009, 25.7% – 2010, 26.35% – 2011) and acquired drug-resistance two times (37.5% – 2003, 38% – 2004, 49.6% – 2005, 50.8% – 2006, 52.3% – 2007, 59.0% – 2008, 64.85% – 2009, 67.8% – 2010, 63.8% – 2011) [3]. MDR-TB represents a global concern and directly threatens disease-control efforts in many countries. Only 30.000 of nearly 500.000 new cases of MDR-TB every year are detected and reported [11]. The misdiagnosed cases contribute to thousands of deaths, nosocomial and community transmission.

No much technology is available for an accurate detection of tuberculosis and its drug resistant forms, this fact being a major obstacle for improvement of tuberculosis control and reduction of the global burden of disease [12]. Microscopy alone, although inexpensive, misses many patients and detects only those with relatively advanced disease. Presently, only 28% of registered tuberculosis cases are detected and reported as smear positive [3]. Undetected cases of tuberculosis increase the risk of disease transmission to the healthy population, endangering tuberculosis control. In low and middle income countries the high rate of HIV infection reduced the sensitivity of microscopy and increased the relevance of rapid diagnostic methods []. In recognition of these issues, substantial efforts were being made to strengthen laboratory capacities to diagnose smear-negative and MDR-TB cases, including the use of solid and liquid culture, conventional drug-susceptibility testing, and line-probe assays. Unfortunately, these tests require extensive laboratory infrastructure and cannot be done outside of reference facilities. Recently, a real-time PCR assay for *Mycobacterium tuberculosis* that simultaneously detects rifampicine resistance was developed on the GeneXpert platform (Cepheid, Sunnyvale, CA, USA), which integrates sample processing and greatly simplifies testing [2]. Expected outcomes ensured by the national implementation of the new Xpert MTB/RIF technique are: early detection of infectious cases, isolation and precocious treatment of early detected new case, especially of MDR-TB, as well as infection control and improving the treatment success rate, that are considered the most efficient tools for interrupting the epidemiological chain of infectious transmission [16]. Starting from December 2010 World Health Organization (WHO) strongly recommends the use of molecular-genetic Xpert MTB/RIF testing as the initial diagnostic test in adults and children presumed to have pulmonary MDR-TB, tuberculosis and HIV coinfection, or

TB meningitis [14]. In addition, WHO established conditional recommendations to use Xpert MTB/RIF test in adults and children presumed to have active TB (not especially MDR-TB), or for testing the non-respiratory specimens targeting the diagnosis of extra pulmonary TB [15]. Always interpretations of this test must be correlated with laboratory and clinical data of the investigated patient. Published data established a high sensitivity among culture positive specimens on average 97.3% and among smear positive patients – 99.5%. The specificity comparing with non-tuberculosis patients was determined to be at 97.9% [2]. Error rates vary from 3 to 4%. The sensitivity is slightly decreased in a single sputum sample. Assessing the threshold of analytical sensitivity it was demonstrated that the biological sample must contain at least 131 colony forming units (CFU) per ml of sample with a confidence interval ranging from 106.2 CFU to 176.4 CFU [2]. In most of cases the detection of mycobacterial DNA depends on the collection procedure, storage, transportation, and technical errors. Insufficient volume of the specimen and insufficient concentration of the viable organisms are the most frequent causes of the negative results [2].

WHO Global TB program provides worldwide leadership in strategic and technical aspects of TB control in order to eliminate TB. In 2011 the Stop TB Partnership started the ongoing of TB Xpert Project that provided 1.4 million Xpert MTB/RIF test cartridges and 220 Xpert instruments in 21 countries, the Republic of Moldova being one of them. Starting from April 2012, the Republic of Moldova received 25 units of Xpert MTB/RIF equipment and 12.000 cartridges. Civilian TB services acquired 21 units, penitentiary TB services – 2 and AIDS services – Xpert MTB/RIF units of equipment. Preliminary results established some problems. The most important is the low sensitivity of Xpert MTB/RIF Assay that is compared with the sensitivity of conventional microbiological methods. More than one half of patients with tuberculosis are diagnosed through clinical-radiological methods. Considering the WHO recommendations for aligning to international quality-assured standards it is necessary to improve the TB diagnostic algorithms.

Aim of the study consisted in evaluation of the Xpert MTB/RIF assay and diseases-related features (clinical and radiological) in patients with pulmonary tuberculosis.

Material and methods

It was performed a retrospective, selective, descriptive and case-control study targeting peculiarities of pulmonary tuberculosis of 361 patients, treated in the Municipal Clinical Hospital of Phthysiopneumology of Chisinau city between 01.01.2014 and 01.01.2015. The total number of cases was distributed in 2 groups: 1st Group (1st Group) included 174 patients with pulmonary tuberculosis assessed by positive Xpert MTB/RIF assay and second group (2nd Group) included 187 patients with pulmonary tuberculosis assessed being negative at Xpert MTB/RIF assay. Including criteria in the 1st group: age > 18 years old; patient with pulmonary tuberculosis was established as a new case; one smear positive GeneXpert MTB/RIF assay detecting MTB DNA; including criteria in the 2nd group: age > 18 years old; patient with pulmonary tuberculosis was established as a new case; one smear negative Xpert MTB/RIF assay. 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, plane tomography, and computer tomography), results of microbiological investigations (bacterioscopy of the smear with Ziehl-Neelson coloration) and bacteriological examinations (culture on classic solid medium Lowenstein-Jensen and liquid medium). Investigations were performed according to the national clinical protocol. Statistical analysis methods used in the study were: comparative, synthesis, discriminate analyses. Mathematical and statistical assessments were carried out by checking the quantitative and qualitative features. Accumulated material was tabled in simple and complex groups. Statistical survey was performed using Microsoft Excel XP soft. The predictability value of each involved factor was calculated using two by two tables. Relative risk and confidence interval were calculated according to the established formula [4].

Results and discussion

Considering the fact that 361 patients were investigated through Xpert MTB/RIF, all of them confirmed with pulmonary tuberculosis and only 174 had positive Xpert MTB/RIF. In the 1st group – 174 positive Xpert MTB/RIF cases included 103 microscopic positive at Ziehl-Neelson staining cases. So, the sensitivity of microscopic method compared with Xpert MTB/RIF assay represents 59.2±3.72%, which means two times lower. The same 1st Group – 174 positive Xpert MTB/RIF cases included 142 culture positive at Lowenstein-Jensen/BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 media. So, the sensitivity of culture method compared with Xpert MTB/RIF assay represents 81.6±2.93%. Rapid detection of rifampicine resistance was established at 63 (36.2±3.64%) cases from 174 positive Xpert MTB/RIF assay cases. On the other hand, in the second group, that included 187 Xpert MTB/RIF negative cases, 9 (4.8±1.56%) cases of them were microscopic positive at Ziehl-Neelson and 28 (14.9±2.60%) were culture positive at Lowenstein-Jensen/BACTEC MGIT media. So, Xpert MTB/RIF assay can't replace conventional microbiological methods in actual epidemiological conditions of the Republic of Moldova.

Case-management of patients with pulmonary tuberculosis

According to the National Tuberculosis Program at least 70% of all new tuberculosis cases must be detected by passive way, through microscopic smear examination of the symptomatic patient. Active way of detection is reserved only to high risk groups, annually investigated by chest Xray examination and also dangerous groups of 3 professional fields: health care staff, public service employees as well as educational staff. National TB Policy approved an examination algorithm of different groups of population (patients, adults and children with TB symptoms, HIV positive patients with TB symptoms,

vulnerable groups – homeless, drug-users, immunosupressed, medical staff, patients with suspected relapse, patients with clinical signs of extrapulmonary TB) for TB diagnosis targeting the appropriate use of Xpert MTB/RIF assay. So, according to this, the sputum smear negative samples in highlighted groups of patients with clinical signs of TB (pulmonary TB or extra pulmonary TB) must be compulsorily investigated by Xpert MTB/RIF.

The biggest rate of patients included in the research was detected by passive way, in the frame of examination of the symptomatic case: 111 (63.8±3.64%) patients of the 1st Group comparing with 87 (46.5±3.64%) in the 2nd Group, p<0.001. By active way of screening (targeted radiological investigation) were detected more frequently the patients from the 2nd Group 100 (53.5±3.64%) comparing with 63 (36.2±3.64%) cases of the 1st Group, p<0.001. However, by passive way were detected more frequently the patients of the 1st Group 111 (63.8±3.64%) comparing with the 2nd Group 87 (46.5±3.64%), p<0.001. Data are shown in the table 1.

Table 1

Distribution of detectional case-management

Screening way	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
Passive	111	63.8±3.64	87	46.5±3.64	<0.001
Active	63	36.2±3.64	100	53.5±3.64	<0.001

In one third of the cases the clinical diagnosis of pulmonary tuberculosis was erroneously omitted due to similar aspects with other pathologies. Distributing patients by clinical onset masks, it was identified that pneumonia mask is more frequently involved in both groups, being more prevalent in the 1st Group 34 (26.4±3.87%) comparing with the 2nd Group 14 (11.2± 2.82%), p<0.05. No statistical differences were identified according to other type of TB masks (table 2).

Table 2

Distribution according to the clinical onset masks

Diagnosis	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
Pneumonia	34	26.4± 3.87	14	11.2± 2.82	<0.05
Bronchitis	6	4.7± 1.85	2	1.6± 1.12	>0.05
Laringitis	6	4.7± 1.85	1	0.8± 0.79	>0.05
Pleuresy	8	6.2± 2.12	1	0.8± 0.79	>0.05
Hemoptysis	4	3.1± 1.52	1	0.8± 0.79	>0.05

Note: LIT - limited pulmonary infiltrative tuberculosis, EIT – extensive pulmonary infiltrative tuberculosis, DT – disseminated pulmonary tuberculosis. FCv – fibro-cavitary pulmonary tuberculosis;

The period of time from the clinical onset till the confirmation of diagnosis was established similar distribution of patients in both groups. The period of one to three months between the clinical onset and Xpert MTB/RIF testing (establishing of TB diagnosis) was more prevalent in both groups: 96 (55.2±3.77%) cases in the 1st Group and 108 (57.8±3.61%) - in the 2nd Group. Other delayed periods from the onset till the confirmation of TB diagnosis were similarly distributed within the groups (table 3).

Table 3

Distribution according to the delayed period till TB confirmation

Diagnosis	1st Group		2nd Group		P
	n	M± m(%)	n	M ± m (%)	
14 days	29	16.7±2.82	24	12.8±2.44	>0.05
1 – 3 months	96	55.2±3.77	108	57.8±3.61	>0.05
3 – 6 months	38	21.8±3.13	43	22.9±3.07	>0.05
>6 months	11	6.3±1.84	12	6.4±1.79	>0.05

All above related data were directly linked to the established clinical-radiological diagnosis of pulmonary tuberculosis. So, pulmonary infiltrative tuberculosis was prevalent form in both groups: 159 (91.4%) of the 1st Group and 180 (96.3%) cases in the 2nd Group. Caseous pneumonia, the severest form of the pulmonary infiltrative tuberculosis was diagnosed in 5 (2.9%) cases of the 1st Group. Disseminated tuberculosis was established in several cases of both groups - 14 (8.0%) cases of the 1st Group compared with 3 (1.6%) cases in the 2nd Group. Less diagnosed were the rest of forms (table 4).

Table 4

Clinical radiological forms of pulmonary tuberculosis

Diagnosis	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
PIT	159	91.4±2.12	180	96.3±1.38	>0.05
PDT	14	8.0±2.06	3	1.6±0.91	>0.05
NT	0	0	4	2.1±1.05	>0.05
FCv	1	0.6±0.57	0	0	>0.05

Note: PIT - pulmonary infiltrative tuberculosis, PDT – disseminated pulmonary tuberculosis. FCv –pulmonary fibrocavitary tuberculosis, NT – pulmonary nodular tuberculosis;

While assessing radio-morphological features of pulmonary tuberculosis it was clearly identified the predomination of the destructive compound of the parenchyma in the 1st Group -58 (33.3%) cases compared with the 2nd Group - 18 (9.6%) cases. The localization of the infectious process in both lungs was also predominated in the 1st group: 105 (60.3%) cases compared with 29 (15.5%) cases in the 2nd Group. The extensibility in more than three segments was argued more often in the 1st Group 104 (59.8%) compared with the 2nd Group 21 (11.2%) (table 5).

Table 5

Morpho-radiological features of pulmonary tuberculosis

Diagnosis	1st Group n=174		2nd Group n=187		P
	n	M± m (%)	n	M ± m (%)	
Destructions	58	33.3±3.57	18	9.6±2.15	<0.001
Dissemination	11	6.3±1.84	4	2.1±1.05	>0.05
Unilateral	69	39.7±3.70	158	84.5±2.64	<0.001
Bilateral	105	60.3±3.70	29	15.5±2.64	<0.001
Limited	70	40.2±3.71	166	88.8±2.30	<0.001
Extended	104	59.8±3.71	21	11.2±2.30	<0.001

Note: LIT – limited pulmonary infiltrative tuberculosis, EIT – extensive pulmonary infiltrative tuberculosis, DT – disseminated pulmonary tuberculosis. FCv – fibrocavitary pulmonary tuberculosis.

Impact of disease related determinants on MTB detection by the positive Xpert MTB/RIF testing

Assessing statistically all above exposed data expressing case management, clinical-radiological characteristics and morpho-radiological features of patients with pulmonary tuberculosis with positive/negative results at the detection of MTB by Xpert MTB/RIF testing through multivariate logistic regression model was identified that all disease related factors have high impact on the positivity of MBT DNA detection: extensive infectious process (involving three and more segments), tuberculosis in both lungs, lung parenchyma destructions. Case management by passive way as well as pneumonia mask were revealed as factors with medium value (table 6).

Table 6

Relative risk forTB factors Xpert MTB/RIF assay positive patients

Factors	Relative Risk	95% CI
Passive way of detection	1.45	1.15-1.82
Pneumonia mask	1.56	1.25-1.94
Lung destruction	1.87	1.55-2.26
Localisation in both lungs	2.47	1.71-3.56
Extensive pulmonary process	5.32	3.49-8.11

Conclusions

Passive case management and pneumonia mask were identified as factors with medium impact on Xpert MTB/RIF assay positive patients.

Extensive tuberculosis (involving three and more segments, localization of pathological changes in both lungs), parenchyma destructions have high impact in Xpert MTB/RIF assay positive patients.

Xpert MTB/RIF assay leads to rapid identifing of possible multidrug-resistant TB (MDR TB) and early starting of effective treatment much sooner than waiting for results from other types of drug susceptibility testing.

References

1. Carriquiry G, Otero L, Gonzales-Lagos E. A diagnostic accuracy study of Xpert MTB/RIF in HIV positive patients with high clinical suspicion of pulmonary tuberculosis in Lima, Peru. *Plos One*. 2012;7(9):e 44626.
2. Cepheid. Xpert MDT/RIF. Two-hour detection of MTB and resistance to rifampicine. Sunnyvale, 2009;22.
3. Centrul Național de Management în Sănătate (National Centre of Health Management). Indicatori în format prescurtat privind sănătatea populației și activitatea instituțiilor medico-sanitare pe anii 2014-2015 (Abbreviated format indicators on health and activity of medical institutions for 2014-2015).
4. Gazi MA, Islam MR, Kibria MG. General and advanced diagnostic tools to detect *Mycobacetrium tuberculosis* and their drug susceptibility. *Eu. J. Clinical Microbiology Infectious Diseaseas.* 2015;34(5):851-61.
5. Hanrahan CF, Shah M. Economic challenges associated with tuberculosis diagnostic development. *Expert Rev. Pharmacoeconomy.* 2014;14(4):499-510.
6. Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and Rifampicine resistance: use of on-demand, near-patient technology. *J. Clinical Microbiology*. 2010;48(1):229-237.
7. Ioannidis P, Papaventsis D, Karabela S. Cepheid GeneXpert MTB/RIF assay for *Mycobacterium tuberculosis* detection and rifampicine resistance identification in patients with substantial clinical indicators of tuberculosis and smear-negative microscopy results. *J. Clinical Microbiology.* 2011;49(8):3068-3070.
8. Moure R, Munoz L, Torres M. Rapid detection of *Mycobacterium tuberculosis* complex and rifampicine resistance in smear-negative clinical samples by use of an integrate real-time PCR method. *J. Clinical Microbiology*. 2011;49(30):1137-1139.
9. Raviglione MC, Pio A. Evolution of WHO policies for tuberculosis control, 1948–2001. *Lancet.* 2002;359(9308): 775-780.
10. Steingart KR, Schiller I, Horne DJ. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicine resistance in adults. The Cochrane database of systematic review. Wiley, 2015;168.
11. Weyer K, Mirzayev F, Migliori B, et al. Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. *Eur. Respiratory J*. 2013;42(1).
12. Wilson ML. Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clinical infectious Diseases*. 2011;52(11):1350-5.
13. World Health Organization. The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis. Geneva, 2011.
14. World Health Organization. Policy Statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicine resistance: Xpert MTB/RIF system. Geneva, 2011.
15. World Health Organization. Xpert MTB/RIF implementation manual. Geneva, 2014.
16. World Health Organization. Towards universal access to diagnosis and treatment of multidrug-resistant and extensively drug-resistant tuberculosis by 2015. Geneva, 2015.

Evaluation of Tetracyclines and Aminoglycosides consumption

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Abstract

Background: Tetracyclines and Aminoglycosides antimicrobials hold the last positions among all main antibiotic subgroups used yearly in hospitals. Nevertheless,without these medical remedies a qualified treatment of patients with different illnesses cannot be well organized or even would be impossible.
Material and methods: For this study we used data of a six-year (2009-2014) period, in the Emergency Medicine Institute and their subdivisions where happened main consumption of antibiotics such as: Reanimation, Therapeutic intensive care, Therapeutic «stroke" intensive care, septic Surgery and septic Orthopedic-Traumatology departments - which show the consumption dynamics of Tetracyclines and Aminoglycosides antimicrobials use in grams and value indexes.
Results: The defined daily doses (DDD) per 1000 occupied-bed days (DDD/1000) in Emergency Medicine Institute in 2009 and 2014 for Tetracyclines registered an increase from 8.2 to 20.8 or a share of 1.24% and 4.48% from the total annual consumption. Aminoglycosides recorded a decrease from 83.1 to 43.7 that represents a share of 12.55% and 9.42% from the total annual consumption. The same data in Intensive care departments recorded for Tetracyclines an increase from 3.61 to 53.98 DDD/1000 or 0.27% and 4.48% and respectively for Aminoglycosides from 494.49 to 134.4 or 15.12% and 11.16%. Septic departments recorded from 3.56 to 17.63 DDD/1000 or 0.57% and 3.44% and respectively for Aminoglycosides from 142.83 to 137.04DDD/1000 or 22.64% and 26.77%. In the mentioned period medium consumption in international hospitals of Tetracycline's increased from 27.00 to 70.00 DDD/1000 or by 2.59 times, and comparatively with the EMI in 2014 was higher by 3.36 times. Aminoglycosides consumption in international hospitals decreased from 50.00 to 40.00 DDD/1000 or by 20% and in 2014 year was similar with the data recorded in EMI. Cost of DDD/1000 in Emergency Medicine Institute during 2009 and 2014 years, for Tetracyclines registered 1.89 and 5.57 lei, for Aminoglycosides respectively 461.19 and 460.83 lei. In Intensive care departments costs recorded 0.98 and 14.44 lei and 2793.61 and 2634.39 lei, and in Septic departments 0.4 and 4.75 lei and 536.61 and 329.23 lei respectively.
Conclusions: We found an increase in consumption of Tetracyclines DDD/1000 during the evaluated period, at the same time consumption in international hospitals in 2014 was in medium 70.00 DDD/1000 or 3.36 times more than 20.8 DDD/1000 recorded in EMI. Aminoglycosides in the evaluated period decreased in DDD/1000 consumption in EMI and international hospitals and in 2014 was around the same.
Key words: antibiotics, tetracycline, aminoglycoside, defined daily dose, consumption, rational use, hospitals.

Introduction

Despite the fact that shares of Tetracyclines and Aminoglycosides in acute-care hospitals hold the last position among other antibiotics subgroups, they remain an important component in patients' antimicrobial treatment. Around 5% of patients admitted to acute-care hospitals acquire nosocomial infections [1, 2]. Antibiotical therapies treatment of severe acute respiratory diseases (SARS), included tetracyclines (91.0%), aminoglycosides (83.3%), quinolones (79.2%); 18.8% of the patients received a combination of tetracyclines and aminoglycosides, while 11.5% received a combination of tetracyclines and quinolones, and 63.5% received a combination of tetracyclines, aminoglycosides and quinolones [3, 4]. The mortality rate of SARS worldwide is approximately 10.5%. The mortality rate of severe acute respiratory syndrome (SARS) patients admitted to the intensive care units (ICU) ranged from 5% to 67% [5, 6]. In international hospitals medium consumption for Tetracyclines recorded 0.5-27-70,00 DDD/1000 and for Aminoglycosides from 40.00 to 50,00 DDD/1000 or no more than 5% of all amount of antibiotics. Consumption in ICU for critically ill patients that received only gentamicin or amikacin is higher and varied between 38%-66% [7, 8, 9]. A higher consumption of Aminoglycosides from 25% to 45% of the total annual amount of antibiotics occurs in septic orthotraumotology department of EMI [10]. The above mentioned supposes surveillance, stringent consumption control and rational antibiotic prescription [11, 12, 13, 14].

The primary aim of the study was to evaluate institutional representative data on Tetracyclines and Aminoglycosides utilizationin in accordance to the World Health Organization (WHO) requirements, directed to determine value of Defined Daily Doses per 1000 Occupied-Bed Days (DDD/1000) [15, 16].

Material and methods

For this study we used the data of a six-year (2009-2014) period, DDD/1000 consumption of Tetracyclines and Aminoglycoside antibacterials in Emergency Medicine Institute (EMI) in used rates are demonstrated. EMI intencive care unit (ICU) – reanimation, therapy intensive care and "stroke" intensive care departments, septic surgical and septic orhtotraumotology departments (SSOD) – which show the dynamics of consumption of antiinfectives for systemic use drugs, as classified by Anatomical Therapeutic Chemical (ATC), classification system of World Health Organization (WHO), indicated in grams and value indexes. Statistical, analytical, mathematical, comparative, logical and descriptive were used as the methods of study.

Results and discussion

For determining the number of DDD/1000 we used data about total annual consumption of Tetracycline's and Aminoglycosides antibacterials and the statistics data concerning the number of treated patients (only patients with health insurance

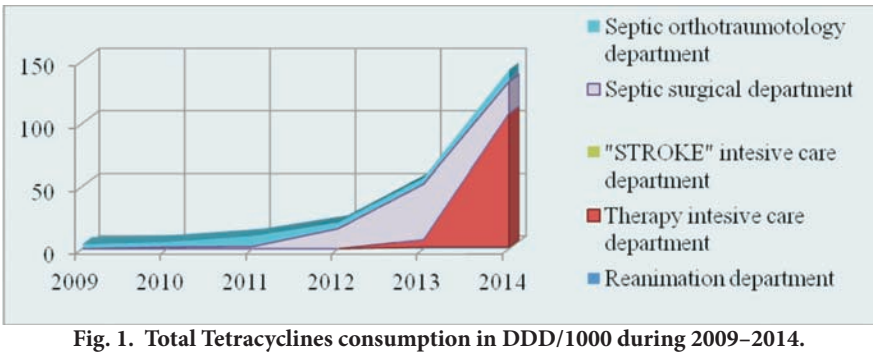

Fig. 1. Total Tetracyclines consumption in DDD/1000 during 2009–2014.

and other free treated by the state categories of citizens). The total number of occupied bed/days in the institution was 188762 in 2009, 191556 in 2010, 186246 in 2011, 199816 in 2012, 193019 in 2013 and 187558 in 2014, and respectively for the corresponding departments of EMI: reanimation intensive care unit (2009 = 3990; 2010 = 6551; 2011 = 6985; 2012 = 9051; 2013 = 7384; 2014 = 7361), therapeutical intensive care (2010 = 2922; 2011 = 3327; 2012 = 3239; 2013 = 3407; 2014 = 3388), "stroke" intensive care (2013 = 2553; 2014 = 4193), septic surgical (2009 = 14030; 2010 = 14212; 2011 = 12875; 2012 = 12372; 2013 = 12464; 2014 = 12104), septic orthopedic-traumotology (2009 =10664; 2010 = 10017; 2011 = 9540; 2012 = 10178; 2013 = 9701; 2014 = 9535) [17, 18, 19, 20]. Total Tetracyclines consumption in DDD/1000 during 2009-2014 is shown in figure 1.

Tetracyclines consumption is characterised by the use of Doxycyclinum with 0.1gr defined daily doses. As can be observed from figure 1 in the evaluated period was recorded an increase of the average consumption annual rate in septic orthopedic-traumotology department from 3.6 to 9.7, in septic surgical department from 1.41 to 25.6, therapeutical intensive care department from 5.9 to 106.6 and reanimation department from 1.35 to 1.36 DDD/1000. In the "stroke" intesive care department consumption of Tetracicynes was not registered at all. In figure 2 the total consumption of Aminoglicosides in DDD/1000 during 2010-2014 is shown.

Aminoglycosides antibacterials are presented with the use of Streptomycinum 1.0, Gentamycinum 0.2, Kanamycinum 1.0 and Amikacinum 1.0 and respectively defined daily doses. Reanimation departament recorded the higher from 494.49 to 219.13 DDD/1000 consumption with a decline by 55.69%. The consumption in septic orthotraumotology department was more stable and varied from 245.2 to 243.52 DDD/1000. The last third positions hold "stroke" intesive care department where was recorded a decline from 206.03 to 124.73 DDD/1000 or by 39.46%, followed by therapy intensive care department with an increase from 35.25 to 59.33 DDD/1000 or by 68.31% and ended the list septic surgical department with a decrease from 40.45 in 2009 to 30.56 DDD/1000 or by 24.45%. The total consumption in intensive care units and septic departments in 2014 was 677.27 DDD/1000, from which the share of reanimation department represents 32.36%, Therapy intensive care department 8.76%, "stroke" intensive care department 18.42%, septic surgical department 4.51% and septic orthotraumotology department 35.96%.

In table 1 comparison of average DDD/1000 consumption of Tetracyclines and Aminoglycosides antibacterials in intensive care and septic departments of EMI is shown.

Table 1

Average DDD/1000 consumption of Tetracyclines and Aminoglycosides antibacterials in intensive care and septic departments of E

Units	2009	2010	2011	2012	2013	2014
Tetracyclines						
ICU					3.61	53.98
SSOD	3.56	2.7	5.13	10.54	24.88	17.63
EMI	8.2	26.8	15.1	7.1	13.5	20.8
Aminoglycoside antibacterials						
ICU	494.49	85.63	174.79	292.74	222.19	134.4
SSOD	142.83	74.13	187.45	93.54	157.84	137.04
EMI	83.1	37.60	76.90	103.20	112.30	43.70
Total antibiotics consumption in EMI						
EMI	662.4	558.2	662.1	542.4	546.9	464.1

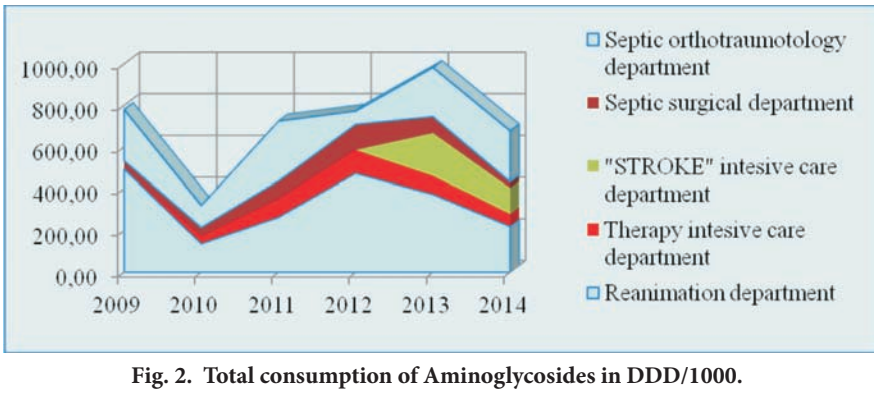

Fig. 2. Total consumption of Aminoglycosides in DDD/1000.

Table 2

Total DDD/1000 consumption of Tetracyclines and Aminoglycosides in EMI and some international acute hospitals

Total DDD/1000 consumption of Tetracyclines in international acute hospitals						
	2009	**2010**	**2011**	**2012**	**2013**	**2014**
Emergency Medicine Institute	8.20	26.80	15.10	7.10	13.50	20.80
Large acute Australian public hospitals [21]	27.00	41.50	52.00	60.00	60.00	70.00
The number of hospitals	16	18	22	32	42	51
Medium acute Australian public hospitals	52.00	42.50	42.00	61.50	71.00	80.00
The number of hospitals	9	9	10	13	24	26
South Australian metropolitan hospitals	40.00	38.70	37.60	41.70	37.20	
SAAUSP 37.2, NAUSP 44.00, NETHMAP 18.00 in 2013; SWEDRES 54.00, DANMAP 15.80 in 2012 [21]						
Total DDD/1000 consumption of Aminoglycosides in international acute hospitals						
	2009	2010	2011	2012	2013	2014
Emergency Medicine Institute	83.1	37.60	76.90	103.20	112.30	43.70
Large acute Australian public hospitals [22]	50.00	50.00	45.00	43.00	43.00	40.00
The number of hospitals	16	18	22	32	42	51
Medium acute Australian public hospitals	53.00	58.00	51.00	50.00	42.00	38.00
The number of hospitals	9	9	10	13	24	26
South Australian metropolitan hospitals	65.50	66.50	64.20	58.70	55.50	
SAAUSP 55.5, NAUSP 42.1, NETHMAP 39.00 in 2013 and SWEDRES 12.00, DANMAP 21.4 in 2013 [22]						

As can be seen from table 1 average DDD/1000 consumption of Tetracyclines antibacterials in intensive care and septic departments in the evaluated period increased respectively by 14.9 (53.98:3.61) and 4.9 (17.63:3.56) times, and totally for the institution by 2.4 (20.8:8.2) times. For Aminoglycoside the data conversely recorded a decrease by 3.6 (494.49:134.4) times and 5% (142.83:137.04), and totally for the institution by 1.9 (83.1:43.7) times. In table 2 is shown Tetracyclines and Aminoglycosides antimicrobial use in EMI and many different international surveillance programs, such as: SAAUSP (South Australian Antimicrobial Utilization Surveillance Program), NAUSP (National Antibiotic Utilization Surveillance Program), NETHMAP (Netherlands Monitoring Antimicrobial Resistance Program), SWEDRES (Swedish Antimicrobial Utilization and Resistance in Human Medicine), DANMAP (Danish Integrated Antimicrobial Resistance Monitoring and Research Program).

From table 2 as one can see in the evaluated period the total consumption of Tetracycline's in large acute international public hospitals increased by 2.59 (70:27) times and respectively in EMI by 2.5 times, at the same time in 2014 these data in EMI were less than recorded in international large acute hospitals in medium by 3–4 times. Aminoglycosides in EMI registered an instable consumption, nevertheless, in the end of the evaluated period comparatively with international acute hospitals recorded in medium the similar data.

In fig. 3 the total DDD/1000 Tetracyclines consumption in lei during 2010-2014 is shown.

As we can see from figure 3 the average consumption annual rate per DDD/1000 in value indexes

- for Tetracyclines in all departments recorded an increment, even so these data are low in the beginning and

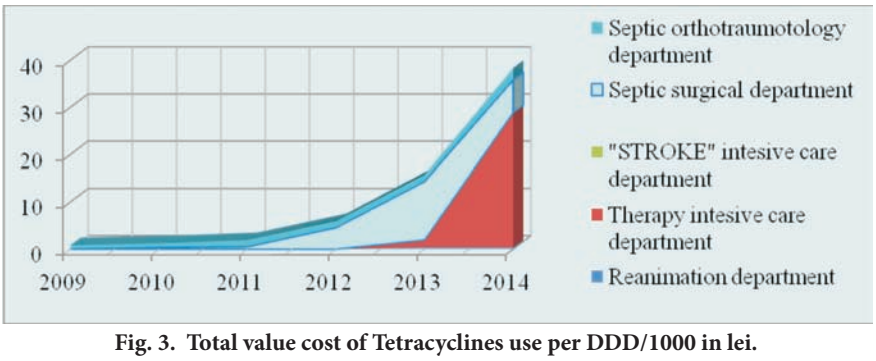

Fig. 3. Total value cost of Tetracyclines use per DDD/1000 in lei.

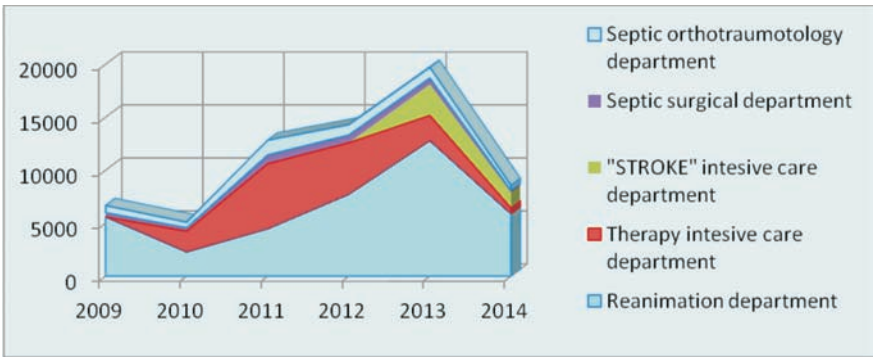

Fig. 4. Value cost of Aminoglycosides antibacterial for systemic use per DDD/1000 in lei.

the end of the evaluated period for therapeutic intensive care department registered

- 1.59 and 28.51 lei, septic surgical department – 4.54 and 6.92 lei, septic orthotramotology department – 0.81 and 2.58 lei as to reanimation department – 0.37 and 0.36 lei.

The value cost of Aminoglycosides antibacterials use per DDD/1000 in lei is presented in figure 4.

As can be observed from figure 4 during the evaluated period the main value cost of DDD/1000 recorded reanimation department from 5587.22 to 5879.70 lei, with the highest results in 2013 of 12780 lei and consequently the second position holds «stroke" intensive care department from 2950.30 lei to 1468.60 lei and respectively therapy intensive care department from 1980.60 to 554.87 lei, with the highest results in 2011 of 6160.50 lei is placed on the third position. The last position, is divided between septic orthotraumotology department with the cost of DDD/1000 from 670.63 to 459.92 lei, with the higher cost of 1395.50 lei in 2011, and respectively septic surgical department from 402.58 to 198.54 lei and the higher cost of 824.77 lei in 2011.

In table 3 is presented the medium cost DDD/1000 of Tetracyclines and Aminoglycosides for ICU and SSOD in lei. To determine the medium cost of DDD/1000 was counted total cost of DDD/1000 separately for ICU and SSOD and divided by the number of these departments (3 and respectively 2) in the evaluated period.

Table 3

Cost of medium DDD/1000Tetracyclines and Aminoglycosides in lei

Tetracyclines						
	2009	**2010**	**2011**	**2012**	**2013**	**2014**
ICU					0.98	14.44
SSOD	0.4	0.61	11.45	2.96	6.89	4.75
EMI	1.89	6.13	3.76	1.98	3.73	5.57
Aminoglycosides						
ICU	2793.6	2126.8	5308.7	6297.5	6036.3	2634.39
SSOD	536.61	419.62	1110.2	845.63	801.64	329.23
EMI	461.19	286.64	664.02	716.28	812.72	460.83

As we can see from table 3 in the evaluated period the medium cost of DDD/1000 for Tetracyclines in 2014 recorded for SSOD 4.75 lei, or less by 3 times than cost of 14.44 lei in ICU and respectively 329.23 lei cost for Aminoglycosides in SSOD in 2014 or less by 8 times than 2634.39 lei recorded ICU.

Conclusions

1. Consumption of Tetracyclines in DDD/1000 in the evaluated period increased in all evaluated departments of EMI as follows: therapeutical intensive care department from 5.9 to 106.6, septic surgical department from 1.41 to 25.6, septic orthopedic-traumotology department from 3.6 to 9.7 and reanimation departmet from 1.35 to 1.36 DDD/1000, that reprezents respectively a share from the total departments consumption of 142.6 DDD/1000 in 2014 of 74.3%, 17.9%, 6.8% and 0.9%. Despite entire institution growth from 8.2 to 20.8 DDD/1000, the difference of consumption is more than 3 - 4 times comparatively with 70 – 80 DDD/1000 recorded in international hospitals.
2. The decrease of the value cost of DDD/1000 in lei for Tetracyclines in the end of evaluated period was recorded for therapeutic intensive care department 28.51 lei, septic surgical department – 6.92 lei, septic orthotramotology department – 2.58 lei as to reanimation – 0.36 lei, when for the entire institution – 5.57 lei.
3. Consumption of Aminoglycosides in the evaluated period was recorded in all departments as follows: reanimation department from 494.49 to 219.13 DDD/1000, septic orthotraumotology department from 245.2 to 243.52 DDD/1000, "stroke" intensive care department from 206.03 to 124.73 DDD/1000, therapy intensive care department from 35.25 to 59.33 DDD/1000 and from 40.45 to 30.56 DDD/1000 septic Surgical department, that represents respectively a share from the total departments consumption of 677.27 DDD/1000 in 2014 of 32.36%, 35.96%, 18.42%, 8.76% and 4.51% respectively. An unstable consumption recorded in EMI from 83.1 at the beginning to 43.7 DDD/1000 in the end of the evaluated period, which in the medium is similar comparatively to international acute hospitals records.
4. The decreased records of value cost of DDD/1000 in lei for Aminoglycosides in 2014 were for reanimation department 5879.70 lei, „stroke" intensive care department – 1468.60 lei, therapy intensive care department – 554.87 lei, septic orthotraumotology department – 459.92 lei, septic surgical department – 198.54 lei.
5. The average DDD/1000 consumption of Tetracyclines in intensive care departments and septic departments during

the evaluated period increased respectively by 14.9 and 4.9 times, and totally for institution by 2.4 times. For Aminoglycoside the data conversely recorded a decrease by 3.6 times and 5%, and for the total institution by 1.9 times.

6. In 2014 the medium cost of DDD/1000 for Tetracyclines in SSOD recorded 4.75 lei, or less by 3 times than cost of 14.44 lei in ICU and respectively of 329.23 lei for Aminoglycosides in SSOD or less by 8 times than 2634.39 lei recorded in ICU.
7. According to some scientific researches antibiotical therapies treatment of severe acute respiratory diseases (SARS), included tetracyclines (91.0%), aminoglycosides (83.3%), quinolones (79.2%); 18.8% of the patients received a combination of tetracyclines and aminoglycosides, while 11.5% received a combination of tetracyclines and quinolones, and 63.5% received a combination of tetracyclines, aminoglycosides and quinolones.

References

1. Swartz M. N. Hospital-acquired infections: diseases with increasingly limited therapies. Proceeding of the National Acadamy of Sciences of the United States of America PNAS. Current Issue. 1994;91(7):2420-2427.
2. Lobritz Michael A, Belenky Peter, et al. Antibiotic efficacy is linked to bacterial cellular respiration. Proceeding of the National Academy of Sciences of the United States of America PNAS. 2015;112(27):8173-80.
3. Wu W, Wang J, et al. A hospital outbreak of severe acute respiratory syndrome in Guangzhou, China. *Chinese Medical Journal*. 2003;116(6):811-818.
4. Finberg RW, et al. The importance of bactericidal drugs: Future directions in infectious disease. *Clin Infect Dis.* 2004;39(9):1314-1320.
5. Manocha S, Walley KR, Russell JA. Severe acute respiratory distress syndrome (SARS): a critical care perspective. *Critical Care Medicine*. 2003;31(11):2684-2692.
6. Franco-Paredes C, Kuri-Morales P, et al. Severe acute respiratory syndrome: a global overview of the epidemic. *Salud publica de Mexico*. 2003;45(3):211-20.
7. Salehifar E, et al. How aminoglycosides are used in critically ill patients in a teaching hospital in North of Iran. *Caspian J Intern Med.* 2015;6(4):238-242.
8. Meyer E, Schwab F, et al. Surveillance of Antimicrobial Use and Antimicrobial Resistance in German Intensive Care Units (SARI): A Summary of the Data from 2001 through 2004. *Infection.* 2006;34(6):303-309.
9. Bernaz EP. Evaluation of the antimicrobials used in defined daily doses in hospitals of the Republic of Moldova. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale [Bulletin of the Moldovan Academy of Sciences. Medical Sciences]*. 2014;44(3):189-200.
10. Bernaz EP. A six year evaluation of antibiotics consumption in DDD in septic orthopedic-traumatology department. *Curierul medical*. 2015;6:10-16.
11. The world medicines situation - 2011. Centre for Drug Statistics in Oslo, Norway. www.who.int/.../WMS_ch14_wRational.pdf: http://www.whocc.no.
12. Surveillance of antimicrobial consumption in Europe 2010. http://ecdc.europa.eu/en/publications/ Publications/antimicrobial-antibiotic-consumption-ESAC-report-2010-data. pdf. 2010;3-59.
13. Esposito S, Leone S. Antimicrobial treatment for intensive care unit (ICU) infections including the role of the infectious diseases specialist. *Int J Antimicrob Agents*. 2007;29:494-500.
14. Paterson DL, Rogers BA. How Soon Is Now? The urgent need for randomized, controlled trials evaluating treatment of multidrug-resistant bacterial infection. *Clin Infect Dis*. 2010;51:1245-7.
15. Bernaz E, Ciobanu Gh, Mişin I, Borovic E, Rusu V. Raţionalizarea consumului de remedii medicamentoase antimicrobiene sistemice în instituţiile medicale spitaliceşti [Rationalisation of consumption with systemic antimicrobial in hospitals]. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale [Bulletin of the Moldovan Academy of Sciences. Medical Sciences]*. 2012;35(3):212-221.
16. Bernaz EP. Evaluation of consumption in DDD of antimicrobial drugs for systemic use in hospitals. *Curierul medical.* 2015;5:10-17.
17. Bernaz EP. Antibiotics consumption evaluation in reanimation department. *Curierul medical.* 2016;1:22-26.
18. Bernaz EP. Evaluation of antibiotics consumption in therapeutic intensive care department. *Curierul medical*. 2016; 2:5-10.
19. Bernaz EP. The Evaluation of Antibiotics DDD Consumption in septic surgical department in the Republic of Moldova. *Journal of Pharmaceutical Sciences and Research (JPSR).* 2016;8(3):141-148.
20. Bernaz EP. Evaluation of antibiotics consumption in therapeutic "stroke" intensive care department. *Archives of the Balkan Medical Union.* 2016;51(1)(supl. 1):216-221.
21. Antimicrobial use in Australian hospitals: 2014 annual report of the National Antimicrobial Utilisation Surveillance Program. Commonwealth of Australia 2015;12-16.
22. Antimicrobial use in Australian hospitals: 2013 annual report of the National Antimicrobial Utilization Surveillance Program. Sahealth. sa.gov.au; 2013;36-37.

Curierul medical, February 2016, Vol. 59, No 1

Segregation of tuberculosis patients by social, demographic and economic features on the model of Chisinau city and the role of community support

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Abstract

Background: One of the most important actions in tuberculosis control represents the improvement of social and economic conditions, as well as nutrition, hygiene, housing and working state of the population. Commission on Social determinants of Health suggests to all tuberculosis burden countries, especially targeting research sector, governments and academia institutions to implement health-oriented interventions, as being the most powerful potential efforts in tuberculosis control. The aim of the study was the segregation of tuberculosis patients according to the social, demographic and economic characteristics revealed at the regional level in Chisinau city for identifying target groups for improving the earlier case-detection.
Material and methods: 185 pulmonary tuberculosis patients diagnosed in the period 01.01.2015-31.12.2015 in Chisinau city were investigated.
Results: While segregating new cases of pulmonary tuberculosis there were identified several high risk groups for active disease: social risk groups with specific features – uninsured, unemployed, living in poor conditions; groups with suppressing medical conditions; epidemiologically endangered groups – homeless, migrants and contacts with tuberculosis patients; persons with harmful habits (alcohol abusers, injection drug users). Due to enumerated risk factors it was identified that one half of patients were late detected as being microscopic positive for acid-fast-bacilli that presented the highest epidemiological danger.
Conclusions: Social and community support must take into account the segregation of TB patients method for improving awareness, education and information of high risk groups and specific groups for tuberculosis.
Key words: tuberculosis, social determinants, risk groups.

Introduction

Tuberculosis represents a classic example of an infectious disease linked with social determinants of the health [13, 16, 18]. As a public health term – social determinants represent a set of factors, that contribute to the social definition of health, disease or illness to which are referred collective determinants. It was established that the decline of tuberculosis epidemiological indices is attributed to the improving of social and economic conditions, rather than to the clinical advances [13]. Additionally, it was identified that the improvement of the nutrition, hygiene, housing and working conditions in highly developed countries contributed to health care progress, more evident in strengthened tuberculosis control. In this context, WHO Commission on Social determinants of Health suggested to all TB burden countries, especially targeting research sector, governments and academia institutions to implement health-oriented interventions, as being the most powerful potential efforts in tuberculosis control. International review identified that in majority of high-income states, the combination of the industrial development progress with the use of anti-TB drugs, associated with the social and infrastructure improvement contributed to the dramatic drop of epidemiological tuberculosis indices [16]. On the other hand, despite improvement of diagnostic and treatment options in low-income and middle-income countries, the major unsolved social determinants make their population continuously vulnerable to the infectious diseases, especially to poverty-related diseases [17]. According to the WHO estimations, diseases associated with the poverty account for 45% of the morbidity in the poor countries, and tuberculosis, malaria and HIV/AIDS together are responsible for 18% of the total morbidity burden [18]. So, although chronic noncommunicable diseases are rapidly emerging in the economically disfavored regions, the infectious diseases still represent a significant proportion of public health burden. It was identified that poverty, illiteracy, gender inequality and rapid urbanization are largely unaddressed even in actual epidemiological context and represent general cause of infectious spreading [18].

Accumulated evidence suggested that not only an effective treatment of tuberculosis is a major issue for TB control actions, but also resolution of social and economic problems, such as improving of housing, transportation, nutrition represent important concerns [12]. For reducing the impact of tuberculosis as a social related disease, it was identified the importance of the approach to the underlying social and economic factors, such as social assistance to vulnerable groups, household conditions, improvement of the general public life style, reducing the harmful habits, recognized as determinants for re-activation of latent tuberculosis infection and active tuberculosis progression [11]. Studies realized in low-income states showed that tuberculosis is concentrated in areas with high density of population, poor environmental and sanitation conditions: poverty, food insecurity, unhygienic living conditions, and lack of pure drinking water [13]. The most affected groups, being assessed as hard-to-reach groups are homeless, migrants, individuals living with HIV, children from poor families, drug injected users.

According to the WHO estimations the Republic of Moldova (RM) remains a high risk zone showing an inadequate concern regarding social determinants that represent main barrier to achieve the health related Millennium Development Goals [15]. In the actual globalization process, the Republic of Moldova is the least economically developed country from the Eastern European Region. According to the classification of World Bank Agency, RM is defined as a lower middle income country, with a Gross Domestic Product of $7.962 billion in 2014 and a population of 3 million 556 thousand individuals. With a total Gross Domestic Product (GDP) of $8.178 billion in 2014, and GDP per capita $4.177, RM shows a big rate of inflation (5.1-10% annually), that continuously worsens the economical state of the population. Despite a continuous diminishing of the poverty headcount ratio: 2009 – 26.3%, 2010 – 21.9%, 2011 – 17.5%, 2012 – 16.6%, 2013 – 12,7% there was not found a similar tendency in the epidemic indices of poverty-related diseases, and this fact endangers the security of public health system. Despite increasing by 5 times the national public budget funding for healthcare sector (1.192 – 2005, 3.846 – 2010, 5.890 million MDL – 2015), the total rate/value reported, the GDP remains constantly low (3.9% - 2005, 6.1% - 2010, 5.7% - 2015) that is reflected by the low coverage of high risk groups by screening procedures [22]. Accumulated evidence suggested that not only the deficiencies in performing an effective antituberculosis treatment is a major issue for the public health system of RM, but also the lack of intervention to resolve social and economic problems of Moldovan patients contributes to the poor recovery of the epidemic state [3].

In this paper we critically describe and segregate tuberculosis patients according to the social, demographic and economic characteristics revealed at the regional level in Chisinau city for identifying target groups for improving the precocious case-detection.

Objectives: 1. Comparative assessment of the epidemiological indices of tuberculosis in Chisinau city and at the republican level; 2. Evaluation of social, demographic and economic characteristics of patients from Chisinau city; 3. Identifying risk stratification of patients diagnosed in Chisinau city through the positive GeneXpert MTB/RIF assay and the role of their support by the community organizations.

Material and methods

It was performed a retrospective randomized, selective, descriptive study targeting social, demographic and economic peculiarities of 185 patients with pulmonary tuberculosis diagnosed in Chisinau city in the period of 01.01.2015-31.12.2015 through the positive GeneXpert MTB/RIF assay. Included criteria were: age > 18 years old, patients with tuberculosis established as a new case, through positive GeneXpert MTB/RIF assay, signed informed consent.

In this context we underline that GeneXpert Xpert MTB/RIF is strongly recommended by the World Health Organization in 2010 for diagnosis of tuberculosis in adults and children presumed to have pulmonary MDR-TB, HIV associated tuberculosis or TB meningitis. In RM it was nationally implemented in 2014 as a compulsory investigation in addition to smear microscopy to all tuberculosis suspects. GeneXpert MTB/RIF is designed as a semi-quantitative, nested real-time polymerase chain reaction (PCR) for detection of *Mycobacterium tuberculosis complex* DNA and rifampicin resistance mutations of the *rpoB* gene [20].

The investigational schedule of the presented research included information data about: sex (male-female), age (distribution in age groups according to the WHO recommendations), demographic characteristics (urban/rural), patient's origin (born in the Republic of Moldova or in other states), educational status (the last level of education), economical status (employed, unemployed, retired, disabled, student), health-insurance state (presence/lack of health-insurance), high risk characteristics (social vulnerability, close tuberculosis contact, migrational and detention history, comorbidities), characteristics of the epidemiological cluster (patient's microscopic status, close contacts: children, pregnant and childbed women, HIV positive family member), health care seeking behavior, way of the patient's detection, medical staff involved in the patient's management.

All selected patients were diagnosed and managed according to the National Clinical Protocol – 123 "Tuberculosis in adults". The informational system for monitoring and evaluation of tuberculosis cases (SIME TB) was used for identifying patients diagnosed at the regional (Chisinau city) level between 01.01.2015-31.12.2015. Data were extracted from the statistic templates filled in the frame of tuberculosis case registration – F089/1-e „Aviz despre bolnavul cu diagnosticul stabilit caz nou/recidivă de tuberculoză activă și de reîncepere a tratamentului și rezultatele acestuia" [Declaration about patient's established diagnosis of a new case/relapse of active tuberculosis and restart of the treatment and its outcomes] and F090 „Fișa de declarare și evidență a cazurilor de tuberculoză" [Declaration and evidence template of tuberculosis cases].

Methodological approach used: social, epidemiological, collection methods, statistical analysis, graphic representation and analytical assessment. Statistic assessment was carried out by checking the quantitative and qualitative features of selected patients. Statistical survey was performed using Microsoft Excel XP soft. Accumulated material was tabled in simple and complex groups.

Results and discussion

For the comparative epidemiological assessment of major tuberculosis indices in Chisinau city and general republican indices was used published data by National Statistical Bureau and National Centre for Management in Health. According to the National Statistical Bureau the stable population of the RM is continuously decreasing. Between 2013 and 2015 the total number of the Moldovan population decreased by 4278 citizens. In 2013 were registered 3.559.497, in 2014 – 3.557.634 and in 2015 3.555.159 citizens. In 65 Moldovan towns, considered as the major infectious cluster were residing 1.492.165 Moldovan citizens (40.67% of the total population)

in 2013, 1.502.996 (42.24% of the total population) in 2014 and 1.507.265 citizens (42% of the total population) in 2015. So, urban area increased its population between 2013 and 2015 by 10.831 people, on the other hand the rural population decreased by 19.428 people in the same period. The difference of 8.597 people between urban and rural population is supposed to emigrate from the RM. As a comparison the population of Chisinau city increased by 9600 people between 2013 and 2015, with 800.600 citizens declared in 2013 (53.65%) 804.500 in 2014 (53.52%) and 809.600 citizens in 2015 (53.71% from the Moldovan urban population) [21].

The study was designed to incorporate health-related issues into demographic measures. In this context we describe Moldovan health care system as being based on the universal access to major services through mandatory health insurance mechanisms. The financing of most health services is performed by the National Health Insurance Company. Uninsured part of Moldovan population ranges 10 to 25% from total population, depending on the demographic region (more frequent in rural area), on the ethnicity (minorities are more frequently uninsured), and other social disadvantaged conditions (unemployment, homelessness). In 2014 were identified 971.331 uninsured persons that represent 27.3% of Moldovan population. Despite free of charge tuberculosis care, the lack of insurance in an insurance-based health care systems determines a low medical coverage of high risk populations, lack of social assistance, deficiency in active screening, and poor tuberculosis control. In RM there are several categories of the population, that receive free insurance coverage: children till 18 years old, students, pregnant women, disabled persons with high and medium degree of disablement, retired persons, unemployed registered at the local territorial agencies, persons who take care of a severely ill person, mothers with 4 and more children, socially disadvantaged families assisted by the state. It is important to underline that all health services, including detection, diagnosis, tuberculosis treatment and hospitalization during the intensive phase are free of charge regardless the health insurance state of the patient, although pathogenesis and immunomodulating treatment, as well as respiratory rehabilitation require health insurance. Regarding specialized in pneumophtysiology medical staff involved in the health care of tuberculosis patients, it can be argued the fact that in RM there were only 219 in 2013 and 216 in 2014 pneumophtysiologists working in specialized services, that corresponds to 0,6 doctors at 100.000 population. On the other hand the primary health care sector, considered the most important chain involved in the detection of symptomatic patients is continuously growing with a total number of 1792 family doctors officially registered in 2014, corresponding to 6,7 family doctors at 100.000 population [21]. With such medical assistance the global incidence (number of new cases and relapses reported at 100.000 populations) and incidence of new cases are the most important epidemiological indicators describing the spread of tuberculosis disease in the general population. According to the published data by the National Centre for Management in Health during the period 2013-2015 it was registered an important decline of the global incidence and the incidence of new cases in urban districts of Chisinau and the increase of both indicators in Chisinau and its suburbs. In this context it is important to enumerate the surrounding villages included in the rural area of Chisinau city in the alphabetic order: Bacioi, Bic, Bubuieci, Budesti, Cheltuitori, Ciorescu, Codru, Colonita, Condtrita, Cricova, Cruzesti, Dobrogea, Dumbrava, Durlesti, Fauresti, Frumusica, Ghidighici, Goian, Gratiesti, Bulboaca, Humulesti, Revaca, Stauceni, Straseni, Singera, Tohatin, Truseni, Vadul lui Voda, Vatra, Vaduleni). So, table 1 shows that the global incidence in Chisinau city decreased by 22.4% between 2013 and 2015 and by -21.3% in RM. A similar vector was established regarding the incidences of new cases, defined as patients that never received tuberculosis treatment or the patient treated less than 1 month. So, between 2013-2015 the incidence of new cases diminished by 18.2% in Chisinau city and by 18.8% in RM [4]. Multiple causes are involved in this rapid decline of registered values: low rate of high risk groups investigated in the frame of active way of screening (annual chest radiological examination), high rate of migration population not accessible for screening procedures (according to mass-media data 1 million Moldovan citizen are migrants), low health care seeking behavior of the population, high rate of citizens with lack of insurance policy.

Segregating data according to the demographic place of patient's residence, it was established a heterogeneous distribution of epidemiological indices both in urban and rural areas.

Table 1

Epidemiological indices of tuberculosis in Chisinau city and the Republic of Moldova

Index	2013		2014		2015	
	Abs.	100.000 population	Abs.	100.000 population	Abs.	100.000 population
Global incidence in Chisinau city	755	94,1	659	81,7	579	71,7
Global incidence in RM	3656	102,7	3305	92,9	2870	80,7
Incidence of new cases in Chisinau city	479	72,1	508	62,9	435	53,9
Incidence of new cases in RM	2968	83,4	2686	75,5	2299	64,6
Global incidence in Chisinau city	659	90,3	561	76,4	479	65,3
Global incidence in urban districts of RM	1371	91,6	1180	78,4	1012	67,2
Global incidence in Chisinau and its suburbd	96	132,7	98	134,0	100	136,7
Global incidence in rural districts of RM	2286	90,5	2125	103,6	1858	90,6

So, in Chisinau the global incidence (new cases and relapses) decreased by 25% from 2013 to 2015 comparing with -24.4% decrease in all urban areas. On the other hand a slow increase of global incidence in rural area (suburb) of Chisinau city with +4 and a stable state in the republican rural areas demonstrated the real epidemiological state. It is important to note that incidence in suburbs of Chisinau was higher comparing with urban districts of Chisinau (+ 42.4% in 2103, +57.6% in 2014 and +71.4% in 2015). Exposed data demonstrated that ambiguous positive trend in Chisinau and RM doesn't reflect adequately the real situation and that epidemiological state of tuberculosis remains tensioned.

Evaluation of social, demographic and economic characteristics of patients from Chisinau city allowed their stratification according to the exposed features. Distributing patients according to their affiliation to local health care institutions it was identified a similar distribution of selected patients (table 2). It is important to underline that patients from Botanica sector were grouped as patients from urban Chisinau and surrounding rural areas: Bacioi, Dobrogea, Revaca and Singera villages were referred to the Medical Territorial Association (MTA) Botanica. Patients from the Centre and rural areas of Cricova, Ciorescu, Vadul-lui-Voda villages were attributed to the MTA Centru. MTA Buiucani offers health services to patients of Buiucani sector and villages: Condrita, Durlesti, Ghidighici, Vatra, Truseni. MTA Ciocana provides health care for patients from Ciocana sector and Cruzesti, Bubuieci, Colonita villages. A major public health issue was caused by a high rate of homeless persons. So, due to the lack of residence visa they can't receive specialized health care, in consequence the Municipal Hospital of Tuberculosis registered them as their own residents. Urban homeless persons constituted 22 (11.9 ± 2.38%) cases from Chisinau city, but the total number of homeless from the suburbs of Chisinau city accountered for 29 (15.7 ± 2.67%) cases. The major part of patients were Moldovan citizens, only a couple (2 (1.1±0.76%) cases) were immigrants from Middle Asia.

Table 2

Residential segregation of patients from Chisinau city according to the referable health care unit

Residential sector	n=185	
	n	M± m(%)
Botanica AMT	27	14,6±2,59
Centru AMT	25	13,5±2,51
Ciocana AMT	32	17,3±2,78
Riscani AMT	30	16,2±2,71
Municipal hospital of TB	22	11,9±2,38
Others	11	5,9±1,74
Homeless patients	29	15,7±2,67

Note: MTA – medical territorial association; others – university clinic, Galaxia and private On-line clinics, homeless patients – tuberculosis cases not referred to any municipal health care unit.

Distributing patients according to the sex it was estbalished the predominance of male sex in comparision with female sex 138 (74.6±3.20%) vs 47 (25.4±3.20%), with a male/female ratio=2,93/1. Repartition of the patients in age groups according to the WHO recommendations, identified that the largest was 35-44 years age group: 52 (28,1±3,30%) patients, followed by the 45-54 years age group – 42 (22,7±3,08%) and 25-34 years age – group 36 (1.5±2.91%) patients. Redistributing patients in two age groups (15-44 years old and >45 years) it was established the predominance of young patients – 112 (60.5±3.59%) comparing with 73(39.5±3.59%) aged more than 45years old. So, segregating patients according to the biological characteristics it was cleared up that men and young individuals must be targeted by the screening methods and risk reduction measures, as well as must be supported by civil organizations in earlier detection. Data are shown in the table 2.

Table 3

Segregating patients in sex and age groups

Biological segregation	Sex	n=185		p
		n	M± m(%)	
Sex stratification	Men	138	74,6±3,20	<0,0001
	Women	47	25,4±3,20	
Young age (reproductive groups)	15-24 years	24	12,9±2,47	<0,0001
	25 – 34 years	36	19,5±2,91	
	35-44 years	52	28,1±3,31	
>45 years old	45-54 years	42	22,7±3,08	
	55 - 64 years	24	12,9±2,47	
	>65 years	7	3,8±1,40	

Segregating patients according to the economic status, was established that employed persons, this way contributing to the health budget by paying taxes, health insurance policy and social benefits were only 25 (13.5±2.51%) in number. Each fourth patient (36 (19.5±2.91%) cases) received specialized health care as being retired, disease disabled or student. Two thirds of patients (124 (67.0±3.46%) were unemployed and without personal financial support for life. The table 4 revealed exposed data.

Table 4

Economic segregation of patients with pulmonary tuberculosis

Type of segregation of economical state		n=185		P
		n	M± m (%)	
Economically stable	Employed	25	13,5±2,51	<0.001
Economically vulnerable	Unemployed	124	67,0±3,5	
	Retired	15	8,1±2,0	
	Students	7	3,8±1,40	
	Disease disability	14	7,6±1,94	
Patients with lack of health insurance		139	75,14±3,18	

Health insurance represents the major condition for accessing health care in RM. Uninsured were the majority of cases (139 (75.14±3.18%) patients). While segregating them according to the biological features was identified that 115 (82.7±3.21%) of them were men, that represent the most im-

portant economic force of the country, and 113 (80.6±3.35%) were young aged patients (<44 years old) in their reproductive period (table 5).

Table 5

Social segregation of patients with lack of health insurance

Biological segregation	Sex	n=139		p
		n	M± m(%)	
Sex stratification	Men	115	82,7±3,21	<0,001
	Women	24	17,3±3,21	
Reproductive stratification	15-24 years	24	17,3±3,21	<0,001
	25 – 34 years	43	30,9±3,92	
	35-44 years	46	33,1±3,99	
>45 years old	45-54 years	38	27,3±3,78	
	55 - 64 years	12	8,6±2,38	

Considering exposed results, *mass media* must inform the general population emphasizing that specialised health care, full accessibility to all related diagnostic tools and specific treatment for tuberculosis is free of charge for all Moldovan patients regaldless their health insurance status.

Assessing last educational level it was established that two thirds of patients 135 [72.9±3.26%] completed the secondary education (secondary school, lyceum or professional school) level of education, and the fifth part of them (38 (20.5±2.97%)) were without any educational level or graduated only primary and incomplete general school (table 6). Considering that tuberculosis is linked with low level of education, were studied the biological characteristics of these patients. Assessing them it was identified that two thirds, 27 (71.1±7.37%) were male and two thirds, 25 (65,8±7,96%) were young (< 45 years old). Exposed data are demonstrated in the table 6.

Table 6

Educational segregation of patients according to the last graduated diploma

Educational segregation	Educationalstatus	N=185		P
		n	M± m (%)	
Low level	Illiteracy	8	4,3±1,5	<0.001
	Primary & general incomplete school	30	16,2±2,71	
Secondary education	General (secondary) school	104	56,2±3,65	
	Professional school (college)	31	16,8±2,75	
Superior level	Superior studies	12	6,5±1,81	

Comparing the number of patients with an optimal (medium) level of education (general school and professional school) with those with low level of graduation it was identified the predominance of patients from the first one (135 (72.9±3.26%) vs 38 (20.5±2.97%)). So, awareness and information about disease signs as well as education for risk reduction measures of persons with low degree of education are the most important tools that must be performed by the civil society organizations and could improve the tuberculosis state at the community level.

Assessing civil status it was identified a similar rate of married and unmarried patients. In the group defined as "others" were included patients- widows and patients in concubine. Regrouping patients in two groups, it was identified that single patients predominated comparing with married patients: 98 (52.9±3.67%) vs 72 (38.9±3.58%). So, the community assistance of single patients must be performed to diminish the impact of lack of family support, in this way improving the patient's social inclusion and adherence to health-related recommendations. Data are shown in the table 7.

Table 7

Segregation of patients according to the civil status

Civil segregation	Civil status	n=185		P
		N	M± m(%)	
Familist	Married	72	38,9±3,59	<0.05
Single civil	Unmarried	66	35,7±3,52	
	Divorced	32	17,3±2,78	
	Others	15	8,1±2,01	

Hierarchy of risk groups according to the widest rate of the selected patients identified that the biggest impact on the risk of developing active pulmonary tuberculosis determine: unemployment and lack of health insurance (two thirds of patients), living in poor conditions (one half), associated diseases (one third), extreme poverty (homelessness), migration and alcohol abuse (the fifth part).The stratification of pulmonary tuberculosis patients established the primary target groups in frame of which must be performed screening awareness, education for risk reduction, and improvement of health behavior as well as the groups for whom methods for active screening are most efficient represent: socially and economically vulnerable persons, comorbid patients, migrants and alcohol abusers. In this context it is important to note a very low rate of family tuberculosis clusters (15 (8.1±2.01%) cases) affiliated to each investigated patient. It is due to a low quality epidemiological cross-examination of the patient, rather than to the lack of close (family) contacts in the patient's environment.

Table 8

Distribution of patients according to the risk groups

Risk groups	Hierarchy of risk groups	n=185		Place
		n	M± m(%)	
Social groups	Lack of health insurance	139	75,1±3,17	
	Unemployment	124	67,3±3,46	
	Poor living conditions	106	57,3±3,64	
Co-morbid groups	Associated diseases	50	27,1±3,26	
Epidemiological groups	Extreme poverty	29	15,7±2,67	
	Migration	24	12,9±2,47	
	Family cluster of TB	15	8,1±2,00	
High risk (specific) groups	Chronic alcoholism	13	7,0±1,88	
	History of detention	9	4,9±1,58	
	Psychiatric diseases	4	2,2±1,07	
	Injection drug use	3	1,6±0,93	

With a lower impact were identified groups of patients with history of detention, chronic alcoholism, close contacts with infectious sources, patients with illicit drug use and psychiatric diseases.

Studying case-management, diagnosis delay, medical staff involved in the patient's detection and clinical-radiological diagnosis it was established that two thirds of patients exposed as a barrier for health care seeking lack of health insurance (139 (75,1±3,18%)), that determined late detection (>60 days after the onset of the symptomatology) of one half of the sample (108 (58.4±3.62%) patients). According to the actual recommendations the major way of new case detection is based on the microscopic examination of the symptomatic patients. So, one half of them were detected by addressing family doctor due to specific symptomatology (passive way) – (103 (55.7±3.65%) patients) and the fifth part (28 (15.1±2.64%)) by active way, through chest X -ray examination of high risk groups. One third of patients (34 (18.4±2.85%)) were diagnosed by direct addressing the pneumophtysiologist with specific signs recognized by them being relevant for pulmonary tuberculosis. In "others" conditions were included patients detected in the frame of investigations performed for the work engagement (10 (5.4±1.66%)).

Table 9

Case-management segregation and disease-related characteristics

	Management characteristics	n=185	
		n	M± m(%)
Case management	Lack of health insurance	139	75,1±3,18
	Late (>60 days)	108	58,4±3,62
	Detected by general practitioner passive way	103	55,7±3,65
	Detected by general practitioner active way	28	15,1±2,64
	Detected by pneumophtysiologist	34	18,4±2,85
	Others	10	5,4±1,66
Para-clinical features	Microscopic positive	101	54,6±3,66
	Extended radiological forms in 1 lung	80	43,2±3,64
	Extended radiological forms in 2 lungs	26	14,1±2,56
	Lung destructions	106	57,3±3,64

Assessing laboratory features of pulmonary tuberculosis it was identified that one half of patients were microscopic positive for acid-fast-bacilli. So, the first criteria that defined the highest epidemiological danger of tuberculosis clusters were identified in one half of patients. Evaluating radio-morphological features of pulmonary tuberculosis, it was identified lung infiltrates complicated with destructions in one half of patients (106 (57.3±3.64%)), unilateral extensive forms of tuberculosis involving 3 and more lung segments in 80 (43.2±3.64%) patients and involvement of both lungs in 26 (14.1±2.56%), the fact reflected due to the late detection of new tuberculosis cases.

Conclusions

The Republic of Moldova shows a continuous decreasing of its population, especially of economical and reproductive active groups. The urbanization is contributing to the segregation of health care services, which are more accessible in urban area.

Moldovan health care system is based on the health insurance mechanisms. Due to the fact that third of the population is uninsured, low health national budget doesn't permit an extensive screening in all high risk groups.

Although it was established a decreasing vector of main epidemiological indices in urban areas of the Republic of Moldova and in Chisinau city, the increase in rural regions, demonstrated that epidemiological state of tuberculosis remains tensioned.

Statistical assessment and segregation of social, demographic and economical features of patients with pulmonary tuberculosis with positive test for *Mycobacteria* DNA (GeneXpertMTB/Rif) identified several high risk groups for active tuberculosis developing: social risk groups with specific features (uninsurance, unemployment, poor living conditions); groups with medical conditions that suppress the immune response and psychiatric diseases; epidemiological groups (homelessness, migration and infectious contact); harmful habits groups (alcohol abuse, injection drug use) and other group (persons with history of detention).

All exposed risk factors reflected that one half of patients were late detected (> 60 days) and were microscopic positive for acid-fast-bacilli that determined the highest epidemiological danger.

Evaluating radio-morphological features of pulmonary tuberculosis were identified lung destructions and extensive forms of tuberculosis in one half of patients.

Community support must take into account the segregation method for improving awareness, education and information of socially risk groups, epidemiologically endangered and specific groups of tuberculosis morbidity.

References

1. Allebeck P. Delay in tuberculosis care: one link in a long chain of social inequities. *Eu J of Public Health*. 2007;5:409-412.
2. Aveyard H. Literature review in health and social care: a practical guide. McGraw-Hill, 2010.
3. Bivol S, Turcanu Gh, Mosneaga A, et al. Barriers and facilitating factors in access to health services in the R. of Moldova. Chisinau, 2012;139.
4. Centrul Național de Management în Sănătate [National Centre for Health Management]. Chisinau, 2015.
5. Hargreaves J, Boccia D, Evans C, et al. The social determinants of tuberculosis from evidence to action. *Am J Public Health*. 2011;101(4):654-662.
6. Hill PC, Jackson-Sillah D, Donkor SA, et al. Risk factors for pulmonary tuberculosis: a clinic-based case control study in The Gambia. *BMC Public Health*. 2006;6:156
7. Holtgrave D, Crosby R. Social determinants of tuberculosis case rates in the United States. *Am J of Preventive Medecine*. 2004;26(2):159-162.
8. Hotărîrea Guvernului RM nr.768 din 12.10.2011 „Cu privire la aprobarea Programului național strategic în domeniul securității demografice a R. Moldova 2011-2015". *Monitorul Oficial*. 2011;182-186.
9. Hill AN, Becerra J, Castro KG. Modelling tuberculosis trends in the USA. *Epidemiology Infectious Journal*. 2012;140:1862-1872.

10. Jenkins H, Ciobanu A, Plesca V, et al. Risk factors and timing of default from treatment for non-MDR TB in Moldova. *Inter J Tuberculosis and Lung Diseases*. 2013;17(3):373-380.
11. Lonnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. *Soc Sci Med*. 2009;68(12):2240-2246.
12. Mikkonen J, Raphael D. Social Determinants of Health: the Canadian Facts. York University School of Health Policy and Management. Toronto, 2010:63.
13. Rasanathan K, Sivasankara K, Jaramilllo E, et al. The social determinants of health: key to global tuberculosis control. *Inter Journal Tuberculosis Lung Diseases*. 2011;30-6.
14. Shivani C, Sharma N, Joshi K, et al. Resurrecting social infrastructure as a determinant of urban tuberculosis control in India. Health Research Policy and Systems, 2014;12.
15. United Nations. Report on Millenium Development Goals. Republic of Moldova. Chisinau, 2013.
16. World Health Organization. Commission on Social Determinants of Health. Action on the social determinants of health. Geneva, 2005.
17. World Health Organization. Human Rights, Health and Poverty Reduction Strategies. Geneva, Switzerland, 2008.
18. World Health Organization. Equity, social determinants and public health programs. Geneva, 2010;219-241.
19. World Health Organization. The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis. Geneva, 2011.
20. World Health Organization. Tuberculosis diagnostics. Xpert MTB/RIF Test, 2013.
21. http://statbank.statistica.md
22. http://www.statista.com/statistics/513335/gross-domestic-product-gdp-per-capita-in-moldova/

Pharmaceutical forms used in the gynecology practice

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Abstract

Background: One of the main indicators of the welfare of a nation is the index of women's health status. Women's health (or health care) is the key factor, on which depends the happiness and wellbeing of each family. During her life cycle, a woman passes through several stages that affect the health of the genital organs: menstruation, fertility, pregnancy, childbirth, and menopause. When ladies are facing these situations, most of them remember that they followed some drug treatments for long period of time. This scientific study represents an analysis of the pharmaceutical preparations and their forms used in gynecology practice. According to records from the State Nomenclature of the Republic of Moldova, research methodology was developed (using the following criteria: anatomical therapeutic chemical classification, dose, division, country and industrial pharmaceutical company producer, producer's price, terms of validity), based on local authors publications. The research study was conducted and reveals the information gathered before (until) 31st of March, 2016.

Conclusion: Taking into consideration all pharmaceutical forms registered in the State Register of the Republic of Moldova, used in gynecological practice can be divided into: a) pills, b) pessaries, c) injection solutions. The Republic of Moldova is located at the top of the list of the 21-producing countries, which have registered pharmaceutical forms, used in gynecological practice. Domestic producers of medicines are covering approximately 10.98% of pharmaceutical products segment.

Key words: pharmaceutical forms, formulations, gynecology, dose, preparations, price, accessibility.

Introduction

One of the main indicators of well-being of a country is the health of women who live in it. And on this factor depends the happiness of every family. The statistic information attests the fact that women's health in the Republic of Moldova is alarming. The number of women of childbearing age suffering from various diseases is growing. At present stage, every second woman reaches reproductive age with seriously modified maternal land [3, 6, 12]. Only in the last 10 years, in the Republic of Moldova, sterile couples increased approximately twice and reached the number of 16.0% [1], one of the main causes of infertility being consequences or complications of gynecological pathologies [5, 7].

During her life cycle, a woman passes through several stages that affect her genital health: menstruation, fertility, pregnancy, childbirth, menopause, and then we should not forget that some of them followed some drug treatments for long period of time.

Some authors state that, in general, gynecological diseases can be divided into three groups: a) inflammatory diseases of the female genitals, b) diseases caused by endocrine disorders, c) hyper-plastic, degenerative and neoplastic diseases in sexual field [4, 13].

Taking into consideration this fact, the multitude of diseases and pathological gynecological conditions, assumes using in their treatment various types of formulations and active ingredients, and not only from the G-code Genitourinary Unit and sex hormones, but some other codes as well, for example – J Anti-infective systemic use, – L Antineoplastics and immunomodulators, etc.

Material and methods

As materials served the information regarding registration of medicinal preparations and their pharmaceutical forms taken from State Nomenclature of the Republic of Moldova (producers analysis results and results of production in this specific segment of medicines, prices of manufactured products and validity period, stated by the producer).

The research methodology was developed based on local author's publications [2, 12] using the following methods: analytical, descriptive statistics, comparison, price analysis, etc. The research study was conducted and reveals the information gathered until 31st of March, 2016.

Results and discussions

According to ATC List with a total of 528 preparations, G-code class Genitourinary Unit and sex hormones, includes 4 sub-codes:

-G01 Gynecological antiinfectives and antiseptics,
-G02 Other gynecological preparations, -G03 Sex hormones and modulators of the genital system,
-G04 medication of urinary system (urological) [8].

The pharmaceutical forms used in gynecological practice, according to the ATC, are shown in figure 1.

As shown in figure 1, according to ATC classification, subcode -G03 Sex hormones and modulators of the genital system accumulate the highest number of registered preparations.

Preparations used in gynecological practice, registered in Moldova, are present in the following pharmaceutical forms: vaginal spray-1-topical solution, oral solution-3, cutaneous solution-2, vaginal solution-1, implants-2, effervescent granules-1, vaginal gel-5, oral gel-1, gel-8, drinkable drops-1, oral paste-1, fragmented vegetal product-3, oral drops, solution-11, syrups-3, dragees-18, ovules -63, pessaries-19, capsules-71 (including, gastro-resistant soft capsule-1, soft capsules-9, capsules with long drug expiration-3capsules with modified release-15, vaginal capsules-14) vaginal cream-5, cream-3, trans-dermal patch-2, tablets-218 (incl. chewable tablets-3, extended release tablets-8, tablets-108, extended release tablets-2, effervescent tablets-1, lozenges-3, orodispersible tablets-6, vaginal tablets-23, homeopath tablets-3), injectable solutions-45 (including injectable solution in a cartridge-6, solution for injection in pre-filled syringe-5, solution for injection in pre-filled pen-5, oil injection-4) lyophilized + solvent / solution for injection-11, powder + solvent / solution for injection-17, suspension for injection-4, solution for infusion-14, powder / vaginal solution-2, lyophilized powder / injection-1, releasing intrauterine system-2, releasing vaginal system-1 [8,10].

According to the records from the State Nomenclature of the Republic of Moldova of March 31st, 2016, from the total share of authorized medicinal products, preparations used in gynecological practice were 5.54%. From these preparations, pharmaceutical forms can be divided into: soluti-

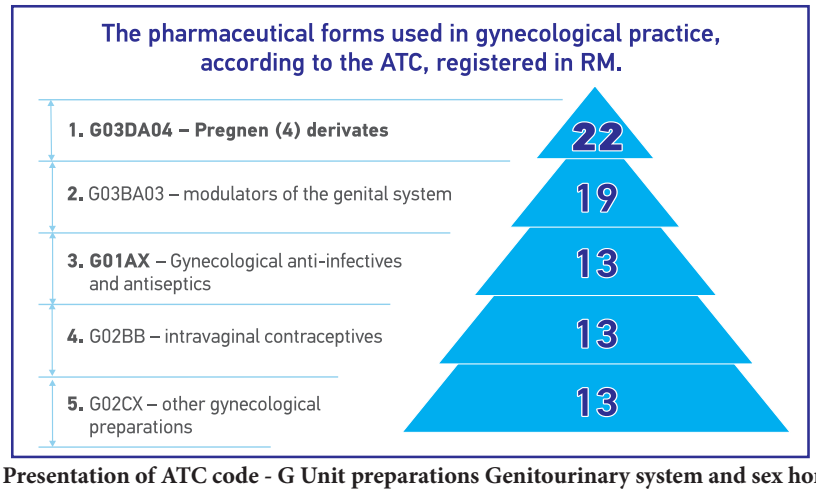

Fig. 1. Presentation of ATC code - G Unit preparations Genitourinary system and sex hormones.

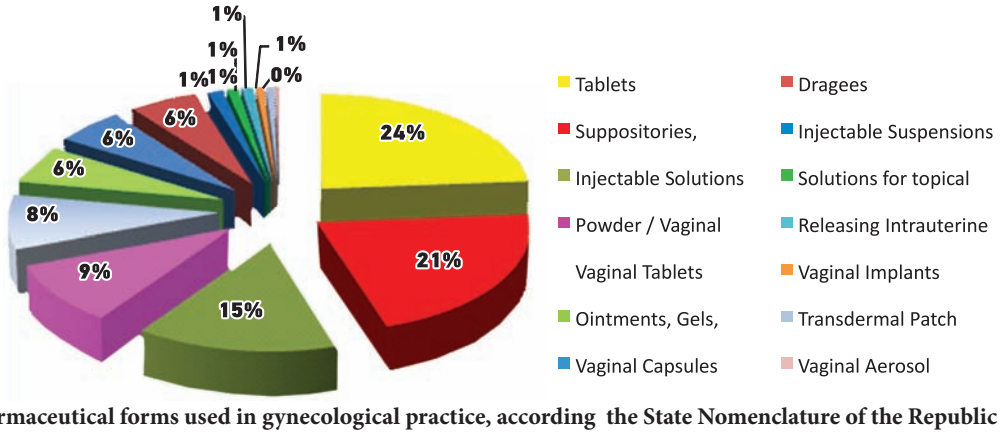

Fig. 2. Pharmaceutical forms used in gynecological practice, according the State Nomenclature of the Republic of Moldova.

ons for topical use, irrigation - 0.95%, vaginal tablets - 7.62%, ointments, gels, vaginal creams - 6.03%, vaginal implants - 0.63%, suppositories, pessaries - 15.53%, releasing intrauterine systems - 0.95%, transdermal patch - 0.63%, vaginal aerosol - 0.32%, vaginal capsules - 5.71%, tablets - 24%, injectable solutions - 15% , injectable suspensions - 1.27%, powder / vaginal solution - 9.21%, dragees - 5.08% [8.10].

Formulations (pharmaceutical forms) used in gynecological practice, according to the State Nomenclature of the Republic of Moldova are presented in figure 2.

The data shown in figure 2 proves that ovules, tablets and injectable solutions are in the top of pharmaceutical forms, used in the treatment of gynecological diseases, recorded in the Republic of Moldova [8, 10].

Medicine producers (Drug makers)

A. Countries (figure 3)

From the number of medicines used in gynecological practice, in Moldova were registered preparations from 21 countries, in the top 10 - the first position is held by the Republic of Moldova - with 18.53% (58medicines - produced and registered), Switzerland holds the second position with 12,78% (40 medicines), on the third place is Hungary and Germany with 11.50% each (36 medicines) [8,10].

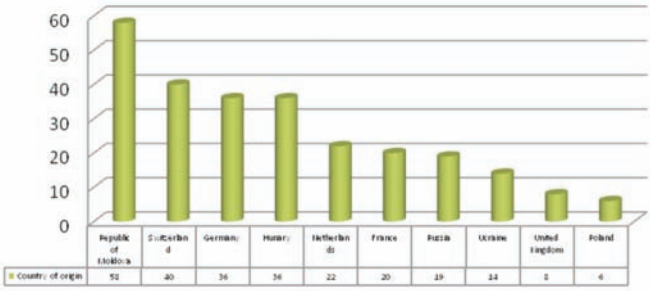

Fig. 3. Top 10 countries producing pharmaceutical forms, used in gynecological practice registered in the Republic of Moldova.

As to information reflected in figure 3, local producers cover production of pharmaceutical forms, used in gynecological practice by 10.98%.

B. Pharmaceutical industry, producing enterprise

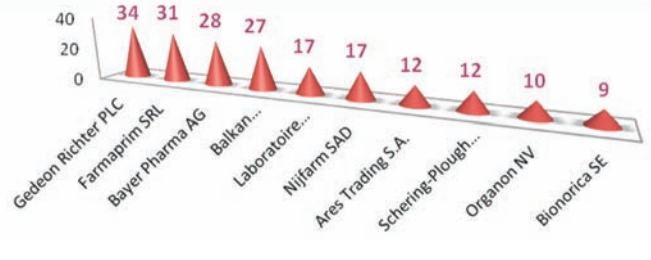

Fig. 4. Top 10 Pharmaceutical industrial producing enterprises, used in gynecological practice, registered in the Republic of Moldova.

The leading pharmaceutical company producing industrial products used in gynecological practice registered in the Republic of Moldova, is Gedeon Richter PLC, Hungary; followed by industrial enterprises: FARMAPRIMSRL, Moldova and Bayer Pharma AG, Germany (fig. 4) [8,10].

Terms of validity (Expiration date)

One of the decisive factors ensuring competitiveness of medicines is their quality. Many pharmaceutical forms are in native equilibrium, but degrade as disposal of free energy, being in a phase when their therapeutic value decreases. Therefore, an important parameter of the quality of the pharmaceutical forms is the term of expiry, determined through different experimental methods, e.g. "Method of accelerated aging" or through other degrading methods [9].

Fig. 5. The expiration date as a parameter of the pharmaceutical forms quality used in gynecological practice, registered in the Republic of Moldova.

As shown in figure 5 the longest expiration date stated by the manufacturer - 60 months, 74 pharmaceutical forms have this validity terms (e.g. suspension for injection - Depo-Provera, 150 mg / mL, 1 mL N1, G03AC06, Medroxyprogesteronum, Pharmacia NV / SA, Belgium); - 12 pharmaceutical forms declared the expiration date of 48 months (e.g., Logest® tablets, 0.02 mg + 0.075 mg, N21, G03AA10, Ethinylestradiolum + Gestodenum, Bayer Schering Pharma AG (Bayer Schering Pharma AG prod.: Germany; Delpharm SAS Lille, France), Germany); - 1 pharmaceutical form announced the expiration date of 40 months (releasing vaginal system - NuvaRing®, 11.7 mg + 2.7 mg, N1, G02BB01, Etonogestrelum + Ethinylestradiolum, Schering-Plough Central East AG (prod.: Organon (Ireland) Ltd., Ireland Organon NV, Netherlands), Switzerland); - 272 pharmaceutical forms stated expiration date - 36 months (e. g. transdermic patch (STT) - Climara®, 3.8 mg (50 mcg / 24 h), N4, G03CA03, Estradiolum, Bayer Schering Pharma AG (prod.: 3M Drug Delivery System, USA), Germany); - 2 pharmaceutical forms stated terms of validity - 30 months; - 163 pharmaceutical forms announced expiration date - 24 months (e.g. Pessaries - Dalacin vaginal pessaries, 100 mg, N3, G01AA10, Clindamycinum, Pharmacia & Upjohn Company, USA); - 2 pharmaceutical forms declared expiration date - 18 months (e.g. Vaginal soft capsules - Polygynax® Virgo, 35000 + 35000 UI UI UI + 100000, N3x2, G01A, Polymyxinum Neomycinum + B + Nystatinum, Laboratoire Innotech International (prod.: Innothera Chouzy, France), France) [8].

Producer's prices

A very important index that describes the accessibility of a proper and efficient treatment (and correlation "cost-effectiveness"), is the price of medicines input by the manufactu-

Fig. 6. Estimation of the preparations used in gynecological practice, registered in the Republic of Moldova, according to price of the producer.

rer. This way, the price declared by the producer in decreasing for some pharmaceutical forms used in gynecological practice (fig. 6):

1. Solution for injection in pre-filled syringe - Elonva®, 150 mcg / 0.5 ml, 0.5 ml, N1, G03GA09, Corifollitropinum alpha, Schering-Plough Central East AG (prod.: Organon (Ireland) Ltd., Ireland; VetterPharma-FERTIGUNG GmbH & Co.KG, Germany), Switzerland, term of validity - 36 months, producer's price (ex works) - € 455.19 or 8835.60 MDL.

2. Solution for injection in pre-filled syringe - Elonva®, 100 mcg / 0.5 ml, 0.5 ml, N1, G03GA09, Corifollitropinum alpha, Schering-Plough Central East AG (prod.: Organon (Ireland) Ltd., Ireland; VetterPharma-FERTIGUNG GmbH & Co.KG, Germany), Switzerland, term of validity - 36 months, producer's price (ex works) - € 412.03 or 7997,83MDL.

3. Solution for injection in pre-filled pen - Gonal-f® 900 IU (66 mcg), 1.5 ml, N1 G03 GA05, Follitropinum alpha, Merck Serono S.A. (Prod .: Merck Serono S.P.A., Italy), Switzerland, term of validity - 24 months, producer's price (ex works) or -310.15 USD 6176.11 MDL.

4. Powder + solvent / solution for injection - Bravelle 75 IU N5x2 + 1ml, N5x2, G03GA04, Urofollitropinum, Ferring GmbH, Germany, term of validity - 24 months, producer's price (ex works) - € 159.03 and 3420.23 MDL.

5. Solution for injection in cartridge - Puregon®, 600 IU / 0.72 ml, 0.780 ml, N1, G03GA06, Follitropi beta-num, Schering-Plough Central East AG (prod.: Organon (Ireland) Ltd., Ireland Vetter FERTIGUNG-Pharma GmbH & Co.KG, Germany), Switzerland, term of validity - 36 months, producer's price (ex works) - € 162.75 or 3159.11 MDL.

6. Tablets - Esmya®, 5 mg, N14x2, G03AD02, Ulipristalum, Gedeon Richter PLC, Hungary, term of validity - 24 months, producer's price (ex works) - € 115.69 or 2591.33 MDL.

7. Releasing intrauterine system - Jaydess®, 13.5 mg, N1, G02BA03, Levonorgestrelum, Bayer Pharma AG (prod.: Bayer Oy, Finland), Germany, term of validity - 24 months, producer's price (ex works) - € 121.30 or 2354.53 MDL.

8. Solution for injection in pre-filled pen - Gonal-f® 300 IU (22 mcg), 0.5 ml, N1 G03 GA05, Follitropinum alpha, Ares Trading S.A. (Prod.: Merck Serono S.P.A., Italy), Switzerland, term of validity - 24 months, producer's price (ex works) - $ 103.35 or 2058.04 MDL.

9. Releasing intrauterine system - Mirena®, 52 mg (20 mcg / 24 hours), N1, G03AC03, Levon-gestrelum, Bayer Oy, Finland, term of validity - 36 months, producer's price (ex works) - 86.5 EUR or 1679.03 MDL.

10. Films - Clostilbegyt, 50 mg, N10, G03GB02, Clomifenum, Egis Pharmaceuticals PLC, Hungary, term of validity - 60 months, producer's price (ex works) - $ 2.76 or 55.18 MDL.

The lowest price can be seen at pharmaceutical form - vaginal tablets - Clotrimazol MCC 100 mg, N6x2, G01AF02, Clotrimazolum, Magistra C & C SRL, SC, Romania, term of validity - 24 months,producer's price (ex works) MDL - 8.35, EUR - 0.43 [8,10].

The number and prices of products manufactured and registered in the Republic of Moldova varies depending on producing enterprises: lower price <50.00 MDL - 37 medicines >50.00 <100.00 MDL – 66 medicines >100 <200 MDL - 76 medicines, > 200 <400 MDL - 35 medicines, > 400 <1000 MDL - 6 medicines, over > 1000 MDL - 16 medicines.

Conclusions

1. In the Republic of Moldova, according to ATC classification code G-Unit genitourinary system and sex hormones, includes 4 sub-codes that represent the following sub-code -G03 Sex hormones and modulators of the genital system and accumulates the highest number of registered medicines, the lowest is sub-code – G 02 Other gynecological medicines.
2. From pharmaceutical forms registered in the State Nomenclature of the Republic of Moldova used in gynecological practice, the largest number goes to: a) tablets, b) ovules and c) injectable solutions.
3. Moldova is located at the head of the 21 producing countries, which have registered pharmaceutical forms used in gynecological practice. Domestic producers of medicines are covering 10.98 % from this segment of pharmaceutical market.

4. The biggest amount of medicines used in gynecological practice and registered in Moldova lies with the company manufacturing industrial medicines Gedeon Richter PLC, Hungary.
5. The term of validity (expiration date) of the pharmaceutical forms used in gynecological practice declared by the manufacturer vary depending on the nature of the active substance and pharmaceutical form from 60 months till 18 months.
6. Producer's price for pharmaceutical forms used in gynecological practice varies between 8.35 MDL and 8835.60 MDL. Prices of the medicines used in gynecological practice produced by domestic medicine manufacturing enterprises have medium range of competitiveness and vary from -13.22 MDL to - 1800.00 MDL.

References

1. Paladi Gh, Stratila M. Main indicators of human reproductive health. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale [Bulletin of the Moldovan Academy of Sciences. Medical Sciences]*. 2009;21(2):106-116.
2. Safta V, et al. Medicine market produced in the Republic of Moldova: situation, problems, solutions. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale [Bulletin of the Moldovan Academy of Sciences. Medical Sciences]*.2010;24(1):6.
3. Cernețchi O, Paladi Gh. Celioscopia in the diagnosis of ectopic pregnancy. Proceedings of the XXII National Congress of Obstetrics and Gynecology. 1998;85-86.
4. Table list of diseases ICD-10-AM. Vol. 1: international statistical classification of diseases and related health problems. Revision 10 Australian modification (ICD-10-AM), ed. 3, 2002.
5. Tihon L. Clinical evolution, diagnosis and treatment of ectopic pregnancy. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale [Bulletin of the Moldovan Academy of Sciences. Medical Sciences]*. 2012;35(3):94-97.
6. Galbur O. Report on demographic evaluation of the population in the republic and analysis of morbidity in the Republic of Moldova, neighboring countries and the European Union, trends and changes in the last 7 years. Chisinau, 2010;33.
7. Cernetskaia O. Modern approach to diagnosis, treatment and rehabilitation of patients with difficult pregnancy: PhD dissertation. Med. sciences. Chisinau, 2000;275.
8. www.amed.md
9. Romanian Pharmacopeea, ed. X, 1993.
10. State Nomenclature of the Republic of Moldova, online edition, 2016.
11. Matcovschi C, Safta V. Pharmacotherapeutic Guide (Medicines approved in the Republic of Moldova). Chisinau, 2010;1296.
12. Tihon-Pascal L. Clinical and medico-social aspects of gynecological states of emergency in the Republic of Moldova: Autoreference PhD thesis in Medical Sciences. 2015;30.
13. http://www.oftalmo.md/bolile-ginecologice-clasificari-simptome-si-tratament.

Application of medical ozone in the complex treatment of the severe chronic marginal periodontitis

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Abstract

Background: The microbial factor plays a major role in the pathogenesis of the periodontal disease. The presence of the periodontal bacteria was demonstrated during numerous researches. The susceptibility of the host in the initial stage of the periodontal disease is considered a mandatory condition.
Material and methods: For this study, there were 96 patients selected, men and women, aged between 41 and 73, diagnosed with severe marginal chronical periodontitis (severe CMP). The patients were divided into two lots – the control lot of 52 patients, who received a classical non-surgical periodontal treatment and the research lot of 44 patients, who received the same treatment complimented with systemic and topic ozone therapy.
Results: The recent researches have shown the other risk factors, such as the formation of free radicals, which are formed internally and externally. Another very important factor, which accumulates free radicals and creates an appropriate environment for the development of the inflammatory phenomena, is the disruption of the microcirculation, which leads to the development of the chronic tissue hypoxia. The interaction of the free radicals with oxygen leads to the creation of the reactive oxygen species (ROS). The excessive production of the ROS is associated with the development of conditions that involve a wide range of affections and degenerative diseases, including periodontitis. The reduction of the oxidative stress plays a critical role in the complex treatment of the periodontal disease. In the last years, the traditional nonsurgical methods for treating periodontitis have lost their effect, because of the number of cases, showing resistance to the antibiotics and increased allergic reactions, in addition to the high number of contraindications and the side effects of the medications. All these factors lead to a new approach and new researches of alternative methods of treatment.
Conclusions: The achieved result led to the rapid reduction of the inflammatory phenomena in the periodontal tissue ensuring a considerable decrease of the bacterial contamination in the periodontal sacks and a stimulation of the repairing processes, which led to the increasing of the health condition maintenance of the periodontal status when comparing it with the traditional treatment.
Key words: severe marginal periodontitis, medical ozone.

Introduction

Marginal periodontitis continues to be a major health problem, because it is one of the most widespread and frequent disease of the human body at any life stage. That is why the main scope in the rehabilitation of the maxillary functions (mastication, phonation and aesthetics) is its prevention, the early stage diagnosis and the performance of a complete and complex treatment, through the integration of all dental specialities [1].

During the periodontal disease pathogenesis (PD), the main role is played by the microbial factor, the presence of the *Prevotella intermedia, Actinobacillus actinomycetemcomitans, Treponema denticola, Bacteroides forsythus, Porphyromonas gingivalis* bacteria was determined by various researches [2].

During the first stage of the PD, the immune inflammatory response of the body plays an essential role, and the susceptibility of the host is a mandatory condition [3]. The reaction to the bacteria aggressiveness is unleashed by a specific mechanism, which is influenced by the genetic predisposition of each individual [4]. For many years, the immune genetic researches are trying to identify various associations between the PD appearance and the existence of any relevant genes. The identification of the genes is based on the analysis of researches over the PD genotype. Studies have demonstrated that the marginal periodontitis is associated with high levels of pro-inflammatory cytokines: interleukin 1 (IL-1) and the tumour necrosis factor - alpha (TNF - alfa), which are the key regulators within the immune response of the host, during the bacterial infections. IL-1 is also a major modulator of the extra cells matrix of the catabolism and of the bone resorption [5]. The researchers in the field claim that these specific genetic markers, which were associated with the IL-1 growth, are a powerful indicator of the susceptibility of the PD occurance.

The recent researches have shown also the role of the risk factors, such as, the formation of the free radicals [6] which originate from the inside environment (phagocytosis, incomplete catabolism, energy production, etc.) and from the outside environment (stress [7], smoking, alcohol, polluted air, processed food, some types of medications, etc.). Another, also, very important risk factor is the periodontal chronical trauma [8]. At the same time, a predisposition role, for the inflammatory phenomena development, into the periodontics, is played by the microcirculation perturbation together with the development of the chronic tissue hypoxia; the role of the hypoxia in the periodontal pathogenesis condition was demonstrated by numerous studies [38]. The settlement and the progression of the hypoxia condition leads to the accumulation of free radicals, which determines the transformation of the aerobic cycles (mainly in the carbohydrates metabolism) in anaerobic cycles and, in consequence, the accumulation, especially, of the lactic and pyruvic acid, leading to the occurrence of the metabolic acidosis into the affected area. There is also an observation of a simultaneous pH decrease, which characterises the degree of hypoxia [39]. The rapid response to the occurrence of the inflammation, resulting from the respiratory enzymes, necessary for the respiratory processes, suddenly decreased, and it can serve as a sign for the early manifestation of the necrosis changes inside the tissue. All the mentioned above factors create favourable conditions for the development of the pathogenic microbes [40].

The main element for the establishment of the diagnosis and for the PD treatment plan is the clinical and radiographical examination. In the last years, the PD treatment has registered success mainly because of the new surgical methods and techniques, the bio stimulator materials and the tissue regeneration, which allow the rehabilitation of the maxillary functions, but they do not eliminate completely the factors that are causing the appearance of the PD. Before not long ago, the scope of the periodontology was to treat and to maintain on the dental arch, by all means, the affected dental periodontal units. Nowadays, the tendency is to maintain and improve the bone tissue, due to unsuccessful periodontal treatments, where the severe atrophies of the alveolar crest make the use of dental implants very difficult or even impossible [9]. Therefore, alongside the surgical treatment, which is a therapeutical stage within the complex treatment of the PD, it is very important to discover and to apply systemic treatments, which could beneficially influence the PD evolution. Thus, in the last years, the traditional methods of the non-surgical periodontal treatment have lost their value. The main cause is considered the increased number of cases, which showed microbial resistance to antibiotics [10]; the frequent appearance of allergies, the high number of contraindications, and the side effects of medications - all these factors lead to a new approach and new researches of alternative methods of treatment.

In this context, ozone therapy deserves to be recognized as one of the simplest and most efficient method [11]. The action of the medical ozone on the human body is very diverse and multidirectional. The medical ozone has an antimicrobial, antioxidant, immunomodulatory [12], antihypoxic, disintoxication, antiviral and antifungal effect, it stimulates the metabolical processes and improves the blood rheological proprieties [13]. If compared with the antibacterial therapy, the ozone therapy has a wider range of therapeutical action and it does not create microbial resistance. Also, it does not produce mutagenic or carcinogenic effects [14], more than that, if there is microflora resistant to antibiotics, the use of medical ozone in antibacterial therapy, leads to the neutralization of the resistance to antibiotics and to the intensification of the antibiotics effect [15].

The efficiency of the use of the medical ozone was demonstrated and proved in various systemic diseases, which mainly have inflammatory character, in both the field of surgery and therapy [16, 17].

The dental practice medical ozone is used only in combination with other treatment methods [18]. Therefore, the maxillofacial surgery is widely using ozonated solutions as antiseptic remedies for the care of local injuries and whole oral cavity (OC) [19, 20, 21]. This, as well, considerably improves the post-surgical convalescence and fastens the epithelialization of the operated wound. The ozone has a beneficial effect over the metabolism and the bone repairing process [22]. It has

been observed that the use of medical ozone in patients with chronic mandibular osteomyelitis normalises more rapidly and completely the unspecific resistance and the T-cell immunity, thus, accelerating the clinical recovery and reducing the incidence of complications [23]. The medical ozone is used, topically, during the treatment of periodontal affections, in the periodontal pockets (PPs), in the form of instillations, solutions or ozonated oils and has a real anti-inflammatory potential, established based on the objective criteria analysis of the diagnosis [24, 25].

Nevertheless, the specialized literature, basically, includes no data about the application of the medical ozone in the form of gas injections (infiltrations) in the submucosa region of the affected marginal periodontium and the data about the application of the semisolid ozonated oil are missing completely.

Therefore, the various use of the medical ozone in clinical periodontology needs a more thorough research.

Material and methods

For this study, there were 96 patients selected, men and women, aged between 41 and 73, diagnosed with severe marginal chronical periodontitis (severe CMP). The patients were divided into two lots – the control lot of 52 patients, who received a classical non-surgical periodontal treatment and the research lot of 44 patients, who received the same treatment complimented with systemic and topic ozone therapy.

- The classical non-surgical treatment included: scaling and root planing treatment (SRP). In order to remove the supra- and subgingival plaque and tartar the ultrasonic NSK – MultiPad Varios170LUX was used instrumentally, through infiltration, under the local anaesthesia. The other parts of the tartar and plaque have been removed through Airflow, by using the NSK Prophy Mate prophylaxis bicarbonate device.

- The complementary treatment with medical ozone included: administration of systemic medical ozone in the form of major autohemotherapy (AHTM) and in the form of gas infiltrations (injections) as well as semisolid ozonated oil as topic applications.

All the patients from the research lot have followed:

Systemically:

-Six AHTM sessions, once in 3 days, with a concentration of 25 - 35 mg/ml O2-O3;

-Six sessions of submucosa infiltrations, once in 3 days, with a concentration of 5 - 10 mg/ml, 0,5 – 1 ml O2-O3.

Topically:

-Six sessions of semisolid ozonated oil applications on the dental arch.

For the generation of medical ozone was used the universal medical ozone generator HERRMANN Medozon (Germany).

In order to pathogenically prove the use of medical ozone in the complex treatment of the severe CMP, the present study has performed the following:

1. Clinical examination of patients, in which periodontal status was recorded: CPITN Index - the Community Periodontal Index of Treatment Needs identifies the periodontal disease, its severity, as well as, the need of treatment. The papillary bleeding index (PBI) - Saxer, Мьhlemann (1975) - is an indicator of the severity of gingival inflammation and allows the individual monitoring of the condition of the periodontium through the evaluation of the papillary bleeding intensity during the examination. The recording of the ZMK periodontal charting form, (School of Dental Medicine, Bern University), allows for the overview of the periodontal status.

2. Radiographic examination of the periodontal modification. For this purpose, ortopantomography was used by applying the cone beam computed tomography device VATECH Pax-Flex 3D. After the treatment, the radiographic analysis indicated the lack of bone tissue regeneration for both of the lots.

3. Laboratory examination which include:

- Identification of germs associated with periodontitis - micro-IDent® plus 11 test, based on polymerize chain reaction (PCR) and represents a high specificity for the identification of the eleven periodontal pathogens bacteria and the establishment of their relative quantity: Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus and Treponema denticola, Peptostreptococcus micros, Fusobacterium nucleatum/periodonticum, Eikenella corrodens, Campylobacter rectus, Eubacterium nodatum and Capnocytophaga spp.
- Evaluating the serum level of the oxidative stress marker - superoxide dismutase (SOD), which was analysed based on blood analysis by using the photometric (enzyme) method.

4. Functional examination, during which the blood microcirculation within periodontal tissue was evaluated, by using the Laser-Doppler (LDF) flowmetry method and the laser LAKK-02, SPE «LAZMA» (Russia) analyser. The status of the blood perfusion in the periodontal tissue was evaluated based on the microcirculation level (M) and on the microcirculation effectiveness index (IEM).

The examination of patients with a severe CMP was dynamically performed before the treatment, after the treatment, after 1 month, 3 months and 6 months after the treatment.

Results and discussion

The clinical picture of the evolution of periodontal disease in both groups of study, before the treatment, did not show significant differences (P> 0, 05). Patients very well tolerated both methods of treatment. The findings of this study showed that patients which received additional treatment with medical ozone, applied systematically and topically, have demonstrated improvement of all clinical parameters, which had maintained during 3 months, then, however returning to initial status within 6 months, with the exception of the average of the total quantity of germs, which returned to the initial status within 3 months. At the same time, the average value of the serum level marker (SOD) has maintained as normal after 6 months.

Taking into account the fact that the infectious factor plays a very important role in the aetiology of the periodontal inflammatory affections, the best method of treatment should include elimination of this factor [26]. Thus, special attention was paid to the control of the oral hygiene status.

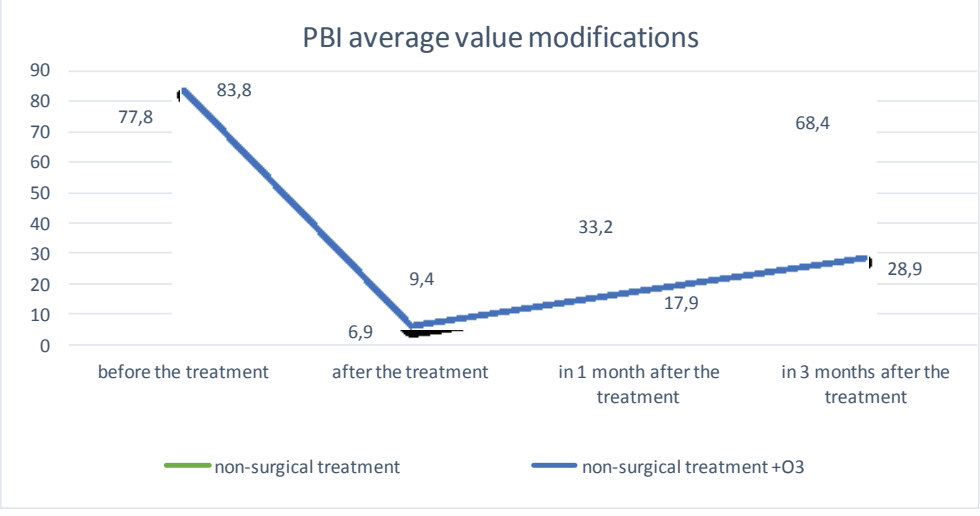


Fig. 1. The comparative analysis of the PBI index average value, between the lots, before the treatment, after the treatment and after 1, and after 3 months after the treatment.

The dynamics of the clinical indices have shown that the complementation of the non-surgical classical treatment with ozone therapy applied systematically and topically, has contributed to a more rapid improvement and longer lasting of the oral hygiene condition in comparison with the non-surgical classical treatment. Moreover, the rapid decrease of the inflammatory phenomena in the periodontal tissues, has allowed the patients with severe CMP to have a better hygiene of the OC, without being afraid of inflicting pain or bleeding during teeth brushing.

The comparative analysis of the papillary bleeding index (PBI) average value, between the lots, has shown the following:

Within the control lot, the average value of PBI was reduced after the treatment from 78 % (<0.01) - to 9 % (<0.001); an improvement which was maintained during one month period, followed by a gradual increase, and after 3 months, coming up, almost to the initial values.

Within the research lot, where patients received additional treatment with medical ozone the average value has shown a higher reduction of PBI, after the treatment, from 84 % (<0.01) to 7 % (<0.001), and an improvement which was maintained for 3 months period followed by a gradual increase, and within 6 months, coming almost to the initial values (fig. 1).

From a bacteriological point of view and taking into account the microbiological research findings on how medical ozone affects bacteria [11, 17, 20, 27], the present study has the scope of researching to what extent the medical ozone has an effect over the periodontal pathogenies bacteria in patients with severe CMP.

In this regard, the micro-IDent® plus 11 test was chosen, used for the identification of the periodontal pathogenic bacteria, based on polymerise chain reaction (PCR) technique and represents a high specificity for the identification of eleven periodontal pathogenic bacteria, and allow the establishment of their relative quantity. Also, this test is more sensitive than the bacterial culture because it identifies the germs according to DNA, regardless of their viability [28].

The comparative analysis of the findings demonstrated a reduction of total quantity of germs:

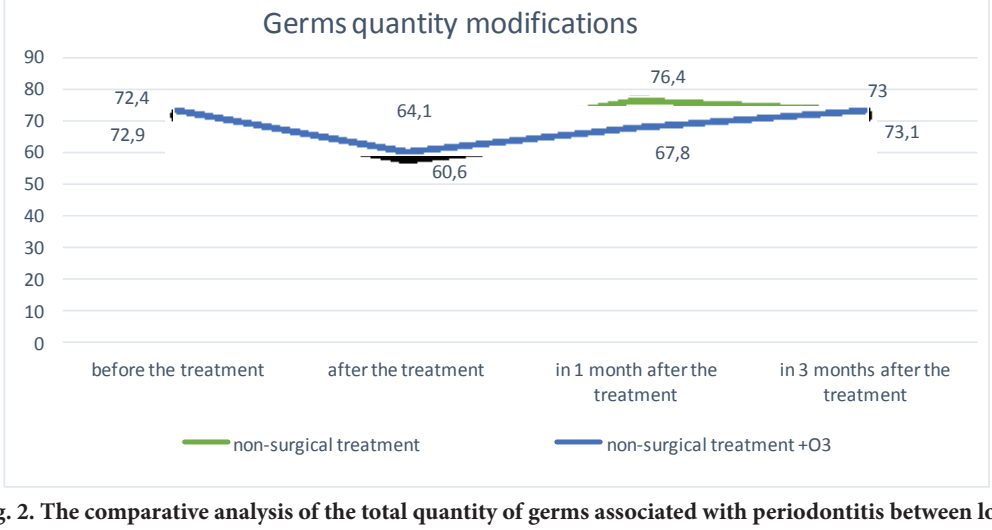


Fig. 2. The comparative analysis of the total quantity of germs associated with periodontitis between lots, before the treatment, after the treatment and after 1 and 3 months after the treatment.

Within the control lot by 11% (P <0, 01) after the treatment, and 1 month after the treatment increasing by 5% (P <0, 01) compared with the initial value.

Within the research lot treated with medical ozone with 17 % (P <0, 01) after the treatment, and in 1 month after the treatment was still decreased with 7 % (P <0,001) compared with the initial values.

On the other hand, there has not been registered any significant difference between the lots in 3 months, after the treatment (P> 0, 05) (fig. 2).

The findings showed the efficiency of complementation of the classical periodontal non-surgical treatment with medical ozone, applied systematically and topically. However, the improvement has lasted for 1 month only, followed by a recolonization of the periodontal pathogenic bacteria, favoured by the PPs depth, which has come, after 3 months, to initial values. A confirmed result of the fact that a wider than 6 mm depth represents an important limitation factor to the adequate cleaning of the root surfaces and the complete elimination of the soft and hard sediments from PPs [29].

As a result, in the case of the patients with severe CMP, the complex treatment of the PD may require also the surgical management of the PPs, which would enable the elimination of this factor.

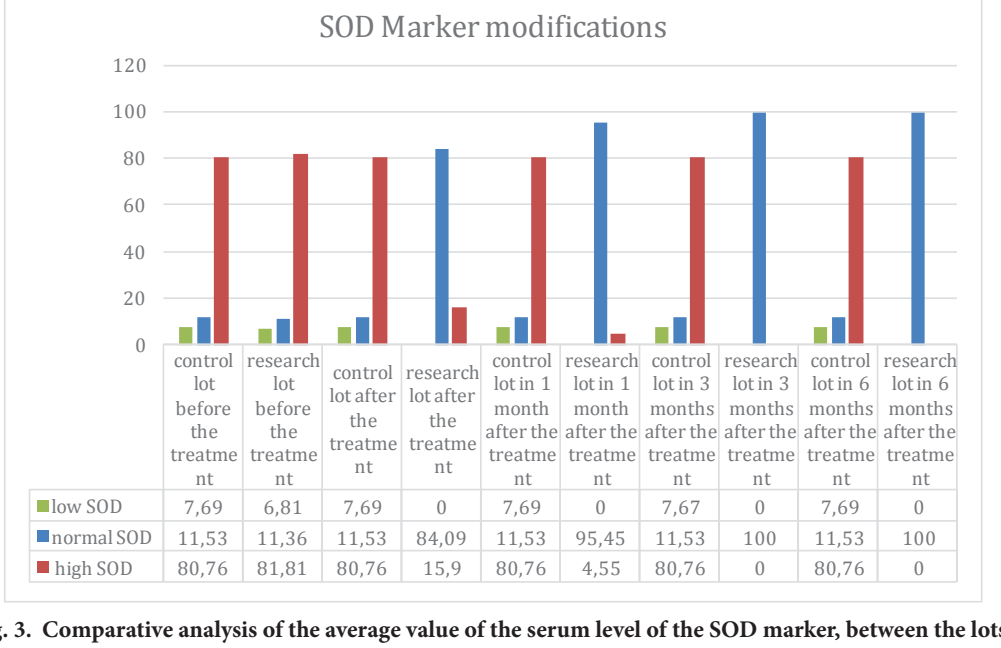
Another objective of this study was the comparative evaluation of the serum level values of the oxidative stress marker - superoxide dismutase (SOD). Together with the glutathione peroxidase and the catalase, the SOD is found inside and outside the cells membranes and they form the primary internal anti-oxidant defence system of the body and plays an important role in the oxidative stress reduction, involved in the development of a wide range of degenerative conditions, which may endanger life [30].

Statistically, the accumulated data, before the treatment, have not shown any significant difference between the two lots (P>0, 05), the average values of the serum level of the SOD marker were normal only within 11%, increased within 81% and decreased within 7% from the total number of patients. After the treatment within the control lot, there has not been registered any evident change of the average value of SOD serum level. On the one hand, within the research lot, after the complementary treatment with medical ozone, there has been registered a significant decrease of the average values of SOD serum level, in patients with high level of SOD marker up to normal values. On the other hand, in patients with decreased values of SOD marker, there has been registered a significant increase of the average value of the serum level of SOD marker, up to normal values. Therefore, the patients, having a normal serum level of the SOD marker, increased from 11% to 84 % (P<0.001) after the treatment and up to 95% (P<0.001) 1 month after the treatment, coming up to 100% (P<0.001) 3 months after the treatment and having maintained its value to normal 6 months, after treatment (fig. 3).

The outstanding results demonstrated the modulation effect of the serum level of the superoxide dismutase, induced by the systemic ozone therapy. The modulation of SOD and of catalase by the medical ozone was observed during other clinical studies and probably involves the modulation of the genes expression [31, 32]. The overall number of these modifications is the metabolic adaptation, which promotes the integration of the homeostatic reactions at different levels and the disorders reduction of the body's self-regulation [33].

Another very important element in the development of the PD is the disruption of the blood microcirculation within the periodontal tissues [35]. During the ozone therapy, both plasma and the erythrocytes are saturated with oxygen [36].

The present study evaluated the medical ozone effect on blood microcirculation within the periodontal tissues in patients with severe CMP by using the Laser-Doppler (LDF) flowmetry method. LDF is a non-invasive, highly informa-



	control lot before the treatment	research lot before the treatment	control lot after the treatment	research lot after the treatment	control lot in 1 month after the treatment	research lot in 1 month after the treatment	control lot in 3 months after the treatment	research lot in 3 months after the treatment	control lot in 6 months after the treatment	research lot in 6 months after the treatment
low SOD	7,69	6,81	7,69	0	7,69	0	7,67	0	7,69	0
normal SOD	11,53	11,36	11,53	84,09	11,53	95,45	11,53	100	11,53	100
high SOD	80,76	81,81	80,76	15,9	80,76	4,55	80,76	0	80,76	0

Fig. 3. Comparative analysis of the average value of the serum level of the SOD marker, between the lots, before and after the treatment, after 1 month, 3 months and 6 months after the treatment.

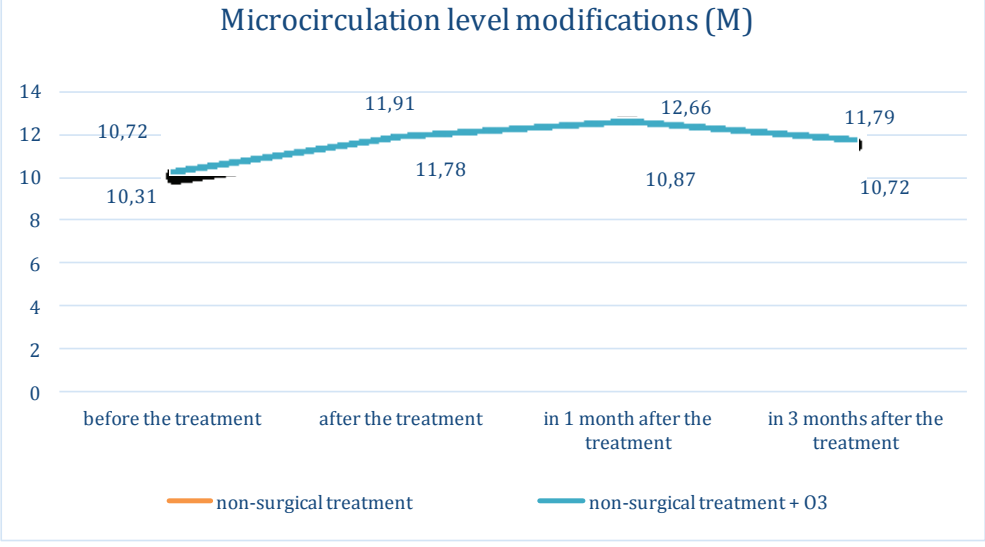


Fig. 4. Comparative analysis of the average value of the capillary blood microcirculation level (M) within the periodontal tissue, between the lots, before the treatment, after the treatment, from 1 and 3 months after the treatment.

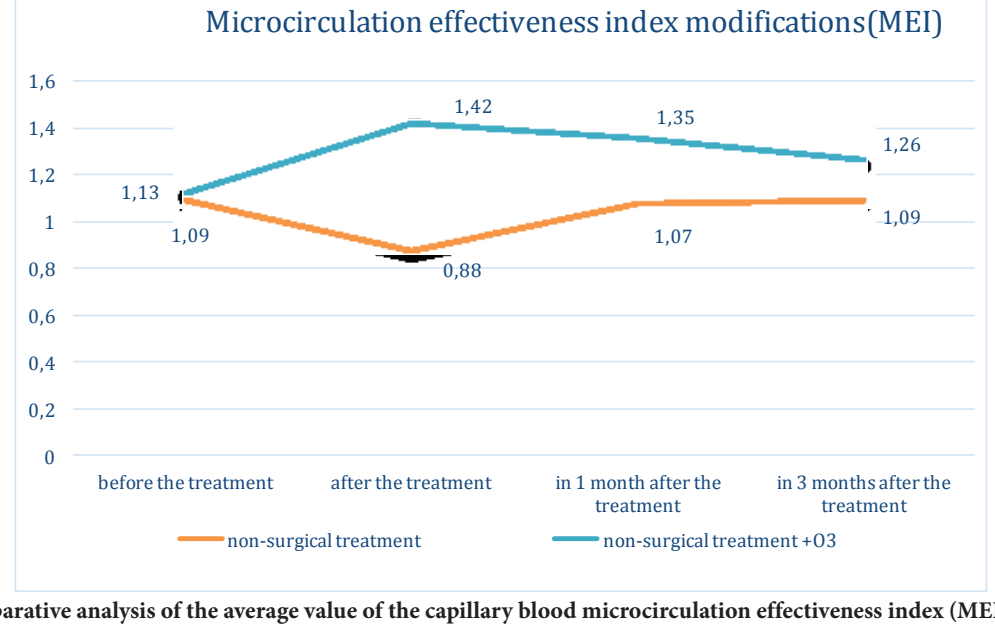


Fig. 5. Comparative analysis of the average value of the capillary blood microcirculation effectiveness index (MEI) within the periodontal tissue, between the lots, before the treatment, after the treatment, from 1 and 3 months after the treatment.

tional and sufficiently sensitive method, used for diagnosis, having a high resolution, for assessing the degree of blood circulation modification, within the tissues, which allows the prominence of the blood flow volume, at the capillary level, the primary symptoms of the affected capillary network, the evaluation of methods and the effectiveness of the chosen therapy [37].

Statistically, the analysis of the microcirculation level values (M) before the treatment did not show, a significant difference, between the two lots (P<0, 05).

Within the control lot, the average value M increased, after the treatment by 10% and after 1 month of treatment, it was increased by 1%, returning almost entirely to the initial values. Respectively, in the research lot, which was treated with medical ozone, the average M values increased after the treatment by 15% and continued to grow, in 1 month after the treatment, up to 23 %; and in 3 months, the values were still increased by 14%, returning within 6 months closely to initial values (fig. 4).

Statistically, the analysis of the microcirculation effectiveness index (MEI) before the treatment did not show a significant difference between the two lots (P> 0, 05).

Within the control lot, the MEI values decreased, after the treatment, by 21% and after 1 month from the treatment, the values were decreased by 1%, returning close to initial values. Within the research lot, treated with the medical ozone, the MEI values increased, after the treatment by 27%, and were increased after 1month after the treatment by 19%, after 3 months by 11%, returning within 6 months closely to initial values (fig. 5).

The spectral dissolution of the LDF - grams allowed the analysis of the flowmotion components of the blood flow. The factors, which are determining the passive mechanism for the modulation of the blood flow within the microcirculation system and specifically the increased maximum amplitude of the high frequency oscillations of the blood flow (AHF), and the decreased maximum amplitude of the pulse oscillations of the blood flow (ACF), lead to the decrease of the MEI average value.

The MEI decrease within the control lot, characterises the development of the vein congestion in the microcirculatory bed of the periodontal tissue due to the minimum traumatic exposure of the periodontium after the classical non-surgical treatment and due to blood flow increase M as well as difficulty of the blood reflux, show the existence of severe hemodynamic disorders at the microcirculation level, within the periodontium in patients with severe CMP. After one month from the treatment, the gradual decrease of the blood flow level led to the return to initial M values and MEI within the control lot.

On the other hand, the research lot treated with medical ozone demonstrated a continuing increase of the average M and MEI values. The MEI values that were obtained immediately after the treatment (1.42±0.02) corresponded to the normal values of the healthy intact periodontium [34]. These values gradually decreased, returning within 6 months after the treatment up to the initial values. This result shows the temporary recovery of the microhemodinamics at the periodontal tissue level.

Conclusions

1. The obtained result led to the rapid reduction of the inflammatory phenomena within the periodontal tissue, ensuring a considerable decrease of bacterial contamination within the periodontal pockets and a stimulation of the recovery processes, which led to the increase of the maintenance period of periodontal health condition, compared with the classical treatment.

2. The comparative analysis of the results showed a reduction of the total quantity of germs and demonstrated the effectiveness of the complementary treatment with ozone therapy applied systemically and topically compared to the classical non-surgical periodontal treatment. Nevertheless, the achieved improvement was followed by a recolonization of the periodontal pathogens bacteria, favoured by the periodontal pockets depth, which in 3 months, came up to the initial values. As a result, the periodontal pockets depth, wider than 6 mm, represents an important limitation factor impairing their adequate cleaning of the root surfaces and thus the elimination of this factor may require surgical management.

3. The increased serum level of the superoxide dismutase showed that the excessive production of the reactive oxygen, involved in the development of certain conditions and degenerative diseases, is associated with severe marginal chronical periodontitis. The dynamic analysis of the serum level of the superoxide dismutase marker showed that the systemic administration of medical ozone represents a key element of the antioxidant therapy and demonstrated an extraordinary effectiveness of the medical ozone. The modulation of the serum level of the superoxide dismutase reduced the oxidative stress, involved in the pathogenesis of severe marginal chronical periodontitis and led to the balancing of the redox homeostasis.

4. The analysis of LDF-grams has pointed to the existence of the severe hemodynamic disorders at the microcirculation level of the periodontal tissue, within patients with severe marginal chronical periodontitis. The systemic and topic application of the medical ozone led to an increase of the average values of the microcirculation level (M) and of the microcirculation effectiveness index (MEI), which characterised the microhemodynamics recovery at the periodontal tissue level. These values have gradually decreased, returning at 6 months after the treatment, almost to the initial values.

5. The obtained therapeutic result has proved the effectiveness of complementing the classical non-surgical periodontal treatment in patients with severe marginal chronical periodontitis with medical ozone applied systemically and topically, in comparison with the classical treatment.

References

1. Dumitriu HT. Periodontology (periodontics). Bucharest: Romanian Medical Life Publishing, 1997;351.
2. Slots J, et al. The occurrence of *Actinobacillus actinomycetemcomitans Bacteroides gingivalis* and *Bacteroides intermedius* in destructive periodontal disease in adults. *J ClinPeriodontol.* 1986;13:570-577.
3. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol.* 2000. 1994;5:78-111. http://www.ncbi.nlm.nih.gov/pubmed/9673164
4. Hodgeand P, Michalowicz B. Genetic predisposition to periodontitis in children and young adults. *Periodontology.* 2001;26(1):113-134.
5. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotypes a severity factor in adult periodontal disease. *J Clin Periodontol.* 1997;24(1):72-7. http://www.ncbi.nlm.nih.gov/pubmed/9049801
6. Nitu Rozalia, Color Delia Irina, Thomas N. Free radicals in biological systems. Cytogenetic effects, Institute of genetics, University of Bucharest, Oranges Alley, no. 1-3, 6th sector, Bucharest; National Institute of R & D for Physics and Nuclear Engineering "Horia Hulubei", Bucharest. http://ebooks.unibuc.ro/biologie/biotehnologie/articolul2.pdf
7. Satheesh Mannem, Vijay K. Chava. The effect of stress on periodontitis: a clinic biochemical study. *J Indian Soc. Periodontol.* 2012;16(3):365-369. http://www.ncbi.nlm.nih.gov/pubmed/23162330
8. Ceban Michael, Postolachi Ilarion. Clinical aspects of chronic periodontal trauma as a risk factor in the etiology of chronic marginal periodontitis. (USMF) Medical University "N. Testemițanu. 2008;4(9):305-309. 6_Stomatologie.doc
9. Vataman Marius Radu. Research on the effectiveness of therapy of the periodontal pockets. Iasi, 2011;3-4.
10. Mărculescu Anca, Cernea M, Nueleanu Veturia, et al. Microbial resistance to antibiotics. University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 2007. http://www.veterinarypharmacon.com/docs/394-ART.11.pdf
11. Bocci V. Biological and clinical effects of ozone: has ozone therapy a future in medicine? *J Biomed Sci.* 1999;56:270-9. http://www.ncbi.nlm.nih.gov/pubmed/10795372
12. Bocci V, Paulesu L. Studies on the biological effects of ozone 1: Induction of interferon on human leucocytes. *Haematology.* 1990;75:510-15. http://www.ncbi.nlm.nih.gov/pubmed/2129118
13. WFOT Review on Evidence Based Ozone Therapy.http://www.wfoot.org/wp-content/uploads/2016/01/WFOT-OZONE-2015-ENG.pdf
14. Caulfield MJ, Burleson GR, Pollard M. Ozonation of mutagenic and carcinogenic alkylating agents, pesticides, aflatoxin B1, and benzidine

in water. *Cancer Res.* 1979;39(6, Pt. 1):2155-9. http://www.ncbi.nlm.nih.gov/pubmed/445412
15. Zullyt B. Zamora, Menéndez S, Bette M., et al. 1. Ozone Prophylactic Effect and Antibiotics as a Modulator of Inflammatory Septic process in Rats. Ozone Research Center, Havana, Cuba. E-mail : ozono@infomed.sld.cu; 2. Institute of anatomy celular and molecular biology, University of Marburg, Germany; 3. Intitute of microbiology, University of Marburg, Germany; 4. Institute of animals laboratory, University od Marburg, Germany. http://lomr.org/ozone-prophylactic-effect-antibiotics-modulator-inflammatory-septic-process-rats/
16. Viebahn-Hansler R. Ozone therapy – the underlying therapeutical concept and models of efficacy. *Erfahrungs Heilkunde*. 1991;4-40.
17. Viebahn-Hansler R. Ozone therapy-the underlying therapeutical concept and models of efficacy. *Erfahrungs Heilkunde.* 1991;4-40.
18. Pirgari AB. Medical ozone in complex treatment of general periodontitis. *Nizhny Novgorod Medical Journal.* 2003;88-189.
19. Haug Karl F, ViebahnRenate. Classic medical ozone text book published, 1987;14-17.
20. Malanchuk V, Kopchak A, Dovbysh N. Ozone therapy in the prevention of inflammatory complications of jaw fractures. 2nd Congress of the Polish association for oral and maxillofacial surgery. Cracow, 1999.
21. Sandhaus S. Ozone therapy in oral surgery and clinical dentistry. *ZahnarztlPrax.* 1969;20(24):277-80. http://www.ncbi.nlm.nih.gov/pubmed/5263394
22. Türk R. Ozontherapie in der zahnärztlichen Chirurgie. *Erfahrungs Heilkunds.* 1976;177.
23. Sanseverino ER. Knee joint disorders treated by oxygenozone therapy. *Eura Medicophys.* 1989;163-170.
24. Menabde GT, Natroshvili ND, Natroshvili TD. Ozonotherapy for the treatment of parodontitis. *Georgian Med News*. 2006;(134):43-6. http://www.ncbi.nlm.nih.gov/pubmed/16783063
25. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. *J Dent.* 2008. http://www.ncbi.nlm.nih.gov/pubmed/18166260
26. Flemmig TF, Petersilka GJ, Mehl A. Working parameters of a sonic scaler influencing root substance removal in vitro. *Clin Oral Investig*. 1997;1(2):55-60.
27. Eberhardt Hans Georg. The Efficacy of Ozone Therapy as an Antibiotic : September 1993: Specialist in Family Medicine. http://www.oxygenhealth.com/oxyfiles/OXY00540.HTM
28. Identification of germs associated with periodontitis. https://www.synevo.ro/identificarea-germenilor-asociati-parodontitei/
29. Papakonstadinu E, Boariu M, Cîrligeriu L, et al. Clinical and microbiological effects of scaling and root planning in periodontal disease. *Experimental & Medical Surgery Researches.* 2008;XV(4):203-209.
30. Vouldoukis I, Conti M, Krauss P, et al. Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress. *Phytother Res.* 2004;18(12):957-62.
31.Sang-Chul Kim, Ok-Su Kim, Ok-Joon Kim, et al. Antioxidant profile of whole saliva after scaling and root planning in periodontal disease. *Periodontal Implant Sci.* 2010;40(4):164-171. Published online 2010, Aug 30. http://www.ncbi.nlm.nih.gov/pubmed/?term=Antioxidant+profile+of+whole+saliva+after+scaling+and+root+planing
32. Leontyeva GV, Kolesova OE, Non-specific mechanisms of sanogenic effect of ozone. Moscow. http://www.ozonterapiklinigi.com/literatur/Rusya%20Ozon%20Birli%C4%9Fi-Proceedings.pdf
33. Martínez-Sánchez Gregorio, Saied M. Al-Dalain, Silvia Menéndez, et al. Therapeutic efficacy of ozone in patients with diabetic foot. Center of Studies for Research and Biological Evaluation (CEIEB-IFAL), University of Havana, Havana 10400; Cuba Ozone Research Center; Cuba Laboratory of Pharmacological Biotechnology; University of Ancona, 60131 Ancona; Italy Department of Chemistry and Medical Biochemistry, University of Milan, Via Saldini, 50-20133 Milan, Italy, 29 September 2005. http://humares.de/files/fallbeispiele/OzonePatientsWithDiabeticFoot.pdf
34. Krechina EK, Maslova VV, Rahimova EN, Shidova AV. Opredelenie gemomikrotsirkulyatsii v tkaneakh parodonta s ispolzovaniem metodov lazernoy i ultrazvukovoy dopplerograf (Determination of hemo microcirculation in periodontal tissues using Laser Doppler and Ultrasound Doppler techniques); II (*ИИ*). 2008;18.
35. Periodontal Disease and Atherosclerotic Vascular Disease: Does the Evidence Support an Independent Association? A Scientific Statement from the American Heart Association http://circ.ahajournals.org/content/125/20/2520.full
36. Rokitansky O. Ozone Oxygen Therapy for Arterial Circulation Disorders. Ozone Science and Engineering. Pergamon Press. *Clinical Consideration and Biochemistry of Ozone Therapy Hospitals.* 1982;53:643.
37. Kozlov VI. The mechanism of modulation of blood flow in the microcirculation system and disorder in hypertension. Materials of all-Russian symposium II "Application of laser Doppler flowmetry in medical practice". Moscow, 2000:5-16.
38. Pinchback JS, Taylor BA, Gibbins JR, Hunter N. Microvascular angiopathy in advanced periodontal disease. *J Pathol.* 1996;179(2):204-9. http://www.ncbi.nlm.nih.gov/pubmed/8758214
39. Vasina TA, Sidorov IA, Zaitsev VYa, et al. Results of application of ozonated solution to the clinic. Proc. I all-Russia scientific-practical conference "Ozone in biology and medicine". N. Novgorod, 1992;58.
40. Kuznetsov EV, Tsarev VI. The microbial flora of the oral cavity and its role in the development of pathological processes. Therapeutic dentistry: Study. - M.: MEDpress-Inform, 2003;178-212.

Curierul medical, February 2016, Vol. 59, No 1

Comparative clinical study of depigmentation products on facial melasma in Latin women

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Abstract

Background: Hyperpigmented lesions need the most frequent dermatological consultations, the acquired ones being more effectively treated. Out of them, the most common is melasma, which is currently treated with hydroquinone. Our objective was to compare the efficacy of a treatment based on Diacetylboldine-DAB, Alpha arbutin and Licorice with hydroquinone 4%.
Material and methods: We carried out a pilot study on 30 Latin patients (skin type III and IV after Fitzpatrick's classification). The product under study was applied on one side of the face and hydroquinone on the other, during 60 days.
Results: The study product demonstrated effectiveness comparable to 4% hydroquinone in the 60 days of monitoring time. The hyperpigmentations are of a chronic type and so, considering the formula of the study product, it has the great advantage of permitting use for a prolonged period of time without the risk of undesirable side effects such as ochronosis. Tolerance for the product was excellent as well, both for daytime and night-time formulations, and neither irritation reactions nor allergic reactions were present during the period of use.
Conclusions: The combined use of active substances is similar and comparable to hydroquinone in a 60 day period. The foundation can be laid for future studies to approach a new investigation with a larger number of patients, in which the use of hydroquinone can be established in comparison with this new treatment, so as to allow a statistically significant relationship to be established. Additionally, by studying a larger number of participants, it would allow this new product to be set up as an effective alternative treatment for melasma.
Key words: facial melasma, antipigment skin agents.

Introduction

Hyperpigmented lesions, especially on the face, are a very common reason for consultation in dermatology. Some spots are of the congenital type and others are acquired; the latter type is relatively more susceptible to effective treatment. Among these we find melasma, ephelides or freckles, lentigines, postinflammatory hyperpigmentations and others [1].

Melasma or facial chloasma is a commonly acquired hyperpigmentation related to an increase in the number and activity of clones of melanocytes, when activated by ultraviolet light. It appears in exposed areas and can be classified, according to location, as centrofacial (the most common), malar or mandibular. It can also occur on the neck and forearms [1, 2, 3, 4].

This pathology is predominant in the female sex, appearing in 10 women for each man, thus highlighting the role played by oestrogen in stimulating melanogenesis, possibly by costimulation in the synthesis of melanosomes [5, 6].

Factors such as exposure to the sun, stress, pregnancy, oral contraceptives, anti-epileptic drugs, endocrine dysfunction, cosmetics, nutritional deficiencies and liver deficiencies have been associated with clinical aggravation of melasma [7].

In the physiopathology of the formation of melasmas in predisposed persons, the action of sunlight on a chemical substrate in areas exposed to light has been established, with presentation increasing during periods of exposure to ultraviolet rays, and decreasing when the patient avoids ultraviolet rays.

Acute exposure to ultraviolet radiation activates one of the most important adaptive mechanisms of the skin, the process of inflammation-tanning, as well as inducing pigmentation in the areas subjected to damage. The increased deposit of melanin can be epidermal, dermal or a mixture of the two [1, 8].

Diagnosis is essentially clinical and it is indispensable that the examination takes place under good lighting. Additionally, other instruments can be used such as a magnifying glass (magnified 7-10x) or a dermatoscope (epiluminescent microscope) using wide field objectives with a focal distance of 10 – 15 cm; magnifications of 10 to 50x are routinely enough to permit observation of dermoepidermal pigmentation lesions, along with a Wood's lamp which is a Hg lamp encased in glass that emits UV radiation with a peak of 360 nm, enough to penetrate into the medium dermis. It allows visualization of the level of pigmentation, differentiating epidermal hyperpigmentation, which increases the contrast in the lesion, from dermal hyperpigmentation, which has no contrast. There is a clinical correlation between this procedure and a histopathological biopsy. Instruments can also be used to evaluate the color of skin, by the presence of melanin, and that allows quantitative evaluation of the treatments used [1, 8, 10].

To treat melasma, suspension of oral contraceptives is recommended, whenever possible, although the pigmentation may last several years in spite of this. Direct exposure to the sun should be avoided, broad-spectrum sunscreen should be used and physical measures, such as wearing a hat, should be taken. Several products exist for topical use, the most common being 2 to 4% hydroquinone, considered the standard in the Western Hemisphere (prohibited in Japan). It can be associated with tretinoin, kojic acid, glycolic acid, arbutin,

ascorbic acid, licorice, ellagic acid, azelaic acid, pycnogenol, etc. [11, 13, 14].

Hydroquinone is approved by the FDA in humans in concentrations equal to or less than 2%, and in higher concentrations only under medical supervision. Other treatments can be added such as chemical and mechanical peelings and laser treatments [15, 16, 17].

General Objective of the Study: to confirm clinical efficacy and tolerance of a treatment based on a formulation with the following components: Diacetylboldine - DAB, Alpha Arbutin, and Licorice (see detailed formula in appendix), to reduce hyperpigmented lesions of the melanosis or facial melasma types, through non-invasive technologies, in Latin women.

Specific Objectives: to compare the efficacy of the product in the study to 4% hydroquinone in a compounded formulation. To evaluate the number of spots and intensity of pigmentation through non-invasive technologies and the cosmetic properties of treatments to be used (self-completed questionnaire).

Material and methods

An open prospective study was performed on 30 volunteers with hyperpigmented lesions that persisted despite receiving other treatments, to confirm the clinical efficacy of a treatment based on a depigmentation product applied at night, formulated with a base of Diacetylboldine - DAB, Alpha Arbutin, Licorice, Glycolic Acid, Ascorbic Acid and Salicylic Acid, plus a depigmentation product applied during the day, formulated with a base of Diacetylboldine - DAB, Beta white®, Vitamin C and 50+ sunscreen.

Patients applied products on one half of the face, to compare their effect with hydroquinone in a 4% compounded formulation on the other half of the face, for a period of 60 days. All patients received UV protection with 50+ broad-spectrum sunscreens (fig. 1). The parameters to be studied were evaluated through clinical examinations, instrumental methods and a self-evaluation questionnaire.

The study was performed over a period of 60 days with a total of 30 female patient volunteers of Latin background. Treatment with the formula took place with applications during the day and at night. The test product was applied according to the manufacturer's instructions and a self-evaluation questionnaire was used for subjective appreciation of the product, cosmetic acceptability of the formulation's appearance, texture and ease of application, presence of greasy residue, perception of odour and subjective evaluation of the efficacy of the product (reduction of spots). Additionally, adverse reactions during the study period (burning, itching and peeling) were evaluated and analyzed to determine any possible relation to product usage. Patients were selected in accordance with the following criteria:

Inclusion criteria – Female volunteer patients aged between 30 and 65 years, diagnosed with moderate to severe melasma, with or without lentigos or ephelides, who presented resistance to other treatments for melasma. They also must have complied with the criteria for being under contraceptive treatment or treatment for menopause, without a change in hormonal therapy, if any, during the 6 months before the study or during the study. They must not be pregnant or breast-feeding, not be using topical or systemic treatments at the start of the study, nor present a history of intolerance to topical products. Also, they were requested to abstain strictly from direct exposure to the sun and to sunlamps during the study period.

Exclusion criteria – patients undergoing treatment for their melasma. Subjects with a history of allergic reactions to depigmentation products were also excluded, as well as women who presented any systemic or cutaneous pathology or who received any medication that might have altered the evaluation parameters during the study period. Patients must not present active facial lesions, or any history of laser treatment, or chemical peeling in the two months prior to the study. Patients who may have used isotretinoin in the 6 months prior to the study, or who may have used steroids, alpha/beta hydroxy acids, or tretinoin in the two months prior to the study, were also excluded.

Patients signed an informed-consent form, notifying them of what to expect from the experience and of the possible consequences of their participation. It is used to give agreement to participate in the study. This consent agreement also informed them that participants' rights would be protected and that the data collected would remain confidential, although it may be used in an anonymous form in scientific studies. The decision to participate was entirely voluntary.

Prospective study that included 30 women volunteers of Latin descent, between 30 and 65 years of age, suffering from moderate to severe melasma on at least both of their cheeks. For the comparison study, a depigmentation product for night-time application was used, which was formulated on a base of diacetylboldine, alpha arbutin, licorice, glycolic acid, vitamin C and salicylic acid (see appendix for formula) along with a depigmentation product for daytime application, formulated on a base of diacetylboldine, Beta White® (a biomimetic peptide encapsulated in liposomes), vitamin C and sunscreen (see appendix for formula). These products were compared with a cream in a compounded formula associated with 4% hydroquinone. Face cleanser, humectant-based cream and 50+ invisible fluid sunscreen were used as part of the regular daily routine. This study was approved by the research ethics committee.

A Wood's lamp was used to determine the type of melasma, and a melanometer (Mexameter) from Khazaka-Courage was used to measure the quantity of melanin and to report the results obtained as a numerical value. The VISIA system was also used to evaluate pigmentation, along with other skin parameters. This system photographs the forehead and right and left sides of the face, which are evaluated by the system's software, which then issues numerical values that relate to average values in the general population of the same age and skin type. It also evaluates the effect of the applied treatments by comparing values issued by the system before and after application of the product in the study.

Digital photos were taken before and after treatment and a subjective evaluation was made by the participating patients

(self-evaluation). Skin phototypes were determined using the Fitzpatrick scale.

Types of skin:

I. Always burns, never tans
II. Always burns, tans slightly
III. Sometimes burns, always tans
IV. Never burns, always tans
V. Always deeply pigmented
VI. Black

Volunteers were evaluated on D 0 and D 60. At the initial visit patients were examined to clinically determine the type of melasma with a Wood's lamp, and parameters were measured by melanometer and with VISIA.

Pigmented lesions present on the right and left cheeks were evaluated clinically and classified as follows:

++++ If pigmented lesions were detected on 75% to 100% of the malar area.

+++ If lesions cover 50% to 75% of the malar area.

++ If lesions cover 25% to 75% of the malar area.

+ If lesions cover less than 25% of the malar area.

Digital photos were taken, and the products included in the protocol were delivered with instructions in their use.

Participants were instructed to report any discomfort that might be due to use of the study product, or if they had questions about their use.

At night, skin cleansing and application of the study product on the right side of the face and the compounded product on the left side, according to the attached diagram.

In the morning, skin cleansing and application of the study product on the right side of the face and base cream on the left side.

50+ fluid sunscreen on the whole face. Make-up as usual. In the examination after 60 days the same parameters were evaluated.

Results

At the beginning of the study the group of patients suffering from facial hyperpigmentation was composed of 30 healthy women, from 33 to 65 years of age, averaging 46,6 years of age, who met the inclusion criteria. 29 of them completed the study. One patient did not attend the examinations because of an illness unrelated to the study in progress.

The Wood's lamp test was applied to the patients, the result of which was compatible with pigmentation mostly of an epidermal character, making clear a major contrast with the pigmented areas when illuminated with this wavelength. Patient 17 did not attend the examinations.

As we see in table 1 of clinical measurement, of the 29 patients who completed the study, 11 patients improved on both sides of the face, 8 patients remained the same on both sides, 5 patients improved only on the right side, corresponding to the study product, 3 patients remained the same on the right side, corresponding to the study product, 2 patients worsened on the right side, corresponding to the study product, 3 patients improved only on the left side of the face, corresponding to hydroquinone, 7 patients remained the same only on the left side of the face, corresponding to hydroquinone. No patient worsened with the use of hydroquinone. An example of the clinical result can be seen in figure 2.

This measurement shows the pigmentation in the evaluated areas in numerical values, numbers that are correlated with clinical evaluation. The pigmented lesions do not disappear, but they do decrease in intensity. The effect can be seen in figure 3 (results with VISIA).

In this table we can see the measurement of melanin on the right and left halves of the face, in which measurements at 4 points on each cheek are averaged. A numerical figure is given that allows comparison of the intensity of melanin pigmentation on the cheek before and after treatment.

A comparative study was made through percentage variance analysis, with the numerical data issued by VISIA (tab. 2, 3). With this data we can emphasise the fact that a 19.7% improvement was made with respect to the initial value in the group of patients who showed improvement with the study product.

By the same analysis, in the patients that improved with hydroquinone, an 18.3% improvement was made with respect to the initial value.

In general, in the self evaluation, patients reported that the melasma affected their quality of life, a significant percentage going so far as to admit they use make-up to try to cover the damaged areas (fig. 4, 5).

Moderate itching and burning were described as adverse reactions to use of the study product, but not to the point of causing participants to decide to discontinue using the product (fig. 6, 7, 8). Furthermore, improvement was shown in areas such as skin texture, increased shine, and improved appearance, even though the hyperpigmented lesions did not necessarily disappear. This effect can also be seen in digital photos as in figure 2.

Even though minor adverse reactions existed that were well tolerated by patients, the majority of them stated that because of the improvement in skin quality from using the product for the established period of time, they would continue to use the product for a longer time. The cosmetic properties of the study product were very well evaluated by the patients.

FORMULAS OF STUDY PRODUCTS

Night time:

- Glycolic Acid 6%
- Lumiskin™ 4% (Diacetylboldine - DAB)
- Vitamin C (ascorbyl tetraisopalmitate) 2%
- Alpha Arbutin 2%
- Salicylic Acid 0.1%
- Licorice 0.1%

Day time:

- Beta white® 5% (Bio-mimetic peptide encapsulated in liposomes)
- Lumiskin™ 4% (Diacetylboldine - DAB)
- Vitamin C (ascorbyl tetraisopalmitate)1%
- Eusolex 15%
- Tinosorb M 9%
- Tinosorb S 1.5%
- Titanium Dioxide 2%

Conclusions

The study product demonstrated effectiveness comparable to 4% hydroquinone in the 60 days of monitoring time.

We know that the hyperpigmentations are of a chronic type and so, considering the formula of the study product, it has the great advantage of permitting use for a prolonged period of time without the risk of undesirable side effects such as ochronosis.

Tolerance for the product is excellent as well, both for daytime and night-time formulations, and neither irritation reactions nor allergic reactions were present during the period of use.

With our study, the foundation can be laid for future studies to approach a new investigation with a larger number of patients, in which the use of hydroquinone can be established in comparison with this new treatment, so as to allow a statistically significant relationship to be established. Additionally, by studying a larger number of participants, it would allow this new product to be set up as an effective alternative treatment for melasma.

References

1. Ortonne JP, Bose SK. Pigmentation: dyschromia. Textbook of Cosmetic Dermatology, terceraedición. Baran R, Maibach H. Taylor&Francis. Abingdon, 2005;393-404.
2. Grimes P. Melasma: etiologic and therapeutic considerations. *Arch Dermatol.* 1995;131:1453-1457.
3. Lage D, Costa A. Melasma. En: Tratado Internacional de Cosmecéuticos, Adilson Costa, Edit. Guanabana Koogan, Río de Janeiro, 2012;534-540.
4. Sánchez N, Pathak M, Mihm M. Melasma: a clinical, light microscopic, ultrastructural, and immunofluorescence study. *J Am Acad Dermatol*. 1981;4:698-710.
5. Cestari T, Arellano I, Hexsel D, Ortonne JP. Latin American Pigmentary Disorders Academy "Melasma in Latin America: options for therapy and treatment algorithm". *JEADV*. 22(7):760-772.
6. Vasquez M, Maldonado H, Benjamin C, Sánchez JL. Melasma in men: a clinical and histologic study. *Int J Dermatol.* 1988;27:25.
7. Inoue K, Hosoi J, Ideta R, et al. Stress augmented ultraviolet - irradiation-induced pigmentation. *J Invest Dermatol*. 2003;121:165-71.
8. Gilchrest BA. Localization of melanin pigmentation in the skin with Wood's lamp. *Br J Dermatol*. 1977;96:245-248.
9. Clarys P, et al. Skin Color Measurements: Comparison Between Three Instrument. *Skin Res Technol*. 2000;6:230.
10. Grimes PE, Camarena E, Elkadi T. Colorimetric Assessment of Pigmentation and Erythema Using the Mexameter MX16 Correlation with Fitzpatrick's Skin Type and Race. Poster Exhibit, American Academy of Dermatology, 2000.
11. Haddad AL, Matos LF, Brunstein, et al. A clinical, prospective, randomized, double-blind trial comparing skin whitening complex with hydroquinone versus placebo in the treatment of melasma. *Int J Dermatol.* 2003;42(2):153-6.
12. Ni Z, Mu Y, Gulati O. Treatment of Melasma with Pycnogenol. *Phytother Res.* 2002;16:567-571.
13. Hurley ME, Guevara IL, Gonzales M, Pandya A. Efficacy of Glycolic Acid Peels in the Treatment of Melasma. *Arch Dermatol.* 2002;138:1578-1582.
14. Yoshimura K, Harii K, Masuda Y, et al. Clinical trial of bleaching treatment with 10% alltrans retinol gel. *Dermatol Surg.* 2003;29(2):155-6.
15. Cotellessa C, Peris K, Fargnoli MC, et al. Microabrasion versus Microabrasion Followed by 15% Trichloroacetic Acid for Treatment of Cutaneous Hyperpigmentation in Adult Females. *Dermatol Surg.* 2003;29:352-356.
16. Kawada A, Shiraishi H, Asai M, et al. Clinical Improvement of Solar Lentigines and Ephelides with an Intense Pulsed Light Source. *Dermatol Surg.* 2002;28:5.
17. Angsuwarangsee S, Polnikorn N. Combined Ultrapulse CO2 Laser and Q-switched Alexandrite Laser Compared with Q-switched Alexandrite Laser Alone for Refractory Melasma: A Split Face Design. *Dermatol Surg.* 2003;29:59-64.

Conflict of Interest

The authors declare that they do not have any conflicts of interest.

The article is offered for publication by pharmaceutical company "Becor"

REVIEW ARTICLES

Novel approaches to the treatment of patients with resistant hypertension: renal sympathetic denervation

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Abstract

Background: Hypertension continues to be a major burden of public health concern despite the recent advances and proven benefit of pharmacological therapy. A certain subset of patients has hypertension resistant to maximal medical therapy and appropriate lifestyle measures. Resistant hypertension continues to pose a major challenge to clinicians worldwide and has serious implications for patients who are at increased risk of cardiovascular morbidity and mortality with this diagnosis. Pharmacological therapy for resistant hypertension follows guidelines-based regimens, although there is surprisingly scant evidence for beneficial outcomes using additional drug treatment after three antihypertensives have failed to achieve target blood pressure. Through modulation of renin secretion, glomerular filtration rate and renal absorption of sodium, the sympathetic innervation of the kidneys plays an important role in the pathogenesis of hypertension. A novel catheter-based technique for renal sympathetic denervation (RSDN) as a new therapeutic avenue has great promise for the treatment of resistant hypertension. Renal denervation has the unique advantage of offering the denervation at the renal level, thus mitigating the systemic side effects. Various trials evaluated the role of renal denervation in the management of resistant hypertension and have found promising results. More studies are underway to evaluate the role of renal denervation in patients presenting with resistant hypertension in different scenarios.

Conclusions: This review included the physiology of the renal sympathetic nervous system and the renal nerve anatomy. Furthermore, the RSDN procedure, technology systems, and RSDN clinical trials as well as findings besides antihypertensive effects were discussed. Findings on safety and efficacy seem to suggest that renal sympathetic denervation could be of therapeutic benefit in refractory hypertensive patients. Despite the fast pace of development in RSDN therapies, only initial and very limited clinical data are available. Large gaps in knowledge concerning the long-term effects and consequences of RSDN still exist, and solid, randomized data are warranted.

Key words: renal sympathetic denervation, resistant hypertension.

Introduction

Arterial hypertension epidemiology and control

The prevalence of hypertension is high and is growing steadily. In 2000, about 1 out of 4 adults (>20 years) were affected worldwide, and its prevalence is expected to increase to 1 out of 3 adults, reaching 1.56 billion in 2025 [1]. Hypertension is an independent major risk factor of cardiovascular events as stroke, heart attack, heart failure and kidney failure, being responsible for 62% of cerebrovascular diseases and 49% of ischemic heart disease [2]. Each increase in systolic blood pressure by 20 mmHg and diastolic blood pressure by 10 mmHg doubles the rate of cardiovascular mortality in 10 years [3].

The American Heart Association [4] and the Joint National Committee [5] define resistant hypertension as persistently high blood pressure (systolic and diastolic blood pressure>140 and 90 mmHg, respectively) despite medication administered, which includes three antihypertensives of different classes in appropriately tolerated maximum doses, one of which is a diuretic. It is necessary to cautiously differentiate resistant hypertension from uncontrolled hypertension caused by sub-optimal pharmacological treatment, non-compliance to treatment and secondary hypertension. The prevalence of resistant hypertension is often underestimated for various reasons, including inadequate sample size and exclusion of patients with resistant hypertension from large clinical trials [6, 7]. Kaplan et al estimated that up to 5% of patients in general clinics and about 50% of patients in clinics for kidney diseases suffer from resistant hypertension [6].

Renal sympathetic nervous system and hypertension

Maintenance of normal blood pressure readings is due to the coordinated action of several systems, the sympathetic nervous system playing an important role among them. The increase in sympathetic activity correlates with all hypertension phenotypes, central sympathetic activity, measured at the level of muscular sympathetic activity, being increased in all grades of hypertension compared to normotensive patients [8].

The important role of the renal sympathetic nervous system (SNS) in hypertension initiation and maintenance has been demonstrated through animal experiments and human experience, either by measuring its activity in hypertensive subjects or by monitoring the changes in blood pressure readings after sympathetic manipulations [9,10].

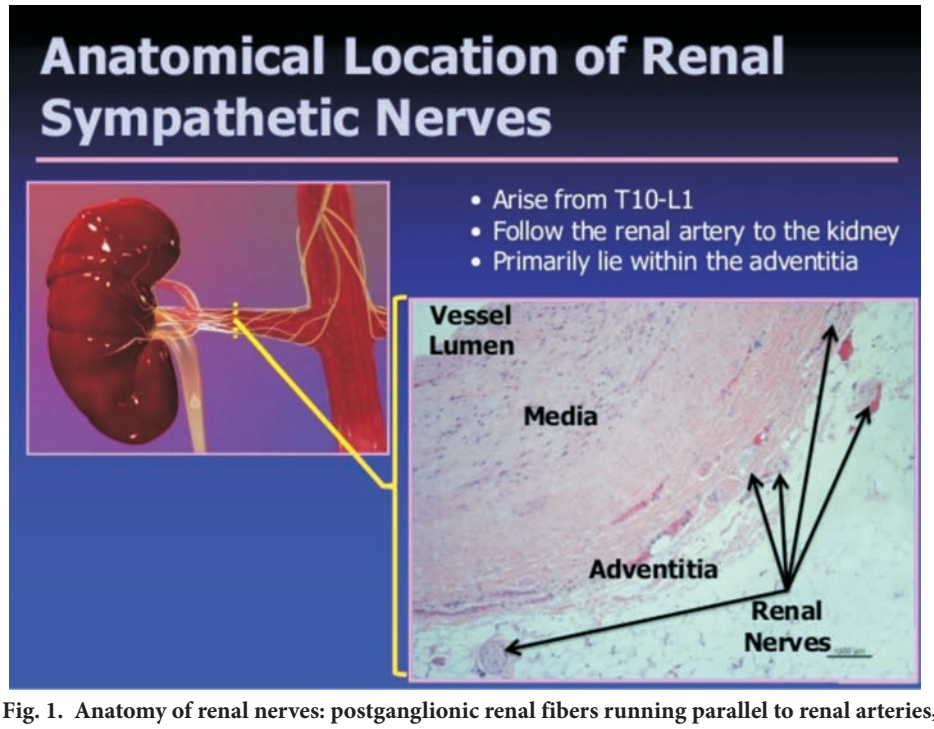


Fig. 1. Anatomy of renal nerves: postganglionic renal fibers running parallel to renal arteries, primarily situated around the adventitia.

The renal sympathetic nervous system is composed of efferent fibers directed from the central nervous system (CNS) to kidneys and afferent fibers having an opposite direction. The preganglionic axons of neurons, that originate in the T10-L2, interact with postganglionic renal nerves at the level of sympathetic pre- and paravertebral ganglia. Postganglionic renal fibers run parallel to the renal arteries, located around the adventitia and enter through the renal hilum to innervate renal tubules, blood vessels and the juxtaglomerular apparatus [11] (fig. 1).

By increasing the production of norepinephrine, these efferent fibers transmit stimuli from the CNS to kidneys and contribute to volumetric homeostasis and blood pressure values, facilitating tubular reabsorption of sodium, followed by hydrosaline retention, and renin secretion, further stimulating the renin-angiotensin-aldosterone system, renal vasoconstriction with the subsequent reduction of the renal blood flow [11, 12, 13, 14]. Oppositely, the kidneys transmit neural responses to the CNS through afferent fibers, also located around the adventitia of renal arteries [15, 16, 17].

Afferent renal sympathetic fibers have an extensive network in the renal pelvis and transmit signals from two types of receptors: mechanical-sensitive receptors that respond to increase in hydrostatic pressure and chemosensitive receptors that are activated by hypoxia and renal ischemia. The signals from these receptors pass through ipsilateral dorsal ganglia to the CNS, especially to the paraventricular nucleus of the hypothalamus [16, 18]. This stimulation of the afferent nervous system leads to increase in blood pressure readings through release of vasopressin, and increase in the systemic vascular resistance. In addition, afferent fibers communicate with the contralateral kidney, thus balancing the unilateral impairment of hydrosaline excretion, underlying the reno-renal reflux [19].

Previous experience of lumbar sympathectomy in the treatment of hypertension

Recognition of the important role that the sympathetic nervous system plays in the pathogenesis of hypertension has led to the development of a surgical approach, known as radical lumbar-dorsal splanchectomy, which interrupts the release of catecholamines [20]. This technique, developed by Smithwick in 1938, reduced blood pressure readings and mortality, but led to severe unacceptable side effects [20, 21]. Several uncontrolled clinical trials on surgical sympathectomy have shown a significant decrease in blood pressure, reduction of heart size, improvement of the renal function and a lower rate of cerebrovascular events [22]. However, the beneficial effects were counteracted by the severe orthostatic hypotension, and the evolution of antihypertensive drugs favored lumbar sympathectomy to be generally removed from medical practice in 1975.

Technique of Renal Sympathetic Denervation (RSDN)

Sobotka and Esler (pioneers in percutaneous renal artery sympathetic denervation) conducted the first studies of catheter-based renal ablation, using radio frequency energy. This procedure involves insertion of an endovascular catheter under fluoroscopic guidance through the femoral artery and its advancement towards a distal renal artery. Sympathetic nerve ablation was performed due to radiofrequency energy applied through an electrode located on the tip of the endovascular catheter. Thus, there were selective thermal injuries at the level of renal sympathetic nerves, while abdominal, pelvic and lower limbs nerves were not affected. Multiple radiofrequency treatments were applied circumferentially, initially in the distal renal artery, and then proximally with catheter withdrawal. The energy applied was lower than that used in electrophysiological studies on the heart; the entire

procedure lasting 30-60 minutes. Selection of patients to be subjected to RSDN is quite scrupulous.

Table 1 shows the main eligibility and exclusion criteria in all clinical studies conducted to date.

Table 1

Eligibility and exclusion criteria in RSDN clinical trials

Eligibility criteria	Exclusion criteria
1. Office SBP≥160mmHg (≥150mmHgin type 2 diabetes), when administering ≥3antihypertensives at maximum tolerated doses (including a diuretic).	1. Secondary hypertension 2. Pseudoresistence 3. Impaired renal function (GFR≤ 45 ml/min/1.73 m2) 4. Renovascular abnormalities: accessory arteries, renal artery stenosis, previous revascularization 5. Pregnancy 6. Type 1diabetes 7. The presence of permanent pacemaker or implantable cardioverter defibrillator 8. Myocardial infarction, unstable angina or stroke in the previous six months

Note: SBP - systolic blood pressure.

It is to be noted that the main renal artery must have a length ≥ 20 mm and have a diameter ≥ 4 mm to avoid structural damage to the arterial wall. Relative contraindications for performing RSDN include visible renal artery stenosis, calcification or atherosclerotic plaque and multiple renal arteries which supply the upper and lower poles of the kidney.

Renal artery denervation in patients with resistant hypertension, using a standard electrophysiology catheter, showed a significant reduction in blood pressure [23]. These data led to the development of numerous catheter systems that are under clinical evaluation. Currently, four systems used in radiofrequency ablation technology have been approved for phase III of the clinical trial in the US, Europe and other countries: Medtronic's Symplicity, St. Jude's EnligHTN, Boston Scientific's Vessix's V2 and Covidien's OneShot.

RSDN preclinical data

The importance of the renal sympathetic nervous system in hypertension has been suggested; when its increased activity was described in rats with spontaneous genetic hypertension compared with the normotensive control group [24]. Several animal models have been used to study the impact of renal sympathetic fibers on blood pressure [25].

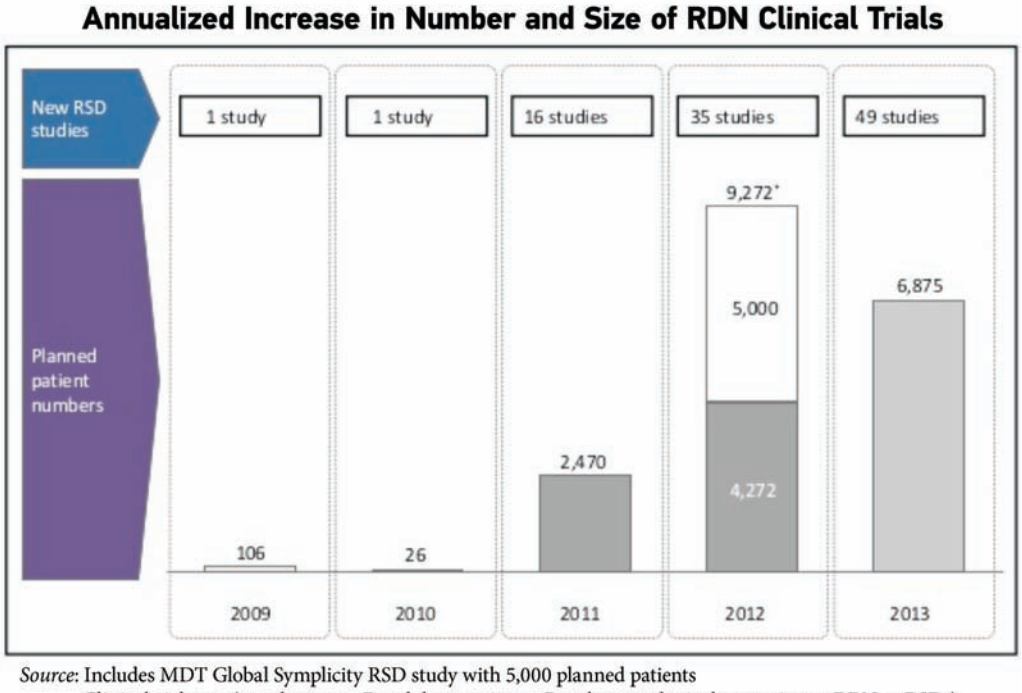
In a large research study including more than 300 pigs, a significant decrease ($p < 0.0001$) of noradrenaline in the kidney tissue was observed in animals subjected to renal denervation compared to the control group. The procedure safety was verified through angiography, as well as histopathologic and clinical data at 7, 30, 60, and 180 days. At all evaluation stages the endothelium was confirmed to be intact, while arterial stenosis was absent.

RSDN clinical trials

To show RSDN efficacy and safety, several clinical trials have been initiated. Thus, in 2009 a clinical trial including 106 patients was initiated; the number of initiated clinical trials reached 49 by 2013, and 6,875 patients were enrolled [26] (fig. 2).

The European Society of Cardiology has developed a Consensus on selection of patients to be subjected to RSDN [27] (fig. 3).

The largest and most important clinical trials according to data obtained are considered those conducted by Medtronic Company (fig. 4) [28].



Source: Includes MDT Global Symplicity RSD study with 5,000 planned patients
Clinicaltrials.gov (search terms: «Renal denervation», «Renal sympathetic denervation», «RDN», «RSD»)

Fig. 2. Annual share of RSDN clinical trials.

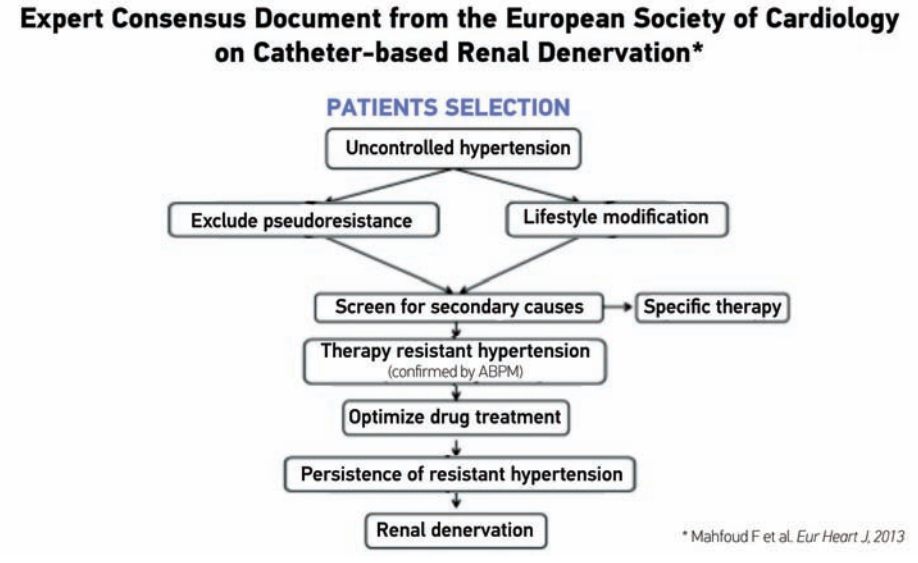


Fig. 3. Selection of patients to be subjected to RSDN.

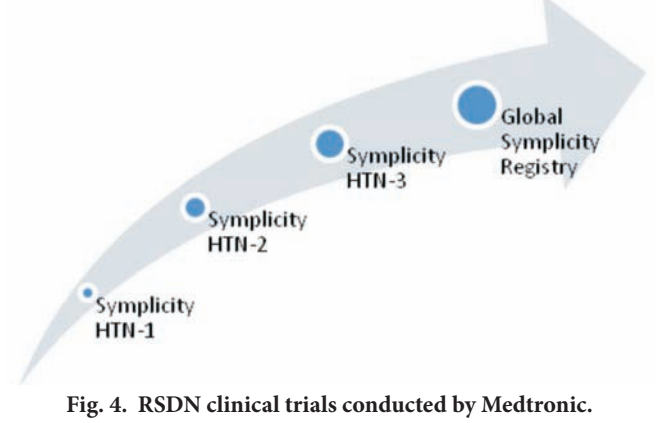


Fig. 4. RSDN clinical trials conducted by Medtronic.

Symplicity HTN-1. Symplicity HTN-1 was a series of pilot studies that included 153 patients. A decrease in blood pressure by -22/-10 mmHg at 6 months after procedure, and by -32/-14 mmHg at 36 months has been reported [29].

Symplicity HTN-2. Symplicity HTN-2 is the first randomized clinical trial including 106 patients with treatment-resistant hypertension, that showed a decrease in systolic and diastolic blood pressure by -32/-12 mmHg, the beneficial effect being maintained for 3 years [30].

Symplicity HTN-3. Symplicity HTN-3 was first blind randomized trial, the results of which were long overdue and veridically much higher than those obtained in previous studies, taking into account the exclusion of many limitations such as small number of the study lot, limited use of ambulatory blood pressure monitoring (ABPM), lack of the blind control lot, etc. After performing the initial screening, patients followed ambulatory treatment with constant blood pressure monitoring at home for 2 weeks.

At the next stage of evaluation, having confirmed treatment-resistant hypertension, renal angiography was performed. During the procedure, depending on the suitability of anatomical eligibility criteria, patients were randomized into two lots – control lot that continued to take medication over the next 6 months (primary end-point), and lot subjected to RSDN [31].

In March 2014, Symplicity HTN-3 trial results were published. They appeared to shatter hopes around renal denervation as a method for the treatment of resistant hypertension. The change in office SBP at 6 months was the primary efficacy end-point, while the change in SBP measured by ABPM was the secondary end-point. At 6 months follow-up, office SBP in patients subjected to RSDN decreased by 14.1 mmHg and by 11.7 mmHg in the control group. The difference of 2.39 mmHg, with p=0.26, was statistically insignificant. The change in ambulatory SBP at 6 months was 6.8 mm Hg in the denervation group and 4.8 in the control group. The difference between the two groups was 1.96, with p=0.98, being also statistically insignificant.

However, the study has reached the point of primary safety, major adverse effects being recorded in 5 patients (1.4%) in the denervation lot and 1 (0.6%) patient in the control lot [32]. This study showed no RSDN benefit, although it demonstrated its safety. These data contradict the data obtained in previous trials that have demonstrated a significant decrease in blood pressure after RSDN. The obvious question arose: why were the Simplicity HTN-3 trial results so different compared to the previous two studies.

Certain assumptions were issued, namely that patients were not adequately stabilized before randomization, given that some drugs require more than 8 weeks to achieve maximum effect and the follow-up in the study was only 2 weeks. Moreover, in this study 40% of patients used direct vasodilators and a higher percentage took spironolactone. The patient population included in the study constituted another difference. In Simplicity HTN-3 one third of subjects were African-American, while Caucasians predominated in previous studies, being recognized a more difficult response of African-American hypertensive patients to therapy. Other observations are related to the absence of randomization in HTN-1, and, though HTN-2 was randomized, it was not blind [33].

Symplicity Global Registry study

Symplicity Global Registry study including patients with resistant hypertension is underway. It collects data on other diseases characterized by increased activity of the SNS such as type 2 diabetes, heart and renal failure, obstructive sleep apnea, etc. Enrollment of 5000 patients to be subjected to RSDN is expected in 231 international centers in 37 countries. After presenting the Symplicity HTN-3trial results, which showed RSDN inefficacy compared to placebo, there are preliminary data obtained from the Symplicity Global Registry, demonstrating significant reductions in both office and ambulatory SBP after 6 months.

These data were presented at the scientific session of the American College of Cardiology on March 30, 2013 [34].

Conclusions

1. Despite the negative results of the Symplicity HTN-3 trial, it is too early to make conclusions that RSDN therapy failed in the management of resistant hypertension.

2. There are sufficient clinical data from multiple clinical trials demonstrating positive effects both in lowering blood pressure and other diseases associated with increased activity of the SNS.

3. A more rigorous selection of patients is necessary to perform RSDN**,** at present the procedure being recommended only for patients with resistant hypertension.

4. RSDN is not a "panaceea" in the treatment of patients with resistant hypertension.

5. Several large randomized clinical trials are necessary.

6. A cost-effectiveness analysis of RSDN would be welcome.

References

1. Kearney PM, Whelton M, Reynolds K, et al. Global burden of hypertension: analysis of worldwide data. *Lancet.* 2005;365:217-223.
2. World Health Organization. World Health Report 2002: Reducing risks, promoting healthy life. Geneva, Switzerland: World Health Organization, 2002.
3. Chobanian AV, Bakris GL, Black HR, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension.* 2003;42:1206-1252.
4. Calhoun DA, Jones D, Textor S, et al. Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation*. 2008;117:e510-e526.
5. The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Arch Intern Med.* 1997;157:2413-2446.
6. Kaplan NM. Resistant hypertension. *J Hypertens.* 2005;23:1441-1444.
7. Sarafidis PA, Bakris GL. State of hypertension management in the United States: confluence of risk factors and the prevalence of resistant hypertension. *J Clin Hypertens (Greenwich).* 2008;10:130-139.
8. Smith PA, Graham LN, Mackintosh AF, Stoker JB, Mary DA. Relationship between central sympathetic activity and stages of human hypertension. *Am J Hypertens.* 2004;17:217-222.
9. Converse RL Jr, Jacobsen TN, Toto RD, et al. Sympathetic overactivity in patients with chronic renal failure. *N Engl J Med.* 1992;327:1912-1918.
10. Abramczyk P, Zwolinska A, Oficjalski P, Przybylski J. Kidney denervation combined with elimination of adrenal-renal portal circulation prevents the development of hypertension in spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol.* 1999;26:32-34.
11. Bertog SC, Sobotka PA, Sievert H. Renal denervation for hypertension. *JACC Cardiovasc Interv.* 2012;5:249-258.
12. DiBona GF, Esler M. Translational medicine: the antihypertensive effect of renal denervation. *Am J Physiol Regul. Integr Comp Physiol.* 2010;298:R245-R253.
13. Schlaich MP, Sobotka PA, Krum H, et al. Renal denervation as a therapeutic approach for hypertension: novel implications for an old concept. *Hypertension.* 2009;54:1195-1201.
14. Grassi G, Seravalle G, Dell'Oro R, Mancia G. Sympathetic mechanisms, organ damage, and antihypertensive treatment. *Curr Hypertens Rep*. 2011;13:303-308.
15. Ditting T, Freisinger W, Siegel K, et al. Tonic postganglionic sympathetic inhibition induced by afferent renal nerves. *Hypertension.* 2012;59:467-476.
16. Stella A, Zanchetti A. Functional role of renal afferents. *Physiol Rev*. 1991;71:659-682.
17. Hausberg M, Kosch M, Harmelink P, et al. Sympathetic nerve activity in end-stage renal disease. *Circulation.* 2002;106:1974-1979.
18. Ciriello J, de Oliveira CV. Renal afferents and hypertension. *Curr Hypertens Rep.* 2002;4:136-142.
19. Kopp UC, Smith LA, DiBona GF. Renorenal reflexes: neural components of ipsilateral and contralateral renal responses. *Am J Physiol.* 1985;249:F507-F517.
20. Evelyn KA, Alexander F, Cooper SR. Effect of sympathectomy on blood pressure in hypertension: a review of 13 years' experience of the Massachusetts General Hospital. *JAMA*.1949;140:592-602.
21. Smithwick RH, Bush RD, Kinsey D, Whitelaw GP. Hypertension and associated cardiovascular disease; comparison of male and female mortality rates and their influence on selection of therapy. *JAMA*. 1956;160(12):1023-1026.
22. Grimson KS, Orgain ES, Anderson B, et al. Results of treatment of patients with hypertension by total thoracic and partial to total lumbar sympathectomy, splanchnicectomy and celiac ganglionectomy. *Ann Surg.* 1949;129:850-871.
23. Prochnau D, Lucas N, Kuehnert H, et al. Catheter- based renal denervation for drug-resistant hypertension by using a standard electrophysiology catheter. *EuroIntervention.* 2012:7:1077-1080.
24. Thorén P. Efferent renal nerve traffic in the spontaneously hypertensive rat. *Clin Exp Hypertens A*. 1987;9(Suppl 1):259-279.
25. DiBona GF, Esler M. Translational medicine: the antihypertensive effect of renal denervation. *Am J Physiol Regul Integr Comp Physiol.* 2010;298:R245-R253.
26. Medtronic Announces U.S. Renal Denervation Pivotal Trial Fails to Meet Primary Efficacy Endpoint While Meeting Primary Safety Endpoint [press release]. 09/01/2014 2014.
27. Mahfoud F, et al. Expert consensus document from the European Society of Cardiology on catheter-based renal denervation. *Eur Heart J.* 2013;34:2149-57.
28. Davis MI, Filion KB, Zhang D, et al. Effectiveness of renal denervation therapy for resistant hypertension: a systematic review and meta-analysis. *J Am Coll Cardiol.* 2013;62:231-41.
29. Symplicity HTNI. Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months. *Hypertension.* 2011;57:911-17.
30. Symplicity HTNI, Esler MD, Krum H, et al. Renal sympathetic denervation in patients with treatment-resistant hypertension (The Symplicity HTN-2 Trial): a randomised controlled trial.Lancet 2010;376:1903–9.
31. Chinushi M, Izumi D, Iijima K, et al. Blood pressure and autonomic responses to electrical stimulation of the renal arterial nerves before and after ablation of the renal artery.Hypertension 2013;61:450–6.
32. Serruys PW. The scientific power of a "sham arm". Euro Intervention 2014;9:1129-31.
33. Persu A, Renkin J, Thijs L, et al. Renal denervation: ultima ratio or standard in treatment-resistant hypertension. Hypertension 2012;60:596–606.
34. Hitesh C Patel, Carl Hayward, Vassilis Vassiliou, Ketna Patel, James P Howard, Carlo Di Mario. Integr Blood Press Control. 2015; 8: 57–69.

Updates in diagnosis and treatment of acute pericarditis

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Abstract

Background: Acute pericarditis is inflammation of the pericardium that begins suddenly, is often painful, and causes fluid and blood components to enter the pericardial space. Acute pericarditis has numerous causes. However, in developed countries, roughly 80 to 90% of cases are idiopathic; that is, no specific cause is identified after routine evaluation. It is assumed that these cases are viral. The remaining 10 to 20% of cases are most commonly associated with post–cardiac injury syndromes, connective-tissue diseases, or cancer [1]. The true incidence of the disease is unknown, it is estimated that it accounts for 5% of emergency department visits for chest pain in the absence of myocardial infarction [2]. New diagnostic strategies have been proposed for the triage of patients with pericarditis and when additional diagnostic investigations are required to perform [3]. Major advances have occurred in therapy with the first multicentre randomized clinical trials.Colchicine has been demonstrated as a first-line drug to be added to conventional antiinflammatory therapies in patients with a first episode of pericarditis or recurrences [3,4]. The information presented here, also contains a clinical case of a patient admited to the cardiology clinic with pericardial effusion in acute pericarditis.
Conclusions: In the field of pericardial diseases there are a limited number of randomized controlled trials. Significant new data have become available since 2004, and the new version of recent guidelines published in 2015 have a great impact for clinical practice.
Key words: acute pericarditis, pericardial effusion, cardiac tamponade, cholchicine.

Introduction

Pericardial diseases may be either isolated disease or part of a systemic disease [4]. The main pericardial syndromes that are encountered in clinical practice include pericarditis (acute, subacute, chronic and recurrent), pericardial effusion, cardiac tamponade, constrictive pericarditis and pericardial masses (tab. 1, 2) [3]. In table 1 is presented a partial list of diseases that can involve the pericardium.

Acute pericarditis, defined as symptoms and/or signs resulting from pericardial inflammation of no more than 1 to 2 weeks duration, can occur in a variety of these diseases (denoted by asterisks), but most cases are considered idiopathic. The term idiopathic denotes acute pericarditis for which no specific cause can be found with routine diagnostic testing [5]. But majority of cases includes an immune-mediated process that is probably triggered by a viral infection in many cases, testing for specific viruses is not routine [6]. The etiology that can be found in a specific setting depends on the epidemiological background. Tuberculosis is the leading cause of pericardial diseases as well as pericarditis all over the world, being the most important etiology in developing countries where tuberculosis is endemic and often associated with HIV infection. On the contrary, tuberculosis is reported in <5% of cases in Western Europe and North America [7].

The true incidence and prevalence of the disease are unknown and there are a large number of undiagnosed cases. However, it may account for up to 5% of presentations to emergency departments for chest pain and up to 0.1% of hospital admissions. In an observational study from an urban area in Northen Italy the incidence of acute pericarditis was 27,7 cases per 100,000 persons per year [8]. A Swedish registry study found an incidence rate of 18,0 per 100 000 for pericarditis in the general population, in retired US military personnel, the incidence rate of pericarditis is 7,4 per 100 000 [9]. Many studies have reported conflicting results on the effect of sex on the risk of pericarditis. A recent randomized trial found 65% of 1361 patients to be male. Furthermore, the incidence rate of acute pericarditis in the general adult population was 2- fold among men compared with women [10]. Reasons for sex differences in pericardial inflammation are unknown, but experimental viral studies of myocardial inflammation have suggested that although genetic differences have some effect, sex hormones are major contributors for sex predisposition. Exogenous testosterone increases viral replication and inflammation in the heart and gonadectomy inhibits cardiac inflammation in experimental viral myocarditis. The in-hospital mortality rate for acute pericarditis is estimated at 1.10%. Female sex is associated with increased mortality in univariate analysis but was not an independent predictor of death in the multivariate model [11].

Case report

A 50 year old female patient M. was admitted to the Cardiologic Clinic on 08.02.2016 with difficulty of breath at low/medium physical effort, dull pericardial chest pain which was relieved by anterior thoracic bending, periodic cardiac palpitations, marked fatigue and light knee pain.

History. The symptoms became evident 2 weeks before when the fatigue and dyspnea progressed and the pericardial thoracic dull pain appeared along with nagging knee pain. During the last year she had 3-4 flu episodes, being liable to oral herpes infection, as well. Moreover, the patient has 1st

Table 1

Etiology of pericardial diseases [3]

A. Infectious causes
Viral (common): Enteroviruses (coxsackieviruses, echoviruses), herpes viruses (EBV, CMV, HHV-6), adenoviruses, parvovirus B19 (possible overlap with etiologic viral agents of myocarditis).
Bacterial: Mycobacterium tuberculosis (common, other bacterial rare), Coxiella burnetii, Borrelia burgdorferi, rarely: Pneumococcus spp, Meningococcus spp, Gonococcus spp, Streptococcus spp, Staphylococcus spp, Haemophilus spp, Chlamydia spp, Mycoplasma spp, Legionella spp, Leptospira spp, Listeria spp, Providencia stuartii.
Fungal (very rare): Histoplasma spp (more likely in immunocompetent patients), Aspergillus spp, Blastomyces spp, Candida spp (more likely in immunocompromised host).
Parasitic (very rare): Echinococcus spp, Toxoplasma spp
B. Non-infectious causes
Autoimmune (common): Systemic autoimmune and auto-inflammatory diseases (systemic lupus erythematosus, Sjögren syndrome, rheumatoid arthritis, scleroderma), systemic vasculitides (i.e. eosinophilic granulomatosis with polyangiitis or allergic granulomatosis, previously named Churg-Strauss syndrome, Horton disease, Takayasu disease, Behçet syndrome), sarcoidosis, familia Mediterranean fever, inflammatory bowel diseases, Still disease.
Neoplastic: Primary tumours (rare, above all pericardial mesothelioma). Secondary metastatic tumours (common, above all lung and breast cancer, lymphoma).
Metabolic: Uraemia, myxoedema, anorexia nervosa, other rare.
Traumatic and Iatrogenic: Early onset (rare): • Direct injury (penetrating thoracic injury, an esophageal perforation). • Indirect injury (non-penetrating thoracic injury, radiation injury). Delayed onset: Pericardial injury syndromes (common) such as postmyocardial infarction syndrome, postpericardiotomy syndrome, posttraumatic, including forms after iatrogenic trauma (e.g. coronary percutaneous intervention, pacemaker lead insertion and radiofrequency ablation).
Drug-related (rare): Lupus-like syndrome (procainamide, hydralazine, methyldopa, isoniazid, phenytoin); antineoplastic drugs (often associated with a cardiomyopathy, may cause a pericardiopathy): doxorubicin, daunorubicin, cardiomyopathy, may cause a pericardiopathy): doxorubicin, daunorubicin, hypersensitivity pericarditis with eosinophilia; amiodarone, methysergide, mesalazine, clozapine, minoxidil, dantrolene, practolol, phenylbutazone, thiazides, streptomycin, thiouracils, streptokinase, p-aminosalicylic acid, sulfadrugs, cyclosporine, bromocriptine, several vaccines, GM-CSF, anti-TNF agents.
Other (common): Amyloidosis, aortic dissection, pulmonary arterial hypertension and chronic heart failure.
Other (uncommon): congenital partial and complete absence of the pericardium.

degree anemia for 5-6 years and periodically uses iron medication for short period courses.

Personal history – normal physical childhood development. Profile history – works as a nurse in the emergency healthcare (physical strain, reduced sleeping).

Clinical examination showed pale pink skin without peripheral edema, and absence of jugular veins turgor. Normal pulmonary clinical examination. The clinical examination of the cardiovascular system showed the increased right and left relative cardiac dullness limits. Rhythmic and clear cardiac sounds with the cardiac contractions frequency of 84 bpm, and a blood pressure of 150/90 mmHg. Well-developed musculoskeletal system with no pain in joint movement. Light hepatomegaly. The thyroid is not palpable.

Investigations. All biological data was in normal range except for the 1st degree anemia (Hemoglobin level 99 g/l, red blood cells (RBC) 3,3x10^12/l) with anisocytosis and hypochromic RBC (microcytes), with a normal serum iron level – 9,9 umol/L, thrombocytes – 412.5x10^9/l, leucocytes – 3.8x10^9/l, and the inflammatory markers: erythrocyte sedimentation rate (ESR) – 40 mm/h, C-reactive protein (CRP) – 24 mg, antistreptolysine O – 200, and a negative latex-test. Troponins – negative, creatine kinase- MB (CK-MB) – 8 U/l, total creatine kinase (CK) – 25 U/l and alkaline phosphatase – 227 U/l.

Electrocardiogram (fig. 1): Sinus rhythm with cardiac contractions frequency – 82 bpm, normal electrical axis of the heart. Flattening of the T waves in all leads.

Thoracic radiography (fig. 2): Pulmonary area with signs of venous stasis. Pulmonary hypertension. Left basal disk shaped atelectasis. Bilateral free costo-diaphragmatic sinuses. Transversally dilated heart.

Echocardiography (fig. 3): Ascending Aorta – 34 mm, left atrium – 44 mm, left ventricle (LV) – 57 mm, right ventricle (RV) – 23 mm, right atrium (RA) – 42 mm, *interventricular septum* (IVS) and *left ventricular posterior wall* (*LVPW*) – 8 mm. *Pulmonary artery pressure* (PAP) – 43 mmHg. Considerable amount of fluid in the pericardium: LVPW – 25 mm, lateral wall of left ventricle (LWLV) – 28 mm, RA basal – 22 mm, anterior wall of right ventricle (AWRV) – 10mm, apex -10 mm. Conclusion: Light dilation of LA, RA, LV. Signs of RA collapse. Mitral valve insufficiency I-II degree, tricuspid valve insufficiency II degree. Preserved cardiac pump function (Ejection fraction (EF) – 65%).

Taking into consideration the clinical data and the investigations there was established the diagnosis: Exudative acute pericarditis, considerable amount of pericardial effusion. Mitral valve insufficiency (II degree), tricuspid valve insufficiency (II degree). Heart failure NYHA II. Anemia of unknown etiology.

Question No 1: What are the diagnostic criteria of acute pericarditis and which one of them is seen in the presented patient?

The classic presentation of acute pericarditis, regardless of its etiology, is a patient with chest pain that is sharp, pleuritic in nature, retrosternal or left-sided in location, that is present in more than 85-90% of cases [3]. The pain is often exacerbated by lying down and is relieved by sitting up or leaning forward. The pain may radiate to the neck, arms, or left shoulder, making it difficult to differentiate from the pain of myocardial infarction. However, pain that radiates to one or both trapezius muscle ridges suggests pericarditis because the phrenic nerve innervates these muscles and crosses the pericardium as well [12]. Acute chest pain may or may not occur in patients with uremic pericarditis or pericarditis associated with rheumatologic disorders, although pleuritic chest pain may be the initial presentation of systemic lupus erythematosus. A pericardial friction rub, which is highly

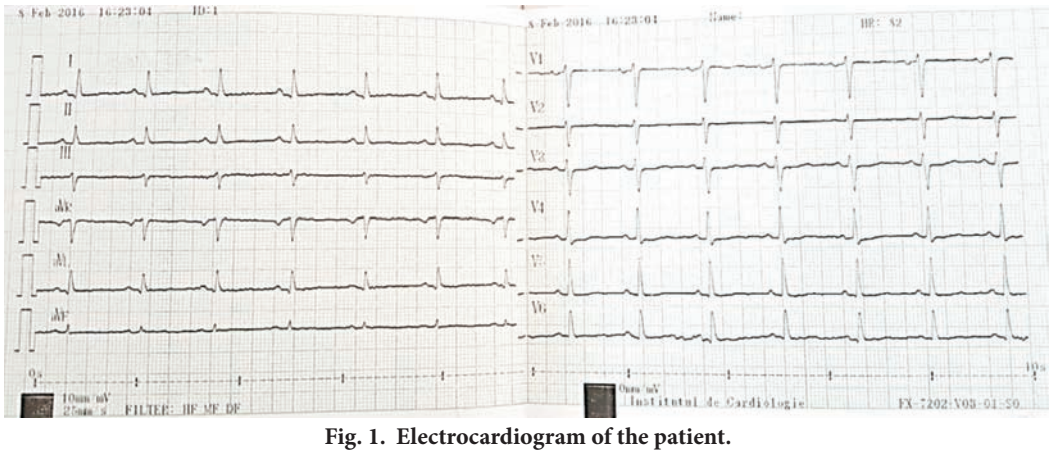

Fig. 1. Electrocardiogram of the patient.

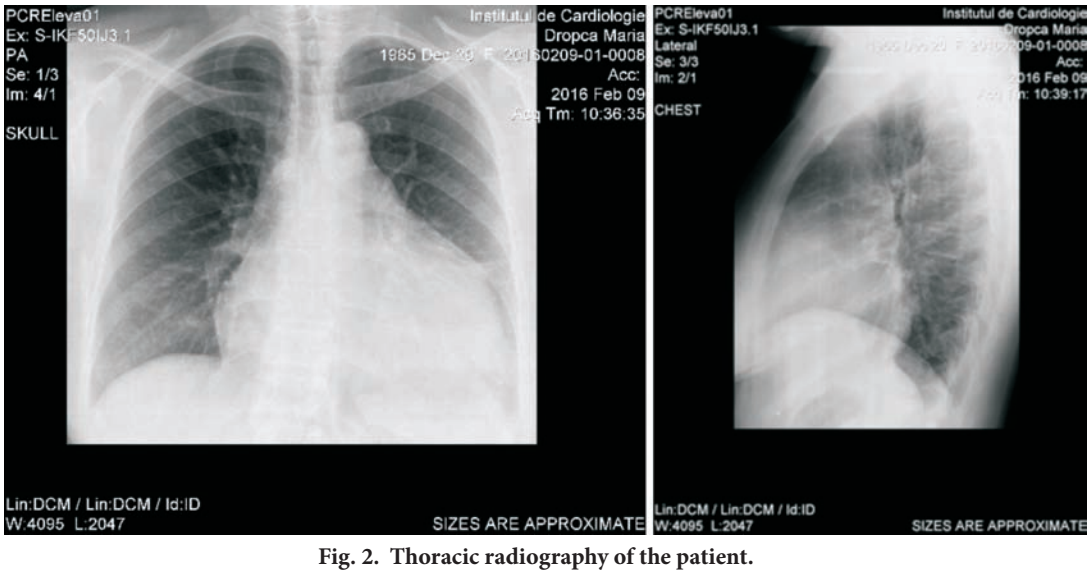

Fig. 2. Thoracic radiography of the patient.

specific and pathognomonic for acute pericarditis, occurs in up to 33% of patients, but its absence does not exclude the diagnosis [3]. According to the European Society of Cardiology, 2015 (ESC), the diagnosis of acute pericarditis is established by the presence of at least 2 of the 4 criteria presented in Table 2 with/ without additional signs.

The electrocardiogram (ECG) is the most important tool in the diagnosis of pericarditis. Typical ECG changes have been reported in up to 60% of cases [13]. It may show sinus tachycardia and widespread ST-segment elevation which has been considered the hallmark of acute pericarditis [14]. The ST segment is usually coved upward and lead involvement in acute pericarditis is more extensive than reciprocal ST-segment depression in ischemia. Another recent criteria to differentiate acute ST segment elevation myocardial infarction (STEMI) from acute pericarditis is the prolongation of the QRS complex and shortening of the QT interval in ECG leads with ST segment elevation which are not the case in patients

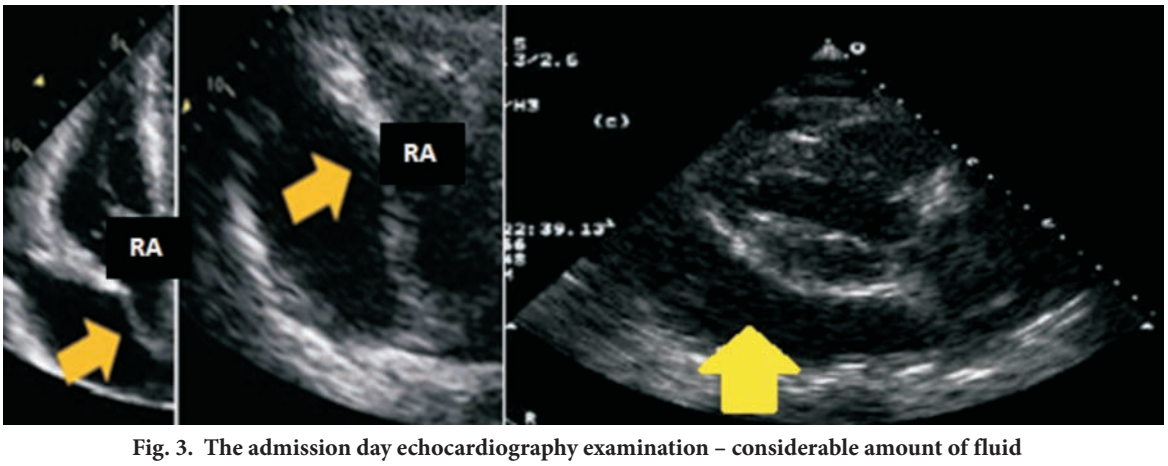

Fig. 3. The admission day echocardiography examination – considerable amount of fluid in the pericardium and signs of RA collapse.

with pericarditis [15]. PR-depression is another feature in the ECG of the patient with pericarditis. According to a study by Porela et al. the most common location for PR depression was lead II (55.9%), while this ECG finding least likely appeared in lead aVL (2.9%). PR depression in any lead had a high sensitivity (88.2%), but fairly low specificity (78.3%) for miopericarditis. The combination of PR depressions in both precordial and limb leads had the most favorable predictive power to differentiate miopericarditis from STEMI (positive 96.7% and negative power 90%) [16]. A useful data suggests how to distinguish by ECG acute pericarditis and benign early repolarization as both conditions are associated with concave ST elevation. Thus, the vertical height of the ST segment elevation (from the end of the PR segment to the J point) is measured and compared to the amplitude of the T wave in V6. A ratio of > 0.25 suggests pericarditis and ratio of < 0.25 suggests benign early repolarization [17].

Other mandatory checks besides auscultation and ECG according to the latest European guidelines on pericardial diseases are echocardiography to rule out effusion concomitant heart disease or signs of myocarditis. Transthoracic echocardiogram (TTE) is the first-line imaging test to detect pericardial effusion and tamponade physiology. Although many patients with acute pericarditis may appear to have normal echocardiographic results, the presence of an effusion is consistent with acute pericarditis [18]. Other findings may include increased pericardial brightness, pericardial thickening, and abnormal septal bounce, suggesting early constriction. A pericardial effusion can be trivial to large and localized, loculated, or circumferential. Importantly, tamponade physiology can be seen in 3% of patients. Intrapericardial fibrinous strands suggest either an inflammatory etiology or clotted blood. TTE may also help differentiate acute pericarditis from myocardial ischemia or injury by excluding wall motion abnormalities, even though a small percentage of patients with acute pericarditis (5%) will demonstrate segmental wall motion abnormalities. If the results of TTE are negative or equivocal in a patient suspected to have acute pericarditis with poor prognostic signs, or evidence of hemodynamic compromise, the most sensitive subsequent test is CMR, which shows edema and inflammation as well as features of constrictive physiology. The choice of CT or CMR should be based on the specific clinical question according to the strength of these modalities. The following are scenarios in which additional imaging with CT or CMR may be considered:

- Inconclusive echocardiographic findings and ongoing clinical concern;
- Failure to respond promptly to anti-inflammatory therapy;
- Atypical clinical presentation;
- Search for a specific cause (i.e., malignancy or tuberculosis);
- Suspicion of constrictive pericarditis or effusive constrictive pericarditis;
- Associated trauma (penetrating injury, chest injury); and
- Acute pericarditis in the setting of acute myocardial infarction, neoplasm, lung or chest infection, or pancreatitis [19].

A chest X-ray is generally normal in patients with acute pericarditis since an increased cardiothoracic ratio only occurs with pericardial effusions exceeding 300 ml [20].

Elevation of markers of inflammation (i.e. CRP and ESR), as well as elevation of the white blood cell count) is a common and supportive finding in patients with acute pericarditis and may be helpful for monitoring the activity of the disease and efficacy of therapy. Patients with concomitant myocarditis may present with an elevation of markers of myocardial injury (i.e., CK, troponin) [21].

Our patient presented 2 of those 4 mentioned criteria: pericardial type pain and pericardial effusion (on TTE and thoracic radiography) and the additional signs found were the inflammatory markers – elevated ESR and CRP levels. Electrocardiogram didn't show typical signs, only flattening of the T waves in all leads.

Table 2

Definitions and diagnostic criteria for pericarditis [3]

Pericarditis	Definition and diagnostic criteria
Acute	Inflammatory pericardial syndrome to be diagnosed with at least 2 of the 4 following criteria: (1) pericarditic chest pain (> 85- 90% of cases) (2) pericardial rubs (≤33% of cases) (3) new widespread ST-elevation or PR depression on ECG (60% of cases) (4) pericardial effusion (new or worsening) (60% of cases) Additional supporting findings: - Elevation of markers of inflammation (i.e. C-reactive protein, erythrocyte sedimentation rate, and white blood cell count); - Evidence of pericardial inflammation by an imaging technique (CT, CMR).
Incesant	Pericarditis lasting for > 4-6 weeks but <3 months without remission.
Recurrent	Recurrence of pericarditis after a documented first episode of acute pericarditis and a symptom-free interval of 4–6 weeks or longer (usually within 18-24 months).
Chronic	Pericarditis lasting for >3 months.

Question No 2: What is the possible etiology of pericarditis in this patient?

In this patient there were evaluated the following possible causes of acute pericarditis:

- Pericarditis in the context of autoimmune diseases (System Lupus Erythematosus, Rheumatoid arthritis, Spondyloarthropathy, etc.), because the patient had joint pain when moving and resting.
- Myxedematous pericarditis
- Viral pericarditis

Pericarditis in the context of systemic diseases was excluded through the evaluation of systemic autoimmune markers, which happened to be negative (tab. 3).

Myxedematous pericarditis was excluded through the evaluation of thyroid hormones, which happened to be in normal range and the thyroid ultrasound examination did not find any pathological changes.

Table 3

Systemic autoimmune markers

Parameter	Results
Anti-ANA, IgG	Negative
dsDNA	Negative
Nucleosomes	Negative
Histones	Negative
SmD1	Negative
PCNA	Negative
PO	Negative
SS-A/Ro60kD	Negative
SS-A/Ro52kD	Negative
SS-B/La	Negative
CENP-B	Negative
Scl-70	Negative
1 snRNP	Negative
AMA M2	Negative
Jo-1	Negative
Pm-Scl	Negative
Ku	Negative
Mi-2	Negative

Frequently, the cause of pericarditis cannot be found, the idiopathic pericarditis having an estimated incidence of 85 – 90 % [22], but many of these cases are probably viral, viral pericarditis being the most frequent cause of infectious pericarditis. Most likely, in the presented case report, the cause of acute pericarditis remains to be viral, as the patient had episodes of flu in the previous period of time, that went through with catarrhal signs (persistent rhinorrhea, viral conjunctivitis) and had a rapid regression. Moreover, denoting that the patient is liable to oral herpes infection.

Question No 3: Is pericardiocentesis a treatment option?

In correspondence with the management algorithm of pericardial effusion mentioned in the ESC 2015 guide for pericarditis, pericardiocentesis or cardiac surgery is a 1C class indication in case of cardiac tamponade, or in case of symptomatic moderate – large pericardial effusion which does not respond to medication or in case of unknown bacterial or neoplastic etiology suspecting (fig. 4). In this case there are no indications for pericardiocentesis, because the patient was hemodynamically stable, without paradox pulse, besides the echocardiographic signs of RA collapse. The diagnosis of cardiac tamponade is a more clinical one and in the absence of hypotension, paradox pulse cannot be established, as well as, during the clinical examination and from the history there was not appreciated the coexistence of a neoplastic process. An eventual pericardiocentesis was difficult to perform because of the possible complications and the technique – since the pericardial fluid was located in the posterior, hard to access space.

Question No 4: What would be the appropriate treatment in this patient?

The medication choice has to deal with the patients' history (contraindications, anterior efficacy or medication

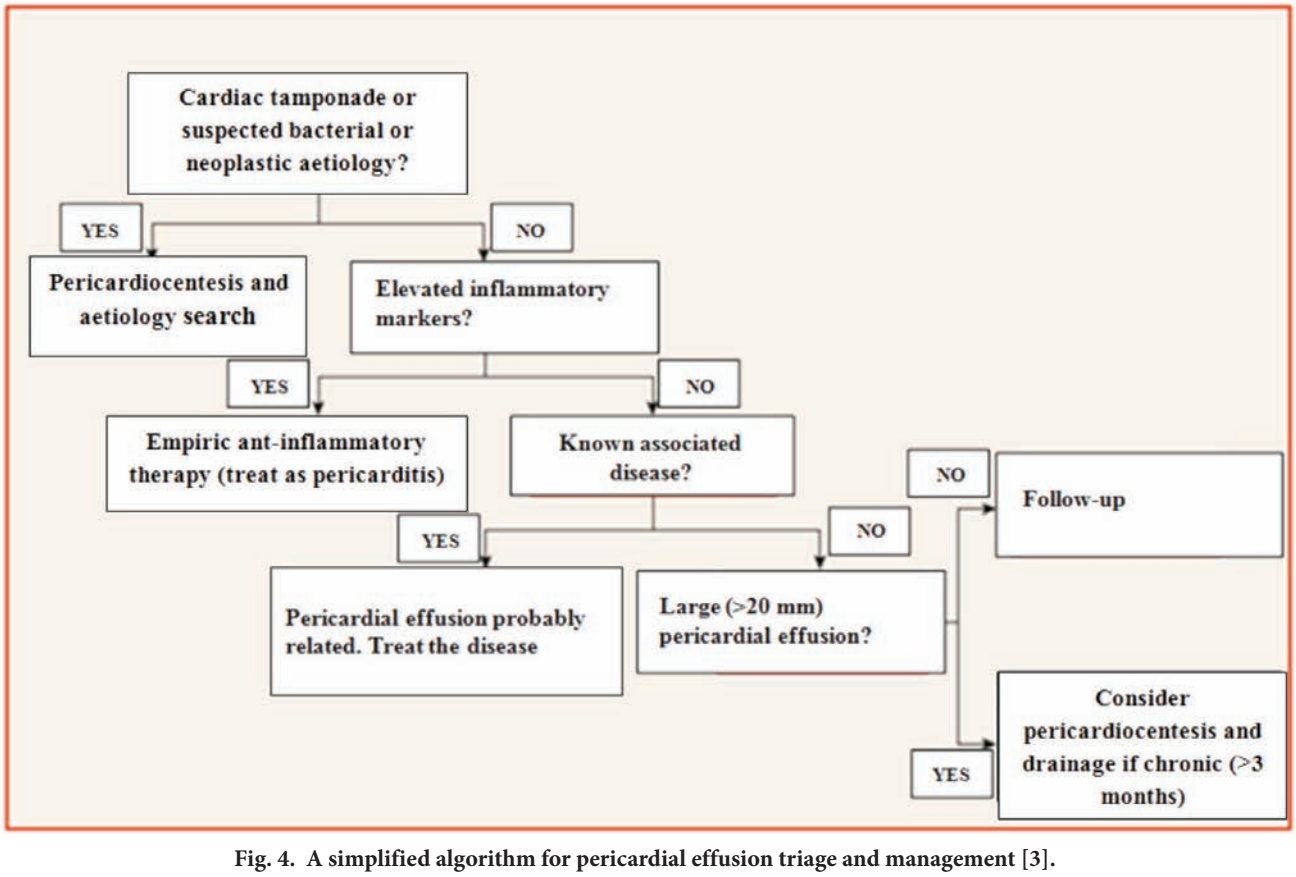

Fig. 4. A simplified algorithm for pericardial effusion triage and management [3].

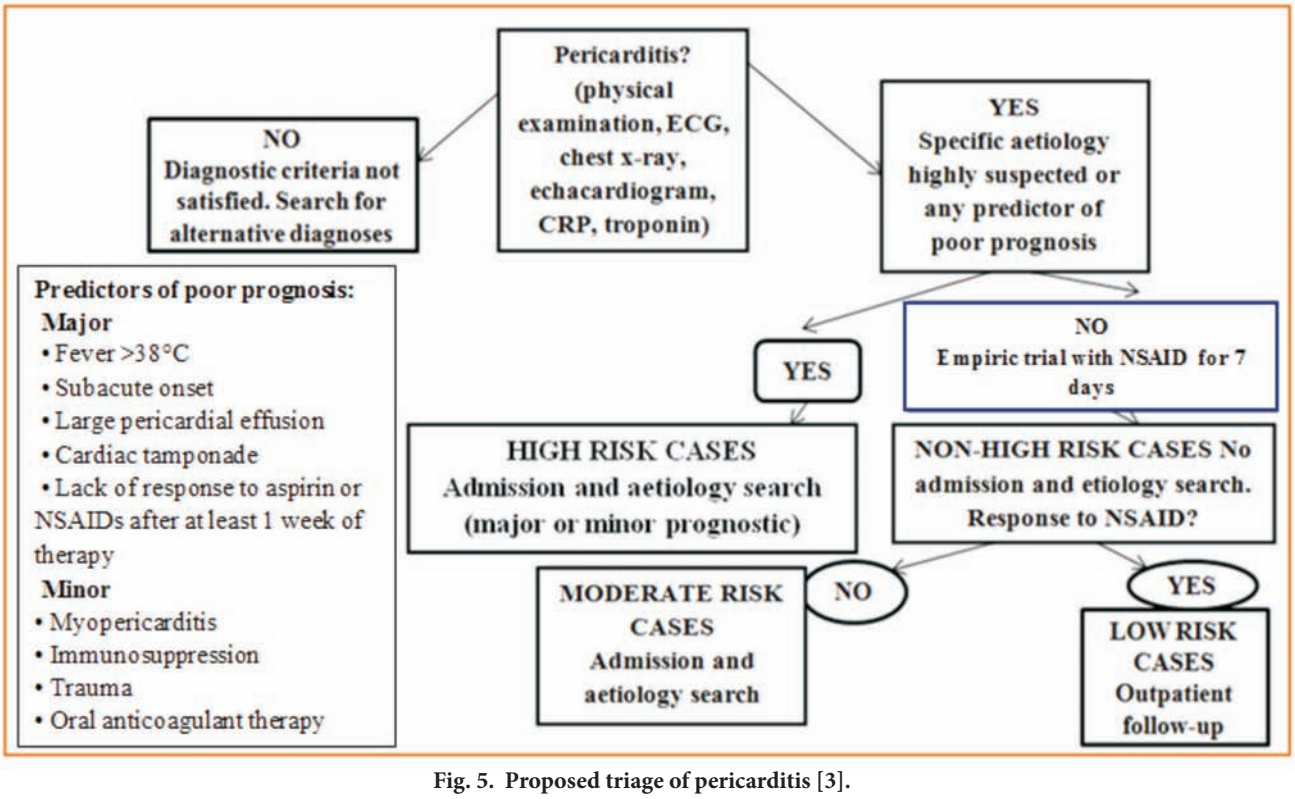

Fig. 5. Proposed triage of pericarditis [3].

side effects), the accompanying diseases [23]. The first non-pharmacologic precaution is avoiding physical effort until stabilization of CRP and the symptoms. Acute idiopathic pericarditis is a self-limited disease with a recurrence rate after an initial episode from 15 to 30% [24]. Because of its excellent safety profile, is preferred ibuprofen (600 mg every 8h). Acetylsalicylic acid (ASA), 750–1000 mg every 8h daily in divided doses, is an alternative and often preferable in patients who require ASA for other indications, as in patients with pericarditis after myocardial infarction because other NSAIDs have delayed infarct healing in animal models and are associated with an increased risk of future coronary events in this population In either case, gastric protection in the form of a proton pump inhibitor should be provided [25]. Colchicine is recommended at low, weight-adjusted doses (0.5 mg once (<70 kg) or 0.5 mg b.i.d. (≥70 kg)) to improve the response to medical therapy and significantly reduced the rate of subsequent recurrences of pericarditis in patients with multiple recurrences. Taken together with results from other randomized controlled trials, colchicine should be probably regarded as a first-line treatment for either acute or recurrent pericarditis in the absence of contraindications or specific indications [26]. Corticosteroids should be considered as a second option in patients with contraindications and failure of aspirin or NSAIDs because of the risk of favoring the chronic evolution of the disease and promoting drug dependence [27].

Thus, in this patient was started empiric treatment (according to the algorithm in fig. 5) with ibuprofen – 1600 mg/day divided in 4 doses and colchicine – 1 mg, ½ tablet x 2/24h, beta – blockers and diuretics. At the 7th day from admission the echocardiography examination was repeated (Fig. 6) to evaluate the pericardial effusion dynamics, where there was found an essential reduction of the pericardial effusion: LVPW – 9 mm, LWLV – 8 mm, RA basal – 3 mm, AWRV – 3mm, apex – 3mm. As well, the inflammatory markers reduced: ESR – 20 mm/h, CRP – 6 mg.

Question No 5: How long does the treatment take?

The ibuprofen recommended treatment duration is 1 – 2 weeks (the patient administered ibuprofen being in the hospital for 8 days, and at discharge she was recommended to continue administering it 6 more days)

Question No 6: Why is the prolonged colchicine treatment necessary?

According to the recent data the simultaneous administration of colchicine for 3 months favors a rapid regression of the symptoms and with fewer recurrences in comparison with patients which administered only NSAIDs and/or colchicine less time [28]. The recurrence rate after an initial episode of pericarditis may increase to 50% after a first recurrence of acute pericarditis in patients not treated with colchicine [29].

Question No 7: What is the prognosis in acute pericarditis?

The prognosis for acute pericarditis is usually good. Although mortality in idiopathic/viral pericarditis is low, purulent pericarditis is always fatal if untreated and carries a mortality of approximately 40% even when treated [29]. In the acute pericarditis it is important to consider the high risk factors for development of complications during the pathologic evolution (cardiac tamponade, recurrence and constriction) [30]. The risk factors associated with a negative prognosis

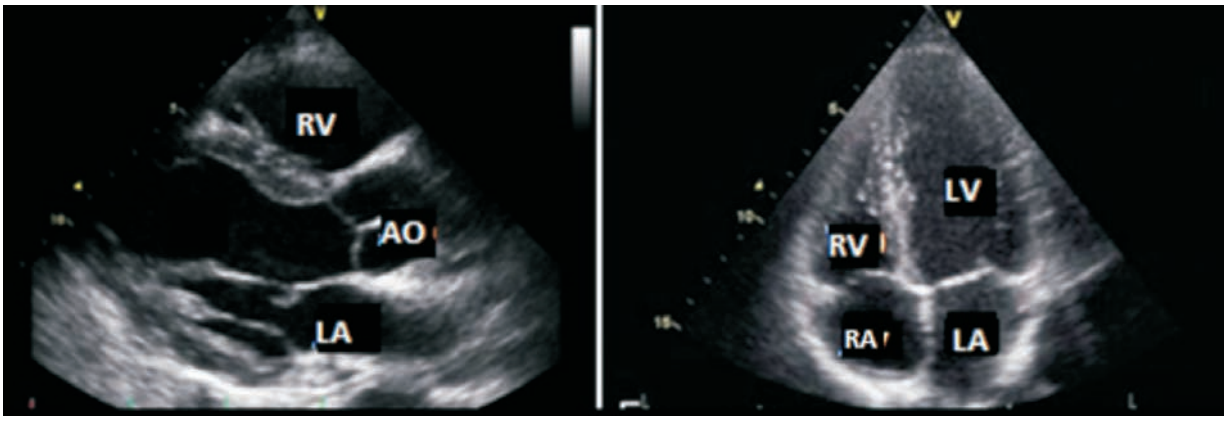

Fig. 6 The echocardiography examination at the 7th day from admission.

are: severe fever (>38°C), subacute evolution, evidence of pericardial fluid in significant amount (> 20 mm), cardiac tamponade, the treatment response absence during a 7 day NSAIDs course, or other minor risk factors as: pericarditis in association with myocarditis, immunosuppression, trauma or oral anticoagulation treatment. Perhaps 15% to 30% of patients with apparent idiopathic acute pericarditis who respond satisfactorily to treatment suffer a relapse. Genetic disorders of the immune system underlie some cases of recurrent pericarditis. A recent study found that 8 of 131 (6.1%) patients thought to have recurrent idiopathic pericarditis had mutations of the TNFRSF1A gene that causes tumor necrosis factor receptor-1–associated periodic syndrome (TRAPS), 25 a monogenic disorder resulting in dysfunction of the innate immune system with periodic fever, rash, abdominal pain, periorbital edema, and polyserositis with pericarditis. Patients with TRAPS respond to corticosteroids but not to colchicine [31]. In another report, 4 of 30 patients with recurrent pericarditis refractory to colchicine were found to have TNFRSF1A mutations. A report that human leukocyte antigen allel patterns are associated with recurrent pericarditis also supports a role of genetic variation in innate immunity as an underlying determinant. The extent to which TRAPS and other innate immune disorders are responsible for recurrent pericarditis merits additional research [32].

In the presented patient there was found a single risk factor – significant amount of pericardial fluid which had a good response to the administered treatment. The majority of patients with acute pericarditis have a long term favorable prognosis. Constrictive pericarditis can appear in < 1% of acute idiopathic pericarditis cases, in about 2 – 5 % of autoimmune pericarditis and about 20 – 30 % of the bacterial ones, especially tuberculous [33].

References

1. Caren G. Solomon. Acute Pericarditis. *N Engl J Med*. 2014;371:2410-2416. DOI: 10.1056/ NEJMcp1404070.
2. Imazio M. Contemporary management of pericardial diseases. *Curr Opin Cardiol*. 2012;27:308-317.
3. 3.Yehuda Adle, Philippe Charron, Massimo Imazio, et al. ESC Guidelines for the diagnosis and management of pericardial diseases. *European Heart Journal*. 2015; doi:10.1093/eurheartj/ehv318.
4. Imazio M, Gaita F. Diagnosis and treatment of pericarditis. *Heart*. 2015;101:1159-1168.
5. Douglas L. Mann, Douglas P. Zipes, Peter Libby, et al. Braunwald's Heart Disease a textbook of cardiovascular medicine, Tenth Edition. *Elisever Sounders*. 2015;1637-1639.
6. Pankuweit S, Stein A, Karatolios K, et al. Viral genomes in the pericardial fluid and in peri- and epicardial biopsies from a German cohort of patients with large to moderate pericardial effusions. *Heart Fail Rev*. 2013;18:329-336.
7. LeWinter MM. Clinical practice. Acute pericarditis. *N Engl J Med*. 2014;371:2410-2416.
8. Herzog E. Management of Pericardial Disease. *Springer INternatiol Publishing. 2014*. DPO 10.10007/978-3-319-06124-5_8.
9. Lin AH, Phan HA, Barthel RV, et al. Myopericarditis and pericarditis in the deployed military member: a retrospective series. *Mil Med*. 2013;178:18-20.
10. Kytö V, Sipilä J, Rautava P. Clinical Profile and Influences on Outcomes in Patients Hospitalized for Acute Pericarditis. *Circulation*.2014;130(18):1601-6. doi: 10.1161/CIRCULATIONAHA.114.010376.
11. Fairweather D, Cooper LT Jr., Blauwet LA. Sex and gender differences in myocarditis and dilated cardiomyopathy. *Curr Probl Cardiol*. 2013;38:7-46.
12. Troughton RW, Asher CR, Klein AL. Pericarditis. *Lancet*. 2004;363:717-27.
13. Bhardwaj R, Berzingi C, Miller C, et al. Differential diagnosis of acute pericarditis from normal variant early repolarization and left ventricular hypertrophy with early repolarization: an electrocardiographic study. *Am J Med Sci*. 2013;345:28-32.
14. Imazio M, Spodick DH, Brucato A, et al. Diagnostic issues in the clinical management of pericarditis. *Int J Clin Pract*. 2010;64(10):1384-92.
15. Rossello X, Wiegerinck RF, Alguersuari J, et al. New Electrocardiographic Criteria to Differentiate Acute Pericarditis and Myocardial Infarction. *Am J Med*. 2013;25; 456(24).
16. Porela P, Kytö V, Nikus K, et al. PR depression is useful in the differential diagnosis of myopericarditis and ST elevation myocardial infarction. *Ann Noninvasive Electrocardiol*. 2012;17(2):141-5.
17. Tikkanen JT, Anttonen O, Junttila MJ, et al. Long-term outcome associated with early repolarization on electrocardiography. *N Engl J Med*. 2009;24;361(26):2529-37.
18. Salisbury AC, Olalla-Gomez C, Rihal CS, et al. Frequency and predictors of urgent coronary angiography in patients with acute pericarditis. *Mayo Clin Proc*. 2009;84:11-5.
19. Allan L. Klein, Suhny Abbara, Deborah A. Agler. ASE expert consensus statement American Society of Echocardiography Clinical Recommendations for Multimodality Cardiovascular Imaging of Patients with Pericardial Disease Endorsed by the Society for Cardiovascular Magnetic Resonance and Society of Cardiovascular Computed Tomography. *J Am Soc Echocardiogr*. 2013;26:965-1012.
20. Imazio M, Adler Y. Management of pericardial effusion. *Eur Heart J*. 2013;34:1186-1197.
21. Imazio M, Brucato A, Maestroni S, et al. Prevalence of C-reactive protein elevation and time course of normalization in acute pericarditis: implications for the diagnosis, therapy, and prognosis of pericarditis. *Circulation*. 2011;123:1092-1097.

22. Masud H. Khandaker, Raul E. Espinosa, Rick A. Nishimura. Pericardial Disease: Diagnosis and Management. *Mayo Clin Proc*. 2010;85(6):572-593. doi: 10.4065/mcp.2010.0046
23. Imazio M, Brucato A, Trinchero R, Spodick D. Individualized therapy for pericarditis. *Expert Rev Cardiovasc Ther*. 2009;7:965-975.
24. Buiatti A, Merlo M, Pinamonti B, et al. Clinical presentation and long-term follow-up of perimyocarditis. *J Cardiovasc Med (Hagerstown)*. 2013;14:235.
25. Olsen AM, Fosbøl EL, Lindhardsen J, et al. Long-term cardiovascular risk of nonsteroidal anti-inflammatory drug use according to time passed after first-time myocardial infarction: a nationwide cohort study. *Circulation.* 2012;126:1955-1963.
26. Brucato A, Cemin R, Ferrua S, et al. Efficacy and safety of colchicine for treatment of multiple recurrences of pericarditis (CORP-2): a multicentre, double-blind, placebo-controlled, randomised trial. *Lancet*. 2014;28;383(9936):2232-7. doi: 10.1016/S0140-6736(13)62709-9.
27. Alabed S, Cabello JB, Irving GJ, et al. Colchicine for pericarditis. *Cochrane Database Syst Rev*. 2014;28(8):CD010652.
28. Imazio M, Brucato A, Cemin R, et al. ICAP Investigators. A randomized trial of colchicine for acute pericarditis. *N. Engl J Med*. 2013;369:1522-1528.
29. Fairweather D, Cooper LT Jr., Blauwet LA. Sex and gender differences in myocarditis and dilated cardiomyopathy. *Curr Probl Cardiol*. 2013;38:7-46.
30. Spodick DH. Acute cardiac tamponade. *N Engl J Med*. 2003;349(7):684-690.
31. Cantarini L, Lucherini OM, Brucato A, et al. Clues to detect tumor necrosis factor receptor–associated periodic syndrome (TRAPS) among patients with idiopathic recurrent acute pericarditis: results of a multicentre study. *Clin Res Cardiol.* 2012;101:525.
32. Cantarini L, Lucherini OM, Muscari I, et al. Tumour necrosis factor receptor–associated periodic syndrome (TRAPS): State of the art and future perspectives. *Autoimmun Rev*. 2012;12:38.
33. Imazio M, Belli R, Brucato A, et al. Efficacy and safety of colchicine for treatment of multiple recurrences of pericarditis (CORP-2): a multicentre, double-blind, placebo-controlled, randomised trial. *Lancet*. 2014;383:2232-2237.

The use of medical ozone in dentistry

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Abstract

Background: Lately, due to a higher incidence rate of infections, as a result of a decreased immuno-inflammatory response capacity of the human body and an expanded role of viral agents in triggering inflammatory diseases, the interest for the use of medical ozone has increased significantly. The reason for this growth of popularity lies in the fact that medical ozone has antimicrobial, antiviral, anti-inflammatory, immunomodulatory, antioxidant and other properties; it is not a pharmacological product, but rather a "hormetic stress" [18], a pure ecologic physico-chemical factor with multiple biological effects [19].

Conclusions: The action of medical ozone on the human body is diverse and multidirectional. The pharmacological effect of medical ozone represents the so-called "hormesis" phenomenon, and this effect is triggered by short chains of hydroxides – hydro-peroxides, also defined as ozone peroxides. Medical ozone possesses antalgic, anti-inflammatory, anti-edematous, antioxidant, myorelaxant, detoxing, immunomodulatory, antimicrobial, antiviral, antifungal effects, it activates the cellular metabolic processes, stimulates oxygen metabolism and improves rheological properties of blood. In dentistry, medical ozone is used on its own or in combination with other treatment methods. At the same time, the aspects of the use of medical ozone in clinical periodontology requires additional research.

Key words: medical ozone, dentistry.

Introduction

The action of medical ozone on the human body is diverse and multifaceted. In various medical fields ozone therapy has been applied for several decades, however the use of ozone in dentistry is relatively recent, since the middle of the 90s. Medical ozone has antalgic, anti-inflammatory, anti-edematous, antioxidant, myorelaxant, detoxing, immunomodulatory [1, 2], antimicrobian, antiviral and antifungal properties; it activates cellular metabolic processes, stimulates oxygen metabolism and improves the rheological properties of the blood [4, 17].

The efficiency of the use of medical ozone has been proven and debated in the treatment of a series of systemic diseases, mainly of inflammatory nature, both in surgical and therapeutic fields [5, 6]. In comparison with the therapy with antibiotics, ozone therapy has a larger spectrum of therapeutic actions, it does not result in microbial resistance and does not trigger mutagenic and carcinogenic effects [7, 8]. Moreover, in case of the existence of microflora resistant to antibiotic treatment, supplementing antibiotic treatment with ozone therapy leads to the neutralization of antibiotic resistance and intensification of the overall effects of antibiotics [9, 10]. In

dentistry, medical ozone is used on its own or in combination with other treatment methods [11, 12, 13].

Historical background

Ozone was mentioned for the first time by the Dutch physicist M. van Marum in 1785, who observed the formation of an oxidizing gas with a characteristic smell during some experiments with electrical discharges. The name ozone, though, comes from the German chemist Friedrich Schonbein in 1840, who discovered for the first time the capacity of ozone to react with biological substrates. In 1857 the German engineer and inventor Werner von Siemens develops the first technical unit of ozone, yet in 1880, the first medical publications appear in America, attesting its therapeutic efficacy. The first generator of ozone was patented in 1896 by the physicist Nicola Tesla and commercialized from 1900. Subsequently, he was the first to ozonize olive oil for medical use. During the First World War, ozone was used for disinfection and treatment of plagues and the treatment of wounds and gangrene. In 1935 the surgeon Erwin Payr presents his publication of 290 pages about the application of ozone in surgery, while the Swiss dentist E. A. Fisch was the first to use ozone in dentistry and is the author of many publications about the use of ozone, defended his thesis on this theme in 1950.

In the following years, due to Dr. J. Hansler, the first medical ozone generator was created. Together with H. Wolff, the author of the book "Medical Ozone", Hansler sets up the Medical Society for Ozone Therapy [14, 14, 16]. In 1973 the International Ozone Association (IOA) was created, and in 2005 the World Federation of Ozone Therapy (WFOT).

Today, after 160 years of use, ozone therapy is recognized and applied in 50 countries [17].

Lately, due to the growth of the number of cases of infections associated with the background of reduction of the immuno-inflammatory response of the body and the increase of the role of viral agents in the outbreak of inflammatory diseases, the interest in the medical use of ozone has considerably increased. The reason for this growth of popularity is that ozone, despite having antimicrobial, antiviral, anti-inflammatory, immuno-modulatory, antioxidant etc. characteristics, is not a pharmacologic substance, but a "hormetic stress" [18], an ecologically pure physico-chemical factor with multiple biological effects [19].

The physicochemical properties and biological effects of ozone

Ozone (derived from the Greek *ozein*, meaning "to smell"), is the second allotrope (active) form of oxygen – and is a gas of a bluish color, with a specific odor; in small quantities it has a pleasant "smell of purified and fresh air", which becomes stingy and intensely irritating when in large proportions.

Its molecule is made of three oxygen atoms (O3), has a strong affinity for the electron (1,9 eV) and has a molecular weight of 48.00 g/mol. In normal temperature and pressure conditions (0°C; 1atm=101.3kPa), the weight of one liter of ozone equals to 2.143g/l. One liter of air weighs 1.429g/l, respectively ozone is heavier than air. The melting point of ozone is minus 192.5°C and it acquires a bluish-dark violet color. The boiling point is minus 119.9°C. The rate of dissolving of ozone in water is 49.4 ml/100ml (0°C; 1atm=101.3kPa), which is 10 times higher than the rate for oxygen [49]. The reaction for the production of ozone is reversible:

$$3O2 + 68kcal \rightarrow 2O3$$

The ozone molecule is unstable and self-decomposes to oxygen, with emission of heat. Additionally, the life of an ozone molecule depends on the temperature, thus at a temperature above 20°C the concentration of ozone decreases by half within 40 min., at 30°C in 25 min., at minus 50°C the concentration of ozone diminishes by half within 3 months [20], and at minus 78°C, in a glass recipient and certain pure plastic and metal recipients, ozone practically does not decompose [21].

The main quality which defines the specifics of the physicochemical properties is as follows:

· A high level of excess energy of the ozone molecule and $O3 \rightarrow {}^{3}/2O2 + 34$ kcal/mol

· the powerful oxidation action; the ozone is the 2-nd powerful oxidant, next to fluorine and persulfate [49, 22].

The ozone oxidizes all types of metals, except for gold and platinum. It is capable to enter in reaction with the majority of organic and inorganic substances. At the thermodynamic level, these reactions may develop up to complete oxidation, i.e. up to the formation of water, carbon oxides and oxides of other elements.

Despite its high potential for oxidation, the interaction pattern of ozone is very selective. The cause of this selectivity is the polar structure of the bond angles of the ozone molecule ($\mu = 0,49$ D) [24], more specifically the positive polarization of the oxygen atom, which confers electrophilic characteristics to the entire molecule, and due to these molecules with a high electronic density become its preferred reactive elements. The components with simple double bonds C=C enter into an immediate reaction, phenols and free amines are oxidized within a few seconds, meanwhile other substances, for example alcohols, become oxidized within several hours.

Of special interest are the reactions of ozone with organic molecules possessing double or triple bonds, which result in understanding the bio-chemical traits of the interaction of ozone with biological objects. The contact with these molecules leads to the creation of complexes and intermediate compounds which are less studied (zwitterione/amphyone, malozonyde, cyclic ozonides), which may be hydrolyzed, oxidized, reduced or thermally disintegrated into multiple substances, and mainly aldehyde, ketone, acids or alcohols (tab. 2) [25].

Ozone reacts with unsaturated hydrocarbons, amines, sulfhydryl groups, organic compounds containing sulfur and nitrogen, and aromatic compounds. Besides unsaturated fatty acids, aromatic amino acids are also sensible to the action of ozone, and mainly the ones containing SH groups. The product of the interaction of the ozone molecule and the bio-organic substrates is the molecule – ozonide [26]. As a result of its dipolar structure, the ozone molecule may lead to

a 1,3-dipolar cyclo-addition of unsaturated bonds, resulting in the formation of a primary ozonide (I), in conformity with the reaction presented in fig. 1, known also as the Crigée mechanism (1953, 1975). Ozonation of aromatic compounds is similar to ozonation of olephyns with creation of polymeric ozonides [26].

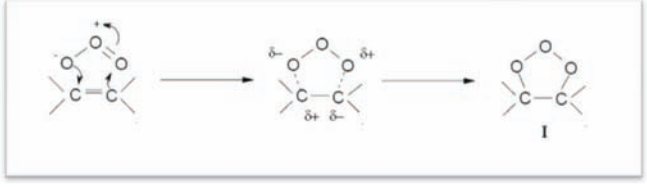

Fig. 1. Formation of primary ozonide.

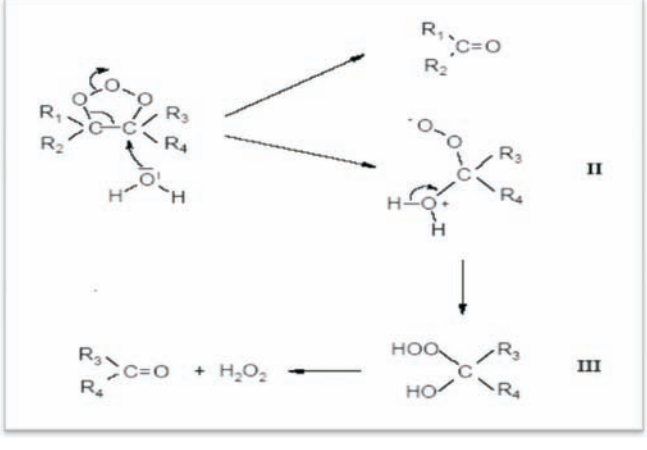

Fig. 2. Primary ozonide is decomposed into a carbonyl compound (aldehyde or ketone) and a zwitterion (II), leading to formation of a hydroperoxide hydroxide (III), the stage which results further in decomposing into a carbonyl group[26].

Ozone may be toxic if inhaled and an irritant for the eyes. Inhaling by humans and mammals of ozone in higher concentrations may lead to its reaction with the mucous compounds of the airways and may result in damaging the substance which covers the interiors of the lungs (pulmonary surfactant), because it has a reduced anti-oxidation capacity. Within the anatomopathological investigations the pattern common in an ozone intoxication is described as follows: blood clotting is disturbed, lungs are affected by a large number of hematomas [27]. Due to this, the maximum allowed concentration of ozone in the air of a working room is about 0,1 ppmv inhaled within one hour, which is 10 times higher than the human olfactory threshold (0,01 ppmv) [28]. The World Health Organization allows for 8 hours of work in a room in which the ozone concentration is 0.06 ppmv, a threshold with an easily-recognizable and quite powerful ozone smell [28]. The conversion is as follows:

1ppmv = 1.0 mcg/ml or 1.0mg/l or 1.0g/m3

Medical ozone is a product of pure oxygen (min. 99,5 %), created in an ozone generator, an apparatus equipped with a tube, in which, through an endothermic process, take place high voltage electrical discharges between the electrodes. In fact, medical ozone is a compound of oxygen and ozone (95% O2 - 5% O3) [37]. Medical ozone cannot be generated from air, due to the presence of nitrogen, which generates toxic nitrogen oxides [29]. Medical ozone is administered externally (on the skin, on the injured surface), internally (per os et per rectum) and parenterally, within the therapeutically allowed concentrations, and does not have a toxic effect on the human body. If used in specific conditions and for the treatment of specific diseases, the therapeutic efficiency of medical ozone is identical or similar around the world. The use of unsuitable administration methods and amounts, is the main cause of the lack of efficiency and adverse effects. Due to this, the medical societies advocating for the use of ozone have established treatment protocols, as a basis for the methodological standards and guidelines, which have been revised and published in compliance with the recent results of research and based on the practice in this field acquired during the last thirty years [29]. These were used to standardize the application, indications, concentration, dosage and frequency of the treatment, based on the mechanism of action and pharmacological properties of medical ozone.

The pharmacological effect of medical ozone reflects the so-called hormesis phenomenon, expressed by the stimulatory or beneficial effect following the exposure to low concentration of a substance, otherwise toxic if used in high concentrations [30]. The concept of ozone applied in low doses (Low-Doze Ozone), with a moderate oxidative stress, becomes nowadays the ideal strategy in the field of ozone therapy [18].

Mechanism of action of medical ozone

Depending on the method of application, the mechanism of action of medical ozone may be included into two major categories:

- topical use (bactericidal action, virucidal, fungicidal);
- systemic use (regulation of the anti-oxidized cellular system, improved emission of oxygen by the RBC and immunomodulation by activating white blood cells (WBC);

Topical use of medical ozone in gaseous state or in the form of ozonized solutions, possesses anti-microbial, antiviral, anti-fungal effect, and the effect of cleansing and treatment of wounds. At the same time, it has a higher efficiency in the aqueous medium, because after decomposing of the ozone in water, a highly reactive hydroxyl radical is generated. In compliance with microbiological studies, ozone has the capacity to destroy all Gram-positive and Gram-negative known bacteria, including *P. aeruginosa*, *Legionella spp*, all lipid and hydrophilic viruses including the hepatic viruses A, B, C, HIV, spores and all known vegetative forms of pathogenic fungi [31, 32]. Among the causes of the bactericidal effect of ozone, most frequently mentioned is the disrupting of cellular membrane activity of the bacteria, by oxidizing the phospholipids and lipoproteins, directly affecting cytoplasmic integrity. Gram-positive bacteria are more sensitive to the action of ozone in comparison to Gram-negative bacteria, perhaps due to the differences in the membrane structure [34].

The attention of researchers regarding the virucidal effect of ozone is centered on the property of the ozone to disrupt the multiple configurations of the lipid molecules. Indeed, if the external lipid membrane of the virus is dislodged, the nuclear DNA or RNA cannot survive. Viruses have no protection against the oxidizing stress, on the other hand, the normal cells of a mammal have the structure of complex enzymes (i.e, SOD, catalase, peroxidase), which neutralizes the negative effects triggered by the reactive oxygen species (ROS) and oxidation disruptions. Viruses which possess, besides nucleocapsid, also an external lipoproteic shell derived from the membrane system of the host-cell (peplos or envelope), are defined as enveloped viruses [34]. The enveloped viruses are much more sensitive to the action of the ozone, when compared to the non-enveloped viruses, namely due to presence of the external glico- and lipoprotein membrane, which interacts easily with ozone [35].

Although the effects of ozone on unsaturated fats represent one of the most documented biochemical actions, ozone is also known to interact with proteins, carbohydrates and nucleic acids. This becomes especially relevant when considering the effect of deactivation of non-enveloped viruses by ozone [36].

In order to explore the utility of ozone therapy in viral diseases, researchers [23] considered the possibility of action of ozone in vivo, because the activity of virucidal ozone becomes insecure when viruses are in biological fluids or, even worse, when they are placed intracellularly (hepatocytes, CD4 + lymphocytes, monocytes, glial and neuronal cells), because, ironically, the powerful antioxidant system of the host cell protects the viral integrity.

The following mechanisms may have some importance:

- A prolonged ozone treatment seems to be able to induce an adaptation to chronic oxidative stress (SCO), and therefore, a balancing of the cellular redox status, which is a fundamental process for inhibiting the replication of HIV, HBV and HCV [35, 37];
- The induction of cytokine synthesis such as interferon (IFN) and interleukine (IL) in ozonized blood has been proved possible. Although ozone is a weak inducer, reinfused lymphocytes and monocytes migrating through the lymphoid system, can activate other cells, which in time will lead to a stimulation of the immune system [38];
- Blood ozone in minor authohemotherapy (AHTm), can induce the oxidation of free virus components, which could become an inactivated and immunogen vaccine [38].

Certainly, ozone improves the oxygenation and the hepatic metabolism and it has always been confirmed that fibrinogen and plasma prothrombin levels have a tendency to normalize infected patients, thus suggesting an improvement of the hepatic protein synthesis. In HIV infection, ozone therapy corrects acquired lipodystrophy and hyperlipidemia that accompany metabolic and cardiovascular complications. Cells with low enzymatic coverage are vulnerable to viral invasions and also susceptible to oxidation and elimination from the body, which allows their replacement with healthy cells [40].

In fungi, ozone prevents the cell growth in certain stages. Fungi called dermatophytes and families of fungi *Candida, Aspergilus, Histoplasma*, *Actinomycoses, Cryptococcus* are destroyed by ozone exposure. The cell walls of the fungi are multilayered and are composed of approximately 80% carbohydrates and 10% proteins and glycoproteins. The presence of many disulfide connections creates the possibility of oxidative inactivation of fungi with ozone. Additionally, ozone is able to diffuse through the fungal wall into the cytoplasm and disturb the cell organelles. The protozoa organisms affected by ozone include *Giardia, Cryptosporidium, Acanthamoeba, Hartmonella* and *Negleria.* The exact mechanism by which ozone exerts its antiprotozoal action has not been discovered yet.

The systemic application of medical ozone through major authohemotherapy (AHTM) and minor authohemotherapy (AHTm), in the form of saline intravenous infusions or as of gaseous mixtures of O2/O3 in rectal or vaginal insufflations, or subcutaneous, intramuscular, para/intra articular injections possess analgesic, antihypoxic, antiiinflammatory, antiedematous, antioxidant, myorelaxant, detoxification and immunomodulator effects.

The following processes are activated after systemic applications of ozone to the human body:

- The activation and induction of biological antioxidants (scavengers) of free radicals;
- The activation of the immuno-competent cells;
- The activation of the metabolism of the red blood cells (RBC).

Antioxidant action. Under normal conditions of temperature and atmospheric pressure, due to its high instability and solubility, ozone is dissolved instantly in water and in biological fluids such as: plasma (90% water from the composition of blood plasma), the same in extracellular fluid, or the thin layer of water covering the skin and the respiratory tract, intestinal, vaginal etc. mucus. Being a strong oxidant, ozone reacts immediately with a number of molecules, that are present in biological fluids and also with antioxidants, proteins and carbohydrates, especially with polyunsaturated fatty acids (PUFAs).

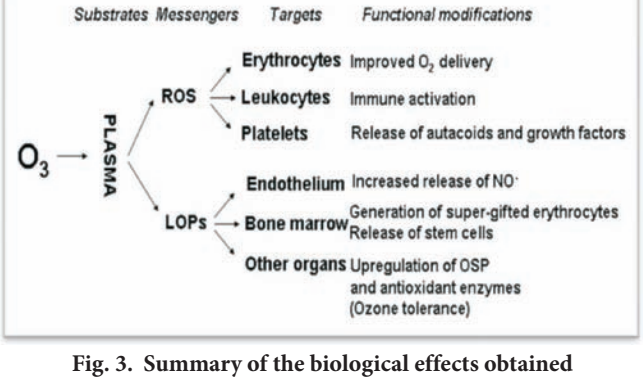

Fig. 3. Summary of the biological effects obtained during AHTM [26].

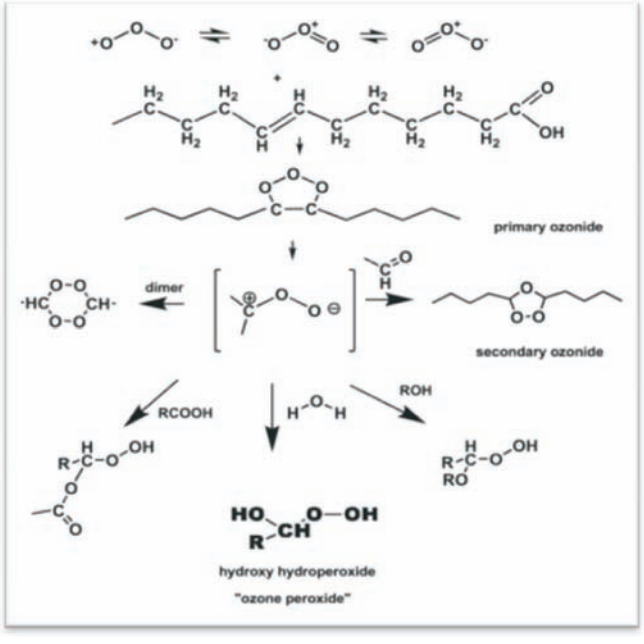

Fig. 4. The reaction of ozone with isolated double bonds (Crigée mechanism). Hydroperoxides, in this case "ozone peroxides", are perceived as active pharmacological substances [18].

The reaction of ozone with so many molecules involves several main processes taking place simultaneously:

· On the one hand, the ozone is inevitably consumed during oxidation of ascorbic and uric acids, sulfhydryl groups (SH) of a reduced glutathione (GSH), the proteins and glycoproteins present in the plasmatic water [43], an important reaction because it generates the reactive oxygen species (ROS), which in turn triggers a chain of biochemical reactions in the blood. ROS are neutralized in the first 30-60 seconds of the antioxidant system (SA).

· On the other hand, the reaction of lipid peroxidation takes place [44]. In the hydrophilic environment of the plasma, one mole of unsaturated olefin and one mole of ozone, lead to the formation of two moles of aldehyde and a mole of hydrogen peroxide (H_2O_2), [26]:

- **o O3 + antioxidants = ROS;**
- **o O3 + arachidonic acid** (or triglycerides and chylomicrons) = **H_2O_2 + LOPs;**

These reactions, consummated within a few seconds, use the entire doze of ozone and generate hydrogen peroxide, which is an oxidant and not a radical molecule (usually included in the ROS group) and a variety of aldehydes, known also as lipid oxidation products (LOPs).

Immediately after the ozone is diluted in the plasmatic water and reacts with the polyunsaturated fatty acids (PUFAs), the concentration of H_2O_2 starts to increase, but decreases equally rapidly, because this non-ionized molecule quickly penetrates the erythrocytes, leukocytes and platelets, activating a series of biochemical reactions and simultaneously is reduced by water, due to the intracellular anti-oxidizing enzymes: glutathione peroxidase (GSH-Px), catalase and GSH. This important moment corresponds to an acute and

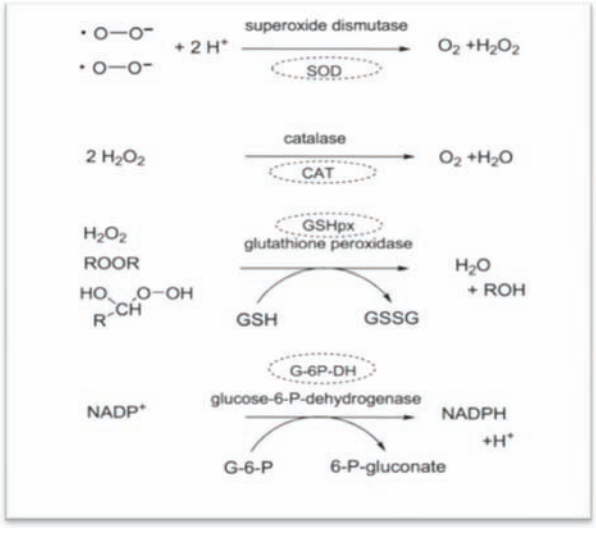

Fig. 5. Situation of oxidation stress, in which the biological anti-oxidation system reduces the ROS. "Ozone peroxides " are controlled by glutathione system, and not by catalase [18].

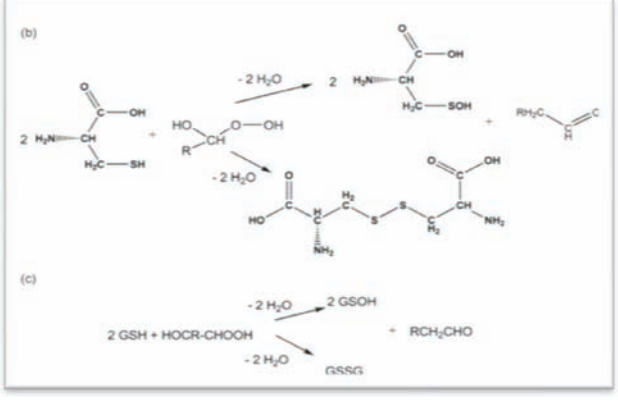

Fig. 6. Reaction of "ozone peroxides" with cystein (residuum) and (c) Glutathione [18].

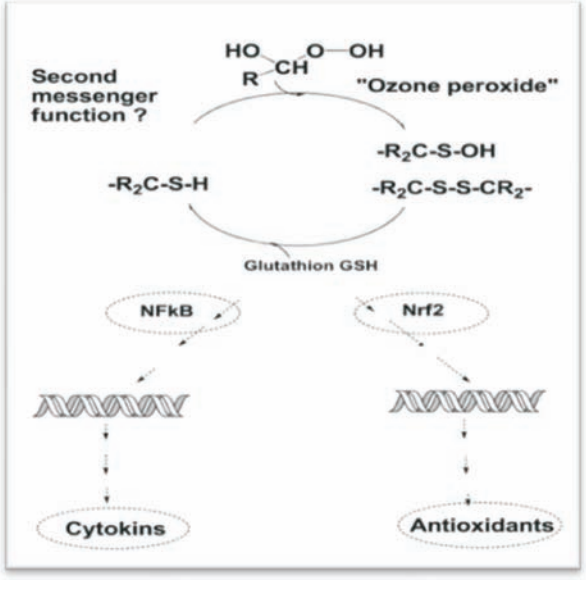

Fig. 7. "Ozone peroxides" as secondary messengers for transmission of an intracellular signal.

transitory controlled oxidation stress, necessary for the biological activation, which excludes any toxicity, provided that the used dose of ozone is compatible with the anti-oxidizing capacity of blood [45]. While the ROS are responsible for the immediate biological effects (fig. 3), LOPs are important due to tardive effectors, which may reach any organ, especially the bone marrow, where after binding to receptors in submicromolar concentration, determines adaptation to repeated acute oxidation stress, a typical feature of the ozone autohaemotherapy [38]. Given its transitory presence in the cytoplasm, H_2O_2 (generated by ozone) acts as a chemical messenger of the ozone. Currently, the H_2O_2 is largely recognized as an intracellular signaling molecule, capable of activating the tyrosine-kinase, which phosphorylates the nuclear transcription factor - kB (NF-kB), allowing for the synthesis of a various number of proteins [46].

Practically, the H_2O_2 functions by oxidizing the cysteine [47], which on its turn influences the mononuclear blood cells [48], platelets, endothelial cells [50] and the erythrocytes [28, 51]. Once inside the cell, the H_2O_2 molecules are almost immediately reduced to water and/or lipoperoxides to hydro-peroxides.

Hydroperoxides or "Ozone peroxides", a term proposed by several authors [28] assume the role of physiologically active ozone metabolites. Due to selective reaction of ozone with the double bonds C = C of essential fatty acids, the classic ozonolysis described by Criegée (fig. 4) becomes the dominant reaction present in physiological conditions with the pH values ≤7,4.

The ozonolysis lasts only fractions of a second, and forms mainly short chains of hydroxi-hydroperoxides in a aqueous medium (ozone peroxides), which are obviously responsible for the pharmacological effect during the systemic treatment with medical ozone.

Ozone peroxides are reactive oxygen compounds, associated with the membranes, which act as second messengers through the residuum of cysteine and/or through reduced levels of glutathione (GSH), in a less aggressive manner compared to the superoxide radicals ·O-O– and the H_2O_2 and are taking over the anti-oxidating regulation, without the need of SOD and catalase, similar to the oxidation stress developed as a result of relevant pathological conditions (fig. 5, 6).

Short chains of Hydroxi-hydroperoxide with a tendency to low radical reactions may result in initiating regulation of the anti-oxidizing protective mechanisms, as a signal for redox (e. g., through the nuclear factor Nrf2 into stress and through the nuclear factor NFkB into inflammatory processes (fig. 7) [18].

Processes for oxidating the residuum of cysteine and glutathione induce regulation of cytokines and of the anti-oxidation system through NFkB, and respectively through Nrf2 [18].

During a lengthy treatment, the activity of LOPs shall culminate in the regulation of antioxidation enzymes, generation of oxidative stress proteins (OSP) (heme oxygenase 1, a typical marker) and probably the release of a stem cell, the essential factors which explain some of the extraordinary effects of ozone therapy.

The therapeutic response obtained after the repeated oxidative stresses suggests a preconditioned effect, eventually capable of restoring the balance of the oxido-reducing tissue system, which has been modified through pathogenic factors [28].

Immunomodulatory action. Presently, the immunomodulatory effects of ozone therapy are related to induction of cytokine by the lymphocytes and monocytes [38], such as interferons (IFN) B and gama, interleukins (IL) 1B, 2, 4, 6, 8 and 10, the tumor necrosis factor (TNFx) and growth factors GM-CSF (granulocyte-macrophage colony-stimulating factor), TGFβ1 (transforming growth factor beta 1) [52], PDGF (platelet-derived growth factors), bFGF (the basic fibroblast growth factor), EGF (epidermal growth factor), KGF (keratinocyte growth factor), thus promoting the synthesis of intracellular matrix and the healing process (fig. 9) [53, 54, 55, 56]. It is possible that these cytokines also activate other lymphoid cells, which leads to immunostimulation without adverse effects [57, 58].

At the same time, the influence of ozone on the phagocyte activity of leukocytes is being studied. Therapy with medical ozone modulates all the dislodged stages of phagocytosis and contributes to rapid elimination of inflammatory processes [59]. Firstly, the time of adherence to the surface of a phagocyte is reduced (PMN, macrophage*)* and secondly, the stage of respiratory explosion becomes specifically pronounced, being determined by the generation of peroxides. Another possible modality for phagocyte activation is the increased synthesis of the phagocyte stimulation factor [60].

Hemorheological action. Erythrocytes contain a large reservoir of GSH (approximately 1 mmol / l), thioredoxin with two available cysteines and powerful antioxidant enzymes (catalase, GSH-Rd, GSH-Px, GSH-Tr and SOD). They may neutralize quickly large quantities of oxidants, such as OH, H_2O_2, OCl^-, $ONOO^-$ and at the same time may recycle back the oxidized compounds [61]. In the erythrocytes, the activation of the oxygen dependent processes are manifested through the increased activity of the glutathione system [4, 38]. Oxidation of sulfhydryl groups (–SH) results in accumulation of oxidized state of glutathione (GSSG) with the modification of the reduced and oxidized GSH [62], which leads to the use of nicotinamide adenine dinucleotide phosphate, in reduced form (NADPH), for recycling of oxidized glutathione (GSSG) at the initial GSH level. NADPH is a coenzyme which serves as an electron donor for various biochemical reactions; additionally the NADPH regenerates also other intracellular antioxidants, especially vitamin E and ascorbic acid [60]. In its turn, the NADP, in its oxidized state is reduced after activation of the pentose phosphate pathway, a reaction in which the glucose-6-phosphate dehydrogenase (G-6PD) is the key enzyme, leading to an increased glycolysis (fig. 8) [38].

Thus, ozone therapy stimulates oxygen metabolism by increasing glycolysis in red cells (fig. 9). On the one hand, this leads to activation of 2,3 diphosphoglycerate (2,3DPG), which in its turn results in an increased quantity of oxygen

released by the oxyhemoglobin to the tissues, which result in improved oxygen levels in tissues:

$$HbO_2 + 2,3 - DFG = Hb\ 2,3 - DFG + O_2.$$

Increased consumption of oxygen by the body has been proven by special measurement methods of the gases contained in blood. The clearest confirmation was the proof of an increased arteriovenous oxygen difference [63]. The partial pressure of oxygen contained in the venous blood, after following a session of ozone therapy, diminishes from 40 mm Hg to 20 mm Hg or even less [60, 64], which means that ozone therapy ensures an increased release of oxygen into the tissues with an insufficient blood supply – an effect impossible to obtain by medication [65, 66].

On the other hand, the increased levels of glycolysis result in activating the Krebs cycle (the aerobe pathway) by increasing the production of the adenosine triphosphate (ATP). The decreased level of the plasma glucose in vitro has been demonstrated by a series of studies [62], and this is especially clear in cases of patients with diabetes [67].

In the process of ozone therapy, the saturation with oxygen of both plasma and erythrocytes takes place. In the presence of ozone, blood is capable to absorb up to 2-10 times more oxygen than normally [68]. At the same time, it becomes possible to maintain the exchange of substances through the intracellular liquid, in conditions of a disturbed vascular tone. All patients who were treated with ozone in the form of AHTM presented a statistically significant increased level of partial oxygen pressure in arterial blood, a decreased partial pressure of carbon dioxide and a raised level of oxyhemoglobin [68]. After concluding the treatment, the maximum time for reduction of oxyhemoglobin decreases very slowly, more precisely within a period of several weeks and even months.

It seems obvious that erythrocytes may be modified by ozone therapy only for a short period of time. However, the repeated therapeutic administrations may allow for the LOPs compounds to permeate the bone marrow and activate the subtle development of an erythropoietic level, favoring generation of "super-gifted erythrocytes" – new erythrocytes with improved biochemical properties [61]. Based on this hypothesis, due to extended ozone therapy, the bone marrow may release a group (approximately 0,9% of the total fund) of new erythrocytes with improved biochemical properties. In fact, the therapeutic benefits do not cease once the treatment is stopped, but rather continue to exist for up to 2-3 months, perhaps in correlation with the life span of circulating super-gifted erythrocytes. It has been demonstrated that after an extended period of treatment with ozone, the fraction of young erythrocytes, isolated by the old erythrocytes [69] by separation in density degree, had a much higher content of G6PD (Glucose-6-phosphate dehydrogenase) [61]. Activation of metabolic processes in the erythrocytes contributes to accumulation of macro energetic compounds ATP [70]. As a result, the transportation function of the cellular membrane is intensified, by activation of ionic pumps ATP-ase Na+ - K+ and, subsequently, the concentration of intracellular cations (K +) and extracellular cations (Na +), the level of resting potential of the membrane and distribution of electrical charges are regulated, and thus the activity of adhesion and aggregation of cells is modified, which determines the rheological properties of blood. Additionally, the generation of a lipid bilayer of peroxides diminished the viscosity of the lipid bilayer membrane, leading in turn to an improved flexibility and elasticity (capacity to deform) of erythrocytes [60].

ROS and LOPs are responsible for the increase of the function of erythrocytes [26], and also for the activation of leucocytes [38], platelets and endothelial cells [50]. This

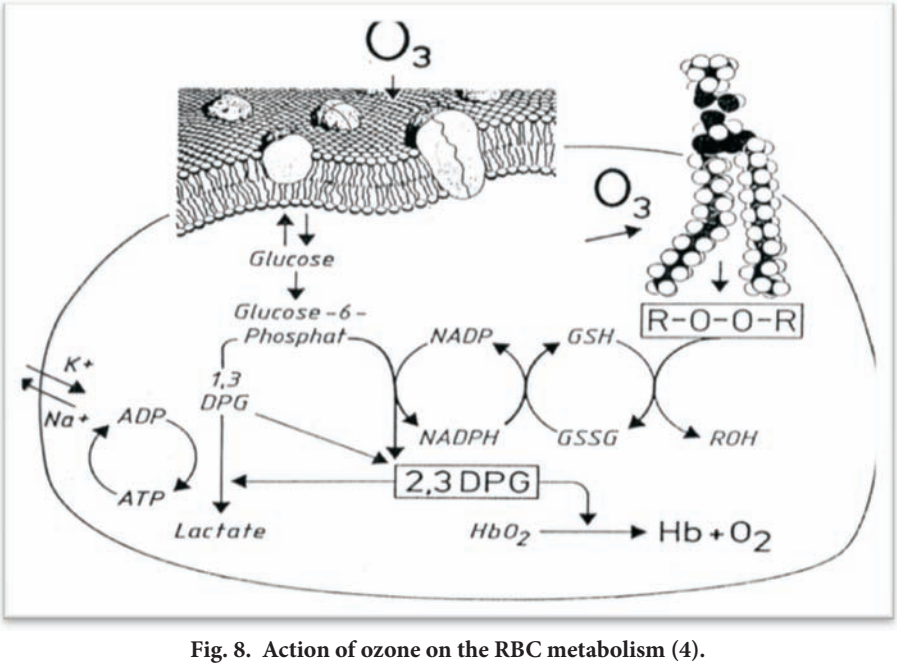

Fig. 8. Action of ozone on the RBC metabolism (4).

multi-faceted and simultaneous activation results in a boosted release of nitric oxide (NO), prostacyclin (through the selective reaction with the double bonds of arachidonic acid, by launching its metabolism), adenosine [71], autacoid and contributes to an improved tissue vascularization [72]. Indeed, through the interaction with the endothelium, the LOPs result in a boosted generation of NO and NO-tiol, which shall result in turn in an increased oxygen input, thus improving the microcirculation. Moreover, the phenomenon of adaptation to chronic oxidative stress implies the fact that repeated ozone treatments induce the synthesis of the oxidative stress proteins, of which the HO-1 (hemo-oxigenase) serves as a primary example. This enzyme induces a boosted level of bilirubin (a lipophilic antioxidant equally powerful to tocopherol) and CO. The enzyme reduces indirectly the vasoconstriction, due to suppression of the endotheline-1 genes and inhibits the proliferation of smooth muscle cells [73]. It is a known fact that the NO, the release of which is boosted by ozone therapy [72], is the most important physiological vasodilator and inhibitor of platelet and leukocyte aggregation and of adhesion to endothelium, which in cooperation with certain traces of CO increases vascular relaxation [38].

The performed studies on ozone therapy effects on hematologic/hemodynamic parameters attest that the treatment with medical ozone, practically, does not modify any levels of the methemoglobin, neither hematocrit levels, because of the absence of any changes in the RBC volume, caused by an edema or lysis, but actively influences the active blood clotting system. The diminished blood and plasmatic viscosity is due to the decreased fibrinogen levels [51].

Fibrinogen is the primary factor for clotting, having the biological property to clot under the influence of the specific enzyme – thrombin. The product of this reaction - fibrin, forms the reticular basis of the thrombus, which clogs up the affected vessel. Fibrinogen plays an important role in the aggregation of erythrocytes and platelets [74]. The increase of the concentration of fibrinogen causes an increase of the viscosity of the blood [75]. Therefore, medical ozone, applied in low concentrations, by reducing the concentration of fibrinogen, lowers the aggregation of blood cells and ameliorates the rheological properties of the blood [76].

The activation of the fibrinolytic mechanism in the hemostasis system prevents the development of blood clots, resulting in partial or total thrombosis, leading in its turn to the fibrinolysis and ensures its elimination from the vascular bed. This is one of the primary mechanisms of revascularization and restoring of the blood flow to the organs and tissues [75].

In high concentration, medical ozone, used only for external applications, has a pronounced hemostatic effect [38].

Therefore, not the ozone itself, but ROS (largely H_2O_2) and LOPs are responsible for the succeeding and multiple biochemical reactions which take place in various cells throughout the body. [38].

Use of medical ozone in dentistry

Normally, the oral cavity hosts about 20g commensal bacteria which are better kept under control by the lymphoid tissue related to the mucous (MALT). However, they can become pathogenic and are largely responsible for tooth decay, which is reported by the Dr. E. Fisch (1899-1966), considered the first dentist to use ozone in his practice and to demonstrate

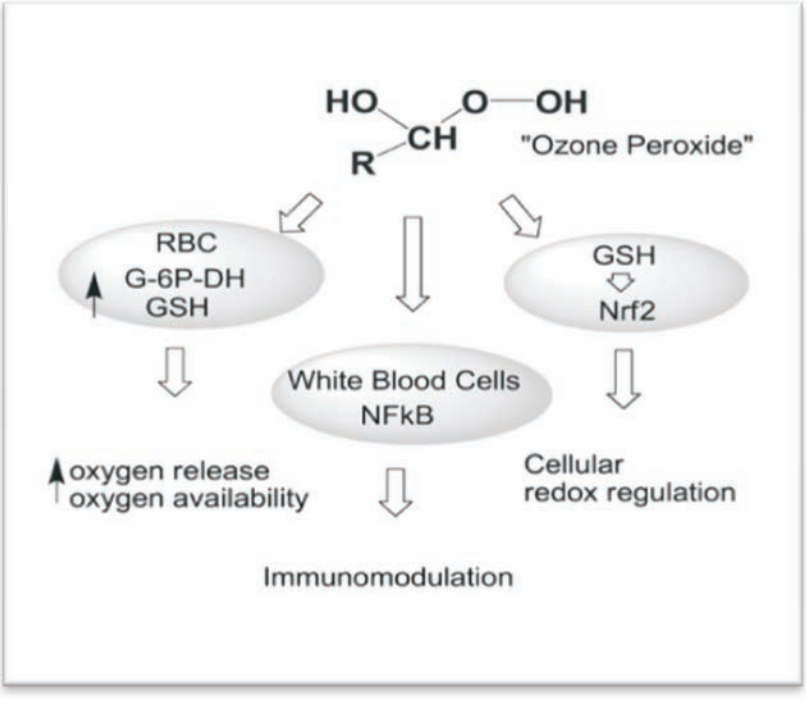

Fig. 9. The three pharmacological effects of medical ozone obtained through peroxides generated by ozone: (1) Improved release of oxygen to the red blood cells (RBC); (2) Immunomodulation through the activation of white blood cells (WBC) and (3) Regulation of the cellular anti-oxidation system [18].

the potential of ozone disinfection activity [38]. Nowadays, medical ozone in dentistry is applied as a complementary treatment or in combination with other methods of ozone therapy [13]. The most frequently used in dentistry and Oral & Maxillofacial surgery (OMS) are the ozonized solutions [77, 78, 79, 80, 81, 82]. At the 15th World Congress organized by the International Ozone Association (IOA, 2001) it was reported that application of ozonized water in oral cavity heals the wounds more speedily compared to placebo treatment. In dentistry the ozonized distilled water is used for cleaning up the oral cavity, antiseptic irrigation of canals and caries. The ozonized water jet fully removes the purulent material and disinfects the affected area [83]. The use of ozone in combination with the dental scaling and professional teeth brushing allows a considerable improvement of oral hygiene and periodontal indices [84, 85].

Although the direct use of ozone in the gaseous form is prohibited (toxic by inhalation), A. Baysan and E. Lynch successfully used a new system of delivering ozone to treat primary root carious lesions (PRCL), capable of avoiding any toxic risk. This system includes a source of medical ozone and a manual dental instrument, on the end of which is fixed a detachable silicone suction cup. Due to its elastic edge, it is sealed to the tooth surface and allows the exposure of the carious lesion to gas. The exposure to ozone for a period of 10-20 seconds, with a concentration of ozone (about 4 mcg / ml) and a gas flow rate of 600 ml / min is enough to destroy all the microorganisms present in the primary root carious lesions. In this case, the most important is to achieve the denaturation of protein and killing the lactobacilli which, following the metabolism of glucose, may result in generation of lactic acid, which in turn favors further demineralization of the dental enamel. The effect of rapid dental surface sterilization by ozone is maintained for about an hour, enough time for the enamel remineralization by calcium phosphate which is present in saliva, thus the tooth becoming stronger and more resistant to bacterial attacks for a period of three months, to the least [86].

The antimicrobial properties of medical ozone, when used for reducing the number of cariogenic bacteria, are also the cause of a significant reduction of microorganisms that are present in BP. From the prospective of a potential antibacterial agent, ozonized water, in most conditions presents less cytotoxicity than ozone gas or recognized antimicrobial agents such as chlorhexidine digluconate, sodium hypochlorite or hydrogen peroxide. As a result, the ozonized water meets the optimal cellular biological requirements regarding biocompatibility in oral application [87].

Some authors recommend complementing OMS methods with ozone therapy. The use of ozone in the pre- and post-surgery allowed for a considerable improvement in postoperative evolution, an acceleration of the operation wound epithelialization and a decreased time of antibiotics administration [88]. In OMS, ozonized solutions are used locally for treating purulent inflammatory processes (periostitis, alveolitis, phlegmon) [89, 78], in open fractures of the mandible and in posttraumatic prophylaxis of inflammatory complications. Ozone has a beneficial influence on metabolism and bone restorative processes [91]. It has been noticed that in patients with chronic osteomyelitis of the jaw, the use of medical ozone normalizes quickly and completely the non-specific resistance and T-cell immunity, thus accelerating the healing process and reducing the incidence of clinical complications [92].

The relevancy of applying ozone therapy in inflammatory processes in the oral & maxillofacial region is determined by the spread of antibiotics resistant forms of microorganisms and the change of the patient's sensibility to this reaction. Several authors mention the necessity of systemic application of ozone therapy, by pleading the immunomodulatory action of medical ozone, which reduces the period of exudation from the wound, accelerates the emergence of the granulation and wounds epithelialization to the patients with phlegmons with torpid evolution in the oral & maxillofacial region [88].

Medical ozone is very effective in endodontics, except one must ensure the correct application of ozone and namely: sufficient concentration, suitable time and the correct method of application in the radicular channels, and certainly, after the procedures of preparation, irrigation and traditional endodontic cleaning have been completed. The studies have demonstrated the potential of using ozone as gas, ozonized water and oil in endodontic therapy. The ozonized water can be used as a final syringe in combination with a ultrasonic apparatus, that produces a very effective "streaming" sound, allowing ozonized water to penetrate many parts of the intra-radicular anatomy, much more efficient than conventional obturation techniques [93].The use of ozone in prevention of peri-implantitis is motivated by ensuring a proper diet and regular control of the bacterial plaque, and additionally, the use of ozone shows a positive healing of wounds due to an increased blood circulation in the tissue. Also, of interest are communications concerning the application in the CMF of ozonated oils, especially in cases of alveolitis [89]. The authors attest the reduction of the healing process in comparison with the traditional therapy duration. In cases of inflammatory diseases of the treatment resistant mucous tissues (i.e. relapsed herpetic stomatitis etc), some authors recommend the combination of the local ozone therapy with the systemic one. Such a treatment scheme allows for the achievement of a stable remission in short term for most patients [94].

The possibility of using ozone as a local antiseptic in treatment of odontogenic purulent sinusitis is of interest likewise. According to studies, ozone therapy is the most effective method when compared to other treatment methods. In case of emphasized intoxication syndrome, which complicates the development of sinusitis, the combination of systemic and local ozone therapy is recommended. This allows for rapid normalization of biochemical blood indicators [95].

Thus, ozone therapy experienced a boosted application in dentistry, including in periodontal dentistry [11, 12, 13].

The ozone acts on several pathogenic links of the periodontal disease, firstly by regulating the system of antioxidants (SA), and secondly by improved oxygen supply and reduction of healing time of periodontal tissues. Certainly, its bactericidal and immunomodulatory abilities are of major importance. In treatment of the periodontal disease, the medical ozone is locally applied into periodontal pockets (PPr), in the form of instillations, solutions or oil drips, and possess a proven anti-inflammatory potential, based on the analysis of objective diagnostic criteria [3, 90, 96, 97, 98].

Conclusions

The ozone molecule is unstable and breaks down into oxygen, succeeded by an exothermic reaction. The basic quality which defines the specifics of the physicochemical properties is the high level of excess energy of the ozone molecule and its strong oxidizing action. The pharmacological effect of medical ozone is represented by the so-called hormesis phenomenon, expressed through the stimulatory or beneficial effect following the exposure to low concentrations of a substance, otherwise toxic in higher concentrations, and this effect takes place due to the short chains of hydroxyl-hydro-peroxide, the so-called ozone peroxide. The mechanism of action of medical ozone is divided into two major categories, depending on the application method: topical or systemic application. The action of medical ozone action on the human body is diverse and multidirectional. The medical ozone possesses analgesic, anti-inflammatory, anti-edematous, antioxidant, muscle relaxant, detoxing, immunomodulatory, antimicrobial, antiviral, antifungal effects, it activates cellular metabolic processes, stimulates oxygen metabolism and improves the rheological properties of the blood. Ozone therapy is a highly effective method for treating many diseases, of which the pathogenesis of the inflammatory syndrome is based on the bacterial etiology and immuno-inflammatory response of the host. At the same time, unlike antibiotic therapy, ozone has a much broader spectrum of therapeutic action, does not create any microbial resistance and has no adverse effects. Medical ozone in dentistry is used in monotherapy or combined with other treatment methods. Meanwhile, the other aspects of the use of medical ozone in clinical periodontology requires additional research.

References

1. Bocci V, Paulesu L. Studies on the biological effects of ozone 1: Induction of interferon on human leucocytes. *Haematologica.* 1990;75:510–15. http://www.ncbi.nlm.nih.gov/pubmed/2129118
2. Washutti J, Viebahn R, Steiner I. Immunological examinations in patients with chronic conditions under administration of ozone/oxygen mixtures. *Ozone Sci Engg.* 1989;11(411):7.
3. Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. *J Contemp Dent Pract.* 2008;9(4):75-84. Review. http://www.ncbi.nlm.nih.gov/pubmed/18473030
4. Viebahn-Hänsle Renate. The Use of Ozone in Medicine, Mechanisms of Action, 2003. http://www.o3center.org/Articles/TheUseofOzoneinMedicine.pdf
5. Viebahn-Hansler R. Ozone therapy-the underlying therapeutical concept and models of efficacy. *Erfahrungs Heilkunde.* 1991;4-40.
6. Maslennikov AV, Kontorshchikova KN. Ozone. Internal Diseases (tutorial). N. Novgorod publishing NGMA, 1999.
7. Burleson GR, Caulfield MJ, Pollard M. Ozonation of mutagenic and carcinogenic polyaromatic amines and polyaromatic hydrocarbons in water. *Cancer Res.* 1979;39(6 Pt 1):2149-54. http://www.ncbi.nlm.nih.gov/pubmed/109190
8. Caulfield MJ, Burleson GR, Pollard M. Ozonation of mutagenic and carcinogenic alkylating agents, pesticides, aflatoxin B1, and benzidine in water. *Cancer Res.* 1979;39(6 Pt 1):2155-9. http://www.ncbi.nlm.nih.gov/pubmed/445412
9. Zullyt B. Zamora, Menéndez S, Bette M., et al. 1. Ozone Prophylactic Effect and Antibiotics as a Modulator of Inflammatory Septic Process in Rats. Ozone Research Center, Havana, Cuba. E-mail : ozono@infomed.sld.cu; 2. Institute of anatomy, celular and molecular biology, University of Marburg, Germany; 3. Institute of microbiology, University of Marburg, Germany; 4. Institute of animals laboratory, University od Marburg, Germany. http://lomr.org/ozone-prophylactic-effect-antibiotics-modulator-inflamatory-septic-process-rats/
10. Pozdnyakov BJ, Velege C, Jadenov II. Wound microflora change of sensitivity to antibiotics in the treatment with complex "Ozone + Poviargol". *Nizhny Novgorod med. Zhurnal.* 2005;169-171.
11. Pirgari AB. Medical ozone in complex treatment of generalized periodontitis. *Nizhny Novgorod med. Zhurnal.* 2003:188-189.
12. Haug Karl F, ViebahnRenate. Classic medical ozone text book published, 1987;14-17.
13. Basrani Bettina. Endodontic Irrigation: Chemical disinfection of the root canal system. 2015;5-286.
14. Viebahn - Haensler R. The Use of Ozone in Medicine. 4th english edition, 2002;24-32.
15. Societatea Ştiinţifica Română de Oxigen – OzonoTerapie (SSROOT). http://www.asociatia-ozonoterapie.ro/
16. Saul Pressman. The Story of Ozone. DCh, LTOH, 1994http://www.spiritofhealthkc.com/wp/wp-content/uploads/2014/03/OZONE3-The-story-of-Ozone-by-Dr.-Saul-Pressman1.pdf
17. WFOT Review on Evidence Based Ozone Therapy. http://www.wfoot.org/wp-content/uploads/2016/01/WFOT-OZONE-2015-ENG.pdf
18. Viebahn-Hänsler Renate, León Fernández Olga Sonia, Ziad Fahmy. Ozone in Medicine: The Low-Dose Ozone Concept. Guidelines and Treatment Strategies Medical Society for the Use of Ozone in Prevention and Therapy, Iffezheim/Baden-Baden, D-76473, GermanyPharmacy and Food Institute, University of Havana, Havana 10 400, Cuba; 408-424.
19. Ozone - a unique treatment method. http://ekoozon.ru/ozonoterapiya/ozonoterapiya-unikalnyi-lechebnyi-metod.
20. Dragan Adriana. Ozonoterapia in bolile reumatice. http://www.romedic.ro/dr-tiron/articol/12053
21. Great Soviet Encyclopedia. http://bse.sci-lib.com/article083854.html
22. Razumovsky SD, Zaikov GE. Ozone and its reactions with organic compounds. Moscow: Nauka, 1974.
http://booksonchemistry.com/index.php?id1=3&category=organik-chem&author=razumovskiy-sd&book=1974
23. Bocci V, Paulesu L. Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes Haematologica. 1990;75:510-515.
24. Viebahn R, Steiner I. The Biochemical Aspects of Central Metabolic Parameters in Ozone/OxygenTherapy. Proceedings of the Ninth Ozone World Congress. 1989;3:88.
25. Lohaus Justus Liebigs, Criegee R. Ozone in polymer synthesis: clean synthesis of telechelic oligomers and block copolymers. *G Ann. Chem.* 1953;583.http://polymerbiomaterials.com/index.php/sample-page-2/ozone-in-polymer-synthesis-clean-synthesis-of-telechelic-oligomers-and-block-copolymers/
26. Holmes Julian. Ozonated Liquids in Dental Practice – a review. Lime Technologies Holdings Ltd, Clinical Director. April 2008. http://www.the-o-zone.cc/research/abstracts/o3liquids/o3lpt08.pdf
27. Ozonom po dyram? [Озоном по дырам?]. http://cleanwater-e.ru/index30.php
28. Bocci V. Ozone - a new medical drug. Springer, 2nd edition. 2011;17-218.
29. Bocci Velio, Borrelli Emma, Travagli Valter, Zanardi Iacopo. The ozone paradox: Ozone is a strong oxidant as well as a medical drug. Article first published online: 3 MAR 2009 http://onlinelibrary.wiley.com/doi/10.1002/med.20150/abstract
30. Bocci V. Oxygen-Ozone Therapy: a Critical Evaluation. Sursa, Anul;234.
31. Cardendale MT, Griffits J. Is there a role for medical ozone in the treatment of HIV and associated infections? Ozone in Medicine. Proceedings of the 11-th Ozone World Congress. San Francisco, 1993;1:32-37.

32. Viebahn-Hansler R. Ozone therapy: the underlying therapeutical concept and models of efficacy. *Erfahrungs heilkunde.* 1991;40:4-9.
33. Bocci V. Ozonization of blood for the therapy of viral diseases and immunodeficiencies. *Medical Hypotheses*. 1992;39:30-34.
34. Caractere generale: virusuri. http://www.justmed.eu/files/ppt/Curs%2012%20AMG%20-%20%20Virusologie.%20Caractere%20generale.ppt.
35. Morisco F, Verde V, Fogliano V, et al. Oxidative status in chronic hepatitis C: 2004, The influence of antiviral therapy and prognostic value of serum hydroperoxide assay. *Free Radic. Res.* Anul;38:573-580.
36. Sunnen Gérard V. The utilization of ozone for external medical applications. 1998. http://www.the-o-zone.cc/book/bookch/ch12.html
37. De Maria N, Colantoni A, Fagiuoli S, et al. Association between reactive oxygen species and disease activity in chronic hepatitis C. *Free rad. Biol. Med.* 1996;21:291-295.
38. Bocci Velio. Ozone - A new medical drug. Springer, 1st edition, 2005;5-250.
39. Kotler DP. HIV infection and lipodystrophy. *Prog. Cardiovasc. Dis.* 2003;45:269-284.
40. Elvis AM, Ekta JS. Ozone therapy: a linical review. *Nat Sci iol Med*. 2011 http://www.ncbi.nlm.nih.gov/pubmed/?term=Elvis%20AM%5Bauth%5D
41. Khalifa AM, El Temsahy MM, Abou El Naga IF. Effect of ozone on the viability of some protozoa in drinking water. *J Egypt Soc Parasitol.* 2001;31(2):603-16. http://www.ncbi.nlm.nih.gov/pubmed/11478459
42. Biologie. lecția 1. http://media.tvrinfo.ro/media-tvr/other/201304/lectia-1_63165600.pdf
43. Halliwell B. Antioxidants in human health and disease. *Ann. Rev. Nutr.* 1996;16:33-50.
44. Pryor WA, Squadrito GL, Friedman M. The cascade mechanism to explain ozone toxicity: the role of lipid ozonation products. Free Rad. Biol. Med. 1995;19:935-941.
45. Stone JR, Collins T. The role of hydrogen peroxide in endothelial proliferative responses. 2002, Endothelium 9:231-238.
46. Baeuerle PA, Henkel T. Function and activation of NF-kB in the immune system. *Ann. Rev. Immunol.* 1994;12:141-179.
47. Rhee SG, Bae YS, Lee SR, Kwon J. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. Sci STKE. 2000;10:53.
48. Reth M. Hydrogen peroxide as second messenger in lymphocyte activation. *Nat. Immunol.* 2002;3:1129-1134.
49. Viebahn-Hänsle Renate. The Use of Ozone in Medicine, 5th English edition, 2007;10-48.
50. Valacchi G, Bocci V. Studies on the biological effects of ozone: 11. Release of factors from human endothelial cells. *Mediat. Inflamm.* 2000;9:271-276.
51. Bocci V. Oxygen-ozone therapy. A critical evaluation. Kluwer Academic Publischer, 2002;440.
52. Bocci V. Ozon general physiology. http://www.ozonterapiklinigi.com/literatur/V-Bocci-ozon-general%20physiology.pdf
53. Pierce GF, Tarpley JE, Tseng J, et al. Detection of platelet-derived growth factor (PDGF)-AA in actively healing human wounds treated with recombinant PDGFBB and absence of PDGF in chronic nonhealing wounds. *J. Clin. Invest.* 1995;96:1336-1350.
54. Beck LS, DeGuzman L, Lee WP, et al. One systemic administration of transforming growth factor- b1 reverses age- or glucocorticoidimpaired wound healing. *J. Clin. Invest.* 1993;92:2841-2849.
55. Schmid P, Cox D, Bilbe G, et al. TGF-bs and TGF-b type II receptor in human epidermis: differential expression in acute and chronic skin wounds. *J. Pathol.* 1993;171:191-197.
56. Sporn MB, Roberts AB. A major advance in the use of growth factors to enhance wound healing. *J. Clin. Invest.* 1993;92:2565-2566.
57. Martin P. Wound healing-aiming for perfect skin regeneration. *Science.* 1997;276:75-81.
58. Slavin J. The role of cytokines in wound healing. *J. Pathol.* 1996;178:5-10.
59. Volozhin AI, Sashka TI, Shulakov VV, et al. The role of activation of phagocytosis in the mechanism of the therapeutic effect of medical ozone in patients with indolent purulent inflammatory processes of maxillofacial soft tissue area. http://www.fesmu.ru/elib/Article.aspx?id=67474
60. Mechanisms of action of medical ozone. http://www.ozonetherapy.ru/ozonoterapiya/mehanizmy-dejstviya-meditsinskogo-ozo/
61. Bocci V, Iacopo Zanardi, Valter Travagli. Ozone acting on human blood yields a hormetic dose-response. *A Journal of Translational Medicine*. 2011;9:91.
62. Rokitansky O. Klinik und Biochemie der Ozontherapie. *Hospitalis.* 1982;52:643-647.
63. Rokitansky O. Ozone Oxygen Therapy for Arterial Circulation Disorders. Ozone Science and Engineering. Pergamon Press. Clinical Consideration and Biochemistry of Ozone Therapy. *Hospitals.* 1982;53:643.
64. Peretyagin SP. Mechanisms of therapeutic action of ozone during hypoxia. Ozone in biology and medicine. Nizhny Novgorod, 1992;4-5.
65. Coppola L, Giunta R, Verrazzo G, et al. Influence of ozone on hemoglobin oxygen affinity in type-2 diabetic patients with peripheral vascular disease: in vitro studies. *Diabete Metab.* 1995;21:252-255.
66. Shiratori R, Kaneco Y, Yamamoto Y, et al. Can ozone administration activate the tissue metabolizm? A study on brain metabolism during hypoxia. *Masui-Japanese J. of Anestesthesiology.* 1993;42(1):2-6.
67. Pavlovskaya EE. Ozone in diabetes: Autoref. N. Novgorod, 1998;24.
68. Aubourg P. L'Ozone Medical: Production, Posologie, Modes d'Applications Cliniques. *Bull Med. Paris.* Anul;52:745-749.
69. Micheli V, Ricci C, Taddeo A, Gili R. Centrifugal fractionation of human erythrocytes according to age: comparison between Ficoll and Percoll density gradients. *Quad. Sclavo. Diagn.* 1985;21:236-248.
70. Mohinder Bansal, Naveen Kaushal.: Oxidative Stress Mechanisms and their Modulation. 2014. https://books.google.md/books?id=6p_cBAAAQBAJ&pg=PA165&lpg=PA165&dq=Oxidative+Stress+Mechanisms+and+their+Modulation+by+Mohinder+Bansal,+Naveen+Kaushal,+2014&source=bl&ots=xMja4fxlef&sig=rIRRvjbubL0QTPbpXYTFCSK4oxQ&hl=en&sa=X&redir_esc=y#v=onepage&q=Oxidative%20Stress%20Mechanisms%20and%20their%20Modulation%20by%20Mohinder%20Bansal%2C%20Naveen%20Kaushal%2C%202014&f=false
71. Riksen NP, Rongen GA, Blom HJ, et al. Potential role for adenosine in the pathogenesis of the vascular complications of hyperhomocysteinemia. *Cardiovasc. Res.* 2003;59:271-276.
72. Jia L, Bonaventura C, Bonaventura J, Stamler JS. S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. Nature. 1996;380:221-226.
73. Morita T, Kourembanas S. Endothelial cell expression of vasoconstrictors and growth factors are regulated by smooth muscle cell-derived carbon monoxide. *J. Clin. Invest.* 1995;96:2676-2682.
74. Johnson PJ. Peripheral circulation. M.: Medicine, 1982;440.
75. Baluda VP. Physiology of homeostasis system. M.: Medicine, 1995;238.
76. Chernukh AM. Microcirculation: monograph. Moscow: Medicine, 1975;456.
77. Sandhaus S. Ozone therapy in odonto -stomatology, especially in treatments of infected root canals. R*ev Belge Med Dent.* 1965;20(6):633.
78. Durnovo NA, Dubova NB. Status of oral antioxidant activity in patients with jaws periostitis when using ozone. Proc : Ozone and methods. efferent therapy in medicine. III all-Russia. scientific-practical. conf. N. Novgorod, 1998;87.
79. Malanchuk V, Kopchak A, Dovbysh N. Ozonotherapy in prevention of inflammatory complications of jaw fractures. 2. nd. Congress. of the Polish association for oral and maxillofacial surgery. Cracow, 1999.
80. Zhahbarov AG. Ozone therapy and hyperbaric oxygen therapy in the complex treatment of patients with acute odontogenic inflammatory processes in maxillo-facial region: Autoref. Almata, 1998;22.
81. Sandhaus S. Ozone therapy in oral surgery and clinical dentistry. *ZahnarztlPrax.* 1969;20(24):277-80. German. http://www.ncbi.nlm.nih.gov/pubmed/5263394
82. Türk R. Ozontherapie in der zahnärztlichen Chirurgie. *Erfahrungs heilkunde.* 1976;177.
83. Filippi Andreas. The Influence of Ozonized Water on the Epithelial Wound Healing Process in the Oral Cavity. In: Proceedings of the 15th Ozone World Congress, 11th - 15th September 2001, Medical Therapy Conference IOA 2001;186.
84. Brauner AW. Ozone application in periodontology, -10''' ozone world congress. March, 1991;55-63.
85. Chekmaev IS, Syrovaya SA, Makarov VA, et al. Ozone and ozone therapy. http://repo.knmu.edu.ua/bitstream/123456789/4013/1/%D0%9E%D0%B7%D0%BE%D0%BD.pdf.

86. Baysan A, Lynch E. Management of root caries using ozone, Division of Restorative Dentistry and Gerodontology, Dental School, Royal Victoria Hospital, Queen's University, Belfast, Northern Ireland, Department of Adult Oral Health, Barts and the Royal London Queen Mary's School of Medicine and Dentistry, London, UK September 2002, http://www.kavo.pl/img_cpm/global/files/global/healozone/19_Management_of_Root_Caries_using_Ozone__A.Baysan_E.Lynch.pdf
87. Sushma Das. Application of Ozone Therapy in Dentistry. http://www.nacd.in/ijda/volume-03-issue-02/122-application-of-ozone-therapy-in-dentistry
88. Malanchuk VA, Tsidelko VD, Kopchak AV, Kuzmichev AI. Ozone therapy in dentistry and maxillofacial surgery. Sursa. 2000;6(20):XI-XII. http://www.umj.com.ua/wp/wp-content/uploads/archive/20/pdf/996_rus.pdf?upload=
89. Cruz O, Menende S, Martinez ME, et al. Application of ozonized oil in the treatment of alveolitis. Ozone-News. 1997;25(4):47.
90. Sanseverino ER. Knee joint disorders treated by oxygenozone therapy. *Eura Medicophys.* 1989;163-170.
91. Agapov VS, Shulakov VV, Fomchenkov NA. Ozone therapy of chronic mandibular osteomyelitis. *Stomatologiia* (Mosk.). 2001;14-17.
92. Freedman George. Contemporary Esthetic Dentistry, 1st Edition. 2011;582-599. http://www.nzimid.org/resources/Freedman_Chapter%2026_SectionA&D%20temp%20file.pdf
93. Lemus L, Ordaz E. Application of Oleozon in the Treatment of Subprosthesis Stomatitis. 1997. http://www.o3center.org/Abstracts/ApplicationofOleozonintheTreatmentofSubprosthesisStomatitis.html
94. Petrov GM, Kudryavtsev BP, Akulich II. Efficacy of ozone products in treatment of paranasal sinusitis. *Military med. J.* 1996;12:26-28.
95. Menabde GT, Natroshvili ND, Natroshvili TD. Ozonotherapy for the treatment of parodontitis. *Georgian Med News.* 2006;(134):43-6. Russian. http://www.ncbi.nlm.nih.gov/pubmed/16783063.
96. Seidler V, Linetskiy I, Hubálková H, et al. Ozone and its usage in general medicine and dentistry. A review article. *Mazánek J. Prague Med Rep.* 2008;109(1):5-13. http://www.ncbi.nlm.nih.gov/pubmed/19097384.
97. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. *J Dent.* 2008; Feb. Review. http://www.ncbi.nlm.nih.gov/pubmed/18166260.

The latest developments in cutaneous homeostasis

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Abstract

Background: The skin is a major actor of human homeostasis mainly due to its important role in body temperature regulation but also through its role of barrier against any external aggression, and as a transmitter of a lot of information to the brain. It is very important that this vital organ can regulate its own homeostasis to be able to assume its role for the rest of human body. It is commonly admitted that cutaneous homeostasis is more or less the barrier effect but the last discovery for the last decade opens new interesting fields of investigation. Degradation of tight junctions with age are well-known. In rosacea, the water permeation in epidermis sever the cells and break the junctions, it is an open door for microbial infections and dramatic dryness. On atopic mice skin model, Yokushi and al. showed in 2015 that tight junctions of atopic skin are more permeable and this is correlated with the filaggrin protein depletion. If junctions still stop microbials and big molecules penetration, they let small molecules under 30 KDalton to penetrate the epidermis. This could be one of the causes of the inflammatory status of atopic skins and of dryness as water permeation is increased as well.
Conclusions: In conclusion, skin homeostasis becomes more and more complex with the last discoveries about skin microbiota. Interactions between sebum, epidermal lipids, epidermal peptides and microbiota are huge. We have an open field to innovate in new treatment taking into account the capability of billions of living cells on our skin surface which talk with our cells all the time and work together to help our skin assume its defense role of the human body.

Key words: cutaneous homeostasis development.

Introduction

The skin is a major actor of human homeostasis mainly due to its important role in body temperature regulation but also through its role of barrier against any external aggression, and as a transmitter of a lot of information to the brain.

It is very important that this vital organ can regulate its own homeostasis to be able to assume its role for the rest of human body. It is commonly admitted that cutaneous homeostasis is more or less the barrier effect but the last discovery for the last decade opens new interesting fields of investigation.

We will study some of the last developments about 4 skin homeostasis mechanisms:

1. Cell cohesion.
2. The stratum corneum lipids and peptides.
3. The hydrolipidic film.
4. Skin microbiota.

Reminder on cell cohesion

From the basal layer to the stratum corneum, keratinocytes are hung together through tight junctions. Hemi-desmosomes hang cells to the dermis, desmosomes hang keratinocytes together, corneo-desmosomes give sealing to corneocytes until desquamation.

Physiology status

By hanging cells together very tightly, junctions filter the entry of external agents and avoid infections and irritation. In

the other way, they slow down water perspiration and participate in a reduced Trans Epidermal Water Loss (TEWL). They are also a very important pathway for cells communication. At least, thanks to proteases activity, their disappearance allows desquamation on the top of stratum corneum. I won't focus on this topic in this article but it is interesting to notice that they are also involved in some skin dysfunctions.

Pathologic status

Degradation of tight junctions with age is well-known. In rosacea, the water permeation in epidermis sever the cells and break the junctions, it is an open door for microbial infections and dramatic dryness.

On atopic mice skin model, Yokushi and al. showed in 2015 that tight junctions of atopic skin are more permeable and this is correlated with the filaggrin protein depletion. If junctions still stop microbials and big molecules penetration, they let small molecules under 30 KDalton to penetrate the epidermis. This could be one of the causes of the inflammatory status of atopic skins and of dryness as water permeation is increased as well [1, 2].

Stratum corneum. Epidermal lipids and natural moisturizing factors

It is impossible to talk about epidermal lipids without speaking about sebum lipids, as even if they are secreted completely separately they mix together on the hydrolipid film.

Their composition is quite similar with both a high content of Free Fatty Acids (FFA) (about 20 to 25%), cholesterol and its esters (richer in epidermal lipids than in sebum), triglycerides for the sebum and ceramides (glycerides and sphingosides derivate) for the Stratum corneum lipids.

The sebum contains two particular molecules: Wax esters and squalene which are specific for human. You won't find them anywhere else in the human body and they are absent of lot of mammalians sebum including big monkeys (but present in rat and mice sebum).

Synthesis of epidermal lipids

On the granular layer of epidermis, the granular keratinocytes show some granules called the lamellar bodies. Those granules are issued from the Golgi corpus and are released very classically by exocytosis. As shown in figure 1, they contain ceramides, and cholesterol. But what it is very interesting to note is that they contain not only the precursors of epidermal lipid such as phospholipids, glucosylceramides, sphingomyelin and cholesterol, but also the enzymes that will transform them after liberation in the extra cellular space. But those granular lipids are real tool boxes as they contain proteases that will act in the stratum corneum and also antimicrobial peptides which will regulate the skin microbiota [3, 4].

Synthesis of natural moisturizing factors (NMF)

In the granular keratinocytes, we found also keratohyalin granules which contain profilaggrin, which is transformed into filaggrin. Filaggrin links to keratin to build the corneocyte cytoskeleton and to involucrin to build the corneo-desmosomes. Filaggrin will at least give birth to the NMF.

The life circle of filaggrin is also a very good example of finest regulation of skin homeostasis. Keratohyalin granules contain profilaggrin. This huge protein (more than 400 KDalton) is made of 10 to 12 filaggrin attached together, and 2 uncomplete filaggrin at each end (one N-terminal and one C-terminal). The N-terminal peptide is separated by enzyme under Ca^{2+} signal. This peptide goes to the nucleus and it is probably one of the signals which lead the keratinocyte into apoptosis. The filaggrin is then dephosphorylated by enzymes. This allows them to link to other proteins (keratin and involucrin). By linking with keratin, they constitute the cytoskeleton of the corneocyte, and by linking to involucrin, they participate in the corneo-desmosmoes which attach corneocytes together and seal the stratum corneum. By these both actions they contribute to decrease TEWL. But it is not their only role, on the last stage of stratum corneum, some new enzymes will deiminate the filaggrin. It allows some other protease to cut the protein into amino acids and acids such as urocanic acid, lactic acid, and urea. These molecules are released into the intercorneocyte space, they are called the Natural Moisturizing Factor (NMF) and they have a key role in cutaneous homeostasis. They have the capability to capture

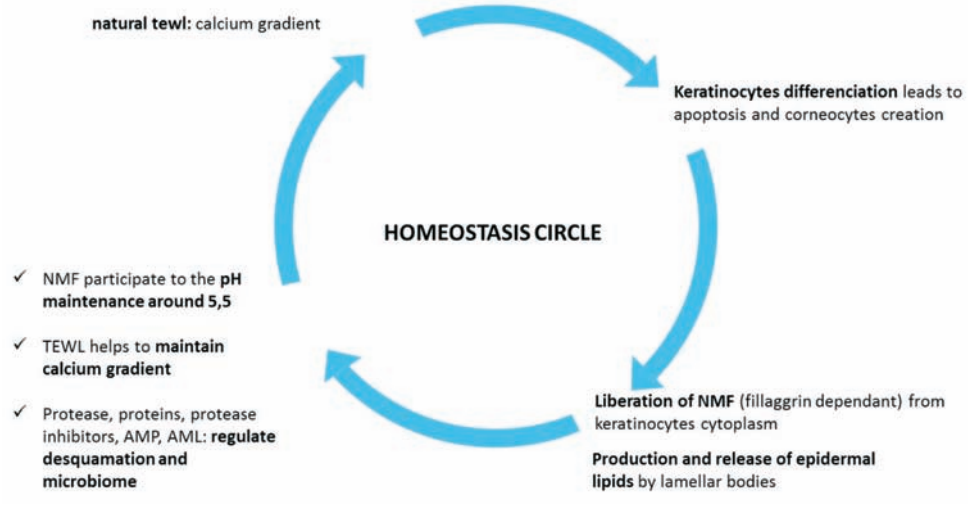

Fig. 1. Homeostasis Circle. Hydrophobic lipid cement decreases TEWL and participates in skin moisturisation. Free fatty acid gives the skin a pH 5.5 and enables the life of commensal bacteria. Fillagrins participates in cell cohesion. The PRO fillagrin triggers apoptosis.

water molecule and, doing that, they will another time reduce the TEWL. As they are low acids, they mix with epidermal and sebum fatty acids, and this mix gives to the skin its very robust buffered pH around 5, 5. This pH is very important as we will see further that it allows the life of commensal bacteria of the skin microbiota. The last but not the least some of these peptides have an antimicrobial action against pathogeneous bacteria, such as *S. aureus* [5, 6].

By considering keratinocyte apoptosis, stratum corneum cohesion, TEWL regulation, living condition of skin microbiota, we can say that profilaggrin and its metabolites are a key factor of skin homeostasis.

In the following drawing, you will find a summary of the regulation role of epidermal lipids and peptides in skin homeostasis (fig. 1).

This is a robust and efficient mechanism, as it is able to repair the barrier function disruption. After a barrier disruption, the brutal change in calcium gradient induces a massive liberation of lamellar bodies. This activates the proteins SREBP and they induce the gene coding for enzymes involved into lipids synthesis such as (HMG-CoA reductase, HMG-CoA synthase, fornesyl synthase, squalene synthase). Following this activation, Free Fatty Acids, cholesterol are synthesized and fill new granular bodies. At the same time, AMP synthesis is also increased to help the epidermis to fight aganst bacterial invasion.

Pathology

Atopy is the more related disease with epidemic lipids and peptides. On human chromosome 1, we find the locus 1q21 with the epidermic differentiation complex. It contains a lot of genes involved into keratinocytes differentiation including seven genes coding for S100 fused Type proteins (SFTPs) and for filaggrin I and II.

A non-sense mutation on filaggrin genes (Kesic and al, Front biosc, 2014) has been shown on Caucasian atopic patient.

A non-sense mutation on Fillggrin-2 gene has been associated with the persistence of atopic bursts on Afro-American patients (Margolis and al., J Aller clin Immunology, 2014).

In addition, the lamellar organization of lipids into lamellar bodies is degraded.

In psoriasis, a lot of genes deficiencies have been observed resulting in abnormal filaggrin expression, excess of involucrin (protein involved into desmosomes) in psoriasic lesions and persistence of cholesterol receptors in upper layer of keratinocytes. All these anomalies show a barrier effect deficiency including both epidermic protein and lipids.

Hydrolipidic film

The hydrolipidic film is a mix between sweat and sebum. Sweat is mainly excreted by eccrine glands. They are mainly present on feet sole and hand palm, armpit and forehead. Apocrine glands always link with hair which is less involved into sweat excretion.

The composition of sweat is very complex and is one of the way for the body for evacuate wastes. It contains a lot of amino-acids, sugars, and urea.

Sebum is excreted by sebaceous glands. These glands are mainly present on head, face, upper chest and back. The composition of sebum is quite similar to epidermal lipids with free fatty acids, triglycerides, cholesterol and its esters. But it contains 2 types of molecules: wax esters and squalene. These types aren't present in other parts of human bodies (fig. 2).

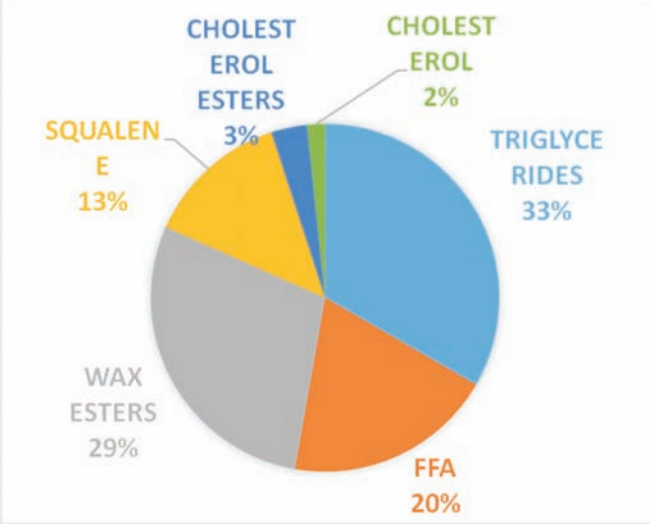

Fig. 2. Hydrolipidic film composition.

If we compare both sweat and sebaceous glands distribution (see below), it is easy to understand that the composition of the hydro-lipidic film will be different regarding the considered part of the body.

On head, it will be richer in sebum, on hand palm and armpit, it will be richer in sweat. In regions poor in both types of glands, the hydro-lipidic film will be the result of the spreading from the wet region giving more or less dry parts of body, and these regions will be more or less richer in sebum or sweat.

But some parameters will be unchanged on body surface: temperature between 32 to 35 °C and a buffered pH around 5.5. This pH is the result of the acidity activity of free fatty acids and weak acids from the NMF (lactic acid, urocanic acid) and molecules of sweat. As this mix has a buffered effect, the pH is relatively stable even when the rate between sebum and sweat changes.

This will have an influence on the efficiency of the barrier effect but a big impact on the last component of skin homeostasis; the microbiota.

Skin microbiota

Cutaneous skin microbiota is a part of human microbiota (mainly located in stomach and gut). Cutaneous microbiota is made of 10^{12} (thousand billions) of bacteria for more than 200 different species. Human microbiome excesses human genoma in cells number and DNA size: 10^{16} microbial DNA copies versus 10^{14} human DNA copies.

This microbiota is a biofilm made of bacteria but also micro-fungis, micromites and micro-nematodes. This biofilm is symbiotic with the skin: it protects the skin from pathogeneous aggression, skin provides nutrients (cellular wastes) and pH around 5,5 which permits life for non-dangerous

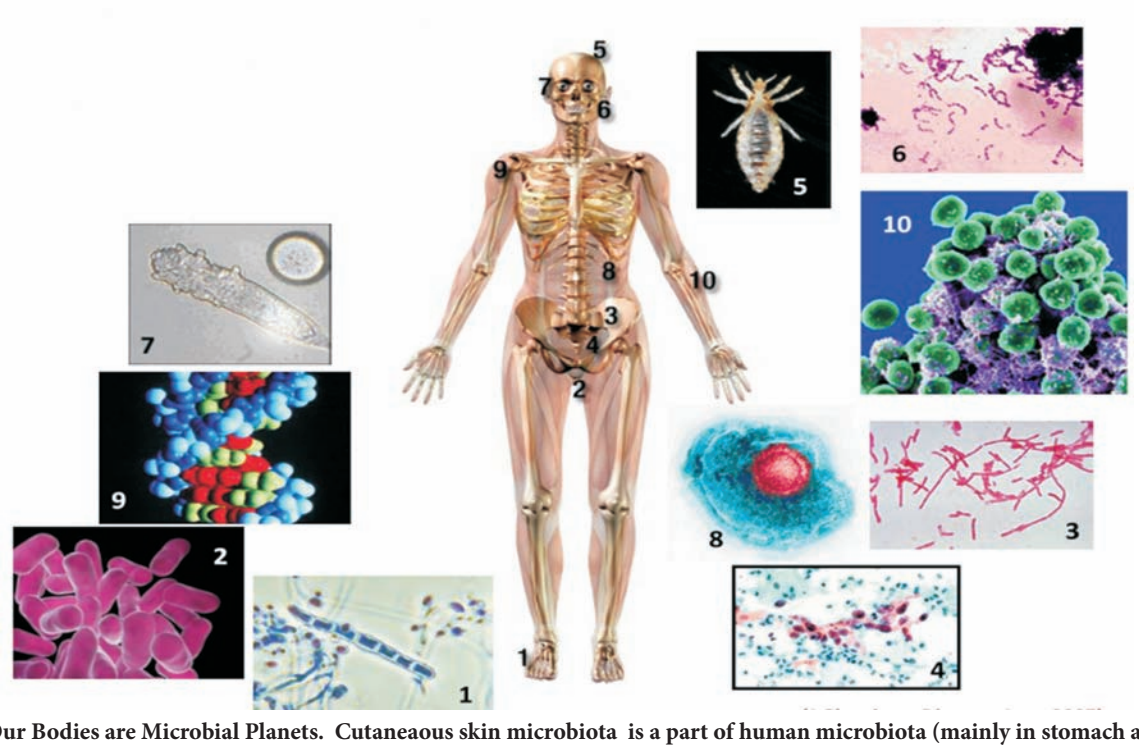

Fig. 3. Our Bodies are Microbial Planets. Cutaneaous skin microbiota is a part of human microbiota (mainly in stomach and gut). Cutaneous microbiota is made of 10^{12} (thousand twelve billion) of bacteria for more than 200 different species. Human microbiome exceed human genoma in cells number and DNA size: 10^{16} microbials cells versus 10^{14} human cells.

bacteria [7, 8]. So we can say that: Our Bodies are Microbial Planets (fig. 3).

The composition of this biofilm evolves all along the skin life from birth to death. It is different for each person and varies with age, sex, activity, and environment. It is also different from one part of the body to another: hand, scalp, armpit, forehead, arm, leg and back are respectively richer in bacteria. Grice and Segre have shown all the parameters modifying the microbiota composition (fig. 4).

Scharschmidt and al. [8] have studied the uptake into the hydro-lipidic film of each bacteria phylum. So knowing that the film composition changes all over the body, it is easy to understand that the composition of bacteria's population will change in the same way (tab. 1).

Table 1

The composition of bacteria's population

Genus	Phylum	Primary nutrient sources in skin
Staphylococcus	Firmicutes	Sweat : Urea, ammonia, AAs, glucose Sebum: AAs SC: peptides
Corynebacterium	Actinobacteria	Sweat : Urea, ammonia, vitamins, glucose Sebum: lipids SC: lipids
propionibacterium	Actinobacteria	Sweat : AAs, glucose Sebum: lipids, AAs SC: peptides, lipids

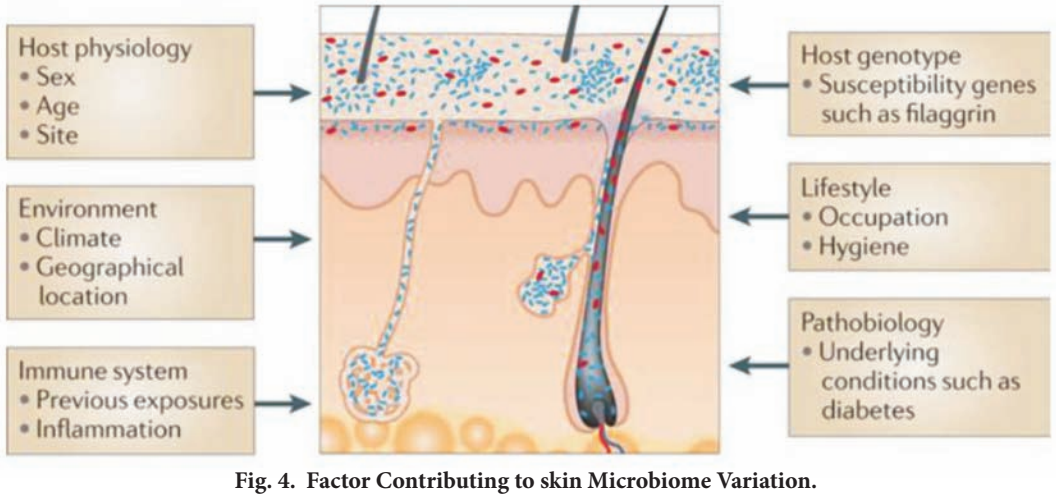

Fig. 4. Factor Contributing to skin Microbiome Variation.

But giving an accurate living temperature and nutrients is not the only role of hydrolipidic film for the microbiota. After millions years of evolution, our microbiota and our immune system have learned to live together. At the early stage of childhood, there will be a lot of exchanges between the bacteria colonization and the innate immune system. Commensal bacteria are presented to immune system inducing a tolerance versus these bacteria.

At the same time commensal microbiota and epidermis will produce a lot of different molecules which will be well-tolerated by commensal bacteria (mainly composed of gram – bacterias) but with anti-microbial activity versus pathogeneous bacterias (mainly composed of gram + bacteria).

These peptides are called Anti-Microbial Peptides (AMP), synthesized by the epidermis and conveyed through the lamellar bodies. It is interesting to note that these AMP are also synthesized by the commensal bacteria. So, microbiota and epidermis work together to avoid pathologic bacteria colonization (tab. 2).

Table 2

Antimicrobial peptides action

AMP	ACTION
Defensines (human β defensin (HBD), human neutrophil peptide (HNP), α defensin and θ defensin	Antimicrobial and anti viral activity
Dermicidine	Stimulate keratinocytes for chemokines and pro inflammatory cytokines production Stimulate neutrophile cells
Psoriasine (S100A15)	
Cathelicidin (LL37)	
Rnase 7	Chemoattractive for lymphocytes, mastocytes and neutrophile cells
Chemokines (CXCL-1)	
Proteins (lactoferrin, lysosime)	

Fats into the hydrolipidic film come mainly from sebum, but also from epidermal lipids synthesized by the lamellar bodies which spread into the film. It has been recently shown that lots of ceramides and sapienic acid (a free fatty acid specific of human sebum) have also an anti-microbial activity which helps the AMP action. It is a new link between microbiota and epidermal components (tab. 3).

Table 3

Antimicrobial lipids action

AML	ACTION
Sphingosine	Inhibits C.albicans, E.coli, S.aureus
Dehydrosphingosin	Inhibits C.albicans, E.coli, S.aureus
Phytosphingosin	Inhibits C.albicans, E.coli, S.aureus
Sapienic acid	Inhibits S.aureus

The last but not the least, we discover during the last decade that the neuro-mediator involved into the stress response or into inflammatory chain reaction has an influence on skin microbiota. It could be a link between inflammatory status and microbial infection and new pathway for treatment in future.

A very interesting example of adaptation between the film and microbiota is the psoriasin. Called psoriasin because it was found in psoriasis lesion at first, psoriasin is a common AMP of healthy skin which has an anti-E.coli activity. It is known that E.coli is a commensal bacterium for gut but could be pathologic for the skin. The way of contamination is mainly provided by the hand and feet. As hands touch the face more than hundred times a day the risk of contamination is high for face as well. If we measure the rate of psoriasin in hydrolipidic film, concentration is ten times more important on hand palm, feet sole and face where the possibility of contamination by *E. coli* is the highest.

We can summarize the balance between hydrolipidic film and microbiota in the following figure. In healthy condition, both systems influence themselves until reaching a balance status. Film provides nutrients to commensal bacteria. Using these nutrients, bacteria reject their wastes in the film. These wastes are mainly polyunsaturated fatty acids which enrich the lipidic film composition and they have usually an anti-microbial action against pathologic bacteria.

But if this balance status is destroyed (for internal reasons that change the composition of hydro-lipid film) or external environmental reasons that change the microbiota composition; this homeostatic circle could be destroyed. Once the pathologic bacteria start to colonize the skin, the wastes given back to the film change with the bacteria species. Opposite to the commensal ones, pathologic bacteria of polyunsaturated fatty acid have an inflammatory effect making the virtuous circle becoming a vicious circle leading to disease and inflammation [9, 10, 11].

The human microbiota has been studied for less than ten years. In 2008, the project human microbiota was launched on the way to explore the composition of this biofilm. The first DNA analysis made after blade sampling showed a majority of human DNA due to cells from stratum corneum removed by the blade. Nowadays, microbiota sampling is made with swabs which allows a mild sampling of bacteria.

DNA is extracted and amplified with 16S RNA. A very rich collection of primer is available and these primers could be specific of the phylum, the genius, the order or the bacteria specie. So thanks to them, it is possible today to make a phylogenetic analysis of each human microbiota. If sampling and sequencing aren't very difficult to do, the more critical in these studies is the data analysis. Knowing that the microbiota of each of us changes from one part of the body to another, that it changes every day according to the environment or our health condition, it needs a lot of sampling and a very fine statistical analysis to obtain relevant results.

We told previously that the hydrolipidic film changes according to glands distribution as shown by Verhulst and al. and that influences the microbiota composition.

Grice and al. showed in 2009 that this hypothesis is true. They study human microbiota on 10 subjects in three areas: sebaceous, moist and dry.

Unsurprisingly, in sebaceous areas a majority of Propionibacterium has been found except on subject 6 who showed a very low level of this genus. In other areas it is more difficult to find a majority genus and the disparity is higher. In these areas we can see the high differences that could occur among people (see especially subject n°5) and this could explain the susceptibility of subjects to diseases.

Following this study, many other researches have been done and we get to know better and better the microbiota composition.

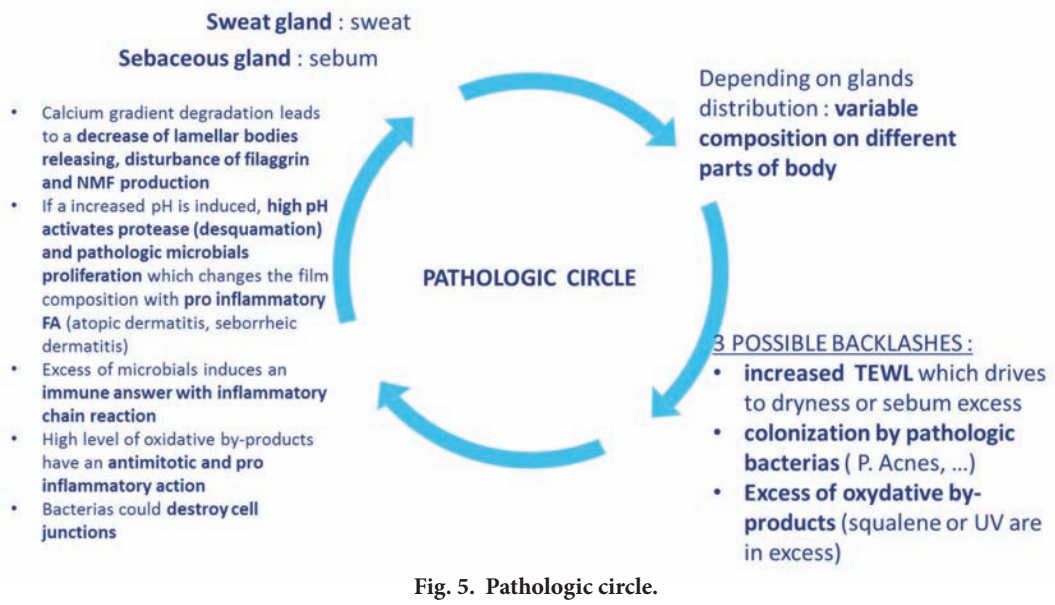

Fig. 5. Pathologic circle.

It is now possible to define that the phyla are more or less specific of a body area and relatively constant from an individual to another one (Spor, 2011; Grice, 2009), even if there is a high interindividual disparity in gender, species and genus are high [12, 13].

What about fungi?

We talked a lot about bacteria but they are not alone in our microbiota. Micronematodes and mites such as Demodex are, of course, present on our skin and important in some pathology such as rosacea. In 2010, Gao and al. made a study to analyze both bacterial and fungal microbiota populations. They confirm the diversity of bacteria's phyla but when we look at fungi, we can see that they are composed of more than 90% by Malassesia spp in humans.

We saw the huge diversity of bacteria species in different parts of the body and in different people but what about the shift occurring in skin diseases.

We can summarize the interactions between hydrolipidic film and microbiota and their results on skin barrier homeostasis (fig. 5).

Pathologic status

In the following figure, you will see the difference between a healthy skin and psoriasic lesions where we can notice a big decrease into Propionibacterium population (fig. 6).

Microbiota has been studied in other skin diseases. It is now well known that Atopic dermatitis is linked with Staphylococcus aureus proliferation.

In acne, microbiota studies have shown that Propionibacterium acne is mainly commensal bacterium. Only on strain of *P. acne* are produced inflammatory fatty acids. It seems that

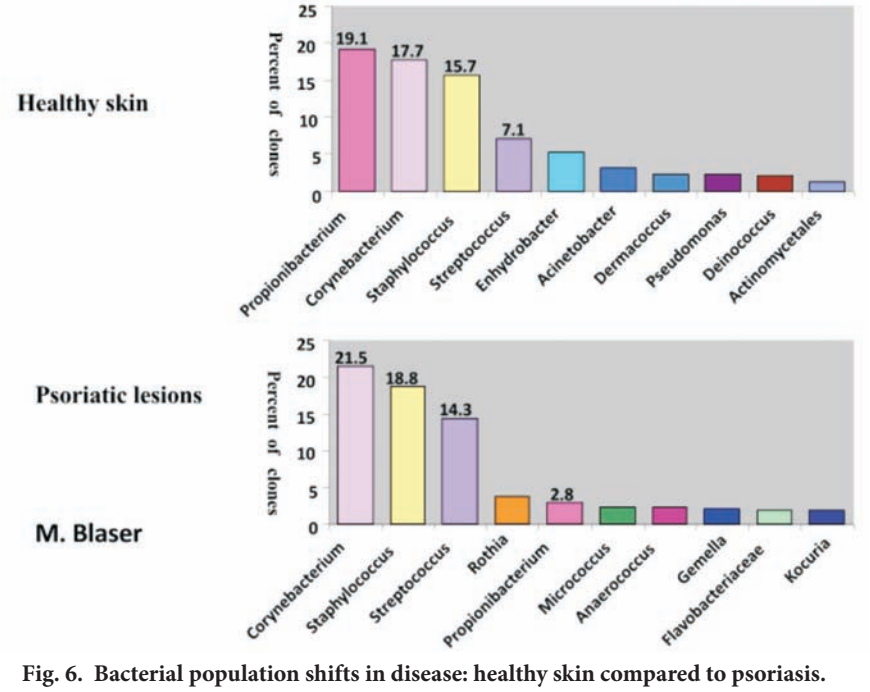

Fig. 6. Bacterial population shifts in disease: healthy skin compared to psoriasis.

it is the combination of this particular strain of *P. acne* with an important production of sebum and oxidized squalene which leads to acne.

In rosacea, Demodex invasion is known for a long time. Recent study (2012) showed that Demodex destroys epithelial cells and allows staphylococcus epidermidis to penetrate into the epidermis and cause pustular rosacea. But we can find lots of other bacteria on Demodex which could be involved into rosacea infections (*P. acne, S. epidermidis, Corynebacterium kroppenstedtii, S. mitis, P. granulosum ans S. anvi*).

For a new therapy

We saw that modified microbiota is involved into a lot of skin diseases. A new way of cure could be to restore microbiota balance. It has been tried in gut with good results. But knowing the complexity of interaction between microbiota and other skin barrier components, and the impossibility to control the environment of skin, adding commensal bacteria to take place of pathologic bacteria won't be a good solution.

But it is possible to influence the microbiota. Seite and al. showed in 2015 that restoring the barrier function by adding an emollient cream leads to a restoration of microbiota balance regarding staphylococcus.

Dr. Reygangne has shown that it is possible to reduce dandruffs caused by Malassesia by food supplemented with lactobacillus paracasei strain NCC2461, ST11.

Conclusions

In conclusion, skin homeostasis becomes more and more complex with the last discoveries about skin microbiota. Interactions between sebum, epidermal lipids, epidermal peptides and microbiota are huge. We have an open field to innovate in new treatment taking into account the capability of billions of living cells on our skin surface which talk with our cells all the time and work together to help our skin assume its defense role of the human body.

References

1. Yokouchi, et al. Epidermal tight junction barrier function is altered by skin inflammation, but not by filaggrin-deficient stratum corneum, *J. dermatol. Sci.* 2015;77:28-36.
2. Apostolos Pappas. Epidermal surface lipids. *Dermatoendocrinol.* 2009;1(2):72-76.
3. Berra Veggetti. Role of epidermal ceramides in barrier function. *J. Appl. cosmetol.* 1996;14:51-57.
4. Sandilands A, et al. Filaggrin in the frontline: role in skin barrier function and disease. *Sci.* 2009;122(9):1285-1294.
5. Brown SJ, McLean WH. One Remarkable Molecule: Filaggrin. *J Invest Dermatol.* 2012;132(3 Pt 2):751-62.
6. Miyagaki T, Sugaya M. Recent advances in atopic dermatitis and psoriasis: Genetic background, barrier function, and therapeutics target. *J. Dermatol. Sci.* 2015;78:89-94.
7. Picardo A, et al. Sebaceous gland lipids. *Dermatoendocrinol.* 2009;1(2):68-71.
8. Scharschmidt TC, Fischbach MA. What lives on our skin: Ecology, Genomics and Therapeutic Opportunities of the Skin Microbiome. *Drug Discov Today Dis Mech.* 2013;10(3-4):e83-e89.
9. Gibbon F, et al. Propionibacterium acnes strain populations in the human skin microbiome associated with acne. *J. Invest Dermatol.*
10. Forton, F. Papulopustular Rosacea, Skin immunity and demodex: Pityriasis Folliculorum as a missing Link. *Journal of European academy of Dermatology and Venerology: JEADV*. 2012;26(1):19-28.
11. Whitfield M, et al. *Staphylococcus epidermidis* : a possible role in the pustules of rosacea. *Journal of the American Academy of Dermatology.* 2011;64;49-52.
12. Weinstock E, et al. Rosacea and small intestinal bacterial overgrowth : prevalence and response to rifaxim. *Journal of the American Academy of Dermatology*. 2013;68:875-876.
13. Murillo C, et al. Microbiota of Demodex Mites from Rosacea Patients and controls. *Microbial Pathogenesis.* 2014;71-72:37-40.

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6. Referințele, în conformitate cu cerințele Comitetului Internațional al Editorilor Revistelor Biomedicale (www.icmje.org, capitolul IV.A.9), se expun în ordinea apariției în text. În lista referințelor titlul articolului, se traduce în limba engleză, poziționându-se în paranteze pătrate. Referințele bibliografice prezentate în grafie chirilică sunt transliterate în grafie latină, utilizând următoarele semne grafice: А–A, Б–B, В–V, Г–G, Д–D, Е–E, Ё–E, Ж–ZH, З–Z, И–I, Й–Y, К–K, Л–L, М–M, Н–N, О–O, П–P, Р–R, С–S, Т–T, У–U, Ф–F, Х–KH, Ц–TS, Ч–CH, Ш–SH, Щ–SCH, Ы–Y, Э–E, Ю–YU, Я–YA; Ь și Ъ se omit. Imediat după transliterare, în paranteze pătrate, se prezintă traducerea titlului articolului în limba engleză. De exemplu: Ivanov IV, Sidorov VM, Kozlov NF. Transplantatsiya organov i tkaney [Transplantation of organs and tissues]. Vestnik Khirurgii [Messenger of Surgery]. 2010; 26(6):45-49.

ГИД ДЛЯ АВТОРОВ

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К публикации принимаются статьи на английском языке. Все статьи направляются на рецензию двум независимым экспертам.

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