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

The Moldovan Medical Journal

The Publication of the Scientific Medical Association of Moldova

Frequency – 4 per year
Category – B+

Vol. 64, No 5
November 2021

This issue is dedicated to the Annual Scientific Conference
of *Nicolae Testemitanu* State University of Medicine and Pharmacy
of the Republic of Moldova
on the occasion of the 76 years of activity

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The Moldovan Medical Journal is an international scientific double-blind peer reviewed periodical edition, 4 per year, of the Scientific Medical Association of the Republic of Moldova designed for specialists in the areas of medicine, dentistry, pharmacy, social medicine and public health. From its debut the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development.

The Editorial Board warmly welcomes both the readers of and the authors for the journal, all those who are enthusiastic in searching new and more effective ways of solving numerous medicine problems. We hope that those who want to make their contribution to the science of medicine will find our journal helpful and encouraging.

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Index for subscription – 32130

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Publisher

"Revista Curier Medical"
192, Stefan cel Mare Avenue
Chisinau, the Republic of Moldova

Printing House "Tipografia Sirius"
2, Lapusneanu str., Chisinau, MD-2004
the Republic of Moldova
www. sirius.md

TABLE OF CONTENTS

ORIGINAL RESEARCHES

REVIEW ARTICLES

ORIGINAL RESEARCHES

https://doi.org/10.52418/moldovan-med-j.64-5.21.01
UDC: 616.12-009.72-036.11-036.22(478)

Clinical-epidemiological aspects of acute coronary syndrome in the morbidity and mortality in the Republic of Moldova

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Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

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Manuscript received August 07, 2021; revised manuscript November 11, 2021; published online November 26, 2021

Abstract

Background: Acute coronary syndrome (ACS) is associated with high costs of hospital care, frequent recurrences and high risks of sudden death and short-term mortality.
Material and methods: The retrospective study was based on the evaluation of 140 patients who met the ACS criteria. Clinical and epidemiological data were obtained based on the analysis of statistical reports of the Ministry of Health during 2016-2020.
Results: The mean age of the patients was 65.0 ± 27.7 years. Incidence of acute myocardial infarction (AMI) in the population increased from 4.7 to 5.2 cases per 1000 inhabitants. The study found the following occurrence of risk factors: history of cardiovascular disease – 42 (91.3 %) patients, hypertension in 35 (76.1%), obesity in 21 (45.6%), diabetes mellitus in 14 (30.4%) and smoking in 13 (28.3%). The share of risk factors in the male group was distributed as follows: history of cardiovascular disease – 82 (87.2%) patients, hypertension – 63 (67.1%) patients, smoking – 40 (42.5%) patients, diabetes mellitus – 27 (28.7%) patients and obesity was established in 24 (25.5%) of men.
Conclusions: ACS affects men more frequently, compared to women, in a ratio of 2.04:1. People under the age of 65 years constitute 57.5%. The results of the study showed that compared to men, women with ACS were older and had significantly more comorbidities.
Key words: acute coronary syndrome, acute myocardial infarction.

Cite this article
Coropceanu I, Ciobanu G. Clinical-epidemiological aspects of acute coronary syndrome in the morbidity and mortality in the Republic of Moldova. *Mold Med J*. 2021;64(5):5-10. https://doi.org/10.52418/moldovan-med-j.64-5.21.01.

Introduction

Cardiovascular disease causes about a third of all deaths in the world, of which 7.5 million deaths are estimated to be caused by ischemic heart disease. Acute coronary syndromes (ACS) and sudden death cause the most deaths related to ischemic heart disease (IHD), which accounts for 1.8 million deaths per year, or 20% of all deaths in Europe, although there are large variations from one country to another. The incidence rate in European countries is between 43 and 144 per 100.0000 inhabitants per year [1-3]. Previous studies suggest that women with ACS have different onset symptoms compared to men. There is a fairly clear tendency for STEMI to occur more frequently in young people than in the elderly and as often in men as in women. The incidence of IHD, in general, and ACS increases with age, although, on average, it occurs 7-10 years earlier in men compared to women [4]. ACS occurs much more often in men than in women under the age of 60, but women represent the majority of patients over the age of 75. The risk of acute coronary events throughout life is related to exposure to traditional cardiovascular risk factors. ACS is a major health problem associated with high costs of hospital care, frequent recurrences and high risks of sudden death and short-term mortality [5]. The frequency of ACS increases with age and is a medical-social problem that increases with the aging of the population of the Republic of Moldova. Mortality in patients with ACS is influenced by multiple factors, including old age, Killip class, delayed treatment, therapeutic strategy, history of myocardial infarction, diabetes, renal failure, number of affected coronary arteries, and left ventricular ejection fraction [6]. Early diagnosis of patients with ACS is important for the selection and success of treatment. Currently, there are limited data on the clinical and epidemiological aspects of ACS in women. Therefore,

the aim of the study was to evaluate the clinical-epidemiological aspects of ACS in the population of the Republic of Moldova.

Material and methods

The retrospective study was conducted based on the evaluation of 156 acute medical unit (AMU) statistical forms (SF No 110/e) approved by the Ministry of Health (MHL) (order No 1079 of 30.12.2016) of patients with ACS during January - July 2020 by the prehospital emergency medical service. Of all the records examined, 140 patients who met the ACS-ST elevation (STE) and ACS- non ST elevation (NSTE) criteria were included in the study and clinically confirmed. Patients with ACS-NSTE were older (P <0.001) than those with ACS-STE. The mean age of the patients included in the study was 65.0 ± 27.7 years, including 46 women with a mean age of 69.7 ± 28.4 years and 94 men with a mean age of 64.6 ± 20.8 years. In order to study the mortality and morbidity of the population caused by ACS, the statistical reports of MHL were also analyzed during the years 2014-2020 (SR No 30-health, ST No 12-health). Statistical analysis of the results obtained was performed using the Statistical Package for Social Sciences (SPSS 19.0) and the Microsoft Excel 2010 version. The confidence intervals were calculated at the level of 95%. A p value below 0.05 was considered statistically significant. Demographic variables and risk factors were also analyzed in terms of frequency and percentage.

Results

According to the statistical data of the National Bureau of Statistics of the Republic of Moldova (2020), presented in table 1, the coefficient of population aging in the period 2014-2020 increased from 17.5 to 21.8, including men from 14.5 to 18.1 and women from 20.3 to 25.1. There was found an acceleration of the aging process of the population, in the referenced time period, and an increase of the aging coefficient by 3.6 in men and 4.8 in women, and the average for both sexes by 4.3.

Table 1. Coefficient of population aging during the years 2014-2020 (on January 1, the number of people aged 60 and over per 100 inhabitants)

Total	2014	2015	2016	2017	2018	2019	2020
Men	14.5	15.0	15.4	16.0	16.7	17.5	18.1
Women	20.3	21.0	21.5	22.2	23.0	23.9	25.1
Both sexes	17.5	18.1	18.5	19.2	20.0	20.8	21.8

Note: According to the J. Beaujeu-Garnier-E. Rosset scale, the value of indicator 12 and above qualifies as "demographic aging".

The study of the morbidity of the population of the Republic of Moldova due to cardiovascular diseases, years 2014–2020 (per 100 thousand population) demonstrates an ascending dynamics, both of prevalence and incidence (fig. 1).

The prevalence of cardiovascular diseases in the population increased from 1604.8 cases in 2014 to 2141.6 cases in 2020, per 100 thousand inhabitants, or by 133.5%. During the reference period, the incidence of the population due to cardiovascular diseases increased from 189.8 cases in 2014 to 258.1 cases in 2020 per 100 thousand inhabitants, or by 136.0%.

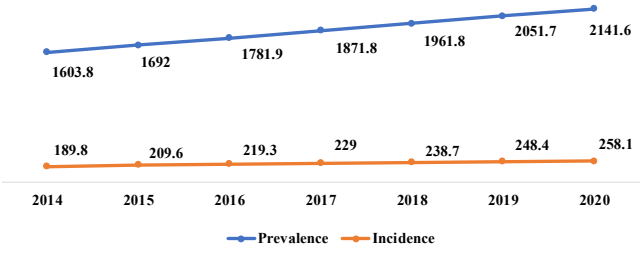

Fig. 1. Prevalence and incidence of cardiovascular diseases in the population of the Republic of Moldova, years 2014–2020 (per 100 thousand population)

The analysis of the morbidity of the population of the Republic of Moldova due to acute myocardial infarction during the years 2014-2020 (fig. 2), established an increase in the prevalence from 4.7 cases to 5.2 cases per 10 thousand inhabitants.

The study of the general mortality and mortality caused by cardiovascular diseases of the population of the Republic of Moldova during the years 2014-2020 shows a high level in 2020 – 1147.9 and 649.3 deaths per 100 thousand inhabitants, respectively (tab. 2).

Table 2. General mortality and through cardiovascular diseases of the population of the Republic of Moldova, years 2014-2020 (per 100,000 population)

Indicators	2014	2015	2016	2017	2018	2019	2020
General mortality	1110.5	1122.8	1083.5	1036.3	1049.3	1037.2	1147.9
Mortality through CVD	642.5	648.2	617.3	605.6	609.4	608.5	649.3
Mortality through IHD	359.5	348.6	314.9	317.7	320.6	313.7	356.6
Mortality through stroke	240.7	206.9	237.8	151.9	147.4	146.1	142.0
Mortality through AMI	51.7	53.2	56.3	51.3	53.2	53.0	49.6

Note: CVD – cardiovascular diseases; IHD – ischemic heart disease; AMI – acute myocardial infarction.

The pathology of the circulatory system continues to remain on the first place in causes of death of the population, constituting 56.6% in 2020. Out of the total 40466 deaths registered in the Republic of Moldova in 2020, 22889 deaths

were caused by cardiovascular diseases, including ischemic heart disease causing 12571 deaths, or 54.9%, which are 356.6 cases per 100 thousand inhabitants. The mortality of the population by myocardial infarction remains at a constant level, constituting 49.6 cases per 100 thousand inhabitants, especially in rural areas. The study of the mortality of the rural and urban population due to cardiovascular diseases highlights an over-mortality in rural areas (tab. 3).

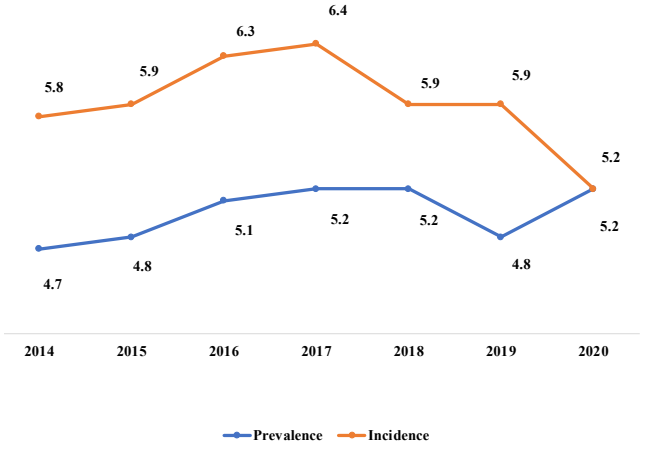

Fig. 2. Prevalence and incidence of acute myocardial infarction in the population of the Republic of Moldova, years 2014–2020 (per 10 thousand population)

Table 3. Mortality of the population of the Republic of Moldova by areas of residence, due to cardiovascular diseases, for the years 2014-2020 (per 100000 population)

Indicators	2014	2015	2016	2017	2018	2019	2020
Urban population	430.4	438.4	427.7	422.5	435.0	417.7	488.4
Rural population	720.0	725.7	687.9	675.2	675.3	682.5	712.6
Total RM	642.5	648.2	617.3	605.6	609.4	608.5	649.3

The mortality rate of the population due to cardiovascular diseases increased from 642.5 deaths in 2014 to 649.3 deaths in 2020 per 100 thousand inhabitants. The mortality of the population due to cardiovascular diseases in 2020 registered 488.4 deaths in urban areas and 712.6 deaths in rural areas, per 100 thousand inhabitants. The distribution of mortality rates of the population due to cardiovascular diseases, in the referenced period, remains on the first place constituting 57.5% in 2014, 58.4% in 2017 and 56.6% in 2020 (fig. 3).

The analysis of the mortality of the population due to ischemic heart disease, by areas of residence established a high level of 359.5 deaths in 2014 and 356.6 deaths in 2020 (tab. 4).

The study showed an increase in the mortality of the urban population due to ischemic heart disease from 211.9 deaths in 2014 to 356.6 deaths in 2020. The mortality level of the rural population due to ischemic heart disease exceeds that of the urban population by 195.2% in 2014 and respectively 146.2% in 2020.

Table 4. Mortality of the population of the Republic of Moldova due to ischemic heart disease, by areas of residence, for the years 2014-2020 (per 100 thousand population)

Indicators	2014	2015	2016	2017	2018	2019	2020
Urban population	211.9	229.1	214.0	215.2	207.6	207.1	267.8
Rural population	413.6	92.9	352.5	356.6	363.3	363.3	391.5
Total RM	359.5	348.6	314.9	317.7	320.6	312.9	356.6

The assessment of the mortality of the population due to acute myocardial infarction, by areas of residence, for the years 2014-2020 established a level of 51.7 cases in 2014 and 49.6 cases in 2020, per 100 thousand inhabitants (fig. 4).

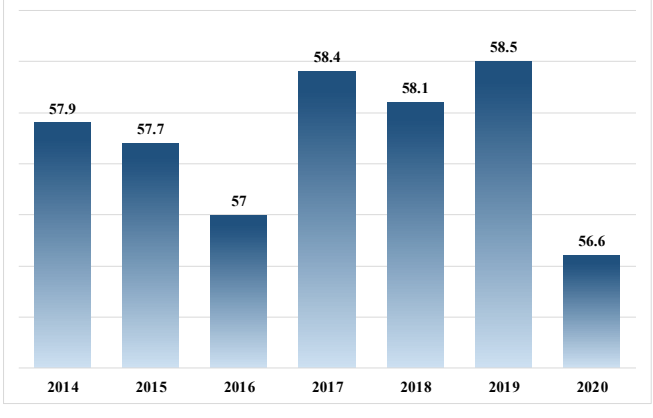

Fig. 3. The distribution of mortality rates of the population of the Republic of Moldova due to cardiovascular diseases (in%), for the years 2014-2020

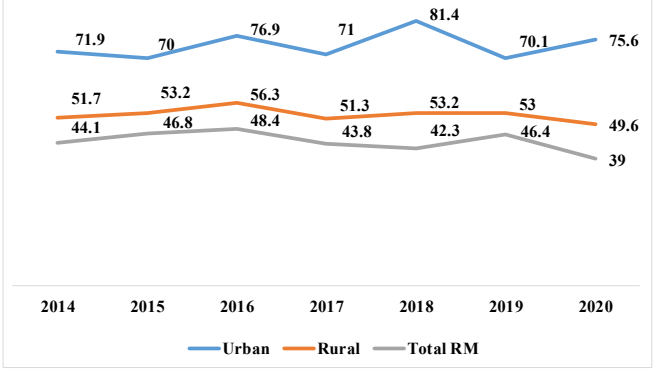

Fig. 4. Mortality of the population of the Republic of Moldova due to acute myocardial infarction, for the years 2014 – 2020 (per 100 thousand population)

The evaluation of 140 application forms for patients with ACS - STE and NSTE showed a total mean age of 65.0 ± 22.7 years, that is 69.7 ± 28.4 years for women and 64.6 ± 20.8 years for men (P< 0.001), (tab. 6).

Table 5. Medical assistance provided to patients with ACS in the Republic of Moldova the years 2015-2020 at the hospitals level

Indicators	2015	2016	2017	2018	2019	2020
Acute coronary syndrome						
Total hospitalization	7276	7838	6694	6408	8771	6338
Transported in the therapeutic window	4758	5464	4362	4499	5764	4987
Coronary angiography was performed	1060	1671	1130	879	2278	2408
Thrombolytic treatment was performed	633	683	502	587	642	310
Percutaneous coronary angioplasty was performed	671	919	682	576	1759	112

Of the group of patients included in the study, 67 (49.3%) patients were under 64 years of age and 71 (50.7%) were patients aged 65 years and over. At the hospital level, ACS-STE was established in 27 (19.3%) patients, ACS-NSTE in 38 (27.1%) and UA in 75 (53.6%). Of 46 (32.9%) women with ACS included in the study, 15 (32.6%) were under 65 years and 31 (67.4%) were aged 65 years and over. The male group consisted of 94 (67.1%) patients, of whom 54 (57.4%) were up to 65 years of age and 40 (42.6%) were aged 65 years and over. In the under 65 age category, ACS is more common in men – 57.4% compared to women – 32.6% (P<0.001). In the over 65 age category, ACS is more common in women, accounting for 67.4% cases compared to 42.6% in men (P< 0.001). The study of risk factors in the general group showed the presence of hypertension in 98 (70.0%) patients, diabetes mellitus in 51 (36.4%) patients, obesity in 45 (32.1%) and history of cardiovascular disease in 128 (91.4%). In the female group, history of cardiovascular disease was present in 42 (91.3 %) patients, hypertension in 35 (76.1%), obesity in 21 (45.6%), diabetes mellitus in 14 (30.4%) and smoking in 13 (28.3%) patients. The distribution of risk factors in the male group was as follows: history of cardiovascular disease – 82 (87.2%) patients, hypertension – 63 (67.1%) patients, smoking – 40 (42.5%) patients, diabetes mellitus – 27 (28.7%) patients and obesity was established in 24 (25.5%) patients. In women, there was a higher level of history of cardiovascular disease (91.3%), hypertension (76.1%), obesity (45.6%) and diabetes mellitus (30.4%) compared to men (P< 0.001).

Discussion

The aim of the study was to evaluate the clinical-epidemiological aspects of acute coronary syndrome in the population of the Republic of Moldova. The study group included 140 patients with ACS, the mean age of the patients in the study was 65.0 ± 27.7 years, among them 46 (32.8%) women with a mean age of 69.7 ± 28.4 years and 94 (67.1%)

Table 6. Clinical-epidemiological aspects of acute coronary syndrome

Indicators	Total		Women		Men		P value
	Abs	%	Abs	%	Abs	%	
Total	140	100	46	32.9	94.	67.1	< 0.001
Mean age	65±27.7		69.7±28.4		64.6±20.8		< 0.001
Age categories							
<45years	12	8.6	3	6.5	9	9.6	< 0.001
46 -64 years	57	40.7	12	26.1	45	47.9	< 0.001
65 – 79 years	50	35.7	22	47.8#	28	29.8	< 0.001
> 80 years	21	15.0	9	19.6	12	12.8	< 0.001
Risk factors							
Smoking	53	37.8	13	28.3	40	42.5	< 0.001
Hypertension	98	70.0	35	76.1#	63	67.1	< 0.001
Diabetes mellitus	51	36.4	14	30.4#	27	28.7	< 0.001
Obesity	45	32.1	21	45.6#	24	25.5	< 0.001
History of CVD	128	91.4	42	91.3	82	87.2	< 0.001
ACS--STE	39	27.8	18	12.8	21	22.3	< 0.001
ACS-NSTE	85	60.7	26	56.5	59	62.8	< 0.001
Killip Class I	109	78.0	32	69.5	77	81.9	< 0.001
Killip Class II-IV	33	23.6	12	26.1#	21	22.3	< 0.001

Note: # – between the men and women; CVD – cardiovascular disease, SCA-STE – acute coronary syndrome with ST-segment elevation; SCA-NSTE – acute coronary syndrome without ST-segment elevation.

men with an average age of 64.6 ± 20.8 years. The ratio of men to women was 2.04:1 [7]. Women were on average 5.1 years older than men (p <0.001). The results regarding the ratio of women to men with ACS were similar to those in the studies conducted by Muherjee S. et al. [8], and Alvi HN. et al. [1]. The majority of ACS cases are registered in men 67.1% and the majority (57.5%) are under the age of 65 [3]. Several studies of the epidemiology, risk factors, and prognosis of ACS have been published in Western countries, which have shown that women with ACS are older and have more comorbidities and risk factors [9-11]. In several studies, smoking, diabetes mellitus, hypercholesterolemia and hypertension are well-established risk factors for the development of coronary heart disease [12-15], which have different characteristics in men and women [15]. Several studies on epidemiology, risk factors and prognosis have been published [5, 16, 17]. The results of the present research showed that compared to men, women are older (69 vs 64 years; P <0.001) and had significantly more comorbidities, such as diabetes mellitus (30.4 vs 28.7%; P <0.001), hypertension (76.1 vs 67.1%: P <0.001), obesity (45.6 vs 25.5%; P <0.001) and history of cardiovascular disease (91.3 vs 87.2%), data correlating with the results of the studies [1, 15]. Men were more likely to have a history of SCA-STE (22.3 vs 12.8%; P = 0.001), SCA NSTE (62.8 vs 56.5%; P < 0.001) and smoking (42.5 vs 28.3%; P < 0.001) [3]. The increasing share of ACS in women is due to an aging population, changing risk factor profiles and changes in diagnostic capabilities [4, 13]. 33 (23.6%) of the patients in the study group had Killip Class II-IV, including 12 (26.1%) women and 21 (22.3%) men, a situation caused by the high frequency of comorbidities and the advanced age of women [18, 19].

Conclusions

1. Acute coronary syndrome is a major health problem for the population of the Republic of Moldova, substantially influencing the rates of morbidity and mortality.

2. The rise in the aging processes of the population, the reduced accessibility to modern methods of diagnosis and treatment, the high share of cardiovascular risk factors will determine a high incidence of ACS in future.

3. ACS affects men more frequently, compared to women, in a ratio of 2.04:1. People under the age of 65 years constitute 57.5%.

4. The results of the study showed that compared to men, women are older (69 vs 64 years; P <0.001) and had significantly more comorbidities, such as diabetes mellitus (30.4 vs 28.7%; P <0.001), hypertension (76.1 vs 67.1%; P <0.001) obesity (45.6 vs 25.5%; P <0.001) and history of cardiovascular disease (91.3 vs 87.2%).

5. Men were more likely to have a history of SCA-STE (22.3 vs 12.8%; P = 0.001), SCA NSTE (62.8 vs 56.5%; P < 0.001) and smoking (42.5 vs 28.3% P < 0.001).

6. The study showed the presence of AMI-STE in 27 (19.3%) patients, AMI-NSTE in 38 (27.1%) patients and UA in 75 (53.6%) patients.

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Funding
The research was provided by *Nicolae Testemitanu* State University of Medicine and Pharmacy and the National Center of Prehospital Emergency Medical Care. The research was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate.
No approval was required for this study.

Conflict of Interests.
There is no known conflict of interests and financial or non-financial support associated with this publication.

https://doi.org/10.52418/moldovan-med-j.64-5.21.02
UDC: 616.728.3-77

The use of the constrained prosthesis in the difficult primary knee arthroplasty

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Manuscript received August 12, 2021; revised manuscript 04, 2021; published online November 26, 2021

Abstract

Background: The constrained knee prosthesis has the basic indication in revision arthroplasty, but the latest literature reveals that it takes place also in primary total knee arthroplasty in cases of knee osteoarthritis associated with major deformities.
Material and methods: Present study is based on the surgical treatment, using the constrained knee prosthesis in the primary total knee arthroplasty, during 2019-2021, of 28 patients with knee osteoarthritis associated with severe deformities in varus or valgus, in the Big Joint Replacement Department, Clinical Hospital of Traumatology and Orthopedics, Chisinau.
Results: In this study, the following criteria were evaluated: the type of deformity – valgus (10 cases) and varus (18 cases); the degree of deformation – for varus knees was on average 30^0, and for valgus knee – 25^0; bone attrition – 11 cases with bone defects where it was necessary to use augmentations; affected side – in 19 cases the right knee was affected and 9 cases the left one; the women/men ratio was 4/1; the mean age of the patients was 67.5 years; average duration of the intervention – 140 minutes; in 5 cases a lateral para-patellar approach was performed, of which 2 cases with tibial tuberosity osteotomy; complications – 1 case with intra-operative periprosthetic fracture and 2 cases with superficial infections of the operated joints.
Conclusions: Osteoarthritis of the knee progresses rapidly, leading to severe deformities, significant bone defects and joint instability, which are indications to use the constrained prosthesis in the primary total knee arthroplasty, long-term follow-up is necessary to obtain the last conclusion, but from this study the constrained knee prosthesis like primary implant for special indication had promising results.
Key words: knee osteoarthritis, difficult arthroplasty, constrained prosthesis.

Cite this article
Iacubitchii V, Betisor A, Erhan N. The use of the constrained prosthesis in the difficult primary knee arthroplasty. *Mold Med J.* 2021;64(5):11-15. https://doi.org/10.52418/moldovan-med-j.64-5.21.02.

Introduction

Knee osteoarthritis (KOA) is a common progressive multifactorial joint disease and is characterized by chronic pain and functional disability [1]. KOA accounts for almost four-fifths of the burden of osteoarthritis worldwide and increases with obesity and age [2]. There were nearly 654 million individuals (40 years and older) with knee osteoarthritis in 2020 worldwide [3]. Up to now, KOA is incurable except knee arthroplasty which is considered an effective treatment at an advanced stage of the disease, however, which is responsible for substantial health costs [4].

Total knee arthroplasty (TKA) is a great success nowadays in modern orthopedics [5, 6] and is a procedure to restore proper function and give pain relief in patients with severe knee osteoarthritis [7].

The use of the constrained prosthesis in the primary TKA was unusual in all the world nearly 15 years ago [8], several years ago the interest in this method appeared in Moldova as well.

The constrained knee prosthesis (CKP) has the basic indication in revision arthroplasty, but the latest literature reveals that it takes place also in primary total knee arthroplasty in cases of severe knee osteoarthritis associated with major deformity with a significant bone defect, stiffness and instability [9]. CKP with its variety of available stems and augments can also help surgeons improve implant stability, optimum alignment, adequate balance and deformity correction [9-12]. Stability is essential for successful TKA [13-16]. By Sabatini et al. the rate of complication is decreased and a good survival rate and functional score results are shown by using the constrained condylar knee prosthesis in primary knee arthroplasty [7, 12, 13].

But anyway, there are some possible disadvantage of CKP which include large bone removal, mechanical loosening due to load transfer to the respective bone ends through an intramedullar extension of the stems leading to early failure and a periprosthetic fracture [9, 14, 17]. Polyethylene insert wearing is another pitfall of CKP [14]. Revision of TKA following CKP is an extremely difficult procedure, as a need for stem removal increases significantly morbidity and operating time [9, 17]. Second generation condylar constrained knee (CCK) prosthesis

reduced some complications to the patella (e.g., fractures, incorrect tracking and osteonecrosis) due to redesigned patellofemoral surfaces [12, 18].

The study aimed to evaluate the method of surgical treatment with constrained prosthesis used in the difficult primary TKA in the clinic.

Material and methods

The study is based on the surgical treatment, using the constrained knee prosthesis (CKP) in the primary total knee arthroplasty (TKA), between May 2019 and June 2021, of 28 patients with severe knee osteoarthritis (KOA) associated with major deformities, significant bone defect, stiffness and instability, in the Big Joint Replacement Department, Clinical Hospital of Traumatology and Orthopedics, Chisinau.

All patients were over 58 years old, with an average age of 67.5 ± 9.95 years (58–77), there were 22 women and 6 men. Nineteen patients had right knee involvement, and 9 – left knee involvement. The mean body mass index (BMI) of the patients was 31.07 ± 1.38 (22.49-39.66). Detailed characteristics of the patients are illustrated in table 1.

Table 1. Characteristics of patients

Demographic parameters (N-28)	Mean ± SD	Range
Age (years)	67.5 ± 9.95	58-77
Women/Men	22/6	
Right/left	19/9	
BMI (kg/m^2)	31.07 ± 1.38	22.49-39.66

N – total sample, BMI – body mass index, SD – standard deviation

The decision to use constrained knee prosthesis was taken pre-operatively in all 28 cases based on the severity of knee osteoarthritis – the major deformities, important bone loss and complex instability assessed clinically and radiographically (fig. 1).

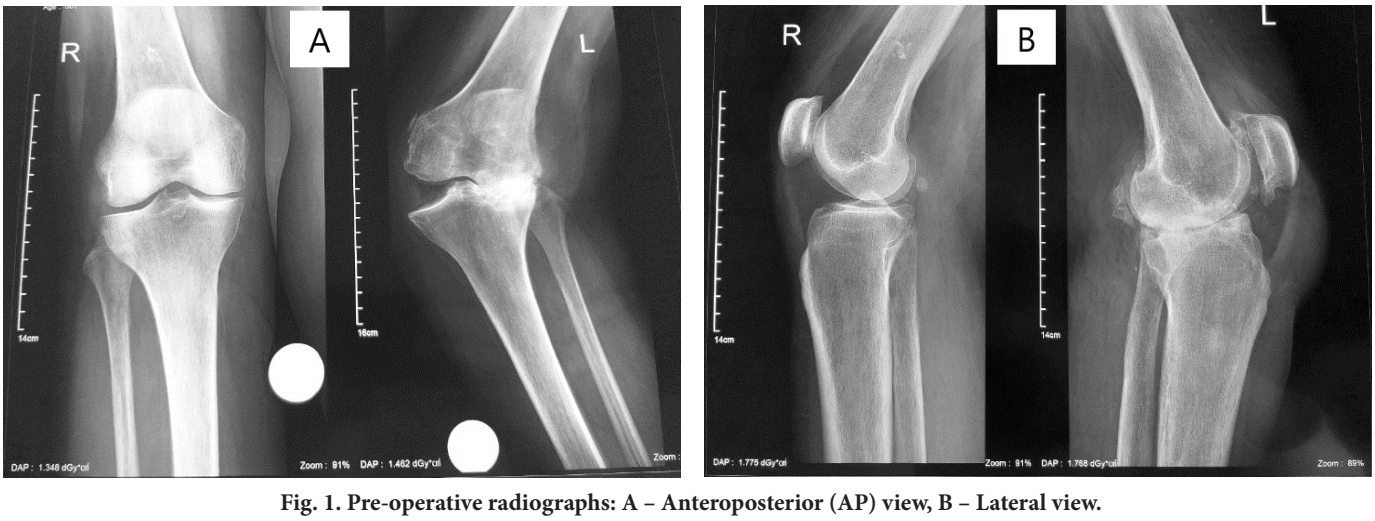

Fig. 1. Pre-operative radiographs: A – Anteroposterior (AP) view, B – Lateral view.

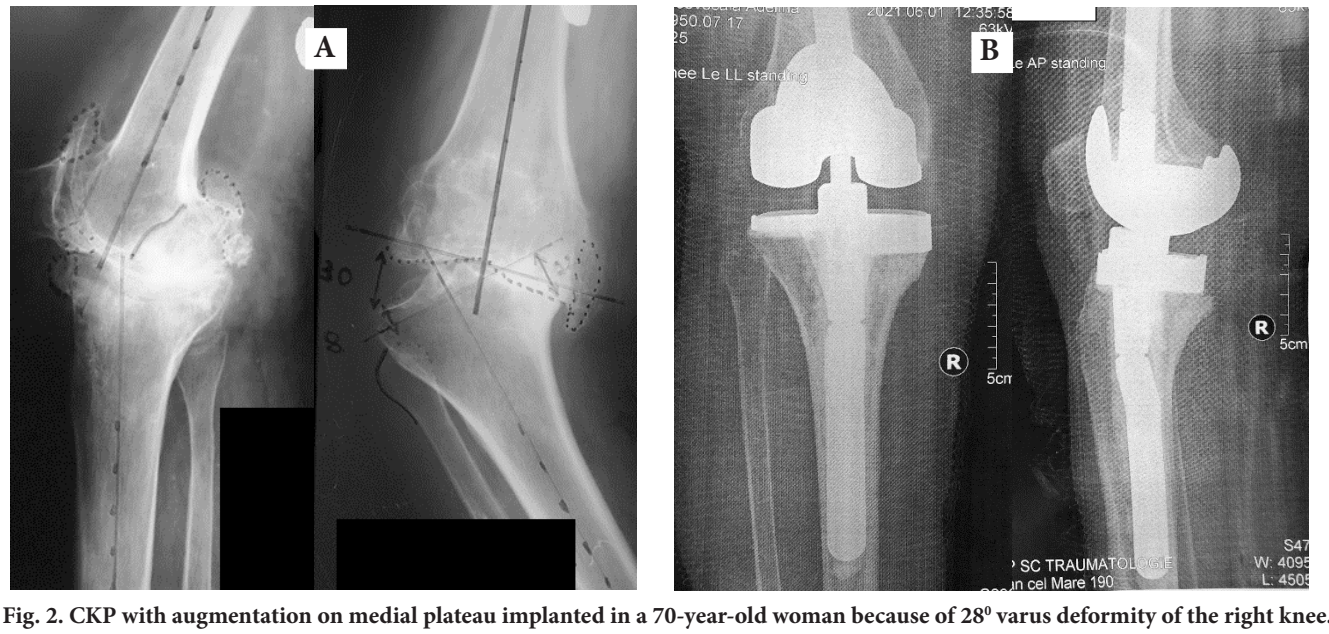

Fig. 2. CKP with augmentation on medial plateau implanted in a 70-year-old woman because of 28^0 varus deformity of the right knee. A – Pre-operative radiographs, B – Radiographs after surgery

Pre-operative planning was made on all 28 knees. The constrained TKA was performed in knees with a varus over 20° or valgus over 15°. Eighteen of 28 knees had a varus deformity (fig. 2) and 10 had a valgus deformity (fig. 3). The degree of deformation for varus knees was on average 30^0 (ranging from 20^0 to 40^0), and for valgus knee – 25^0 (ranging from 15^0 to 35^0);

Another indication for the use of CKP is the advanced bone defect, based on Ahlbäck classification [19]:

1. Grade I: joint space narrowing (less than 3 mm).
2. Grade II: joint space obliteration.
3. Grade III: minor bone attrition (0-5 mm).
4. Grade IV: moderate bone attrition (5-10 mm).
5. Grade V: severe bone attrition (more than 10 mm).

Eleven cases were with severe bone loss and it was necessary to use augmentations, with thickness from 5 to 15 mm, respectively 9 on the medial (fig. 2), 1 on the lateral (fig. 3), and 1 on the entire surface of the tibial plateau. In one case, with moderate bone attrition, was used 1 screw on the medial compartment for better support of the tibial component (fig. 4).

The mean operative time was 140 min (85–195). A pneumatic tourniquet was used during the surgeries, when the allowed time had elapsed; the tourniquet was deflated for a short period, then inflated again.

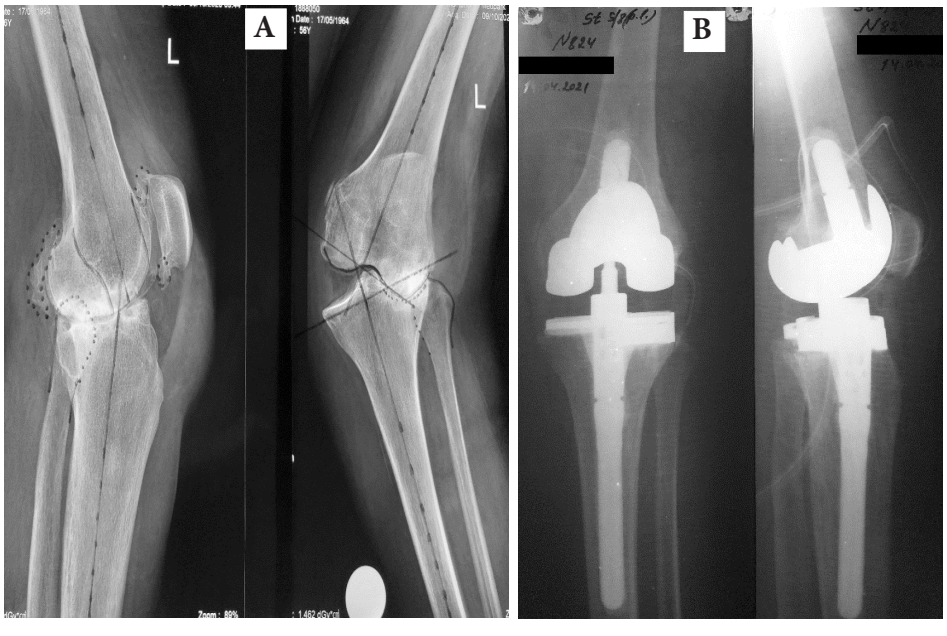

Fig. 3. CKP with augmentation 10 mm on lateral plateau implanted in a 56-year-old woman with a valgus deformity of 30^0 of the left knee. A – Pre-operative radiographs, B – Radiographs after surgery

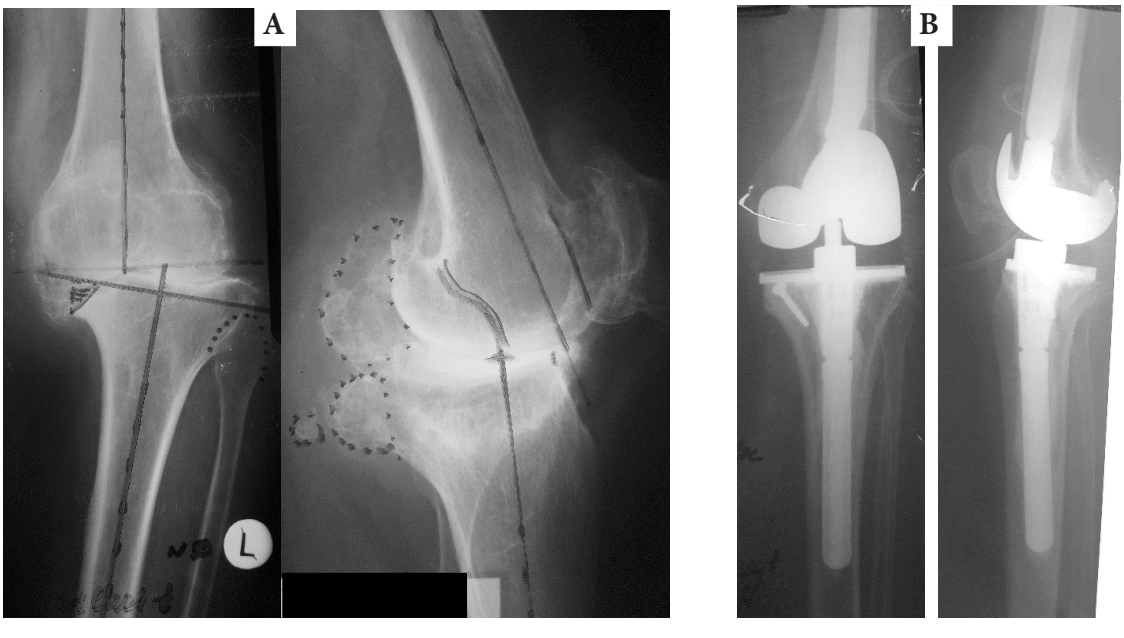

Fig. 4. CKP in a 76-year-old patient with a varus deformity of 15^0 of the left knee. A – Pre-operative radiographs, B – Radiographs after surgery

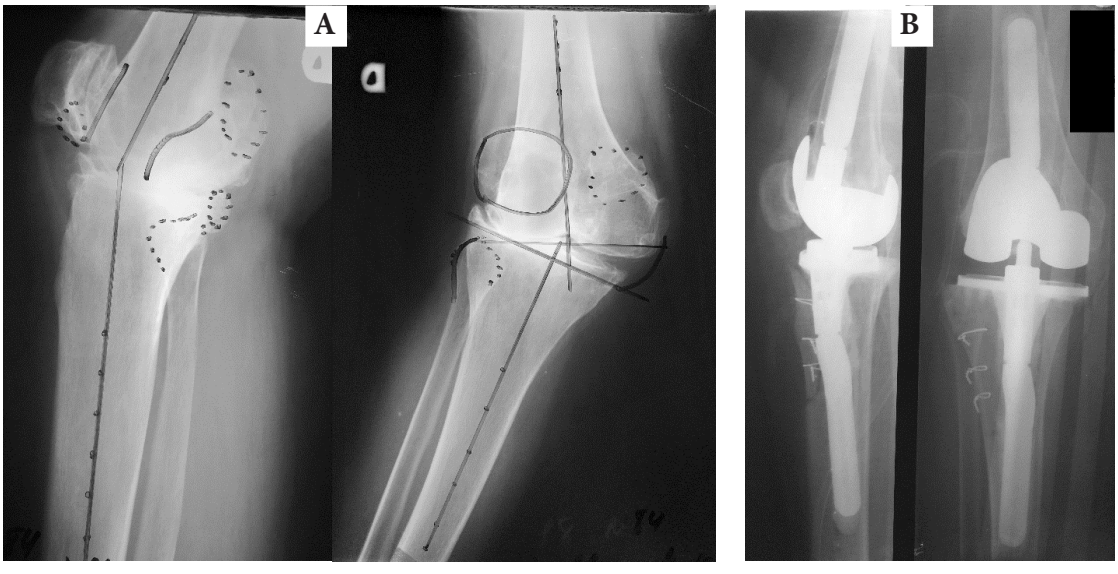

Fig. 5. CKP in a 67-year-old patient with a valgus deformity of 23⁰ of the right knee. A – Pre-operative radiographs, B – Radiographs after surgery

The following approaches were used: medial parapatellar – 21 cases; lateral as described by Keblish [20] – 5 cases; and mid-vast [21] – 2 cases (in moderate knee deformations). To achieve sufficient exposure in 2 cases were performed tibial tuberosity osteotomy (TTO) (fig. 5). The plasty of the articular capsule involving part of Hoffa's fat pad was performed in 3 cases with severe valgus deformity.

In one case, intra-operatively with periprosthetic lateral femoral condyle fracture, the result was achieved by osteosynthesis with one screw. In another case, intra-operatively has been determined on medial femoral condyle a subchondral cyst, size 2x3x2.5 cm, which was supplanted with autologous bone grafting.

The intramedullary femoral and tibial guide was used routinely, stem extensions were always used and all components were cemented. Routine patella resurfacing was not performed in these cases; however, patella denervation with electrocautery was performed in all 28 knees.

Results

28 knees with constrained knee prosthesis were reviewed as the first implant. Ten patients were evaluated clinically and radiologically at 2-, 6-, 12-month after surgery, 14 patients at 2-, 6-month after surgery, and 4 patients at 2-month after surgery.

Knee Society Score (KSS) functional score was used to assess the patients [22]. The mean KSS improved from 25 points pre-operatively to 91 (74-100) points at the last follow-up. All patients recovered full extension during the follow-up.

Radiographs showed no radiolucent lines in all knees neither within the femur nor within the tibia. No component loosening or periprosthetic fracture was reported after the surgery. There were 2 cases with superficial infections of the operated joints, carried out by early irrigation and debridement (I&D).

Five patients suffered from thigh pain, solved after 2-3 months of physiotherapy and rehabilitation. No revisions or reoperations were performed. 16 patients experienced numbness on the lateral side of the knee. There were no important neurovascular injuries in this experience.

Discussion

The need of a semi-constrained implant in primary TKA is rare due to ligament instability or significant bone defects; different recent works yet recommend to take into account the use of a CKP when it is particularly complex to gain adequate soft tissue balance. Insall et al. (1976) and Donaldson et al. (1988) had already described indications to CKP replacement among which are included severe axial deformities, collateral ligaments insufficiency and severe bone loss [12].

Negatives about the use of CKP include larger bone removal, polyethylene insert wearing, mechanical loosening due to extension of the stems which lead to early failure and a periprosthetic fracture [9, 14, 17].

The most important deduction of this study was that the use of the constrained prosthesis in the primary total knee arthroplasty corrected the severe deformity with major bone defects, stiffness, and instability of the knee joint restoring excellent clinical outcome and recovered the needed function.

Using KSS after CKP, 11 cases were rated as excellent and 17 as good. Considering this report, it can be concluded that CKP as a primary implant is effective and justified option for the treatment of difficult KOA. However, it's not without some complications.

Conclusions

Osteoarthritis of the knee progresses rapidly, leading to severe deformities, significant bone defects and joint insta-

bility, which are indications to use the constrained prosthesis in the primary total knee arthroplasty, long-term follow-up is necessary to obtain the last conclusion, but from the present study the constrained knee prosthesis like primary implant for special indication had promising results.

Rigorous selection of patients, pre-operation planning, compliance with surgical techniques according to the algorithm allows to obtain good functional results in the majority of cases.

Constrained prosthesis in the primary total knee arthroplasty allows the correction of deformity, gives stability, removing the pain syndrome, improving mobility in the joint, the relatively rapid resumption of function during the postoperative period and considerably improves the quality of life at the patients.

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Authors' contributions
VI designed the trial and interpreted the data. AB and NE revised the manuscript critically. All the authors approved the final version of the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 02, of 23.10.2017).

Conflict of Interests
No competing interests were disclosed.

DOI: https://doi.org/10.52418/moldovan-med-j.64-5.21.03
UDC: 616.379-008.64:616.24-002.5

The effect of diabetes mellitus on evolution and outcome of tuberculosis in a prospective study

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Manuscript received September 10, 2021; revised manuscript November 08, 2021; published online November 26, 2021

Abstract

Background: In the Republic of Moldova almost 5% of the cases with tuberculosis are diagnosed annually among diabetic patients. The aim of this study was to assess the impact of diabetes mellitus on the evolution and anti-tuberculosis treatment effectiveness in a prospective study.
Material and methods: A prospective, longitudinal and case-control study, which included a total number of 252 patients diagnosed with pulmonary tuberculosis and distributed in a study group, consisting of 93 patients diagnosed with diabetes mellitus and a control group, consisting of 159 patients without glycemic disorders, was performed.
Results: This study identified that one half of the group with diabetes was detected by active screening and one third received anti-tuberculous treatment before actual episode. A similar rate of diabetic and non-diabetic patients was microbiologically positive, as well confirmed with drug-resistance. The anti-tuberculous treatment effectiveness was lower in diabetic patients, the death rate and the low treatment outcome (lost to follow-up and failed) were higher than in non-diabetic patients. The main causes of unfavorable evolution were: glycemic disorders (hyperglycemia), diabetes complications and the history of the anti-tuberculous treatment in the anamnesis.
Conclusions: The individualized approach and a tight follow-up should be performed regularly in all patients with glycemic disorders and tuberculosis for the improvement of the disease outcome.
Key words: tuberculosis, diabetes, risk factors, outcome.

Cite this article
Malic A, Lesnic E. The effect of diabetes mellitus on evolution and outcome of tuberculosis in a prospective study. *Mold Med J.* 2021;64(5):16-20. https://doi.org/10.52418/moldovan-med-j.64-5.21.03.

Introduction

Diabetes mellitus (DM) is an important public health issue worldwide [1]. It is one of the four priority non-communicable diseases targeted by the sustainable development goals. Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from the disturbances in insulin secretion, insulin action or both [2]. The chronic hyperglycemia contributes to the long-term damage, dysfunction of different organs, increased risk for infections of lower respiratory tract and tuberculosis [1]. The risk for tuberculosis is increased 1.5-7.8 times in patients with uncontrolled hyperglycemia due to high levels of pro-inflammatory mediators (IL-6, TNF-α) released by the infection with *Mycobacterium tuberculosis* in the lung tissue [3, 4].

Patients with both types of diabetes are one of the most important risk groups for tuberculosis and are exposed to the annual radiological investigation [5]. The association of diabetes and pulmonary tuberculosis is most commonly established in patients who have been diagnosed with diabetes [6, 7]. If tuberculosis and diabetes are detected simultaneously, diabetes worsens the evolution of tuberculosis and decreases the treatment effectiveness [8-10]. The factors that contribute to the development of tuberculosis in diabetic patients are the disturbances of the innate resistance, cellular immunity, and the alveolar macrophage dysfunction [1, 3]. Late detection, the glucose metabolism disorders during the anti-tuberculous treatment, the high rate of the adverse drug reactions and acquired MDR-TB due to the low absorption of the drugs from the gastrointestinal tract contribute to the unfavorable evolution of tuberculosis [11, 12]. Antidiabetic therapy in patients with tuberculosis should be frequently revised due to the high frequency of glycemic balance disturbances and adverse reactions to anti-tuberculous drugs. In severe forms of tuberculosis, the association of the insulin with metformin is recommended for maintaining the control of the glucose level [12]. The rate of the adverse anti-tuberculous drug effects in diabetic patients is high and ranges between 20% and 54% in MDR-TB patients [12]. The most frequent adverse drug reactions effects are: peripheral neuritis caused by Isoniazid, toxic hepatitis determined by Isoniazid, Rifampicin, Pyrazinamide, renal toxicity of Aminoglycosides (Streptomycin, Kanamycin, Amikacin) and optic neuritis due to Ethambutol [8]. In consequence, the hospitalization of the diabetic patients with tuberculosis is recommended for initiation of the anti-tuberculous treatment [5].

Tuberculosis in diabetic patients is often misdiagnosed and several factors contribute: low specificity of the clinical signs and atypical localization [13]. If the relevant localization in typical tuberculosis is in the segments I, II, VI and X, in DM patients the localization is more often in the segments III, IV and V. Also, severe, extensive destructive process is a common funding in new diagnosed cases of tuberculosis and diabetes. Despite including the patients with DM in the risk groups for annual screening, they are regularly detected with severe forms of tuberculosis with chronic evolution [13]. The late detection and late onset of the antituberculous therapy, dietary errors and inadequate antidiabetic treatment represent the causes of premature death in diabetic patients with TB [8-13]. Considering all the abovementioned data, the study has been performed with the aim to assess the impact of diabetes mellitus on the evolution and anti-tuberculous treatment outcome.

Material and methods

The research was prospective, selective, descriptive and cross-sectional. It included a series of 252 patients diagnosed with pulmonary tuberculosis during the period 01.01.2017-31.12.2017 in the Republic of Moldova. The inclusion criteria, which determined the selection of the patients in the research, were: age over 18 years old, TB diagnosed by a phthisiopneumologist and the signed informed consent. The cases were distributed in two groups. In the study group were selected 93 patients in which the main inclusion criteria was the DM diagnosed by an endocrinologist. In the control group were selected 159 patients without DM or history of DM. The diagnosis of pulmonary tuberculosis was confirmed through the criteria provided by the National Clinical Protocol. The sputum examination by Xpert MTB/Rif test, Ziehl-Neelsen staining, culture on Lowenstein-Jensen and liquid BACTEC media and chest X-ray investigations were performed in all patients from both groups.

The methods used for the diagnosis of DM were: capillary glucose concentration, the fasting venous plasma glucose (FVPG) done after 10-14 hours of fast and the glycated albumin (HbA1c). The capillary glucose concentration was measured with a glucometer in a blood sample obtained from the fingertip. Glycated albumin was calculated, as the percentage of glycated albumin in the total albumin and the value ≥6.5% (48 mmol/Mol) and was a diagnostic indicator. The investigation schedule included the following data about the patients: biological and social peculiarities, high-risk characteristics (homelessness, migration, history of detention, the contact with a TB patient), case-management, and features of tuberculosis, glycemic indicators, anti-tuberculous treatment, antidiabetic treatment and outcome. The research was approved by the bioethics committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy on 21st of November 2017.

The statistical analysis was performed using EpiInfo software. The data were appreciated as nominal or quantitative. The frequency and percentage were reported for nominal data, and the mean and standard deviation were reported for continuous data. The statistical analysis of the differences between normally distributed continuous variables was tested with the t-*Student* test. A p-value of <0.05 was considered statistically significant.

Results

The distribution of the patients, according to the sex established a higher rate of men compared with the women rate in the control group (2.9) compared with the study group (2.1). The repartition of the patients, according to the age established that the young groups aged between 18 and 44 years prevailed in the control group – 100 (63%) vs 18 (19%) cases in the study group. A similar rate of patients aged between 45 and 54 years was determined in both groups. The patients older than 55 years statistically predominated in the study group – 53 (57%) vs 22 (14%) cases in the control group (tab. 1). So, young individuals aged younger 44 years, prevailed in the control group and older than 55 years in the study group. The patients' average age in the study group was 59±8 years old, and in the control group – 38±5 years old.

Table 1. Distribution of patients by sex and age

Sex Age groups	Study group N=93 (M%)	Control group N=159 (M%)	P-value
Men	63 (67)*	118 (74)*	>0.05
Women	30 (33)	41 (26)	>0.05
18-24 years	0	20 (13)	**<0.001**
25-34 years	8 (8)	40 (25)	**<0.001**
35-44 years	10 (11)	40 (25)	**<0.001**
45-54 years	22 (24)	37 (23)	>0.05
55-64 years	37 (40)	18 (11)	**<0.001**
65+ years	16 (17)	4 (3)	**<0.001**

The applied statistical test: the paired sample T-test, M – Mean. *Absolute numbers and percentages per column (in brackets).

According to the type of glycemic disorders, in the study group, the type 1 diabetes was diagnosed in 17 (18%) patients and type 2 diabetes in 76 (82%) patients, which was complicated in 39 (42%) patients. The value of the FVPG exceeded the normal limit of 6 mmol/L in 68 (73%) diabetic patients. Reporting to the total number of patients with hyperglycemia, the fasting venous plasma glucose was established in the range between 6.1 and 8 mmol/L, assessed as a low increased level in 31 (46%) patients. In 18 (26%) cases the value ranged between 8.1 and 12 mmol/l and was assessed as middle increased value. Higher than 12 mmol/L was established in 19 (28%) patients and was defined as uncontrolled hyperglycemia. So, in the most of the cases the fast venous plasma glucose exceeded the normal limit and in less than one half, in 39 (42%) cases, was within the normal range being under medication control. The concentration of the glycated albumin was assessed in 68 (73%) diabetic patients. The normal HbA1c concentrations detected

in the range between 4.8 and 5.6%, considered as normal values, were found in 14 patients (20% of the investigated ones). Those patients were identified with low increased hyperglycemia. The values included in the range between 6 and 6.4% were found in 27 (40%) patients. In 27 (40%) cases the HbA1c concentrations exceeded 6.4% and in all cases the FVPG exceeded 12 mmol/l.

The insurance provided the free of charge screening procedures. The health insurance was identified in 61 (66%) cases of the study group vs 72 (45%) cases of the control group. Homeless or people without a place of permanent residence, neither urban nor rural, predominated in the control group 39 (25%) vs 4 (4%) patients in the study group ($p<0.05$). A similar rate of the economic migrants, who returned from abroad in the last 12 months were identified in both groups. A couple of patients from the control group had a life history of detention. The individuals with tuberculous contact were detected in a low proportion in both groups – 5 (5%) in the study group and 15 (9%) in the control group. In 71 (76%) patients of the study group were diagnosed several associated diseases, among which one was diabetes, complicated in every third case ((39 (42%) patients). In the control group comorbidities were diagnosed in 24 (15%) patients. So, the rate of the patients with associated diseases statistically predominated in the study group ($p<0.001$). Data are shown in the figure 1.

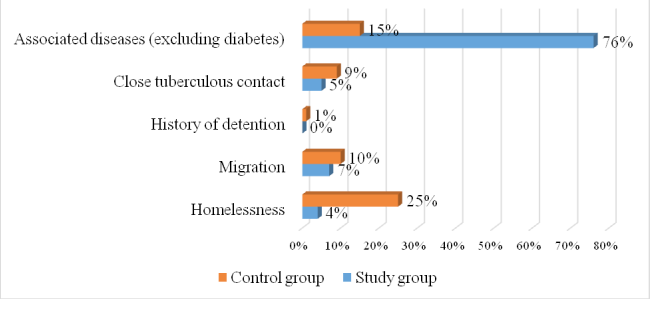

Fig. 1. Distribution according to the risk factors

The TB case-management was particularly for diabetic patients. Symptomatic screening performed by the general practitioner detected tuberculosis in a higher proportion in the control group – 68 (43%) compared with the study group, 20 (21%) cases. The active screening of the risk groups detected a higher proportion of the patients in the study group 49 (53%) compared with 34 (21%) cases in the control group. The rate of the patients detected through the active screening realized by the medical specialists was statistically higher in the study group 26 (28%) compared with the control group – 10 (6%). The rate of the patients detected through the symptomatic screening realized by the medical specialists was insignificantly higher in the study group – 23 (25%) compared with the control group 24 (15%). Only patients from the control group came directly to the Pneumophthisiology hospital and avoiding the primary health-care facilities. Thus, the specialized therapeutic intervention was more accessible for diabetic patients and the direct addressing to the pneumophthisiology hospital was preferred way for patients without glycemic disorders.

Evaluating the disease history, it was established that patients previously treated with anti-tuberculous drugs were found more often in the study group 32 (34%) compared with 31 (19%) patients in the control group. The major causes of the treatment interruption in the study group were the low treatment clinical tolerance and in the control group the treatment incompliance. The assessment of the laboratory results identified a similar rate of the patients that were positive on Ziehl-Neelson staining and conventional cultures in both groups. The drug-resistant TB was diagnosed through Xpert MTB/RIF assay in 20 (23%) cases in the study group and 44 (28%) cases in the control group and was confirmed through the conventional cultures (tab. 2).

The distribution of the patients according to the clinical and radiological criteria resulted in similar rates of the patients diagnosed with pulmonary infiltrative tuberculosis and the predomination of the fibro-cavernous form in the study group. All patients with chronic evolution had been

Table 2. Methods of detection, case-types and microbiological features

Reference	Case characteristics	Study group N=93 *(P%)*	Control group N=159 *(P%)*	P-value
Primary health care providers	Detected by GPs – symptomatic patients	20 (21)*	68 (43)*	<0.001
	Detected by GPs - screening of HRG	23 (25)	24 (15)	>0.05
Specialized health care level	Detected by SP – symptomatic patients	24 (26)	35 (22)	>0.05
	Detected by SP - screening of HRG	26 (28)	10 (6)	<0.001
	Addressed directly to the specialized TB hospital	0	22 (14)	<0.001
Case type	New cases	61 (66)	128 (80)	<0.001
	Previously treated for TB	32 (34)	31 (19)	<0.001
Microbiological results	Positive on AFB microscopy	38 (44)	79 (50)	>0.05
	Positive on conventional cultures	46 (53)	94 (59)	>0.05
	MDR-TB	20 (23)	44 (28)	>0.05
Clinical-radiological forms	Infiltrative tuberculosis	85 (91)*	144 (90)*	>0.05
	Disseminated tuberculosis	1 (1)	14 (9)	**<0.001**
	Fibro-cavernous tuberculosis	7 (8)	1 (1)	**<0.001**

The applied statistical test: the paired sample T-test, GP – general practitioner, SP – specialist, HRG – high risk group. *Absolute numbers and percentages per column (in brackets)

treated with anti-tuberculous drugs, before actual episode was diagnosed.

The standard treatment for drug-susceptible TB, according to WHO recommendations is recommended in the Republic of Moldova since 2000 and all selected patients were treated according to it. The treatment for the drug-susceptible tuberculosis was administrated in 73 (78%) cases of the study group and in 115 (72%) cases of the control group. The treatment for drug-resistant tuberculosis was provided in 20 (23%) patients of the study group and in 44 (28%) patients of the control group. The treatment success rate, which included cured and those who completed the treatment was statistically higher in the control group – 140 (88%) compared with 66 (71%) patients in the study group. The death rate was statistically higher in the study group – 14 (15%) compared with 1 (1%) in the control group. A similar rate of patients in both groups was lost to follow-up or failed the treatment (fig. 2).

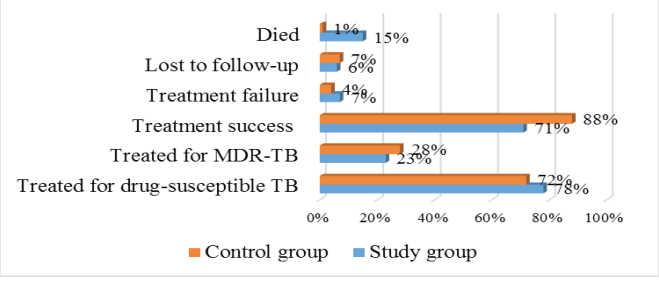

Fig. 2. Distribution according to the risk factors

Discussion

This research established that type 2 diabetes was diagnosed more often, even if the patients with type 1 diabetes were more vulnerable to infection and the occurrence of tuberculosis. The research established that tuberculosis appeared in diabetic patients with increased glycaemia and with associated complications. Similar results confirmed that uncontrolled blood glucose level increases the risk for the occurrence of tuberculosis, as the consequence of the immune disturbances [8-10, 12]. Studies showed that men are significantly more at risk for contracting and dying from tuberculosis than women, as peculiarity of the high burden country [6]. The conducted research established a similar distribution of the diabetic patients in groups according to the sex, which demonstrated the impact of the immune disturbances on the risk for acquiring tuberculosis, more than the social risk factors. Most of the studies carried out in high tuberculosis burden countries demonstrated a high prevalence of tuberculosis in young age groups [14]. This research concluded that diabetic patients are often older 55 years which constituted a risk for acquiring type 2 diabetes. This study and the several cohort studies as well established that the social and economic vulnerability constituted a risk factor for tuberculosis in patients with DM [8-10, 12]. As a result, the research established a high rate of the diabetic patients with free access to the screening procedures, due to specific conditions, like having been retired or disabled, which is characteristic for our country. The low rate of tuberculosis detected within the investigation of the tuberculous clusters in diabetic and non-diabetic groups, reflected poor quality of the contacts' examination done in the frame of the primary health care sector. It was confirmed that the annual radiologic investigation of diabetic patients was an efficient way for tuberculosis detection in the Republic of Moldova, as being recommended by the National Clinical Protocol. The result has been sustained by another study [11]. In case the active radiological screening was routinely used, the tuberculosis with chronic evolution, such as fibrocavernous type was more often diagnosed in diabetic patients. The literature explained the effect of the uncontrolled glucose level in the blood on the releasing of the pro-inflammatory cytokines and occurrence of fibrotic changes in the lung tissue with chronic inflammation [9, 14]. Furthermore, in the reported research the rate of previously treated for tuberculosis patients was high, which explained the fibrotic changes of the lung parenchyma in diabetic patients with long-lasting disease. A high rate of the tuberculosis recurrence and lost to follow-up among diabetic patients were shown by other researches [8-11]. The main causes contributing to the recurrence or relapse of tuberculosis in diabetic patients are: short duration of the standardized anti-tuberculous treatment, high rate of MDR-TB and adverse drug reactions, detection at the late stage, associated complications and dysfunction of the immunity [3, 4, 8-14].

The standard treatment for drug-susceptible and MDR-TB was applied in a similar proportion in both groups. However, there are studies demonstrating a higher rate of drug resistance in diabetic patients [14, 15]. The Republic of Moldova ranks in the list of the countries with a high prevalence of drug resistance, which explained that every fourth patient in both selected groups was confirmed by culture with MDR-TB [6]. The anti-tuberculous treatment effectiveness was considerably lower in the diabetic patients. The mortality rate was higher in diabetic patients, compared with non-diabetic patients, which was confirmed by other studies [3, 4, 8-10, 12-16]. This study is the first to identify the peculiarities of the patients diagnosed with tuberculosis and diabetes in the Republic of Moldova and to clarify the evolution and outcome of the patients with both diseases. The limitations of this study should be also considered, due to a small number of patients included. Further studies on a larger scale are needed to establish the risk factors of both diseases.

Conclusions

The main peculiarities of diabetic patients with tuberculosis are: the average age 59±8 years old, social-economic vulnerability, diagnosis of type 2 diabetes, which was complicated in every third case and high level of blood glucose. The present study identified that one half of the diabetic group was detected by active screening and one third received anti-tuberculous treatment before actual episode. A

similar rate of diabetic and non-diabetic patients was microbiologically positive, as well confirmed with drug resistance. More frequently diabetic patients have been diagnosed with fibro-cavernous tuberculosis. The anti-tuberculous treatment effectiveness was lower in diabetic patients, the death rate and the low treatment outcome (lost to follow-up and failed) were higher than in non-diabetic patients. The main causes of unfavorable evolution were: glycemic disorders (hyperglycemia), diabetes complications and the history of the anti-tuberculous treatment in the anamnesis. So, the individualized approach and a tight follow-up should be performed regularly in all patients with glycemic disorders and tuberculosis for the improvement of the disease outcome.

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Authors' contribution
AM collected the data; EL designed the research, reviewed the statistics and interpreted the data, drafted the manuscript. All the authors revised and approved the final version of the manuscript.

Funding
The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval
This study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova (No 44 of 26.05.2025).

Conflict of interests
No competing interests were disclosed.

https://doi.org/10.52418/moldovan-med-j.64-5.21.04
UDC: 616.127-005.4+616.132.2-004.6

Correlations of myocardial bridges with left ventricle myocardial hypertrophy and prepontin coronary atherosclerosis

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Manuscript received August 20, 2021; revised manuscript November 10, 2021; published online November 26, 2021

Abstract

Background: Of particular interest are the studies researching the correlations of myocardial bridges with hypertrophic cardiomyopathy and correlations of thick myocardial bridges with the development of coronary atherosclerosis in the proximal to the bridge arterial part.
Material and methods: Assessment of the correlation between myocardial bridges, coronary atherosclerosis, and the degree of hypertrophy of the left ventricle was performed by retrospective analysis of 6168 coronary angiography protocols (2012-2019) and echocardiographic data from patients' clinical records.
Results: Moderate systolic compression predominated, and the number of patients detected with severe under the bridge systolic coronary stenosis was double as in patients with nonsignificant coronary atherosclerosis. From the total number, patients with myocardial hypertrophy and myocardial bridges were twice less when compared with the patients with the normal myocardial thickness. The comparative research did not show any interdependence between the degree of vascular compression and the degree of left ventricular myocardial hypertrophy. Proximal to the bridges atherosclerosis was detected in 32% of cases without correlation with the force of the myocardial bridge.
Conclusions: The study showed the absence of the correlation between the degree of arterial stenosis caused by the bridge and the degree of hypertrophy of the ventricular myocardium as well as the degree of proximal to the bridge atherosclerosis. Important finding was that the degree of coronary systolic compression is higher in patients with moderate and severe proximal to the bridge atherosclerosis.
Key words: myocardial ischemia, coronary angiography, myocardial bridge.

Cite this article
Tasnic M, Revenco V, Catereniuc I. Correlations of myocardial bridges with left ventricle myocardial hypertrophy and prepontine coronary atherosclerosis. *Mold Med J.* 2021;64(5):21-26. https://doi.org/10.52418/moldovan-med-j.64-5.21.04.

Introduction

The term myocardial bridge means the situation when a portion of the coronary artery with a typical subepicardial trajectory is covered, over a certain length, by a myocardial band, being included into variants of the intramural trajectory of the heart arteries [1, 2].

In patients without obstructive atherosclerotic lesions, in 50% of cases, the cause of myocardial ischemia is the myocardial bridge [3].

A myocardial bridge is a congenital anomaly, which is developing in a close correlation with adjacent vessels [4] and can cause local systolic compression of varying degrees of the subpontine vascular segment [5].

Of particular interest are the studies researching the involvement and impact of thick myocardial bridges in the occurrence of acute cardiac events without associated obstructive coronary artery disease (MINOKA), in myocardial infarction caused by under the bridge or distal to the bridge coronary thrombosis or prolonged coronary spasm [6, 7], sudden death of young people caused by major physical exertion [8], with an intact coronary arteries – especially in case of high-performance athletes [9]or hypertrophic cardiomyopathy [10].

Despite of multiple hypotheses regarding pontine etiopathogenesis, Li J. ed. (2008) stated that the ways in which myocardial bridges cause clinical manifestations are still unknown, as well as the optimal methods of their detection and treatment [11].

Myocardial bridges can have an evident impact on the patient's quality of life, considering the angina pectoris they may induce [10].

Proximal to bridge atherosclerosis [12], vasospasm [8, 13], thrombosis [14], are important etiopathogenetic links of the genesis of acute coronary syndrome.

Another specific feature of the intramural trajectory of the large coronary arteries, which is the subject of controversy, is the protective role of the myocardial bridge in preventing atherosclerosis of the under the bridge vascular segment [15].

In contrast to the subpontine antiatherogenic effect, the

frequency of atherosclerotic lesions in prepontontine vascular segment is increased [16].

Material and methods

Assessment of the correlation between myocardial bridges and the degree of hypertrophy of the left ventricular myocardium, correlation between myocardial bridges and coronary atherosclerosis was performed by retrospective analysis of 6168 coronary angiography protocols performed between 2012 and 2019 at Medpark International Hospital, Department of Cardiology and Interventional Cardiology.

The myocardial bridges were detected in 331 of the 6168 evaluated reports, representing 5.3% of the total number of cases.

Results

In 96.4% of cases the myocardial bridges were detected on the left anterior descending (LAD) artery; on the rest of coronary arteries, myocardial bridges were described in 3.6% of cases: the right coronary artery – 0.6%, the circumflex branch – 0.3%, the diagonal branches – 1.8 %, the marginal branches – 0.6%, the posterolateral branch – 0.3%. The existence of several myocardial bridges in the same patient was detected in 1.8% of cases.

The degree of subpontine arterial systolic stenosis varied within 10-95%. Of the total described myocardial bridges, in 50% of cases, they caused an insignificant systolic compression of the artery, reducing the lumen of the vessel up to 50% of the initial value (visually appreciated) and only in 16% of cases the degree of compression exceeded 75%.

In patients with coronary arteries not severely affected by atherosclerotic pathology, insignificant systolic stenosis caused by myocardial bridge of the subpontine arterial segment predominated, while in the second group of research (patients with moderate and severe atherosclerotic lesions), moderate systolic compression predominated and the number of patients detected with severe subpontine systolic stenosis was double as in the first group of study (fig. 1)

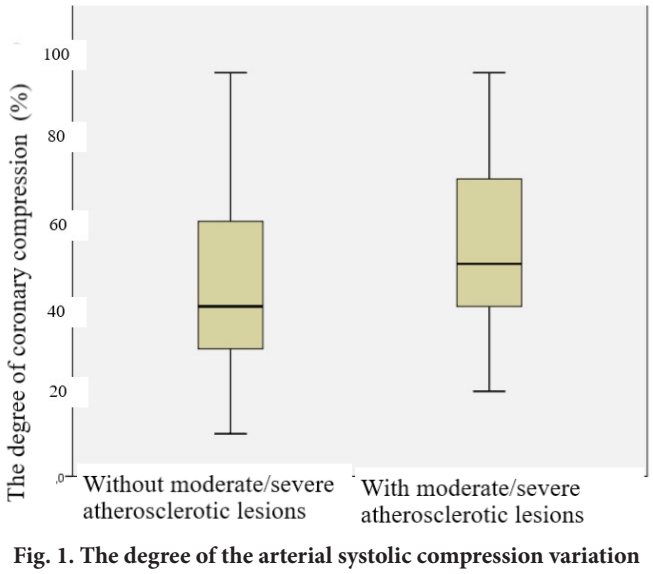

Fig. 1. The degree of the arterial systolic compression variation in the group of patients without atherosclerotic lesions and with moderate and severe coronary lesions

Another aspect, widely discussed in the scientific literature, and thoroughly analysed, is the interdependence of the degree of systolic stenosis of the anterior interventricular branch and the degree of hypertrophy of the ventricular myocardium.

In this study, patients were divided into: the group of patients with myocardial bridges and left ventricular myocardial hypertrophy, and the group with myocardial bridges without left ventricular myocardial hypertrophy.

From the total number, patients with myocardial hypertrophy and myocardial bridges were twice less when compared with the patients with the normal myocardial thickness.

The comparative research did not show any interdependence between the degree of vascular compression caused by myocardial bridge and the degree of left ventricular myocardial hypertrophy excepting the category of patients with myocardial hypertrophy and systolic stenosis lower than 50% (fig. 2). Thereby, in patients with myocardial hypertrophy, were determined 10% more cases of vascular systolic stenosis under 50% of the vessel lumen than in patients with the same degree of vascular compression but without myocardial hypertrophy (fig. 3).

The analysis of the dependence between the myocardial bridges and proximal to the bridge coronary atherosclerotic lesions, showed in 32% of cases atherosclerotic plaques at various distances to the bridge and only in one case (0.5%) – atherosclerotic plaques with location immediately after the bridge.

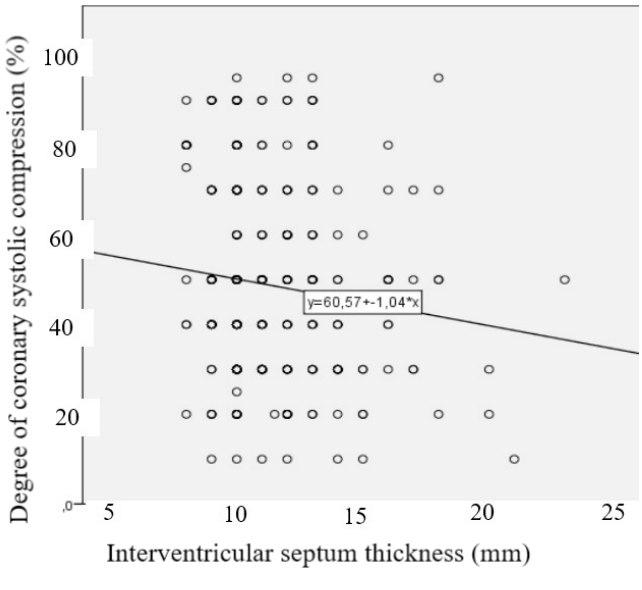

Fig. 2. Lack of direct correlation between the degree of left ventricular myocardial hypertrophy and the degree of compression of the subpontine coronary segment in the general group of research

No cases with under the bridge coronary atherosclerosis were detected.

The research did not determine the interdependence between the degree of dynamic coronary stenosis caused by bridge and the degree of proximal to the bridge atherosclerosis (fig. 4).

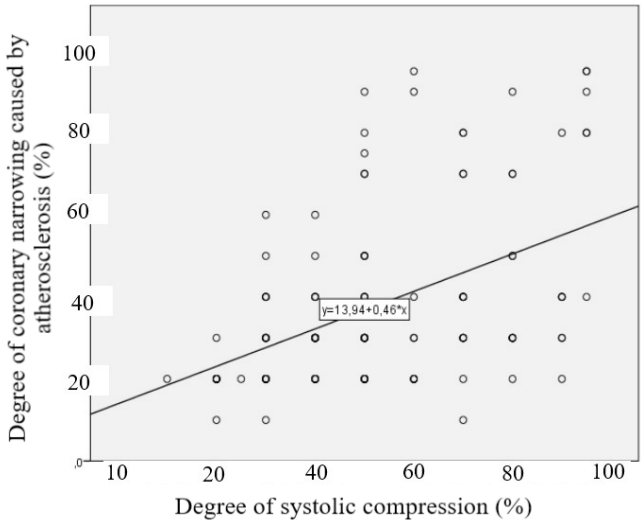

Fig. 4. Lack of direct correlation between the degree of prepontine atherosclerotic lesion and the degree of subpontine systolic stenosis

Discussion

Within the performed research, no cases were detected with atherosclerotic lesions under the myocardial bridge. The obtained data is consistent with the reports published in the medical literature. Thus, some authors attribute to myocardial bridges antiatherogenic functions [17], while the others claim the possibility of forming, in their presence, atherosclerotic plaques under the bridge in 6.7% of cases [18], thus denying the antiatherosclerotic role of the bridges [19].

Therefore, the opinions promote the idea that the association of the myocardial bridge with the atherosclerosis under the bridge is very rare [20] or that this association presents a major risk in the occurrence of acute myocardial infarction [12]. Other authors mention that if the atherosclerosis is detected under the myocardial bridge, its degree of lesion is clearly lower than the proximal to the bridge [21].

As to the protective antiatherosclerotic mechanisms, both are described, the morphological features specific to the subpontine endothelial layer, resistant to atherosclerotic lesions, and the pulsating character of the coronary vessel under the bridge [22]. In this aspect, the essential endothelial changes were highlighted, which allowed the authors to assume that they play a protective role against atherosclerosis [23].In contrast to the subpontine antiatherogenic effect, in the proximal to the bridge vascular segment, the incidence of atherosclerotic lesions is increased [16].

Within the performed study, the atherosclerotic lesions visualized on angiography in the proximal to the bridge arterial portion were detected in 32% of cases. However, a correlation between the degree of coronary systolic stenosis and the of plaques, and the degree of proximal to the bridge atherosclerotic stenosis, was not found.

The obtained results did not show a definite correlation between the presence of myocardial bridge and prepontine coronary atherosclerosis.

In the recent publications of Dr. Ishikawa, he affirms that myocardial bridges can be considered as risk factors for the development of atherosclerosis in certain segments of the coronary arteries, especially the proximal to the bridge ones, and in the genesis of myocardial infarction [9].

The incidence of prepontine stenosis is lower in this study than in other published data. Thus, multiple studies denote the predisposition, in 50% up to 90% of cases, of the respective segment in the formation of atherosclerotic plaques [24], increasing thereby the risk of acute coronary syndrome [25]. In this context, cases of complications of proximal to the bridge coronary atherosclerotic plaques by rupture of the fibrous capsule and acute thrombosis are reported [1].

Though, no correlation was detected between the degree of myocardial bridge compression and the incidence of atherosclerotic coronary lesions and their degree. Some authors used IVUS (intravascular ultrasonography) during the diagnostic coronary angiography and established the direct correlation between the degree of pontine compression of the vessel and the development of atherosclerotic plaque in the proximal to the bridge part of coronary artery, the degree of systolic compression being a more influential factor in the development of atherosclerotic plaque than the length, thickness and location of complete myocardial bridges [4]. Therefore, the degree of prepontine coronary damage can serve as an indicator of the severity of the myocardial bridges and the choice of the optimal treatment tactics [26].

The use of intravascular imaging methods is much more sensitive in detecting of minimal atherosclerotic lesions in the prepontine coronary segment than the conventional angiography used in the study.

As well as the dependence between the degree of coronary artery disease and the degree of coronary systolic stenosis, the causes of atherosclerotic prepontine lesions remain unclear.Some authors assume the multifactorial influence of the local, proximal to the bridge action of the blood flow, of the parietal stress, which leads to the mechanical lesion of the vascular wall [1].

This mechanical factor is caused by a retrograde blood flow, the result of the systolic expulsion of blood column from the intramural portion of the vessel.

The intima lesions resulted from the meeting of retrograde blood flow with the anterograde one, facilitate the rupture of atherosclerotic plaque and the appearance of acute coronary syndromes [1].

However, it is mentioned the existence of differences in proximal to the bridge and intramural parietal stress forces.

Thus, under the myocardial bridge the parietal stress is higher than in the proximal to the bridge coronary segment, leading to increased transendothelial distribution of lipids [1].

The mentioned effects are aggravated by the turbulent circulation of blood in the proximal to the bridge portion, at the place of meeting of the anterograde blood flow with the retrograde one [28].

These processes are confirmed by intracoronary studies by Doppler investigations [27].

A particular feature, found in the current study, is the predominance of moderate and severe systolic stenosis caused by bridges in case of proximal to the bridge atherosclerotic plaques that reduced the coronary lumen more than 50%, and the number of patients with severe subpontine systolic stenosis was double compared to the group of patients without moderate and / or severe prepontine coronary atherosclerotic lesions.

The obtained results could be explained by the intracoronary pressure and the lower intracoronary resistance in case of coronary bed severely affected by atherosclerosis plaques located proximal to the bridge with the reduction of the coronary flow proximal to the bridge and increasing compressive effect of the bridge. Consequently, it could explain why myocardial bridges bring ischemic suffering in older people and not from childhood or youth. At the same time, this peculiarity would strengthen the idea of the existence of anti-compressive mechanisms at the level of arteries and myocardial bridges, which could prevent arterial systolic stenosis, but with the progression of atherosclerosis of the coronary artery the effect of these mechanisms could be annihilated. In this case, the intracoronary pressure decreases because of proximal to the bridge coronary lesions that diminish the coronary flow.

A second explanation, hypothesis, which could explain the predominance of a more pronounced degree of under the bridge arterial compression in the group of patients with moderate and severe coronary atherosclerotic lesions, would be the medication that patients with severe ischemic heart disease are taking as chronic treatment, including nitrates, which is increasing the degree of systolic arterial stenosis reducing the intracoronary pressure [19].

The above-mentioned explanations resonate with the concepts identified in the literature, as well as the idea that nitrates could accentuate the degree of systolic stenosis of the under the bridge vascular segment by reduction the intracoronary pressure [27].

Although, some authors consider that the bridge is also protecting the distal to the bridge vascular segment from atherosclerosis [28], the results of some studies denote the possibility of atherosclerotic lesion of this segment [29].In 25%-30% of cases, atherosclerosis can affect the postpontine portion of the vessel [30]. Within the present study, these kinds of lesions were detected in only one case.

Another aspect, widely discussed in the literature and analysed in this study, is the interdependence between the degree of systolic stenosis of the anterior interventricular branch and the degree of myocardial hypertrophy [30].

Thereby, it is mentioned that the myocardial bridges are more common (up to 30%) in patients with myocardial hypertrophy [31].

At the same time, in the literature were found discrepancies regarding the influence of myocardial bridges in patients with hypertrophic cardiomyopathy and the severity of clinical manifestations.

According to some studies [30], the myocardial bridges in people with hypertrophic cardiomyopathy are worsening the clinical picture and can lead to angina pectoris, ventricular tachycardia, cardiac arrest. However, according to other authors, there is no correlation between myocardial bridges, hypertrophic cardiomyopathy, and the severity of the clinical picture [32]. There is evidence that in patients with hypertrophic cardiomyopathy, the degree of compression is increased by the myocardial bridge [30]. The importance of this correlation could explain why in certain periods of life the myocardial bridges, which are present from birth, could cause myocardial ischemia [30].

From the total number, patients with myocardial hypertrophy and myocardial bridges were twice less, in comparison to patients with normal myocardial thickness. The results contradict the literature references that myocardial bridges are more common in people with myocardial hypertrophy, assuming that hypertrophy increases the compressive effect on the vessel of the myocardial bridge. The comparative research did not highlight any relationship between the degree of under the bridge vascular compression and the degree of left ventricular myocardial hypertrophy in the general study group, except for the category of patients with myocardial hypertrophy and systolic stenosis lower than 50%.

Thus, in patients with myocardial hypertrophy, were detected 10% more cases of vascular systolic stenosis below 50% of the vessel lumen, than in patients with the same degree of vascular compression, but without myocardial hypertrophy.

It should be noted that in the performed study there were no patients with diagnosed hypertrophic cardiomyopathy. Patients from the researched group, in most cases had myocardial hypertrophy up to 15 mm, assessed by echocardiography.

This phenomenon could be explained by the fact that in case of hypertrophy below 15-20 mm, possibly, the myocardial bridge does not always have enough force to overcome the intracoronary pressure, opposed to the degree of myocardial hypertrophy encountered in hypertrophic cardiomyopathy [33].

Conclusions

1. Active systolic myocardial bridges, with potential to induce myocardial ischemia, are detected in 5.3% of patients.

2. There is no correlation between the degree of stenosis caused by the bridge and the degree of hypertrophy of the ventricular myocardium in patients without hypertrophic cardiomyopathy, as well as the degree of stenosis caused by the bridge and the degree of proximal to the bridge atherosclerotic stenosis.

3. Important finding was that the degree of coronary systolic compression is higher in patients with moderate and severe proximal to the bridge atherosclerosis.

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MT conceptualized the idea, conducted literature review, wrote the manuscript, revised, and approved the final text; VR added clinical relevance; IC designed the research and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. It was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 55 of 03.06.2016).

Conflict of Interests
No competing interests were disclosed.

DOI: https://doi.org/10.52418/moldovan-med-j.64-4.21.05
UDC: 616.98:578.834.1

The peculiarities of patients with COVID-19 infection

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Manuscript received July 30, 2021; revised manuscript November 10, 2021; published online November 26, 2021

Abstract

Background: The surge demand and decreased availability of the health workers determined the designation of the Clinical Municipal Hospital of Phthisiopneumology (CMHP) into a hospital for the case-management of COVID-19 patients during the period of December 2020 – April 2021 for the treatment of the mild cases. The aim of the study was to assess the main peculiarities of the patients with COVID-19 through a cross-sectional study developed during the period February-April 2021 in the CMHP.
Material and methods: A cross-sectional, analytical and prospective study was realized, which included 145 patients with COVID-19 hospitalized in the CMHP during February-April 2021. The inclusion criteria were: patient older 18 years, COVID-19 infection diagnosed by the pulmonologist with the positive polymerase chain reaction result for SARS-CoV-2 (COVID-19) and signed informed consent.
Results: The peculiarities of patients with COVID-19 were dominated by the female sex, age over 60 years, residence in the urban districts of Chisinau and the infection acquired in the Republic of Moldova. Common clinical manifestations were: high temperature, profuse sweats, asthenia and headaches, productive cough, dyspnea, loss of smell or taste, and sore throat. The typical radiological findings such ground-glass pattern or consolidation with bilateral multifocal involvement were most frequent. Were cured most of the patients and the death rate was low.
Conclusions: Peculiarities and clinical manifestations of the patients admitted in CMHP were typical for the COVID-19 infection. These findings did not show the real outcome of the standard case-management of the COVID-19 due to the restricted admission criteria.
Key words: COVID-19, risk factors, management.

Cite this article
Ungurean A, Malic A, Osipov T, Lesnic E. The peculiarities of patients with COVID-19 infection. *Mold Med J.* 2021;64(5):27-32. https://doi.org/10.52418/moldovan-med-j.64-4.21.05.

Introduction

The Republic of Moldova (RM) registered 258.624 coronavirus cases, of which 6.243 died and 251.489 recovered till 26th July 2021 [1]. According to the national regulation the first contact point with the patients with suspected COVID-19 infection are the ambulance service or the primary healthcare providers. The regulation recommends that at the country border (Chisinau Airport and Moldovan border entries) the police are responsible for identifying the suspected cases through the temperature screening and completion of the epidemiological cards. If suspected for COVID-19 infection case is found, the patient is transferred through the Emergency medical service (ambulance) to one of the designated for COVID-19 hospitals, depending on the severity of the clinical manifestations. The cases known for the community transmission of the infection, are quickly redirected for the screening to the Triage Center for testing through the polymerase chain reaction for SARS-CoV-2 (COVID-19) and the positive for COVID-19 cases are referred to the designated hospitals. The National Clinical Protocol Infection with Coronavirus the new type (COVID-19) PCN-371 was adopted on 31st March 2021 and was based on the World Health Organization (WHO) clinical guidelines [2, 3]. Several comprehensive clinical standards were developed by the Moldovan specialists in the case-management in the Intensive Care Units, in pediatric service and at the level of the Primary Healthcare.

The physical infrastructure of the medical institutions and the redirection of the workforce capacity was continuously adapted by the Ministry of Health, Labour and Social Protection of the Republic of Moldova according to the evolution of the COVID-19 outbreak [4, 5]. On 4th March 2020 four hospitals in Chisinau, which integrated 512 health workforce were designated for the treatment of COVID-19 patients, among them 34 Intensive Care Unit beds and 134 beds for mild and moderate cases. Due to a surge demand and a decreased availability of the health workers the Clinical Municipal Hospital of Phthisiopneumology, which specialised in the case-manegement of tuberculosis patients, was designated as a hospital for COVID-19 patients during the period of December 2020 till April 2021 [5]. Since December 2020 the mild and moderate COVID-19 cases of Chisinau residents were hospitalized in medical subdivisions of the Clinical Municipal Hospital of Phthisiopneumology. The hospital's requirements were adapted and were procured the medical devices (ventilators, pulse oxymeters, oxygen

concentrators), medicines, laboratory tests and consumables. The medical personnel was trained. At the beginning there was a shortage of the medical devices and antiviral drugs, but these problems were solved through the public procurement and donations. Since the state of emergency for COVID-19 was declared, 49 training sessions on infection prevention and control, psychological intervention, clinical management of moderate, severe and critical cases were held with the support of the WHO in the Republic of Moldova. The medical and non-medical staff of the Clinical Municipal Hospital of Phthisiopneumology was trained before the initiation of the treatment management as well.

A wide range of researches confirmed the infection caused by the new coronavirus SARS CoV-2 (COVID-19) varies from asymptomatic or mild form, which counts two thirds of the cases of severe pneumonia associated with acute respiratory distress syndrome (ARDS) / acute respiratory failure with hypoxia and/or hypercapnia. A range from 16 till 35% of COVID-19 infected patients develop severe pneumonia and 2–17% of them require invasive mechanical ventilation (VMI), of which up to 15% require oxygen therapy [6-9]. In severe forms of COVID-19 the excessive inflammation leads to the onset of the massive vascular inflammation, intravascular disseminated coagulation, shock and acute respiratory distress syndrome which contributes to the death [10]. However, in majority of cases, COVID-19 infection is self-limiting with gradual spontaneous resolution [8].

The aim of this study was to assess the peculiarities of the patients with COVID-19 through a cross-sectional study developed during the period February-April 2021 in the the Clinical Municipal Hospital of Phthisiopneumology.

Material and methods

The research was cross-sectional, analytical and prospective study, which included 145 COVID-19 patients hospitalized in the Municipal Clinical Hospital of Phthisiopneumology (SCMF), Chisinau, the Republic of Moldova during February-April 2021. The inclusion criteria of the patients in the research were: age older 18 years, COVID-19 infection diagnosed by the pulmonologist, positive polymerase chain reaction (PCR) result for SARS-CoV-2 (COVID-19), and signed informed consent. The protocol schedule included the following data about the patient:

1. Biological and social peculiarities: sex (male-female rate), age (distribution in age groups through decades), demographic characteristics (urban/rural residence).

2. Economical peculiarities: economical status (employed, unemployed, retired, disabled) and health-insurance coverage (presence/lack of health insurance).

3. Characteristics with high risk: comorbidities, migration or history of returning from abroad to the Republic of Moldova within the last 2 weeks.

4. Case-management peculiarities: health care seeking behavior and addressability, methods for the detection and medical staff involved in the case-management, comorbidities and complications.

The statistical analysis was performed using EpiInfo software. Data were appreciated as nominal or quantitative. The frequency and percentage were reported for nominal data, and the mean and standard deviations were reported for continuous data. The statistical analysis of the differences between normally distributed continuous variables was tested with the Student T-test. A p value of <0.05 was considered statistically significant.

Results

While distributing patients according to the sex it was established a higher rate of women 90 (62%) compared with 55 (38%) men with women/men rate=1.7/1. Repartition of the patients, according to the age established that the elders aged 60 years and more constituted the largest part – 89 (61%) cases. A lower rate was constitued from the patients aged between 50 and 59 years –20 (14%) cases. A similar low number of 14 (10%) patients were between 40 and 49 years ol d, between 30 and 39 years old were 12 (8%) patients and between 18 and 29 years old were 10 (7%) cases. The average age was 68±14 years.

While assessing the risk factors for contracting COVID-19 infection it was establisehd that those who returned from abroad within the last 2 weeks before the sickness constituted 12 (8%) cases. Demographic distribution identified that 132 (91%) patients came from urban sectors of Chisinau and 13 (9%) patients from rural localities. No homeless or without a stable living residence were established (fig. 1).

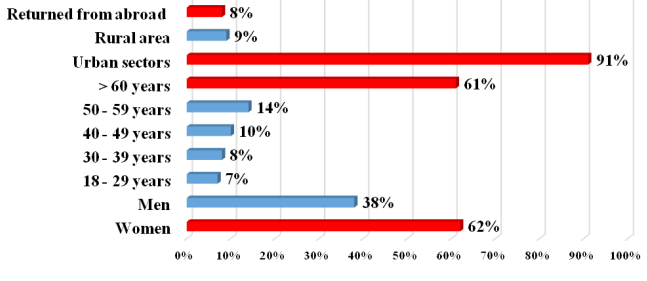

Fig 1. Distribution of the patients according to the sex, age and residence (%)

When distributing patients according to the economic status, it was established that employed persons, which were contributing in this way to the health budget by paying taxes, health insurance policy and social taxes were only 48 (33%) cases. Disabled patients were 7 (5%). Due to the predominance of the patients older 60 years the retired statistically prevailed compared with other economical groups – 78 (54%) cases. There were 12 (8%) unemployed. Health insurance represents the major condition for accessing health care and free of charge medical procedures (radiological investigations, laboratory tests). Due to a higher rate of retired and disabled patients the health insurance coverage supported by the public authority was

established in 90 (62%) cases and was supported by the beneficiary 48 (33%) cases. The distribution of patients in economical groups established that the largest group was represented by the patients in the economical vulnerable state (unemployed, retired, disabled), which were 97 (67%) cases. Lack of the health insurance, which represents the major barrier for accessing the specialized health treatment, was identified in 12 (8%) cases. Assessing the harmful habits, it was established 18 (12%) cases of active smokers and 3 (2%) alcohol abusers. Personal medical history suggestive of allergy had 15 (10%) patients (fig. 2).

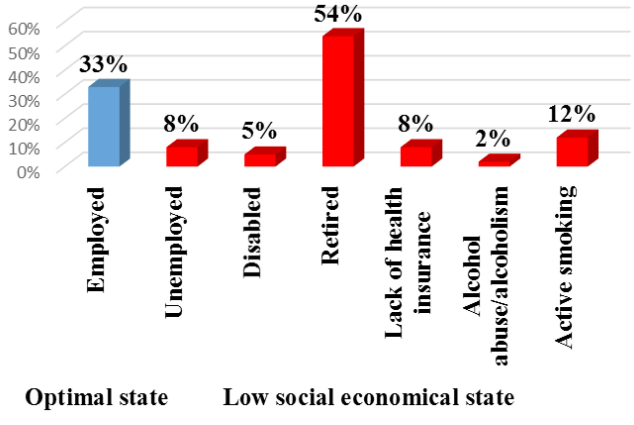

Fig. 2. Distribution of the patients according to the social and economical peculiarities (%)

The poor evolution of COVID-19 infection in comorbid patients is well recognised. Most of admitted patients, 108 (74%) had associated diseases, of which 106 (73%) had arterial hypertension, diagnosed, when blood pressure was 140/90 mmHg or higher. Grade 2 arterial hypertension was diagnosed in 84 (57%) patients and grade 3 in 12 (8%) patients. Arterial hypertension was complicated with tubulointerstitial nephritis in 16 (11%) cases and with congestive heart failure in 4 (3%) cases. In 4 patients type 2 diabetes mellitus was diagnosed. Those patients were diagnosed with arterial hypertension, as well. In 8 (6%) patients chronic respiratory disease was diagnosed mainly linked with the active smoking. Hyperthyroidism was diagnosed in 4 (3%) patients. Gastrointestinal disorders were established in 5 (3%) patients.

At the hospital admission the common symptoms such as fever was detected in 127 (87%) cases and feverish in 18 (12%) cases. The general state was moderately disturbed in all admitted cases. No severe forms, requiring the intensive care were admitted. The high temperature was associated with profuse sweats in 90 (62%) cases and tremor in 24 (16%) cases. The productive cough in 113 (78%) cases was associated with dyspnea in 86 (59%) cases, among which the 1st degree dyspnea was diagnosed in 36 (25%) cases and the 2nd degree in 50 (34%) cases. Asthenia and fatigability were confirmed in 82 (56%) cases, associated with anorexia in 6 (4%) cases and headache in 126 (87%) cases. Peripheral edema was established in 4 (3%) cases. Nausea and vomiting were identified in 12 (8%) cases associated with diarrhea in 24 (16%) cases. Arthralgia was established in 18 (12%) cases (fig. 3).

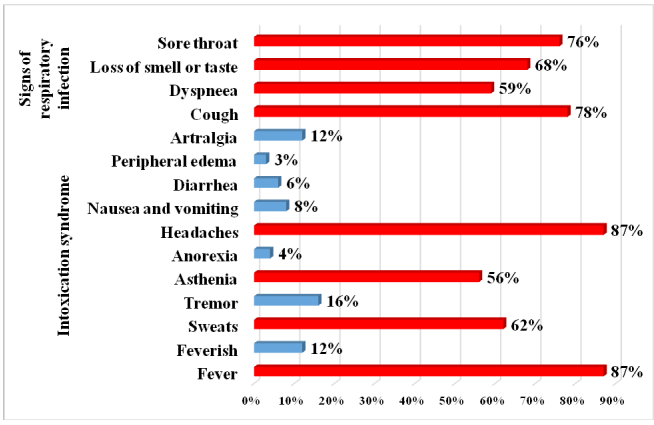

Fig. 3. Distribution of the patients according to the clinical manifestations

According to the pulse oximetry, the peripheral saturation with O_2 was between 99% and 94% in 24 (16%) cases and between 94% and 89% in 130 (88%) cases. Patients with the peripheral saturation with O_2 lower than 89% in the CMHP were not admitted. At the hospital admission the arterial tension ranged within the normal limits in 78 (54%) cases and the arterial hypertension was confirmed in 67 (46%) cases, among which the 1st class arterial hypertension was diagnosed in 31 (21%) cases, the 2nd class in 16 (11%) cases and the 3rd class in 20 (14%) cases. The respiratory rate was between 16 and 19/minute in 126 (87%) cases and between 20 and 25/minute in 19 (13%) cases. Loss of smell or taste was established in 99 (68%) cases. Sore throat (acute nasopharyngitis) at the onset of the disease was diagnosed in 110 (76%) cases.

Inflammatory biomarkers, blood count, liver enzymes (AST, ALT) and products of protein metabolism (urea, creatinine) in the serum were evaluated, as a compulsory laboratory investigation of COVID-19 patients. Erythrocyte sedimentation rate (ESR) in 114 (79%) cases was increased. Fibrinogen exceeded the normal threshold (4 g/L) in 132 (91%) cases. The alpha-amylase in 12 (8%) cases was increased. AST was increased in 78 (54%) cases, including 18 (12%) patients were diagnosed with severe hepatotoxicity with the value of AST exceeding 100 U/L. ALT was increased in 31 (21%) patients and in 15 (10%) patients was diagnosed severe hepatotoxicity. Creatinine was higher than the normal level in 65 (45%) patients. Assessing the leucocyte count was established leukocytosis in 62 (43%) cases. The lymphocyte count revealed increased number (lymphocytosis) in 58 (40%) patients and diminished number (lymphopenia) in 28 (19%) cases. No pathological deviations in the number of the thrombocytes and eosinophils were established (fig. 4).

The conventional radiological investigation of the chest in an anteroposterior projection revealed the ground-glass pattern or consolidation with unilateral involvement

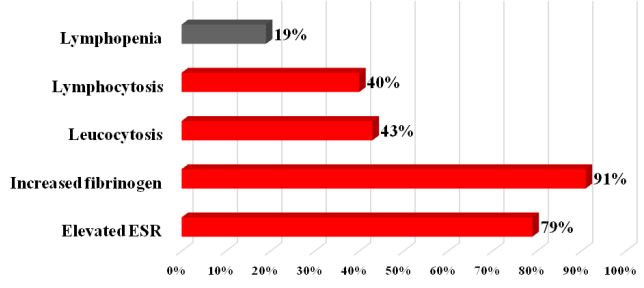

Fig. 4. Pro-inflammatory biomarkers in patients with COVID-19 (%)

in 25 (17%) cases. Bilateral multifocal involvement, seen as symmetrical localized ground-glass opacities and consolidations in lower fields were observed in 120 (83%) cases. Reticular pattern due to interstitial involvement was confirmed through the computed tomography in 12 (8%) cases. During the hospitalization the radiological findings extended in several days after the onset of the symptoms and progressed into a diffuse disease in 3 (2%) patients in which the pneumonia progressed in acute distress syndrome. Was assessed the severity of pulmonary involvement by the chest X-ray. Using a vertical line (along the spine) and the horizontal line (along the lower edge of the left main bronchus) 4 quadrants were obtained. Each quadrant was assigned with a score of 0–4 depending on the extent of consolidation or ground-glass opacities: 0 = no findings; 1 < 25%; 2 = 25%–50%; 3 = 50%–75%; 4 > 75%. In 72 (50%) cases the score was equal 1 (<25% involvement of the lung parenchyma), in 61 (42%) cases the score was equal 2 (25-50% of the lung parenchyma involved) and 12 (8%) cases the score was equal 3 (>75% of the lung parenchyma involved). Data are shown in figure 5.

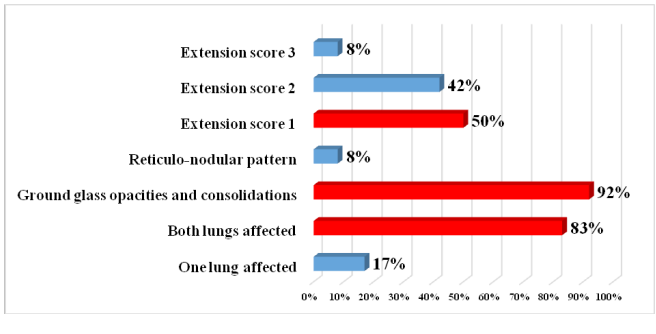

Fig. 5. Distribution of the patients according to the radiological findigs

The patients were treated according to the national regulation (National Clinical Protocol 371) and the average duration of the hospitalization was 8 days. Were discharged as cured 135 (93%) patients, 3 (2%) died due to ARDS and for the mechanical ventilation in the Intensive Care Unit 7 (5%) cases were transferred.

Discussion

Similar studies performed in different countries since the declaration of the emergency state of COVID-19 outbreak established that the main peculiarities of the patients diagnosed with mild COVID-19 infection are female sex, older age and people with underlying chronic conditions, which increase the risk for severe infection [6-9]. This research established that two thirds of the investigated group constituted women, with the age older 60 years and diagnosed with associated diseases. Among the comorbidities, arterial hypertension was diagnosed in most of the investigated cases. One half of the group was identified with arterial hypertension at the admission. Several studies linked the predisposition to acquire severe COVID-19 infection in patients with arterial hypertension and a high risk for death [10]. One tenth of COVID-19 patients had infection complicated with the tubulo-interstitial nephropathy, which was recognised as an acute kidney injury associated with COVID-19 by a wide range of papers [11-14]. The World Health Organization's technical guidance and Clinical Management Guideline made an overview on COVID-19 and influenza similarities and differences according to the clinical aspects. While both diseases have multiple similar clinical presentations: a sore throat, fatigues, chills, muscle or body aches, headache, there are important clinical differences between the two viral infections [3]. According to the WHO Guideline the clinical manifestations with high specificity for the pneumonia caused by COVID-19 infection are: high fever, dry cough, shortness of breath (dyspnea) and hypoxemia [3, 6-9]. The conducted study proved that the evaluated patients were more frequently complaining of the high fever, sweats, headaches, cough, sore throat and loss of smell or taste, which were reported by the clinical papers [6-8, 11, 13-15]. According to the WHO study about 15% of COVID-19 cases are severe and about 5% of people get acute respiratory distress syndromes (ARDS) requiring the ventilation [3]. A true rate of severe COVID-19 infection-related pneumonia couldn't be established through the discribed research because of the admission criteria in the hospital. Only 7% of the admitted patients with mild COVID-19 infection developed ARDS and severe hypoxemia requiring mechanical ventilation. The obtained results were not reflecting the overall state of the COVID-19 pneumonia.

Evaluating the economical consequences it was identified that two thirds of the cases, which included the retired, unemployed and disabled patients had a vulnerable social and economic state. The proportion of the patients without health insurance was low. The health insurance provided a fair healthcare in all cases. The obtained data could not be compared with other studies, as no relevant information was found.The patients with harmful habits, such as active tobacco smoking and alcohol drinking were in a lower proportion. These findings can be attributed to the high proportion of women and elders, in which the prevalence of harmful habits is low.

The conventional radiological investigation of the chest revealed the ground-glass pattern or consolidations with bilateral multifocal involvement in most of the cases. The consensus statement endorsed by the Society of Thoracic Radiology and the American College of Radiology classified the radiological patterns of COVID-19 in typical, indeterminate and atypical appearance [16]. The patterns of the typical appearance are: peripheral bilateral ground-glass opacities with or without consolidation, multifocal ground glass with rounded morphology and signs of the organizing pneumonia. Indeterminate appearance is established for multifocal, diffuse, peripheral or unilateral ground-glass opacities, without a specific distribution. The atypical appearance is the isolated lobar or segmental consolidation without ground-glass opacities and small nodules. The radiological appearance in selected patients was typical for COVID-19 infection. A retrospective study identified that the most common laboratory findings in patients are: lymphopenia, thrombocytosis, increased prothrombin time, increased lactate dehydrogenase, mild elevated inflammatory markers (protein C reactive and erythrocyte sedimentation rate), elevated D-dimers, mildly elevated serum-amylase and elevated liver function enzymes [17]. Inflammatory biomarkers (erythrocyte sedimentation rate and fibrinogen) were elevated and showed a moderate inflammation in most of the selected COVID-19 cases. Leucocytosis and lympchocytosis were more frequently established in this research, than lymphopenia. It can be explained by the secondary bacterial infection, which develops on the background of the decreased host immunity, confirmed by other studies [18].

Conclusions

The peculiarities of patients with COVID-19 hospitalized in the clinical subdivisions of the Clinical Municipal Hospital of Phthisiopneumology during 2 months were dominated by female sex, age over 60 years, residence in Chisinau city and the infection acquired in the Republic of Moldova. Clinical manifestations were specific for COVID-infection: high temperature, profuse sweats, asthenia, headache, cough and dyspnea, loss of smell or taste and sore throat. The radiological findings demonstrated typical appearance with ground-glass pattern or consolidations with bilateral localization. The therapeutical approach contributed to the curing of most of the patients and the death rate was low. The treatment outcome should not be generalized as a characteristic outcome for the COVID-19 infection. It could be the consequence of the limitations due to admission criteria of the patients with mild forms of the COVID-19 infection. Large-scale studies should be developed including all forms of the COVID-19 infection.

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AU collected the data; AM collected and analised the data, TO conducted the clinical and laboratory work; EL conceptualised the project and designed the research, reviewed the statistics and interpreted the data, drafted the manuscript, revised the manuscript critically, All the authors revised and approved the final version of the manuscript.

Funding
The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval
This study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova (No 44 of 26.05.2025).

Conflict of interests
No competing interests were disclosed.

https://doi.org/10.52418/moldovan-med-j.64-5.21.06
UDC: 616.62-006.6-089.87

Endourological treatment of non-muscular-invasive bladder tumors

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Manuscript received August 27, 2021; revised manuscript November 04, 2021; published online November 26, 2021

Abstract

Background: Transurethral resection of the bladder is one of the essential methods in the diagnosis, treatment and management of non-muscular-invasive bladder cancer. The purpose of the procedure is to remove completely all visually detected tumors with a following establishment of a very precise histological diagnosis. The aim of the study is to compare the results of conventional transurethral endoscopic treatment and the En-bloc resection method using different types of energy sources in the treatment of bladder tumors.
Material and methods: A total number of 88 patients underwent endourological interventions. Regarding the distribution, 23 patients had conventional transurethral resection, 22 – En-bloc monopolar resection, 21 – En-bloc bipolar resection and 22 – En-bloc with Thu:YAG laser. Clinical data, intraoperative and postoperative data and also the histopathological examination results were compared.
Results: The compared groups were heterogeneous by age, sex, tumor characteristic (size, number, location). No significant differences were observed during the operations, comparing the intraoperative and postoperative complications of the studied groups. The detrusor musculature was detected in 74% of cases after conventional transurethral resection, in 91% of cases of En-bloc monopolar resection, in 95% of cases of En-bloc bipolar resection and in 96% of cases of En-bloc Thu:YAG laser.
Conclusions: The En-bloc resection technique of non-muscular-invasive bladder tumors is a safe and effective method comparing with the conventional transurethral resection; it allows more favorable postoperative results and obtaining better quality tumor samples which allow establishing correct diagnosis of the disease.
Key words: en-bloc resection, laser, non-muscle-invasive bladder cancer.

Cite this article
Vladanov I. Endourological treatment of non-muscular-invasive bladder tumors. *Mold Med J.* 2021;64(5):33-37. https://doi.org/10.52418/moldovan-med-j.64-5.21.06.

Introduction

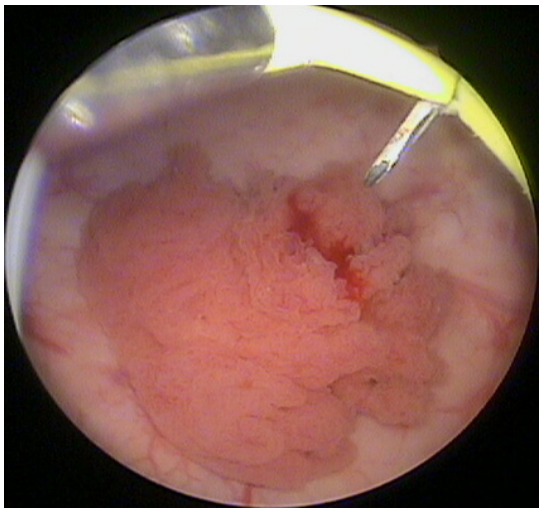
Worldwide bladder cancer is considered to be one of the most common cancers of the urinary tract and is the 11th most common malignancy. It is divided in two types: non-muscle-invasive bladder cancer (NMIBC), accounts about 75% and muscle-invasive bladder cancer (MIBC) about – 25% [1].

Transurethral resection of bladder tumor (TURBT) using white light cystoscopy (WLC) has been the essential procedure in bladder cancer diagnosis, removal, and local staging, since the first endoscopic descriptions of fulguration of papillary bladder tumors in 1910 (Beer E. 1983). The aim of TURBT is the complete resection of the total amount of papillary tumors parallel conducting biopsy of the suspicious flat lesions. For non-muscle-invasive bladder cancer (NMIBC), a high quality TURBT is essential in reducing tumor recurrence and its progression [2].

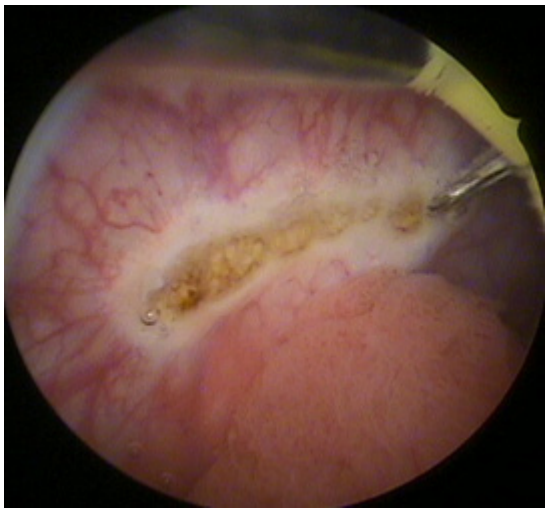
It must be taken into account the fact that after TURBT recurrences are often developed. Tumor understaging is another danger which should be taken into consideration. In order to overcome these limitations, the surgical strategy was optimized leading to technological improvements, including new energy sources etc. [3]. There is no doubt that the En-bloc resection (fig. 1) represents a promising surgical technique. It involves a circular incision of the mucosa at a safe distance from the lesion, followed by preparation and removal of the whole tumor, including the underlying detrusor muscle. A common opinion in the recent literature sources is that this 'no-touch' principle leads to a better specimen quality, as also to an improved surgical radicality, and a reduced recurrence rate [3].

For choosing the treatment strategies an exact pathologic staging of NMIBC is important. For achieving a good prognosis, a complete and correct resection is required [2, 4, 5]. An indicator for a higher risk of residual disease and also for early recurrence is the absence of detrusor muscle in the specimen [6]. The absence of detrusor muscle also represents a poorer surgical experience [6, 7].

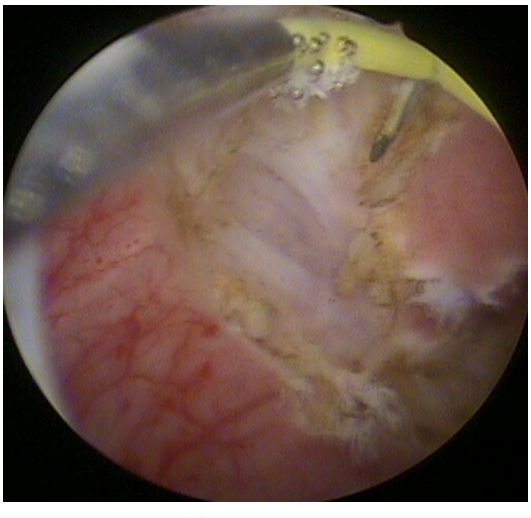
The aim of the study is to compare the results of conventional transurethral endoscopic treatment and the En-bloc resection method using different types of energy sources (fig. 2) in the treatment of bladder tumors.



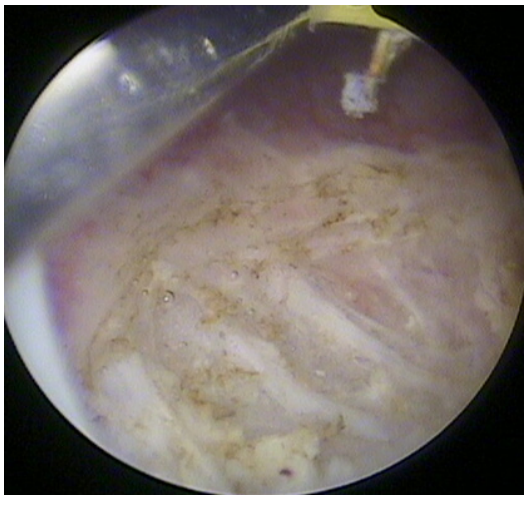
A. Tumor visualization



B. Marking of the resection area



C. Tumor resection



D. Assessment of the post-resection area

Fig. 1. En-bloc resection technique

Material and methods

The study was performed between February 2017 and February 2020, within the Department of Urology and Surgical Nephrology of *Nicolae Testemitanu* State University of Medicine and Pharmacy, in *Timofei Mosneaga* Republican Clinical Hospital. 88 patients were surgically treated with bladder tumor pathology. A transversal descriptive study was performed. The patients were selected from the total amount of bladder tumor patients treated in the department by En-bloc resection using different types of energy sources and transurethral resection of the bladder tumors, according to the following criteria. The inclusion criteria: non-muscular-invasive bladder cancer, patients aging over 18 years old and the Eastern Cooperative Oncology Group (ECOG) score 0-2. The exclusion criteria: other non-urothelial tumors, severe comorbidities, ECOG score ≥ 3 and pregnancy. 88 patients were divided in four groups: 23 patients had conventional transurethral resection (TURBT), 22 – En-bloc monopolar resection, 21 – En-bloc bipolar resection and 22 – En-bloc with Thu:YAG laser resection. The obtained data were comparatively analyzed. Descriptive statistics was applied. The results of this study are demonstrated as absolute and relative values.

Results

The study was done on 88 patients treated endourologically by En-bloc resection and TURB. Intraoperative hemorrhage during the procedure was carried out endoscopic and no blood transfusion was required. These four groups had comparable clinicopathological characteristics: age, gender, tumor size, tumor grade, tumor multiplicity, postoperative complications and histological outcomes (tab. 1, 2).

By gender patients were distributed as follows, from 88 patients included in the study, 73 (83%) were men and 15

Table 1. Patient and tumor demographics

Parameters	Categories	TURBT (n=23)	En-bloc M (n=22)	En-bloc B (n=21)	En-bloc L (n=22)	Total (n=88)
Gender:	Men, n (%)	19 (83%)	18 (82%)	18 (86%)	18 (82%)	73 (83%)
	Women, n (%)	4 (17%)	4 (18%)	3 (14%)	4 (18%)	15 (17%)
Age, years	Mean age (CI 95%)	64,8 (29-82)	65,3 (34-87)	65,6 (27-85)	66,2 (31-83)	65,5 (27-87)
Tobacco/Smoking	Yes, n (%)	12 (52%)	9 (41%)	11 (50%)	11 (50%)	43 (49%)
Tumor size:	< 3 cm, n (%)	18 (78%)	17 (77%)	14 (67%)	16 (73%)	65 (74%)
	≥ 3 cm, n (%)	5 (22%)	5 (23%)	7 (33%)	6 (27%)	23 (26%)
Number of tumors:	Single tumors, n (%)	15 (65%)	14 (64%)	14 (67%)	14 (64%)	57 (65%)
	≥ 2 tumors, n (%)	8 (35%)	8 (36%)	7 (33%)	8 (36%)	31 (35%)

Note: CI – Confidence Interval, TURBT – Transurethral resection of bladder tumor, En-bloc M – En-bloc monopolar transurethral resection of bladder tumor, En-bloc B – En-bloc bipolar transurethral resection of bladder tumor, En-bloc L – En-bloc Thu:YAG laser transurethral resection of bladder tumor.

Table 2. Surgical and histological outcomes

Parameters	Categories	TURBT (n=23)	En-bloc M (n=22)	En-bloc B (n=21)	En-bloc L (n=22)	Total (n=88)
TNM	Ta, (Tis), n (%)	14 (61%)	12 (55%)	12 (57%)	14 (63%)	52 (59%)
	T1, n (%)	9 (39%)	10 (45%)	9 (43%)	8 (37%)	36 (41%)
Detrusor muscle	**n (%)**	**17 (74%)**	**20 (91%)**	**20 (95%)**	**21 (96%)**	**78 (89%)**
Histopathology grade	Low-grade, n (%)	11 (47%)	10 (45%)	10 (48%)	11 (50%)	42 (48%)
	High-grade, n (%)	12 (53%)	12 (55%)	11 (52%)	11 (50%)	46 (52%)
Clavien-Dindo	CD grade I	2 (9%)	3 (14%)	2 (10%)	2 (9%)	9 (10%)
	CD grade II	4 (17%)	4 (18%)	3 (14%)	2 (9%)	13 (15%)
Complications	ONR, n (%)	4 (17%)	3 (14%)	3 (14%)	0 (0%)	10 (11.5%)
	BP, n (%)	3 (13%)	3 (14%)	2 (10%)	1 (5%)	9 (10%)

Note: TURBT – Transurethral resection of bladder tumor, En-bloc M – En-bloc monopolar transurethral resection of bladder tumor, En-bloc B – En-bloc bipolar transurethral resection of bladder tumor, En-bloc L – En-bloc Thu:YAG laser transurethral resection of bladder tumor, Low-grade – Low-grade papillary urothelial carcinoma, High-grade – High-grade papillary urothelial carcinoma, Tis (CIS) – Carcinoma in situ, "flat tumor", Ta – Noninvasive papillary tumor, T1 – Invades subepithelial connective tissue, CD – Clavien-Dindo, ONR – Obturator nerve reflex, BP – Bladder perforation.

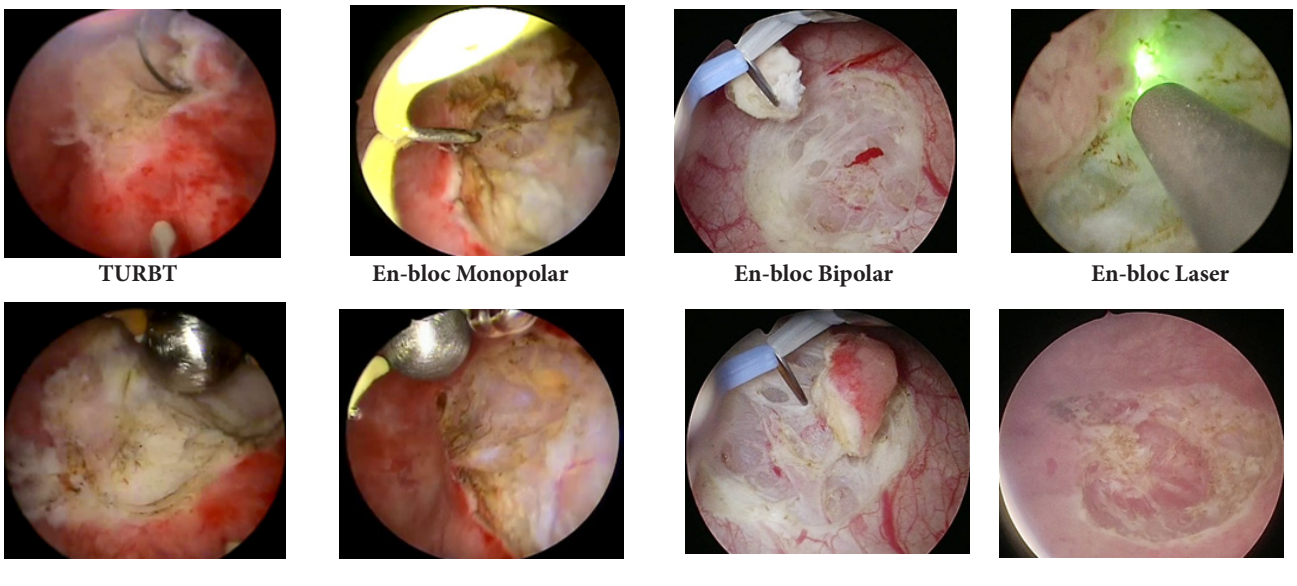


Fig. 2. Resection techniques using different types of energy sources

(17%) – women. The age varies between 27 to 87 years, the mean age encounted 65.5 years, majority of the patients were over 60 – 73 years (69%).

Tumor analysis (tab. 1) showed that in majority of the cases tumors were localized on lateral urinary bladder walls, single bladder tumors were detected in 65% of patients, tumor sizes up to 3 cm were detected in 74% of patients included in the research. An important risk factor for the development of bladder cancer is tobacco abuse which is detected in 49% (from 88 patients) of cases.

According to the Clavien-Dindo classification for surgical complications, only grade I and grade II complications occurred in the study groups (tab. 2). Intraoperative obturator nerve reflex occurred in 17% in the TURB group, 14% in case of En-bloc monopolar resection, 14% – En-bloc bipolar resection and did not occur in En-bloc with Thu:YAG laser. Bladder perforation, hematuria was managed by catheterization for 3-4 days. Histopathological examination showed that fragments of detrusor muscle were detected in 74% of cases after conventional transurethral resection, in 91% of cases of En-bloc monopolar resection, in 95% of cases of En-bloc bipolar resection and in 96% of cases of En-bloc Thu:YAG laser.

Discussion

Since 1997 (Kawada T.) ERBT has been available as a concept for the current-based En-bloc resection. A lot of publications of the last years suggest that transurethral En-bloc resection can potentially change the approach to endoscopic manipulations in the removal, diagnosis, and assessment of NMIBC [8, 9].

Recently an alternative to the standard monopolar TURBT is the bipolar, or plasmakinetic TURBT. There is a couple of advantages comparing them both – the bipolar resection allows the use of isotonic irrigation fluids for decreasing the risk of TUR syndrome. This improves potentially the safety profile comparing with monopolar energy [10]. A lot of studies compared bipolar with monopolar TURBT. It was found that bladder injury decreases and is associated with obturator nerve reflex [11, 12] as also an improved detrusor sampling was observed [13]. The advantages of bipolar resection are limited to safety of the surgical procedure and not to clinical outcomes, the reason is that most of the studies did not find significant effects of bipolar resection on recurrence rates [11, 14].

Pathological interpretation of TURBT samples is a well-known challenge, because the resected tumor fragments are the source for cautery damage, crush artifacts, tangential sections, and lack of spatial orientation which is caused by a random embedding of bladder tissue [15]. En-bloc resection technique improves the pathological assessment due to better preserving tumor architecture and orientation. En-bloc resection can be performed using standard electrocautery, lasers, or water jet combined with monopolar energy [16].

Laser-based En-bloc resection ensures not only a better preservation of tissue orientation, but also reduces surgical morbidity. The reason therefore is the decreasing of bladder perforation through obturator nerve reflex and post-operative bladder irritation [17].

The En-bloc resection has not only the important advantage of conserving the presence of detrusor muscle within specimens, but it also improves the detection of muscularis mucosae or other muscular layers in ERBT specimens. As a consequence these facilitate an accurate T1 substaging. A retrospective comparison between En-bloc resection with the use of green KTP laser and standard monopolar TURB in T1 bladder tumors is reported by Liang et al. [18]. As the primary endpoint the detection of muscularis mucosae in the specimen was regarded. So better outcomes with the En-bloc laser technique (30.7% for En-bloc resection with monopolar electrode versus 11.4% for TURBT) were demonstrated. They also found due to their multivariate logistic regression analysis that the KTP laser resection method is characterized by the presence of muscularis mucosae in specimens. The fact that En-bloc resection leads to a better quality of specimens reveals a more convenient clinical application due to its prognostic possibilities of several pathologic parameters including T1 substaging.

Cheng et al. showed that in spite of the positive surgical margin rate which serves as another indicator of resection quality [19], no significant difference in the positive surgical margin rates between En-bloc resection (98.9%) and TURBT (94.8%) could be demonstrated.

Complications of grades I and II according to Clavien-Dindo complication classification were registered in both groups (tab. 2). The following complications were observed: hematuria of different intensity, obturator nerve reflex, urinary tract infection, bladder perforation and urinary retention which was managed successfully with a standard approach.

The aim of En-bloc resection is not only to improve the resection quality, but also to reach three other goals: to decrease the recurrence rate, to reduce the perioperative complication rates and to decrease the number of repeated resections [15].

Conclusions

The En-bloc resection technique of non-muscular-invasive bladder tumors is a safe and effective method comparing with the conventional transurethral resection. It allows the achievement of more favorable postoperative results and the obtaining of better quality tumor samples. All mentioned above leads finally to the establishment of correct diagnosis of the disease.

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IV conceptualized the idea, conducted literature review, wrote the manuscript, revised and finalized the text.

Funding
The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 4 of December 16, 2019).

Conflict of Interest
The author has no conflict of interests to declare.

https://doi.org/10.52418/moldovan-med-j.64-5.21.07
UDC: 616-001.21-08

Treatment approaches of electrical injuries

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Manuscript received October 15, 2021; revised manuscript November 09, 2021; published online November 26, 2021

Abstract

Background: The purpose of this study was to explore the differences in prehospital care, admission characteristics, burn intensive care, surgery and outcomes in patients requiring admission to a burn intensive care unit.
Material and methods: The study was conducted on a group of 31 patients, who were hospitalized within the Clinical Hospital of Orthopedics and Traumatology in the period 2015-2019. The data analysis was carried out on the applied method of surgical treatment, the associated postoperative complications, antibiotic therapy applied, etc.
Results: 27 out of the total number of patients underwent necrectomy and extensive wound debridement surgery in the first hours upon admission. 14 patients required additional decompression incisions in the underlying skin and fascia. 4 patients out of the total number presented visible signs of carbonization on the affected areas upon admission, therefore they were subjected to an emergency amputation. Out of 27 patients classified as "delayed emergency" cases, 13 patients were subjected to amputation of the corresponding segments.
Conclusions: Electrical injuries are a severe cause of disability, as well as a challenging issue for reconstructive surgery, which is concerned with restoring the damaged structures with prosthetic amputation abutments, therefore improving the aesthetic and psychological appearance of the patients.
Key words: electrical injury, electric shock, reconstructive surgery, high and low voltage burn injuries.

Cite this article
Taran A. Treatment approaches of electrical injuries. *Mold Med J.* 2021;64(5):38-41. https://doi.org/10.52418/moldovan-med-j.64-5.21.07.

Introduction

Electrical injuries are classified as extremely serious injuries, commonly resulting in a poor life and poor functional prognosis, which can only be improved by complex general and local treatment, tailored to the individual characteristics of each case while considering the extreme urgency [1]. The prognosis of electrical burns depends on various factors:

– Burn surface area;
– Degree of the burn;
– Age;
– Possible complications of the burn;
– Patient's health before the accident;
– Occurrence of concomitant injuries (particularly, lung injuries);
– Early treatment and quality of treatment [2].

The only factor that can be corrected from the aforementioned ones is the "early and quality treatment" for the patient with an electric burn being admitted to a medical facility, thus the present issue requires an in-depth research approach.

The electric burn treatment should start immediately after it occurred and continue during the recovery period [2]. Any electric burn is an immediate surgical emergency, hence the surgical treatment will be carried out together with other drug therapies, which will significantly increase the chances of a quality recovery [2].

Successful treatment is based on a detailed clinical examination, which is actually a real strategy in this case, requiring knowledge on the multiple peculiarities of this type of lesion [3]. During the clinical examination, special attention is focused on the following areas:

– Electric entry mark and electric exit mark;
– Injuries caused by the electric path;
– Thermal burns caused by electric flames or ignition of clothing [3].

Following a complete initial assessment of the affected lesions, emergency treatment will be initiated. Local treatment involves a surgical intervention which is a critical component in the acute phase of electrical shock [4]. Emergency surgery is aimed at removing dead tissues and cleaning the associated burn injuries, on the one hand, as well as clearing the blood flow by preventing or combating the development of acute peripheral ischemia syndrome, on the other hand [1].

Necrectomy and extensive wound debridement are the primary goal of local treatment. Basically, the extended pressure sore excision and deep cleansing of devitalized tis-

sues might eliminate the main source of toxic substances (myoglobin, denatured proteins, cellular metabolites), as well as all the sepsis-causing bacteria [1]. Although a conservative treatment is commonly applied, in cases when sepsis can be life-threatening, the amputation will be performed as quickly as possible. Direct trauma in electrical injuries causes gradual ischemia, forming thrombi in the small artery or arteriole, constriction of blood vessels slowing blood flow and causing tissue necrosis, being a decision factor for amputation. To protect viable tissue in the initial stage of treatment is fasciotomy decompression of the involved compartment. The next step is to approach the methods of reconstruction from simple to compound by using skin grafts, already regional or free flaps need to be used in severe lesions (local flaps, removed flaps and free flaps). The local treatment should be combined with general resuscitation treatment aiming to implementing therapeutic shock recovery and rebalancing the hemo-hydro-electrolytic levels, on the one hand, as well as preventing or managing complications, on the other hand [5, 6].

In this context, the present research paper aims to highlight the peculiarities of the clinical evolution of the patients after electrical injuries depending on the surgical strategies used.

Material and methods

The study group involved 31 patients admitted to hospital in the period 2015-2019 at the Clinical Hospital of Traumatology and Orthopaedics.

The main study inclusion criterion was the electrical injury.

The characteristics determined for each patient comprised:

– Age;
– Gender distribution;
– The surgical approach applied;
– Antibiotic therapy;
– Length of hospital stay;
– Associated complications.

Results

By gender distribution, the groups were divided as follows: 21 cases (67.74%) – men and 10 cases (32.25%) – women (fig. 1). Of the total number of 31 cases: 4 cases (12.9%) – B third-burn degree, 7 cases (22.58%) – AB third-burn degree and 20 cases (64.51%) – fourth-burn degree (fig. 2).

The distribution of hospitalized patients by age groups was as follows: 21-45 years – 11 (35.48%) patients, 46-60 years – 17 (54.83%) patients, over 60 years – 3 (9.67%) patients (fig. 3).

Upon the first hours after hospitalization, 27 patients (87.09%) out of 31 underwent necrectomy and extensive wound debridement. In the other 4 cases (12.9%) radical intervention was performed by emergency amputation (fig. 4). Out of 4 patients who were amputated upon the first hours of hospitalization, the lower limb was amputated in 3 cases and the upper limb was amputated in only one case.

Of the 27 patients who underwent necrectomy and surgical wound debridement upon the first hours of hospitalization, 8 patients lost their sensory function and pulse in the distal part of the limb during the following hours, whereas the other 6 patients had an increased limb circumference due to massive edema. Thus, 14 out of 27 cases required additional decompression incisions in the underlying skin and fascia.

Finally, of the 27 patients categorized as delayed emergency cases, a decision on segment amputation was made in 13 cases.

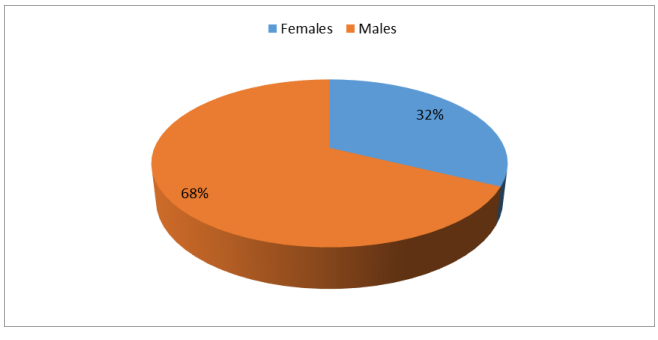

Fig. 1. Gender distribution of patients

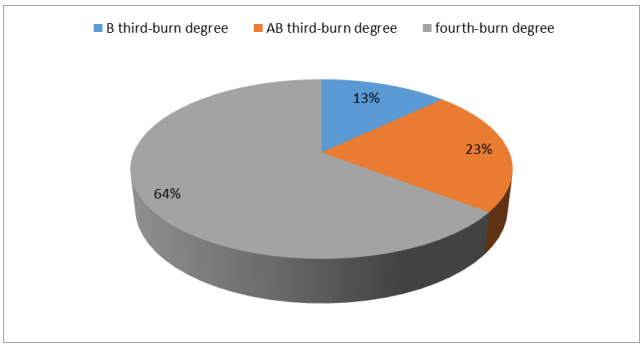

Fig. 2. Distribution of patients by burn degree

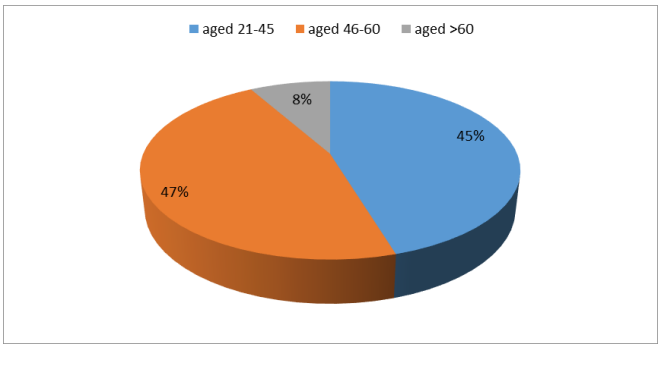

Fig. 3. Distribution of patients by age groups

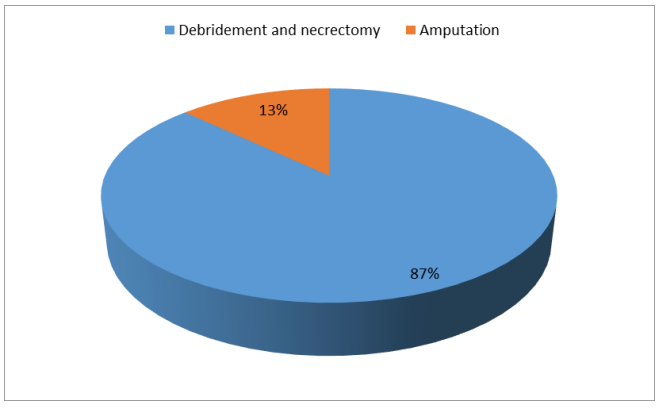

Fig. 4. Distribution of patients by type of intervention performed upon the first hours of admission

Out of 13 patients who managed to save the limb affected by the electric shock, a deficiency of optimal wound coverage was reported in 9 cases, therefore epithelization was stimulated via skin and flap autografts.

The average length of hospital stay was 33.7 days.

As regarding the antibiotic therapy, two or more associated antibiotics were administered in all 31 cases. The primary purpose was the administration of cephalosporins combined with fluoroquinolones (levofloxacin) or beta-lactam penicillin group (amoxicillin). To cover the group of anaerobic bacteria, Metronidazole was administered in all cases.

Complications associated with electrical injuries were grouped into the following categories: acute renal failure – 5 cases; atrial fibrillation – 3 cases; atrioventricular block – 2 cases; pneumonia – 2 cases.

Discussion

Treatment of electrical injuries is difficult, complex and should be tailored according to the particularities of the patient. The effectiveness of early decompression incisions, necrectomies and surgical debridement has been proven, thus ensuring the removal of necrotic detritus, relieving skin tension, improving blood flow, including peripheral blood circulation and eliminating edema. However, the procedures were not adequate for all cases, thus requiring additional decompression incisions. For this reason, it was concluded that a longer time of vascular compromise might lead to greater incidence of deep subfascial edema, similar to crush syndrome. Along with decompression incisions, deep exploration incisions are also important for assessing the condition of neurovascular elements, bones and muscles.

Regardless of the voltage that caused the electric burn (low or high), patients should be treated in the same way as for high voltage injuries as they may manifest as loss of consciousness, compartment syndrome, myoglobinuria and hemoglobinuria [7].

In 4 cases, when emergency amputation was performed, the patients showed signs of limb carbonization even upon admission to medical institution, therefore, appropriate interventions were performed to avoid life-threatening risks. The "delayed emergency" cases resulted in amputations due to the patient's unfavorable clinical development, as well as being associated with septic complications and progression to wet gangrene of the corresponding limb.

Electrical injuries are the result of preventable accidents. The transformation of electricity into thermal energy through the direct effect in association with blunt mechanical trauma can lead to tissue destruction and organ dysfunction, thus requiring trauma management and thorough clinical management, which will establish the stages of surgical treatment in several stages (fasciotomy, skin graft, long-term supportive care) [8].

The optimal wound coverage is another key point in the treatment of electrical injuries, since it should be performed as early as possible to prevent superinfection of the wound accompanied by secondary necrosis.

Conclusions

1. Electrical injuries are serious emergencies that can lead to patient's death unless proper medical or surgical management is provided.

2. The patient suffering from an electric shock should be treated comprehensively and by a multidisciplinary team, in order to prevent life-threatening conditions due to both direct consequences of electrical injury and commonly associated general complications.

3. The surgical intervention is the treatment of choice, however if patients present all the indications for segment amputation, it should be inevitably performed so as not to endanger the patient's life.

4. Treatment of a patient with electrical injury continues despite the correction of the underlying problem. It continues during the rehabilitation period and involves numerous reconstructive operations aimed at restoring the damaged structures and prosthesis of amputation abutments, as well as improving the aesthetic appearance.

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AT conceptualized the project, designed the research, interpreted the data and wrote the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the author's initiative. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 2 of 23.10.2017).

Conflict of Interests
No competing interests were disclosed.

DOI: https://doi.org/10.52418/moldovan-med-j.64-5.21.08
UDC: 618.14-007.66:614.21(478)

The incidence of uterine abnormalities

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Manuscript received October 15, 2021; revised manuscript November 15, 2021; published online November 26, 2021

Abstract

Background: Congenital uterine abnormalities result from abnormal formation, fusion or resorption of the Mullerian ducts during fetal life. These abnormalities have been associated with an increased rate of miscarriage, preterm birth, and other fetal adverse outcomes.
Material and methods: Was performed a clinical observational retrospective study of uterine malformations, diagnosed in the Republic of Moldova. Pelvic MRI (1.5-3 tesla) with contrast and without were examined, from 01.01.2016 to 20.11.2016. During this time, 190 MRIs were performed according to the program, 167 MRIs were included in the study, 23 MRIs were excluded, having total or partial hysterectomy performed. The age of the examined persons is between 81 years and 6 months.
Results: 15 uterine malformations were detected, which represent 11.13%. Among the uterine abnormalities were detected 6 cases of bicorn uterus, 3 cases of didelph uterus, 2 cases of uterine agenesis and septate uterus and one case of arcuate uterus, and unicorn.
Conclusions: In this study it was determined that the prevalence of uterine malformations in an unselected population in the Republic of Moldova is 11.13%, and that of the septate uterus is 1.2%, data that are similar and correspond to the international literature.
Key words: septate uterus, uterine malformation, Mullerian duct.

Cite this article
Toncoglaz C, Toncoglaz S, Toncoglaz A. The incidence of uterine abnormalities. *Mold Med J.* 2021;64(5):42-46. https://doi.org/10.52418/moldovan-med-j.64-5.21.08.

Introduction

The uterus is responsible for many of the most crucial steps in the process of reproduction. Sperm migration, embryo implantation, fetal nourishment, development and growth, and finally, the process of labor and birth are all reliant on the existence of a structurally normal and functionally competent uterus [1]. Malformations of female genital organs account for 4% of all congenital malformations. They are detected in 3.2% of women of reproductive age [2]. Malformations of the genitourinary system occupy the 4th place (9.7%) in the structure of all developmental anomalies of modern humans [3]. Congenital uterine abnormalities are a heterogeneous group of uterine configurations that may adversely affect reproductive potential. Although subtle variations can occur, the more common abnormalities fall into two broad categories of unilateral development or failure of midline fusion. These abnormalities have been well described for over a century although the mechanisms of their unfavorable impact on fertility and clinical management have not been systematically studied. Although exact aetiologies remain unclear, the association of congenital uterine abnormalities and anatomical abnormalities in other systems of mesodermal origin suggest a possible shared cause. The proposed explanations for these organizational defects have ranged from teratogenic injury to heredity. Neither has been conclusively proven [4]. Theoretically, teratogenic interference by any agent during the developmental period of 6–10 weeks of gestation could influence any of the mesodermal systems, although convincing evidence for a well-defined teratogen is lacking. Uterine abnormalities may be familiar and be associated with defects in other mesodermal-derived systems and may be part of a more complex genetic syndrome [5]. These systems include renal, alimentary, cardiac, skeletal and auditory abnormalities. Although none have been identified, this genetic hypothesis has appeal in providing an explanation to the multiple system abnormalities across several mesodermal derivatives. The appearance of these abnormalities within several families has given credibility to a genetic basis although no specific gene defect has been identified. The exact etiologies remain undefined despite attractive hypotheses. In 1962, Pendleton Tompkins suggested that these abnormalities be viewed on a spectrum from clearly normal to clearly abnormal [6]. He suggested that the case for or against intervention rested on subtle distinctions. No debate can be mustered, for example, when there is no question that a cavity is at either end of the normal–abnormal spectrum. However, debate exists over the management of the configurations in between. Subjective standards continue to be used to differentiate normal from abnormal: what may be septate

to one examiner may be arcuate to another. To address the need for standardization and provide a basis for reliable, one-to-one comparison of outcomes, several classification schemes have been proposed since Jarcho J. suggested one of the more specific systems in 1946 [7, 8]. These classifications are intended to provide a means of assessing the likely impact of a specific abnormality on reproductive outcome and the basis for deciding when and which intervention would improve outcome. Variable definitions have evolved from broadly based and nearly useless terms such as 'double uterus' or 'duplicated uterus' to more precise definitions included in the various classification schemes. For purposes of this communication, the term 'unicornuate uterus' refers to unilateral uterine development with unilateral tube and ovary. There may be an associated uterine remnant (or rudimentary horn) with variable tubal and ovarian development and location. The term 'septate uterus' refers to a division of the uterine cavity by a midline septum that variably penetrates the cavity from one to two centimeters, resulting in partial division to the entire length of the cavity or resulting in complete division and two separate cavities. The external serosal surface of the septate uterus is normal in configuration [9]. The term 'bicornuate uterus' refers to division of the uterine cavity into two separate cavities and an associated cleft in the midline fundus, resulting in two anatomically distinct structures. There are two terms used in this setting: 'bicornuate bicollis', in which the two cavities are separate divided external uterine structures that persist throughout and are associated with two cervices with a possible longitudinal vaginal septum, and 'bicornuate unicollis', which refers to a divided uterus with one cervix and persistence of the internal division through the entire cavity up to the endocervical canal (complete) or through only part of the cavity (partial). A longitudinal vaginal septum may be associated with these abnormalities and depending on clinical scenario intervention may be required, but the first attempt to classify female congenital anomalies goes back to the beginning of the 20th century; Strassmann described septate and bicornuate uterus and some subgroups of the disorders in 1907 [10]. However, the first classification system for categorization of congenital uterine malformations was that of the American Fertility Society (AFS) published in 1988, mostly based on the previous work of Buttram V.C. and Gibbons W.F. [8, 11]. Almost 15 years later, Acien P. et al. [4] proposed another option for the classification of congenital female malformations using the embryological origin as the basis of the system. A newer version of this classification has been published recently [12]. Furthermore, Oppelt P. et al. [13] published a very detailed classification system based on the Tumor Nodes Metastases (TNM) principle in oncology and known as vagina, cervix, uterus, adnexae and associated malformations (VCUAM) classification system. It is also interesting that, apart from these alternatives for the classification of the female genital malformations in general, some other subdivisions for certain categories of anomalies have been published. AFS classification received wide acceptance and it is still the most broadly used system [14].

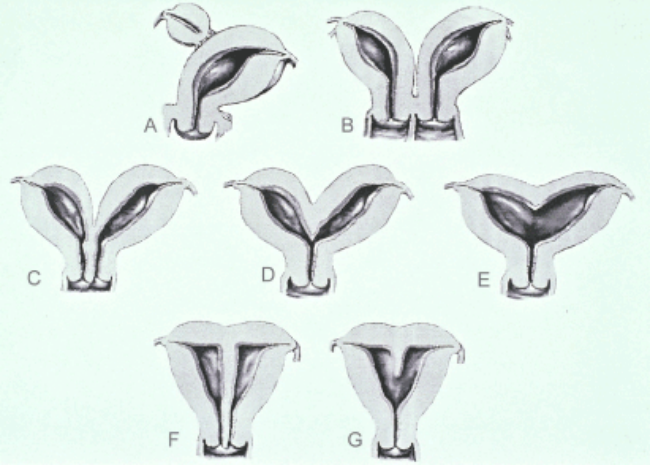

Fig. 1. Main groups of uterine anomalies.

A – Unicornuate uterus with rudimentary horn, B – Didelphic uterus, C – Complete bicornuate uterus, D – Partial bicornuate uterus, E – Arcuate uterus, F – Septate uterus, G – Subseptate uterus. Uterine agenesis and diethylstilbestrol – related T-shaped uterus are not presented [15].

Septate uterus is one of a variation of uterine abnormalities that have as their commonality a failure of midline fusion. The incidence is 5–35% in the infertile population (excluding the milder and normal variant of arcuate uterus). The identification of a septate uterus as contributing to poor reproductive outcome dates to the turn of the 20th century, with various practitioners identifying uterine duplication as contributing to first-trimester pregnancy loss. According to the recent ESHRE-ESGE classification, a septate uterus is defined as a congenital uterine anomaly with an abnormal resorption of the midline septum, a normal outline of the uterus and an internal indentation at the fundal midline exceeding 50% of the uterine wall thickness, regardless of the size of the septum [16]. Women with a septate uterus are at increased risk of miscarriage, preterm delivery, and fetal malpresentation and have lower clinical pregnancy rates [17]. In women with a septate uterus, resection of the septum is performed as standard worldwide. Observational studies suggest that surgery improves reproductive outcome in women with a septate uterus, but this has yet to be substantiated by randomised controlled trials that are currently underway [18, 19]. The rationale behind septum resection assumes that the septum is composed of an entirely different structure than the normal uterine wall [20, 21]. Therefore, implantation in the septum would hypothetically lead to poorer reproductive outcome in comparison to embryos that implanted in the lateral uterine wall. The foundation for this theory is meagre as knowledge about the pathophysiology of the intrauterine septum and its possible relation to impaired reproductive outcomes in women with a septate uterus is limited [22].

The unicornuate uterus is unique among Müllerian abnormalities in that the influence for abnormal development is unilateral (fig. 1) [12, 17]. The unicornuate uterus is the rarest of the uterine abnormalities accounting for 0.3–4%

of the uterine abnormalities. This broad range is due to the varying clinical presentations and symptoms leading to evaluation. Asymptomatic patients are frequently undetected. One series of asymptomatic patients studied hysteroscopically for nonreproductive loss describes an incidence of 0.3%. The incidence increases to 3.5–4% with increasingly significant history. The unicornuate uterus and rudimentary uterine horn are frequently detected as part of an evaluation for infertility and repeated pregnancy loss or the assessment of chronic and recurrent pelvic pain when an obstructive uterine horn is present [23].

Rudimentary uterine horn. The presence of a rudimentary uterine horn does not influence pregnancy outcome when the pregnancy is in the unaffected hemiunicornuate uterus [24]. Management of the uterine horn depends on the presence or absence of functioning endometrium within the horn [25]. Conventional teaching held that a rudimentary horn regardless of functionality should be removed because of an adverse influence on pregnancy outcomes when the pregnancy occurred in the normal hemiuterus. Contemporary management is more selective and based on better, outcome-related data. Excision of the uterine horn without a functioning endometrium attached to the unicornuate uterus merely to enhance obstetric outcomes is not warranted. When no functioning endometrium is demonstrated, no intervention is warranted. When functioning endometrium is present, excision should be considered particularly if there is a haematometra. The uterine horn may end bluntly or taper and end in an atretic cervical stump without a functional endocervical canal. These obstructions may also be part of a more complex and broader spectrum of anatomical changes [26].

Bicornuate uterus. A word-regarding nomenclature describing this configuration is warranted. There are three variations in this configuration and include one or two cervices (bicornuate unicollis and bicornuate bicollis, respectively) and a variable degree of cavity division (partial or complete) associated with the external configuration of two distinct structures. The term 'didelphys' is sometimes used as a generic term and refers literally to two horns. Precise nomenclature regarding this classification of uterine malformations should include a designation regarding the status of the cervix [27]. The degree of failed unification can be quite variable when there is partial fusion of the uterine horns and persistence of a cleft or division along the external contour of the uterus (bicornuate, or literally two horns). This separation may be restricted to the uterus with a single cervix (bicornuate unicollis) or extend throughout the uterus, cervix and vagina (bicornuate bicollis). These distinctions are recognized in most classification schemes and are subtle but important. The incidence of bicornuate uterine abnormalities in all categories ranges from 5 to 30%, depending on history, intensity of evaluation and imaging technique used [28].

Material and methods

Was performed a clinical observational retrospective study of uterine malformations, diagnosed in the Republic of Moldova. The examination center was selected based on high access to medical data at a German Medical Center in Chisinau. Inclusion mandatory criterion was feminine gender; exclusion criterion was a person with surgical intervention on pelvic floor. Medical data of all patients with pelvic MRI (1.5-3 tesla) with contrast and without were retrospectively reviewed, from 01.01.2016 to 20.12.2016. The MRI is also associated with a high sensitivity and specificity. During this time, 190 MRIs were performed according to the program, 167 MRIs were included in the study, 23 MRIs were excluded, having total or partial hysterectomy performed. Data were collected from the Clinic of internal health informational system. The incidence of uterine malformations per 100000 of population was calculated, population data were extracted from the national bureau of statistics (the Republic of Moldova). In 2016 the national statistic bureau registered 1465175 females.

Results

The age of the examined persons in this study varies between 6 months and 81 years, with a median age of 32 years. From total of 167 investigated people 15 uterine malformations were detected, which represent 11.13%. Among the uterine abnormalities were detected 2 cases of septate uterus and uterine agenesis, 6 cases of bicorn uterus, 3 cases of didelph uterus and one case of arcuate uterus, and unicorn.

The incidence of uterine malformations is 1.02 cases per 100000 population. Among disaggregated data of uterine malformations, the highest incidence was bicorn uterus with an incidence of 0.41 cases per 100000 of population. The lowest incidence was calculated for arcuate and unicorn uterus with an incidence of 0.07 cases per 100000 of population (tab. 1, fig. 2-8).

Table 1. Incidence of uterine malformation

Uterine malformations	% from total studied population	Absolute	Incidence per 100000 population
Arcuate uterus	0.60	1	0.07
Unicorn uterus	0.60	1	0.07
Septate uterus	1.20	2	0.14
Uterine agenesis	1.20	2	0.14
Didelph uterus	1.80	3	0.20
Bicorn uterus	3.59	6	0.41

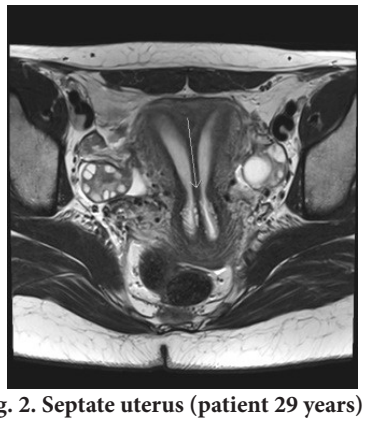

Fig. 2. Septate uterus (patient 29 years)

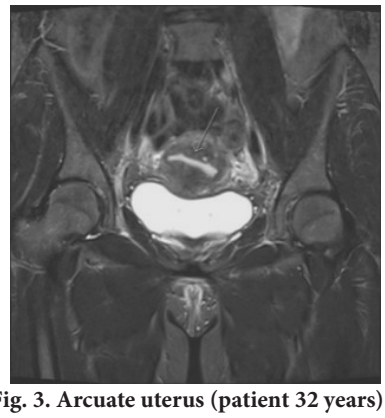

Fig. 3. Arcuate uterus (patient 32 years)

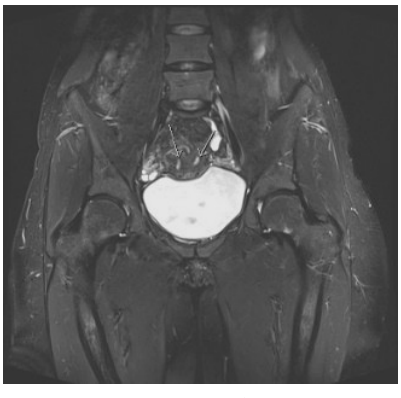

Fig. 4. Didelph uterus (patient 38 years)

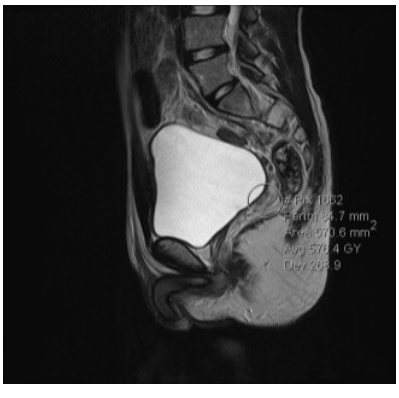

Fig. 5. Uterine agenesis (patient 1 year)

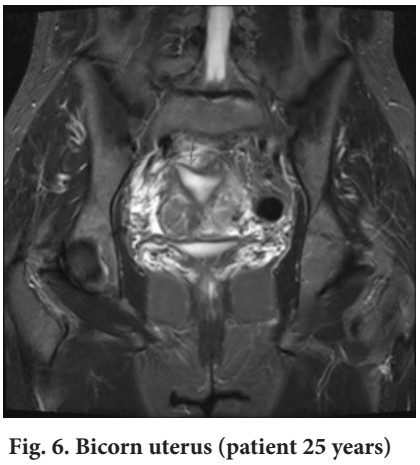

Fig. 6. Bicorn uterus (patient 25 years)

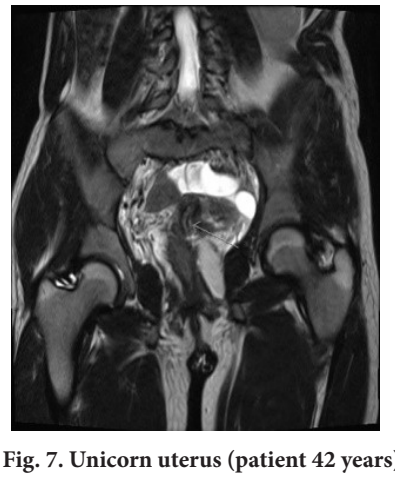

Fig. 7. Unicorn uterus (patient 42 years)

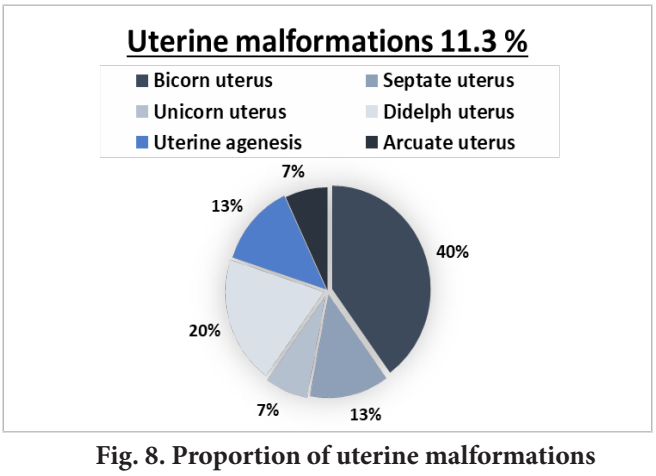

Fig. 8. Proportion of uterine malformations distributed by type

Conclusions

The incidence of septate uterus is 0.14 cases per 100 thousand of population comparing with the cumulative incidence of 1.02 cases per 100 thousand of population of uterine malformations in the Republic of Moldova. It was determined that the proportion of uterine malformations in an unselected population in the Republic of Moldova is 11.13%, and that of the septate uterus is 1.2% from total investigated persons, data that are similar and correspond to the international literature. MRI seems to be a very useful diagnostic tool because it can provide detailed information about the anatomical condition of the female genital tract.

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Authors' contributions

CT designed the study, conducted the laboratory work; ST drafted the first manuscript; AT interpreted the data and critically revised the manuscript. All the authors reviewed the material and approved the final version of the manuscript.

Funding

This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The review study was the authors' initiative.

Ethics approval and consent to participate

Agreement was obtained from the German Diagnostic Center and from patients for the use of the data.

Conflict of Interests

The authors declare no competing interests.

https://doi.org/10.52418/moldovan-med-j.64-5.21.09
UDC: 616.233-003.6-07-053.2

Clinical and imaging interrelationships in the diagnosis of foreign body aspiration in children

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Manuscript received October 18, 2021; revised manuscript November 16, 2021; published online November 26, 2021

Abstract

Background: Foreign body aspiration (FBA) is a typical occurrence in children. The clinical signs are influenced by various causes, and the differential diagnosis is important, especially when the suffocation crisis is not recognized. The aim of this study was to evaluate the clinical and imaging symptoms in children with FBA.
Material and methods: A retrospective study is provided of 156 children who were hospitalized and examined (clinical and paraclinical tests) in the Pneumology Clinic between 2011 and 2020 after having a foreign body removed from their airways, using rigid tube bronchoscopy or fibrobronchoscopy.
Results: The most affected age group was 1-3 years, which constituted 77.6% (95% CI 70.2% -83.8%). The most common symptoms were: cough – 98.7% (95% CI 95.4%-99.8%), dyspnoea – 94.2% (95% CI 89.3%-97.3%), wheezing – 61.5% (95% CI 53.4%-69.2%). Chest radiography was relevant for foreign body aspirations in 55.8% of cases (95% CI 47.6%-63.7%). The foreign body was extracted from the right bronchus in 32.1%, from the left bronchus in 21.8% of cases, from the lobar / segmental bronchi – 22.5%, and in 21.2% – multiple locations. The etiological structure of the endobronchial foreign body was dominated by the organic ones – 96.8%.
Conclusions: Cough, dyspnoea, and wheezing are suggestive of this pediatric emergency. Chest radiography provides diagnostic information only for every second child.
Key words: foreign body, children, bronchoscopy.

Cite this article
Rotaru-Cojocari D, Rascov V, Selevestru R, Sciuca S. Clinical and imaging interrelationships in the diagnosis of foreign body aspiration in children. *Mold Med J.* 2021;64(5):47-50. https://doi.org/10.52418/moldovan-med-j.64-5.21.09.

Introduction

Foreign body aspiration is a common pathology in the pediatric population, with various forms of presentation ranging from acute symptoms accompanied by life-threatening respiratory failure due to total airway obstruction to mild recurrent respiratory manifestations, with exacerbations that alternate with periods of apparent health [1]. It is critical in both situations to make a differential diagnosis of airway blockage, which might be caused by a foreign body aspiration, infections, or an allergic bronchopulmonary disease [2, 3].

The clinical picture of foreign body aspiration events in the airways is determined by several parameters, including the degree of installation in the respiratory system, the type and pathogenic mechanism of the blockage, the nature of the foreign body, and the time between diagnosis and treatment [4-7]. Fixation of the foreign body to the tracheal bifurcation results in abrupt death in the middle of severe suffocation or cardiorespiratory arrest. Furthermore, the progression of this pediatric emergency differs depending on whether the bronchi are partially or completely blocked, or if the valve mechanism is used. Organic foreign bodies create greater problems since they are non-contrasting to chest radiography most of the time, undergo putrefaction processes over time, and are difficult to remove using bronchoscopic methods. Sharp foreign bodies, particularly metal foreign bodies formed of hard plastic, can cause mechanical damage to the bronchial shaft, increasing the likelihood of developing catarrhal-purulent endobronchitis and granulations, and in some circumstances can breach the bronchial wall.

With a sensitivity of 96% and a specificity of 76%, a suffocation episode is one of the most reliable clinical indicators of foreign body aspiration [8, 9]. However, only a tiny number of patients exhibit the typical triad, which includes suffocation, coughing, and wheezing, and the penetration event frequently goes undetected or ignored, complicating the diagnosis of foreign body aspiration [6, 10, 11]. In the absence of particular therapy, such as bronchoscopy and foreign body extraction, it encourages the development of complications, some of which are followed by persistent broncho-pulmonary sequelae, bronchopulmonary processes with chronic characteristics [12-16].

Table 1. General and clinical characteristics of pediatric patients with foreign body aspiration

Age group	No of cases	%	95% CI	Gender	No of cases	%	95% CI
<1 year	20	12.8	8.0-19.1	Male	89	57.1	48.9-64.9
1-3 years	121	77.6	70.2-83.8	Female	67	42.9	35.1-55.1
>3 years	15	9.6	5.5-15.4				
The main symptoms							
Symptom	**No of cases**	**%**	**95% CI**	**Symptom**	**No of cases**	**%**	95% CI
Stridor	9	5.8	2.7-10.7	Cough	154	98.7	95.4-99.8
Fever	64	41.0	33.2-49.2	Wheezing	96	61.5	53.4-69.2
Perioral cyanosis	59	37.8	30.2-45.9	Dyspnoea	147	94.2	89.3-97.3

Material and methods

The study group included 156 children hospitalized in the Pneumology Clinic of Institute of Mother and Child, in a period of 10 years (2011-2020). The investigation was conducted retrospectively and was based on the review of patient observation records. A total of 66 patients were targeted with suspicion for FBA, and the diagnosis was suspected and confirmed in 90 patients while they were in the hospital. Age, gender, the reason for hospitalization, time from aspiration to diagnosis, clinical symptoms, and radioimaging characteristics were all tracked for all patients. Endobronchial foreign body was diagnosed using rigid tube bronchoscopy (Karl Sorz 3.5–6.0mm) or fibrobronchoscopy (BF3C160, BF1TQ170 Olympus 3.8-6.0mm), depending on the case.

Results

The examination by age groups in the study group revealed a net prevalence of children aged 1-3 years – 121 cases (77.6%), and babies in 20 cases (12.8%). Aspiration of foreign bodies in the respiratory system had only a modest, statistically insignificant male predominance: 89 cases in boys (57.1%) and 67 instances in girls (42.9%) (tab. 1). In terms of location, 68.58% of patients (107 children) are from country areas, whereas 31.41% (49 patients) are from city areas.

There was a varying time delay for each case, varying from one hour to 6 months, between the time the foreign body was aspirated and the request for a medical consultation. Only 66.0% of cases (103 children) had the penetration syndrome recognized anamnestically, but only one-third of these patients presented to the hospital during the first three days of the aspiration incident. In these cases, the diagnosis was simple and quick to confirm, and the treatment entailed the removal of foreign bodies from the bronchial tree as soon as possible.

The most common symptoms seen in the clinical presentation of foreign body aspiration cases were: cough, which was present in 154 instances (98.7%), dyspnoea of different severity verified in 147 children (94.2%), and wheezing in 96 cases (61.5%) (tab. 1). Bronchopulmonary physical examination revealed: decreased vesicular resonance in 75 cases (48.07%), wet rales in 88 cases (56.41%), wheezing rales in 111 cases (71.15%), and no stetoacoustic changes in 4 cases (2.56%).

The radiological examination was performed in all patients, but no radiopaque foreign materials were detected, and the radiography in 13 cases did not demonstrate pathological changes, with a normal image. In the majority of instances (55.8 % (95% CI 47.6%-63.7%)), indirect radiological signals were indicating the presence of a foreign body in the bronchial tree, such as atelectasis in 25% of cases, localized emphysema in 48% of cases, mediastinal deviation in 19.2% (tab. 2). One of the most dangerous situations is an obstruction through the valve mechanism, which causes localized hyperinflation with diaphragmatic flattening and mediastinum displacement to a healthy hemithorax with significant risks of pneumothorax (fig. 1, 2).

Table 2. Pulmonary radiological changes in children with foreign body aspiration

Chest radiography	No of cases	%	95% CI
Atelectasis	39	25.0	18.4-32.6
Mediastinal deviation	30	19.2	13.4-26.3
Localized emphysema	75	48.1	40.0-56.2
Pneumonic opacities	120	76.9	69.5-83.3
Normal aspect	13	8.3	4.5-13.8

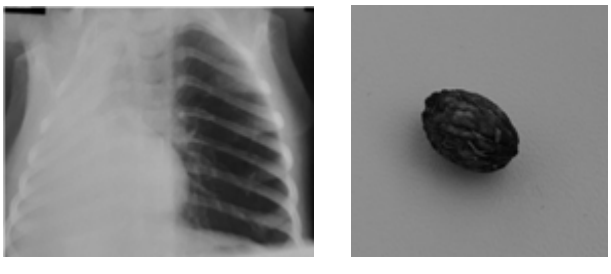

Fig. 1. Valve mechanism with left lung hyperinflation, significant deviation of the mediastinum to the right, and flattening of the left hemidiaphragm in olive kernel aspiration, located in the left main bronchus

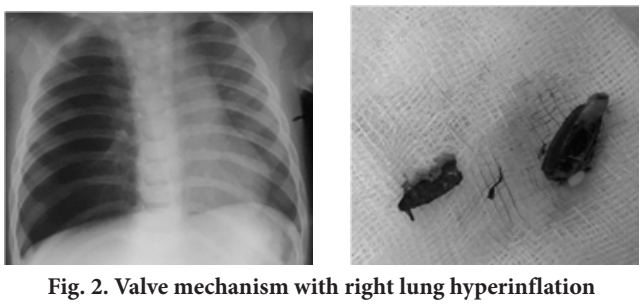

Fig. 2. Valve mechanism with right lung hyperinflation and mediastinum deviation to the left after sunflower seed aspiration, positioned in the right main bronchus

In the study the following foreign bodies were localized in the respiratory tree: trachea – 2 cases (1.3%), main right bronchus – 50 cases (32.1%), left main bronchus – 34 cases

(21.8%), lobar/segmental bronchi – 35 cases (22.5%), and multiple locations – 33 cases (21.2%), as confirmed by bronchoscopic examination.

The nature of foreign bodies was very diverse, the organic ones predominating – 96.8% (fig. 3). Foreign body was removed during the first bronchoscopy in 94.87% of patients. The remaining foreign body fragments were removed from the bronchial tree in 14.74% of cases at the endoscopic reassessment after 3-5 days. The most common complications were infectious, these patients subsequently requiring antibiotic therapy.

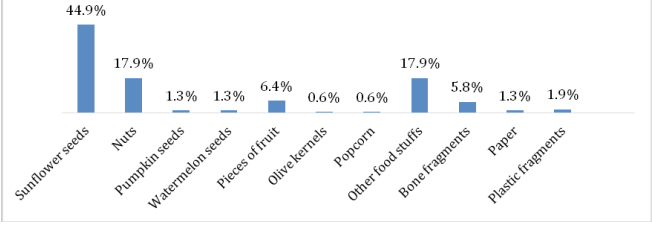

Fig. 3. The etiological structure of foreign bodies extracted from the bronchial tree in children

Discussion

The 156 cases reported over ten years indicate that this occurrence is relatively common among children. The maximum incidence aligned to information in the literature, which stated that approximately 80% of pediatric episodes of FBA occur in children under the age of three, with a maximum incidence between the ages of one and two years [17-21]. There is a variety of reasons that support aspiration, most of which are tied to this age, as youngsters are especially vulnerable at this period due to increased activity and interest, as well as reduced parental monitoring as they grow up. Additionally, young children have a high level of activity and distraction while eating and tend to explore the world with their mouths while playing. Anatomical and morphological characteristics are also important. The lack of molars increases the danger of foreign body aspiration. Because airway resistance is inversely related to the cross-sectional radius, children's airways have a stronger proclivity to impede airflow, even though small foreign bodies [6].

The gender difference was statistically insignificant, with boys having a little predominance. However, studies from the literature reveal a net frequency in males of almost 2:1 [18, 20, 21]. Tomaske M. et al. on a group of 370 children with an aspiration of foreign bodies found 242 cases (65.4%) in males [22]. Another recent study showed that 67.9% of the 316 children with foreign bodies in their airways were boys [19]. In a larger group analysis (1027 patients with foreign body aspiration), 626 boys and 401 girls were found to have them [23, 24].

When patients present to the emergency department with a clear history of foreign body aspiration, the diagnosis is usually clear. Difficult situations are represented by those cases in which the moment of aspiration goes unnoticed by parents or supervisors, and the child begins to develop complications (usually trenant pneumonia). When present in the anamnesis, penetration syndrome is highly suggestive of a diagnosis. In the studied group it was found in 103 cases (66.0%). Other authors report this syndrome in 77% of cases [25]. However, it is not always visible, which causes a delay in diagnosis. Cough, dyspnea, and wheezing were the most prevalent symptoms among the patients in the study. According to the study more than 1000 patients had cough (84.3%), dyspnea (13.1%), and cyanosis in 7.7% of cases [24].

When there is even the slightest suspicion of foreign bodies in the airways, a radiological scan is required. Children in the study group had indirect indicators: atelectasis, mediastinal shift, and localized emphysema. The authors of the previously referenced study discovered emphysema in 707 patients (68.8%) and atelectasis in 137 patients (13.3%) [24]. Normal radiological result was found in 13 patients (8.3%). A normal chest X-ray cannot rule out the existence of a foreign body [26]. In the event of a strong, clinical, or anamnestic suspicion, it is required to repeat the radiograph and maybe do tomography, which can provide more information.

Both the study and the information in the literature show that foreign bodies are mostly found in the right bronchus. The higher frequency has anatomical explanations: the right bronchus is closer to the tracheal axis than the left bronchus, it has a larger caliber, the suction force of the right lung is greater, and the carina is to the left [12, 24].

The nature of the aspirated foreign body differs based on geographical area, environment, culture, and eating habits, with numerous researches indicating that biological foreign bodies are more common [27]. In the Republic of Moldova, it is also observed that the aspirations of vegetable foreign bodies predominate, namely sunflower seeds and nuts.

The main cause of late arrival in specialized services is misdiagnosis due to the multiple pathological entities with which foreign body aspiration can be confused, and extraction usually takes place after prior hospitalization in pediatric wards or after inefficient and inadequate outpatient treatments with diagnosis. The progression and prognosis of foreign body aspiration are dependent on the timing of diagnosis and therapeutic intervention, and so any suspicion based on anamnesis, clinical data, and imaging data should be followed by tracheobronchoscopic investigation.

Conclusions

1. Foreign body aspiration into the airways is rather prevalent in children, particularly those aged 1-3 years.
2. The presence of penetration syndrome, followed by cough, dyspnoea, and wheezing, is very suggestive for establishing the diagnosis.
3. At the smallest suspicion of foreign body aspiration a radiological study is required, which may be indicative of unilateral pulmonary hyperinflation or lobar/segmental atelectasis.

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DRC designed the research, reviewed statistics and interpreted the data, drafted the manuscript; VR collected the data, did statistical analysis; RS interpreted the data, revised the manuscript critically; SS conceptualized the project and designed the research, revised the manuscript critically.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 41 of 25.05.2021).

Conflict of Interests
No competing interests were disclosed.

https://doi.org/10.52418/moldovan-med-j.64-5.21.10
UDC: 613.6:371.12

Assessment of teachers' health in relation to working conditions

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Manuscript received October 25, 2021; revised manuscript November 16, 2021; published online November 25, 2021

Abstract

Background: Teaching in schools has been defined as the profession with the most diverse risk factors that affect health. With almost 48000 people currently working in educational institutions, teachers make up the largest occupational group in the Republic of Moldova.
Material and methods: The study involved 519 teachers from pre-university educational institutions. For the self-assessment of the health status, a questionnaire was created which was applied online. The subjective assessment was performed by researching the entries in the medical cards.
Results: The majority of respondents were women (91.4%), with a mean age of 45.6±7.5 years and a working experience of 22.9±3.2 years. In 75.7% of them health is satisfactory, only 3% have serious health problems and would require hospitalization. In 35.8% of cases, the presence of chronic diseases is registered, their top being led by diseases of the gastrointestinal tract (33.8%), the cardiovascular system (29.2%) and endocrine (27.2%). The most common risk factors mentioned were: high levels of stress (53.5%); increased number of tasks (23.4%); lack of support from family and colleagues (22.9%).
Conclusions: The health of teachers is influenced by a variety of risk factors. There were no correlations between the type of institution and the subject taught, which highlights the importance of studying the influence of factors in the occupational environment and the development of prevention measures.
Key words: teachers, health status, risk factors, preventive measures.

Cite this article
Cheptea D. Assessment of teachers' health in relation to working conditions. *Mold Med J.* 2021;64(5):51-55. https://doi.org/10.52418/moldovan-med-j.64-5.21.10.

Introduction

Human health is considered one of the most important tasks of the society of any state in the world. In this context, at the current stage of development, the problem of teacher's health is mentioned as a global problem [1, 2]. The health of a modern individual is influenced by a multitude of physical, chemical, biological, social factors, and reflects the integral system of material and spiritual relationships existing in society [3]. It largely depends on the quality of the environment, the level of development of society, working conditions, professional level, material condition, the organization of health care.

The teacher, in his professional and social role is a key figure in the educational process, a bearer of special knowledge, as well as a model of behavior and attitude towards health. Currently, the activities of teachers in the Republic of Moldova are not limited to teaching lessons in classrooms. In addition, they must prepare for lessons, evaluate students' exercises, carry out guidance activities, prepare for external school reviews, participate in continuous professional development, and meet the demands of management [4]. Addressing the issue of the influence on health of different categories of factors, it is necessary to address the aspects of the professional activity of teachers. As a result, teachers may suffer from mental and physical health problems due to the diversity of functions and extended overtime.

Even among young teachers there is a large percentage of patients with chronic diseases, and the leading one is cardiovascular pathology [1, 2, 5]. And only 9% of teachers are healthy, they maintain a relatively high level of efficiency and don't present a chronic disease. Most of them have "iron nerves" by nature, or they have learned to quickly recover mentally after a shift of home rest [5].

Consequently, the profession of a teacher comes first in terms of work intensity and the presence of harmful factors in the workplace [5]. The main harmful factors of pedagogical activity are: physical, emotional overstrain, a high degree of tension of the sensory load on the organs of sight and hearing, a heavy load on the laryngeal vocal apparatus, a high density of epidemic contacts, and the effect of electromagnetic waves [6].

Another risk factor that has a major action on health is the rapid change of modern lifestyle which in recent times is associated with increasing requirements to have new skills, the need to adapt to new types of work as examples of online teaching, the pressure of higher productivity and quality of work, the pressure of time indicating the need for priority action in improving the psychosocial environment of jobs [7].

Epidemiological, clinical and experimental studies show that high levels of acute or chronic stress adversely affect the functions of the human body. Stressors have a significant impact on the physical and emotional well-being of teachers. There is a strong relationship between stress and

several inflammatory, metabolic, reproductive, autoimmune disorders. It is well known that all the biological systems of the human body are affected by stress [8]. The main chronic diseases of our time appear against the background of emotional experiences, acute or chronic stress. According to the literature, up to 70% of diseases are associated with emotional stress. More than a million people die in Europe each year due to influence of stress on cardiovascular system [8, 9].

The aim: to assess the teachers' status of health working in general primary and secondary educational institutions.

Material and methods

In order to achieve the proposed goal, a descriptive cross-sectional study was carried out, with some prospective elements. The initial stage consisted of the elaboration of a structured questionnaire that was applied to teachers working both physically and online during the COVID-19 pandemic. The questionnaire included 14 chapters, such as: general data, working and living conditions, eating behavior, subjective assessment of health and objective assessment of changes caused by online teaching. This questionnaire contains a total of 159 questions, of which 96 are required to determine the demographic grade and general clinical condition of each person (general health, living and working environment, family environment). The remaining questions are grouped as follows: 37 refer to teachers' nutrition and 24 were organized in the form of a psychological questionnaire in which respondents rated statements with grades from 0 to 5. The questionnaire includes closed questions (yes/no type), as well as questions with a variant or variants with multiple answers.

The questionnaire was applied to teachers who meet the following participation criteria: teachers from general primary and secondary education institutions who signed the informed consent and agreed to complete the questionnaire; people of any gender (men, women); people from urban and rural areas without ethnic restrictions; people up to 63 years old.

Study variables

The influence of occupational environmental factors on health such as occupational stress and burnout was analyzed as follows.

Assessment of psychological burnout

The Maslach Burnout Inventory (MBI) was used to assess the level of burnout. The instrument measures three constructs: Emotional Exhaustion (EE) (9 items), Depersonalization (DP) (5 items) and Lack of Personal Accomplishments (8 items). Thus, a high level of burnout involves subjects getting high scores on exhaustion subscales (e.g., I feel exhausted from work, I feel like I am run out of power), and depersonalization (e.g., I do not really care about what happens to some of my students).

Assessment of occupational stress

To assess the level of occupational stress, the "Impact of Event Scale – Revised (IES-R)" tool was applied, which is a short self-report questionnaire that is easy to administer, it has 22 questions. The tool, an appropriate instrument to measure the subjective response to a specific traumatic event in the older adult population, especially in the response sets of intrusion (intrusive thoughts, nightmares, intrusive feelings and imagery, dissociative-like re-experiencing), avoidance (numbing of responsiveness, avoidance of feelings, situations, and ideas), and hyperarousal (anger, irritability, hypervigilance, difficulty in concentrating, heightened startle), as well as a total subjective stress IES-R score.

The collected data was entered in Microsoft Excel 2007 and statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 23. Qualitative variables, such as gender, socioeconomic status and morbidity profile were summarized as frequencies and percentages. Quantitative variables, such as age and hours of work were expressed as mean and standard deviation.

Results

Of the 750 teachers included in the study, 519 people completed the questionnaire, which represents a response rate of 69.2%. The sample consisted of teachers, department heads and principals from a single school board, with a mean age of 45.6 ± 7.5 years and a working experience of 22.9 ± 3.2 years. The selection of teachers was carried out randomly so as to cover the entire territory of the Republic of Moldova. Table 1 depicts the distribution of baseline sociodemographic characteristics and occupational characteristics among the sample.

Table 1. General characteristics of study subjects (N=519)

Variables	Frequency (n)	Percentage (%)
Sociodemographic characteristics		
Age:		
>25 years	9	1.7
25-30 years	34	6.6
30-35 years	71	13.7
35-40 years	91	17.6
40-45 years	81	15.6
45-50 years	112	21.4
50-55 years	58	11.2
55-60 years	36	6.9
<60 years	27	5.2
Gender		
Male	475	91.5
Female	44	8.5
Marital status		
Currently married	442	85.2
Single	29	5.6
Widowed	20	3.9
Divorced	28	5.4
Educational status of participants		5.0
Undergraduate	26	82.1
Postgraduate	426	12.9
Others	67	

Socioeconomic status (Modified BG Prasad classification) [10]		
Upper class	56	10.8
Upper middle class	421	81.1
Middle class	35	6.7
Lower middle class	7	1.4
Occupational characteristics		
Teaching experience (years)		
>5	48	9.3
5-10	66	12.7
10-15	79	15.3
15-20	80	15.4
20-25	73	14.1
25-30	75	14.5
30-35	60	11.6
>35	38	7.1
Classes taught		
Primary grade	24	4.6
Middle grade (gymnasium)	271	52.2
Higher secondary class	224	43.2
Number of workdays/weeks		
4	133	25.6
5	386	74.4
Academic hours of work/day (1 academic hour = 45 astronomical minutes)		
≤ 6	279	53.8
7 to 8	211	40.7
≥ 8	29	5.6
Commuting time to work		
10 min	245	47.2
30 min	222	42.8
60 min	40	7.7
>60 min	12	2.3
Type of transport to work		
Walking	346	66.6
Personal car	48	9.2
School bus	125	24.1
Hours spent in job-related work at home/ day		
≤ 2 hours	45	8.7
3 to 6 hours	387	74.6
> 6 hours	87	16.8

In order to identify the main risk factors in the occupational environment, the answers of the respective category were analyzed. With the help of the questionnaire we proceeded to the subjective evaluation of the conditions of the occupational environment. Of all the participants in the study, 197 people (38%) reported an orthostatic position (standing) throughout the work period, 20.6% – sitting, 41.4% – with class trips. Brightness level assessment more than 82.3% rated a sufficient level of light, and only 13.5% accused insufficient lighting levels. Unstable summer-winter temperature levels accounted for 10.8% of teachers. In order to have a more general overview, the answers to the statements about the physical environment were scored, the positive perception being assigned a score of «1» and the negative perception assigned a score «0», so that the higher the score, the perception of the physical environment at work is more positive. The average score was 6.25±1.82.

Study participants in 75.7% of cases report a satisfactory health status and only 3% have serious health problems and would need hospitalization. Unfortunately, a large number of teachers (7.5%) do not know their health status because they are not provided with quality primary health care. Analyzing the share of chronic diseases, it can be concluded that most teachers do not suffer from chronic diseases (52.2%). Chronically ill people (35.8%) usually get sick more in the winter-spring period (46.7%) and the autumn (38.8%). Again, it becomes a problem that 11.9% of teachers do not know whether or not they suffer from any chronic diseases, the reason being the lack of specialists in the region, the impossibility to go to a private clinic and the insurance of the rural medical system. Among the participants suffering from chronic diseases, 29.2% of them suffer from diseases of the gastrointestinal tract, 16.8% – the articular system, 29.4% – cardiovascular system, 33.6% – the endocrine system, 21.0% – respiratory system and 12.6% – nervous system. Table 2 shows the distribution of workplace-related morbidity experienced by the participants in the last year.

Table 2: Workplace-related morbidity profile of study subjects in the last year year (N=519)

Variables	Frequency (n)	Percentage (%)
Injury	29	5.58
Respiratory symptoms	477	91.9
Sleep disturbances	378	72.8
Eye strain	463	89.2
Voice strain	176	33.9
Headache	507	97.7
Ear pain or hearing disturbances	466	89.8
Musculoskeletal disorders:		
Ankle/ feet	117	22.5
Knees	59	11.4
Lower back	93	17.9
Neck	72	13.9

Analyzing the structure of morbidity among teachers it can be concluded that diseases of the gastric tract are predominant (33.8%) followed by diseases of the cardiovascular system (29.2%). This can be explained by analyzing the nutritional style as well as the nature of the work performed. Increased levels of stress due to poor nutrition are the main predictors of this structure. Allergic diseases also play a key role. Their appearance is usually due to the presence in the air of the occupational environment of a stable concentration of dust produced by wiping the classrooms.

For the subsequent characteristic of the influence of risk factors in the occupational environment and their action on teachers' health, a correlation analysis was used. To estimate the simultaneous comorbidity of different diseases at the individual level, the dichotomized responses were further combined into three ordered categories: 0 = subjects who report a low level of frequency of acute diseases or low level of chronic diseases (referents); 1 = subjects who report either a high frequency of acute diseases or a high frequency of chronic diseases (one of the 2 dimensions); 2 =

Table 3. Mean, standard deviations (SD), and latent correlation between all factors

Variable	Mean	SD	1	2	3	4	5	6	7	8	9
1. Gender	1.42	0.49	-								
2. Years of professional experience	22.9	3.20	0.23*	-							
3. Number of working hours	6.9	1.34	0.09*	-0.71**	-						
4. Number of working days	4.8	0.37	0.05*	-0.39*	-0.12	-					
5. Increased number of tasks	4.3	2.2	0.05**	0.37*	0.34	0.05	-				
6. Sudden change of teaching type (switching to online teaching only)	1.7	0.52	0.60	-0.74**	0.64*	0.39**	0.05*	-			
7. Occupational stress	11.9	4.99	0.015**	-0.05*	0.31*	0.03*	0.12*	0.77*	-		
8. Burnout (MBI score)	58.3	17.78	-0.18*	-0.11*	0.45*	0.29*	0.51*	0.61*	0.11**	-	
9. Health status	2.3	1.08	-0.22*	0.65**	0.12	0.31*	0.22	-0.07*	0.56*	0.71*	-

Note: *p<0.05, **p<0.001

subjects who report a high frequency of acute diseases and / or a high frequency of chronic diseases (all 2 dimensions). Table 3 demonstrates the average, standard deviation and correlation relationships between the main risk factors in the occupational environment and the incidence of various diseases in teachers.

Discussion

This study expands previous research literature on teachers' health status and the influence of occupational factors, such as emotional exhaustion by examining the direct and indirect relationships between health status and perceived stress and burnout in a sample of teachers. The basic reasoning was that health status can be improved by reducing perceived stress, which in turn would promote lower levels of teachers' exhaustion, regardless of gender, age, and level of teaching.

A series of empirical evidence has been accumulated proving that deficits in emotional abilities are directly related to increased incidences of both chronic and acute illnesses, due in large part to the influence of psychosocial factors and especially burnout [2, 9]. Consistent with these findings, this research provided evidence that health was directly correlated with both perceived sense of stress and burnout symptoms. Specifically, teachers who report being emotionally poor in their perception and management of emotions also tend to report that they feel more exhausted and more cynical about their work and the students they work with, less sense of personal fulfillment. This in turn tends to be a strong predictor of the installation of various disorders of the central nervous system, demonstrated in previous studies.

One explanation for these findings is that the skills of teaching professionals to effectively manage emotional challenges are characterized by more constructive thought patterns, and it is easier for teachers to catch and identify misjudgments and correct misinterpretations in the workplace. In addition, it is possible that teachers who are more emotionally competent would also feel that they have more control over their stressful environment because they can confer sense and manage their negative moods associated with stress more adaptively.

According to previous studies, it was established that about 70% of respondents reported deviations in their health. The most common pathological forms were: visual disorders – 68%; cardiovascular diseases – 48%; musculoskeletal disorders – 44% [11]. There is also a high incidence of polyorganic deficiency syndrome [12]. These pathologies are largely caused by prolonged exposure to stress, sedentary lifestyle and other social factors.

Proceeding from this, the professional activity of a teacher in modern conditions does not contribute to the preservation and strengthening of his health, as evidenced by the following data: 60% of teachers constantly experience psychological discomfort during work; 85% are in a constant state of stress: for 85% of women teachers, their activities are a factor that negatively affects family relations, as a result of which 1/3 of teachers have nervous system disorders [5]. Only 10.4% did not demonstrate health problems.

The workload of teachers at this stage includes many tasks, such as teaching students, preparing lessons, evaluating students' homework and extracurricular activities. As a result of these extended duties, school teachers may have physical and mental health problems. Musculoskeletal pain is the main reason for absenteeism and early retirement for teachers in Saudi Arabia [13].

The present study shows that most teachers have faced at least one physical health problem at work in the last year and most teachers have experienced moderate and high levels of stress due to the environment at work. Such results have been demonstrated by other authors, such as Geetha Mani in her study "Occupational health assessment of teachers in selected schools in Vellore district", Tamil Nadu: "Role of workplace environment in health" [7]. Also, in the same study it was highlighted that occupational stress is identified

among the main factors predisposing to the occurrence of musculoskeletal disorders.

Arvidsson I. and the authors also came to the conclusion about the negative action on the effectiveness of teachers in the study "Burnout among Swedish school teachers – a cross-sectional analysis" [14] where it was identified that high levels of emotional exhaustion had a direct correlation with the occurrence of health problems characteristic also for teachers from the Republic of Moldova.

Given the limitations of the present study, future research should include longitudinal models to test the stability over time of received findings. Although the obtained results do not lend themselves to deducing causality, they suggest that training the above risk factors and their influence on teachers' health could help alleviate the psychological symptoms associated with work-related stressors. In addition, it is recommended to include additional variables (e.g. resilient adaptation or positive affect) to gain a better understanding of the mechanism by which they, especially psychosocial factors, can function in improving the health and well-being of teachers. It was also argued that self-reporting of health is not always in correspondence with the entries in medical cards, which proves that the latter are not completed qualitatively. This sample was similar to previous heterogeneous and large samples; however, studies with larger samples are needed that can compare the results according to the level of teaching, because the predictive value of this factor allows the design of more effective intervention programs on emotional preparation.

Conclusions

This research showed a high prevalence of chronic as well as acute diseases in teachers, largely due to the pandemic situation. Another problem was the high levels of stress that most often or due to too many activities in which teachers are involved and insufficient rest. Another factor with a strong influence was the high levels of burnout which was directly correlated with the occurrence of health disorders in both young specialists and those with more work experience. Work experience has been shown to be a protective factor in the occurrence of stress at early levels, but which has no statistical connection with teachers' health disorders.

Some useful preventive measures can be implemented at school level to reduce the high prevalence of chronic diseases, such as a proportional reduction in workload for older teachers, optimizing working hours per day and the availability of comfortable furniture.

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Author's contribution
DC conceptualized the idea, conducted literature review, collected the data, interpreted the data, and wrote the manuscript.

Funding. This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the author's initiative. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 17 of 14.04.2019). An informed consent from all participants in the study was obtained.

Conflict of Interests . There is no known conflict of interests to declare.

https://doi.org/10.52418/moldovan-med-j.64-5.21.11
UDC: 616.428-006.441-08

Impact of targeted treatment in non-Hodgkin's lymphoma with primary lymph node involvement

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Manuscript received August 14, 2021; revised manuscript November 10, 2021; published online November 26, 2021

Abstract

Background: Non-Hodgkin's lymphomas (NHL) are malignant tumors that develop from lymphoid tissue. Primary lymph node (LN) involvement is the most common localization (52-70%). The integration of Rituximab (R) in the NHL treatment represented a turning point. The aim of this study was to evaluate the therapeutic impact of the use of R in combination with conventional polychemotherapeutic (PChT) in the treatment of nodal onset NHL.
Material and methods: A descriptive cohort study was performed on 80 patients diagnosed with NHL.
Results: In the study participated: men – 39(48.8%), women – 41(51.2%). The mean age of the patients was 56.09 ± 13.6 years. The onset of NHL occurred in peripheral l/n in 85.0% of cases, in mediastinal LN – 7.5%, and abdominals in 7.5%. Stages I-II were identified in 21(26.2%) patients, stages III-IV in 59(73.8%) cases. Aggressive NHLs were diagnosed in 54(67.5%) patients, indolent NHLs in 26(32.5%) cases. In 61(76.3%) patients, first-line R+PChT treatment was applied – group 1(G1), and in 19(23.8%) cases conventional PChT was applied – group 2(G2). The overall response rate (ORR) in G1 was 86.8%, in G2 – 63.1%. Complete remissions (CR) were obtained in G1 in 63.9% of patients, in G2 – 47.3% of cases. Progression-free survival (PFS) in G1 had a median of 20 months, and in G2 the median was 12 months ($p < 0.05$).
Conclusions: The use of Rituximab increased the ORR rate (86.8% vs 63.1%), the frequency of CR (63.9% vs 47.3%) and PFS (20 months vs 12 months ($p < 0.05$).
Key words: Non-Hodgkin's lymphoma, lymph nodes, treatment.

Cite this article
Tomacinschii V, Robu M, Buruiana S, Finciuc V, Grecu A, Dudnic C, Urescu D, Suschevici M. Impact of targeted treatment in non-Hodgkin's lymphoma with primary lymph node involvement. *Mold Med J.* 2021;64(5):56-61. https://doi.org/10.52418/moldovan-med-j.64-5.21.11.

Introduction

Non-Hodgkin's lymphomas (NHL) are malignant tumors of lymphatic tissue [1, 2]. They are some of the most common malignant hematological disorders having a proportion of 3.1-4.3% of the structure of malignant tumors, being ranked as the 5th to 9th most common cancer in most countries worldwide [3-5]. NHL morbidity in the Republic of Moldova is estimated to be 4.1 cases per 100000 inhabitants[1,6]. NHL can develop at any age group, however NHL morbidity increases with age, with a maximum incidence between 45 and 65 years, thus affecting the working age population [3, 7].

These malignancies arise from the malignant transformation of mature and immature cells of immune system, affecting either B lymphocytes – representing around 86% of all NHL, and a smaller proportion of T- and natural killer (NK) cells – 14% in developing regions [5]. A series of prognostic factors are recognized for the influence of NHL evolution, among them: age, sex, clinical stage, LDH level, etc. NHL prognosis is also influenced by the location of the primary affected area [1, 8].

The onset of NHL can occur in any organ and tissue. More often primary NHL develops in lymph nodes (52-70%). Among other risk factors the progression of NHL is also influenced by the location of the primary affected area.

Rituximab represents a humanized chimeric anti-CD20 monoclonal antibody, that is a powerful tool for treating B-cell malignancies being licensed for the treatment of non-Hodgkin's lymphomas, chronic lymphocytic leukemia, Waldenstrom macroglobulinemia, etc. [9]. Direct signaling, complement-mediated cytotoxicity (CMC), and antibody-dependent cellular cytotoxicity (ADCC) all appear to play a major role in rituximab efficacy in association with chemotherapy in treatment of NHL [9, 10].

Since the approval of rituximab, anti-CD20 monoclonal antibodies have revolutionized the treatment of B cell NHL and have become a cornerstone of modern gold standard practice [11, 12].

The aim of the paper is to identify and evaluate the clinical and evolutionary features of non-Hodgkin's lymphomas with primary involvement of peripheral lymph nodes under the influence of chemoimmunotherapeutic treatment with rituximab in combination with conventional chemotherapy.

Material and methods

The clinical aspects and the evolution of the disease were studied in 80 patients with NHL with primary lymph node (LN) involvement, aged between 22 and 83 years. The diagnosis was morphologically confirmed by lymph node biopsy. The spreading degree of the tumor process was determined according to the International Clinical Classification adopted in the city of Ann Arbor (USA), in 1971 [13].

For the staging of the tumoral process and determination of the degree of initial expansion of NHL, were used data received from: clinical examinations, imaging and ultrasound investigations, bone marrow aspiration, bone marrow biopsy, endoscopic or radiological research of the gastrointestinal tract (if necessary), fibroepipharyngoscopy (if necessary).

For the statistical analysis of the data, the standard descriptive statistics kit was used through the data analysis of Microsoft EXCEL and on IBM SPSS Statistics 26.0. The use of the standard descriptive statistics kit facilitated the calculation of mean, median and p value. To assess patient survival, the Kaplan-Meier's life-table method of forming survival curves, was used.

Results

Out of the 80 patients participating in the study, 39 (48.8%) were men and 41 (51.2%) women. The mean age of the patients was 56.09±13.6 years. The age categories most often affected by NHL in the study group were: age group 61-70 years – 25 patients (31.25%), age group 51-60 years – 22 patients (27.5%) followed by age group 41-50 years – 14 cases (17.5%), (fig. 1).

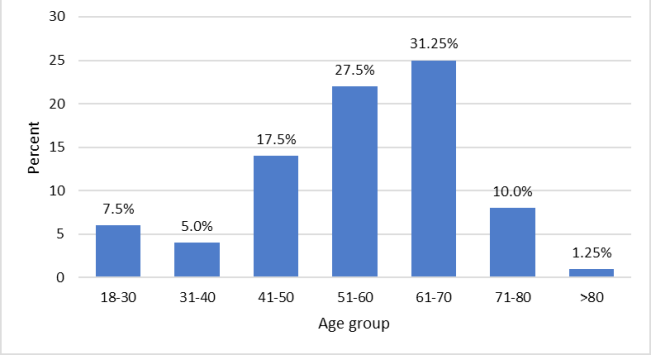

Fig. 1. Distribution of patients in the study group according to age categories

The analysis of the statistical distribution of the study group in correspondence with the area of the LN primary involved showed that more often the NHL onset occurred in: peripheral l/n in 85.0% of cases, in mediastinal LN – 7.5%, and in abdominals LN in 7.5%, (tab.1).

Table 1. Distribution of patients in the study group according to area of lymph nodes primary involved in the tumoral proliferation

Area of lymph nodes primarily involved	Patients	Frequency (%)
Peripheral LN	68	85.0
Mediastinal LN	6	7.5
Intrabdominal LN	6	7.5
Total	80	100.0

Table 2. Distribution of NHL patients with primary lymph node involvement according to the localization of the primary involved area and age category

Age, years	Count / %within age category	Onset in the area of the lymph nodes						Total
		Cervical	Axillary	Inguinal	Mediastinal	Intra-abdominal	Supraclavicular	
18-30	Count	1	0	0	3	1	1	6
	%	16.7%	0.0%	0.0%	50.0%	16.7%	16/7%	100.0%
31-40	Count	2	1	0	0	0	1	4
	%	50.0%	25.0%	0.0%	0.0%	0.0%	25.0%	100.0%
41-50	Count	8	2	2	0	1	1	14
	%	57.1%	14.3%	14.3%	0.0%	7.1%	7.1%	100.0%
51-60	Count	7	4	3	0	2	6	22
	%	31.8%	18.2%	13.6%	0.0%	9.1%	27.3%	100.0%
61-70	Count	11	5	2	2	2	3	25
	%	44.0%	20.0%	8.0%	8.0%	8.0%	12.0%	100.0%
71-80	Count	3	2	0	1	0	2	8
	%	37.5%	25.0%	0.0%	12.5%	0.0%	25.0%	100.0%
>80	Count	1	0	0	0	0	0	1
	%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%
Total	**Count**	**33**	**14**	**7**	**6**	**6**	**14**	**80**
	%	**41.3%**	**17.5%**	**8.8%**	**7.5%**	**7.5%**	**17.5%**	**100.0%**

Among the peripheral lymph nodes, the cervical lymph nodes served, most frequently, as the area of onset of the tumor process – 33 (41.3%) cases, followed by the supraclavicular and axillary area with 14 cases each (17.5% each), whereas the onset of lymphoma occurred in the inguinal lymph nodes in 7 (8.8%) patients. The analysis of the distribution by age categories shows that the area of onset of lymphoma proliferation starting from the cervical lymph nodes was predominant in all age groups, except for the age category 18-30 years where in 50% of cases the onset of tumor proliferation began in mediastinal lymph nodes (tab. 2).

According to the International Clinical Classification, approved in Ann Arbor (USA), most patients were diagnosed in generalized stages: stage III – 17 (21.3%) cases, stage IV in 42 (52.5%) patients. Localized stages were diagnosed in 26.3% of cases, from which: stage I – 7 (8.8%) patients, stage II – 14 (17.5%) cases.

The histological examination of the tissue samples has determined aggressive types of NHL in 54 (67.5%) cases. However, it should be mentioned that indolent NHLs were also found quite frequently, in 26 (32.5%) patients. Aggressive non-Hodgkin's lymphomas were the only types of NHL (100%) that were detected in cases of primary mediastinal localization of the tumor process. Aggressive NHLs also predominated in patients with primary involvement of the inguinal, cervical, abdominal, and axillary lymph nodes (85.7%, 72.7%, 66.7%, and 57.1%, respectively). Indolent NHLs were recorded more frequently in patients with primary supraclavicular lymph node involvement (57.1%) cases (tab. 3).

Table 3. Distribution of NHL patients with primary lymph node involvement according to evolutionary type of NHL and primary involved area

Area of l/n primarily involved	Count / within area of l/n primarily involved	Evaluative type of NHL		Total
		Aggressive	Indolent	
Cervical	Count	24	9	33
	%	72.7%	27.3%	100.0%
Axillary	Count	8	6	14
	%	57.1%	42.9%	100.0%
Inguinal	Count	6	1	7
	%	85.7%	14.3%	100.0%
Mediastinal	Count	6	0	6
	%	100.0%	0.0%	100.0%
Intra-abdominal	Count	4	2	6
	%	66.7%	33.3%	100.0%
Supraclavicular	Count	6	8	14
	%	42.9%	57.1%	100.0%
Total	Count	54	26	80
	%	67.5%	32.5%	100.0%

Aggressive NHLs constituted the majority of NHL types in patients diagnosed in localized stages: stage I – 7 (100%) cases; stage II – 10 (71.4%) of patients. In the case of generalized stages, the ratio between aggressive NHL vs indolent NHL looks like this: stage III – 47.1% vs 52.9%; stage IV – 69% vs 31% (tab. 4).

Symptoms of general intoxication were present in 42 patients (52.5%), mostly (73.8%) in the generalized stages of the disease. Symptoms of intoxication were present in 100% of cases in the primary involvement of the mediastinal lymph nodes, 64.3% in the primary involvement of the axillary lymph nodes, and 51.5% in the primary involvement of the cervical lymph nodes. The primary involvement of intrabdominal and supraclavicular lymph nodes in half of the cases (50%) was associated with B symptoms, in contrast, the onset of lymphoma in the inguinal lymph nodes was associated with B cell symptoms in only 14.3% of patients.

Table 4. Distribution of NHL patients with primary lymph node involvement according to clinical stage and evaluative type of NHL

Disease stage	Count / % within disease stage	Evolutive type of NHL		Total
		Aggressive	Indolent	
I	Count	7	0	7
	%	100.0%	0.0%	100.0%
II	Count	10	4	14
	%	71.4%	28.6%	100.0%
III	Count	8	9	17
	%	47.1%	52.9%	100.0%
IV	Count	29	13	42
	%	69.0%	31.0%	100.0%
Total	Count	54	26	80
	%	67.5%	32.5%	100.0%

Depending on the type of treatment given to patients, the group of patients was subdivided into 2 subgroups: subgroup 1 – subgroup of patients, whose conventional polychemotherapeutic (PChT) treatment (CHOP, COP; FC, etc., in accordance with the provisions of the national clinical protocol) was associated with the administration of rituximab, and subgroup 2 – the subdivision of patients who, for various reasons (medical contraindications, personal allergic history, personal choices of the patient, etc.), followed the treatment only with the use of conventional polychemotherapy. In subgroup 1 (R + PChT) were included 61 (76.3%) patients, and the subgroup 2 (PChT alone) included 19 (23.8%) cases.

Analyzing the treatment efficacy it has been observed that the overall response rate (ORR) in subgroup 1 constituted 86.8%, compared with subgroup 2 where the ORR was equal to 63.1%. Complete remissions (CR) were obtained in subgroup 1 in 63.9% of patients, and in subgroup 2 in 47.3% of cases (fig. 2).

Progression-free survival (PFS) in subgroup 1 had a median of 20 months, and in subgroup 2 the median was 12 months (p=0.001) (fig. 3).

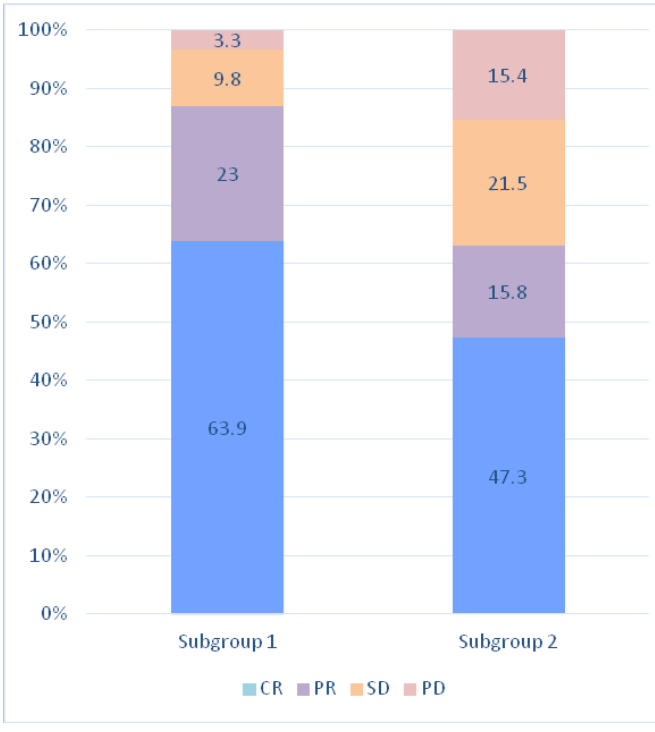

Fig. 2. Effectiveness of treatment within study subgroups. CR – complete remission, PR – partial remission, SD – stable disease, PD – progressive disease

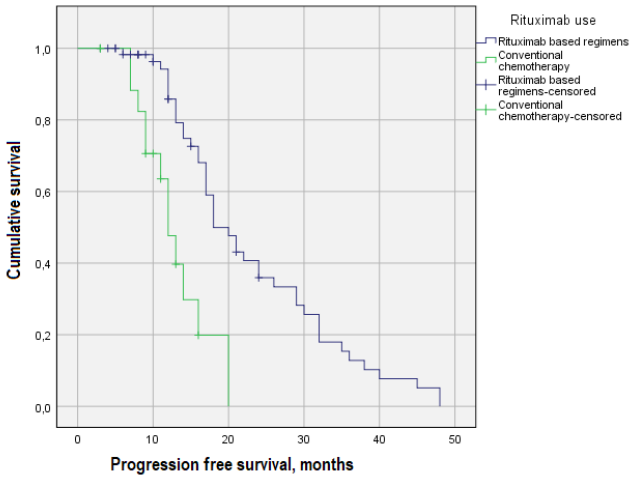

Fig. 3. Kaplan Meier estimates of progression free survival (PFS), within the study subgroups

Discussion

As a result of this study, it was found that NHL with primary lymph node involvement more frequently developed in people aged between 61-70 years (31.25%), and 51-60 years (27.5%), which corresponds to the literature data [3, 14-16]. At the same time, this study confirmed the primary predilection for involvement of non-Hodgkin's lymphomas in the peripheral lymph nodes, with 85% of cases beginning here.

Cervical and supraclavicular lymph node area served as area of origin of tumoral expansion in 41.3% and 17.5%, respectively. Large descriptive studies that have been realized, previously have also shown that the most common sites of involvement are localized in cervical and supraclavicular area. The percentage reported by the literature data varied in the case of primary cervical lymph node involvement ranged from 31 to 37% [17, 18], and the supraclavicular area served as the area of primary involvement in the lymphoma process in 11%, more or less similar to our data [18]. Similarities between data from other scientific articles and our data were also found in case of primary involvement of the axillary lymph nodes (17.5% – our data vs 11.9% in the literature data [18]. Meanwhile, must be paid attention to a slight dissonance in the case of primary involvement of the inguinal lymph nodes. Our data determined a primary involvement of the lymph nodes in 8.8%, while studies show that the groin area is involved in approximately 16.4-18% [17-19]. The correlation of the primary location of the tumor localisation according to the age category did not show discrepancies compared to the general frequency of the primary implications of non-Hodgkin's lymphomas, the cervical area remaining predominant. The exception was the age category 18-30 years, in which 50% of the tumor's process onset served the mediastinum area. Similar data regarding the onset of non-Hodgkin's lymphoma in correlation to age can be found in several studies of the literature [20-22]. The group of patients was homogeneous, as a confirmation comes the mutual ratio between the percentages of aggressive non-Hodgkin's lymphomas vs indolent non-Hodgkin's lymphoma (67.5% vs 32.5%). Epidemiological studies showed similar values in terms of the frequency of detection of aggressive lymphomas (59-65%) vs indolent (35-41%) [16, 23-25]. The correlation between the clinical stage of non-Hodgkin's lymphomas and their evolutionary type determines another peculiarity, namely, the only or most aggressive NHLs are determined localized in stages I and II (100% and 71.4% respectively) while in generalized stages the ratio between aggressive NHL vs indolent NHL is more or less equal and constitutes: 47.1% vs 52.9% in clinical stage III and 69.0% vs 31.0% in clinical stage IV. These data can be explained by the earlier addressing of patients with aggressive NHL who have a more overt clinical picture [23].

Treatment with the monocolonal anti-CD-20 antibody, rituximab, increased the overall response rate. Thus, within subgroup 1 there was an ORR of 86.8% compared to subgroup 2 where ORR constituted 63.1%. Specialized analyses performed on various treatment groups in terms of structure and demography provided results comparable to those obtained by us, ORR ranged from 79-92% in treatment groups using rituximab, compared to 57-75% in groups where best available treatment was used [26-30].

PFS in the study subgroup using rituximab in combination with conventional chemotherapy had a median of 20 months (p=0.001) at a 36-month follow-up period. PFS in the subgroup treated with rituximab in our case is slightly shorter compared to previously published data [12, 26, 28]. A possible explanation would be the comorbid status,

which required a decrease in the total therapeutic doses of the drugs used.

Conclusions

1. NHL with primary involvement of peripheral lymph nodes developed more often in the age category 61-70 years (31.25%).

2. The onset of NHL occurred more frequently in the peripheral lymph nodes (85.0%), in which the involvement of the cervical lymph nodes predominated (41.3%).

3. The frequency of aggressive NHL was 67.5%. Indolent NHL was diagnosed in 32.5% of cases.

4. The use of rituximab in combination with PChT increased the ORR rate (86.8% compared to 63.1%), the frequency of CR (63.9% as opposed to 47.3%) and PFS (20 months compared to 12 months).

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Authors' contribution
VT designed the research, reviewed statistics and interpreted the data, drafted the manuscript; MR conceptualized the project and designed the research, revised the manuscript critically; SB interpreted the data, revised the manuscript critically; VF, AG, CD, DU and MS collected the data, made statistical analysis. All the authors revised and approved the final version of the manuscript.

Funding
The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 1 of July 03, 2020).

Conflict of Interests
There is no known conflict of interests and financial or non-financial support associated with this publication.

https://doi.org/10.52418/moldovan-med-j.64-5.21.12
UDC: 616.124.2-008.6:616.74-007.17-053.2

First manifestation and evolution of early left ventricular dysfunction in children with Duchenne muscular dystrophy

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Manuscript received October 03, 2021; revised manuscript November 10, 2021; published online November 26, 2021

Abstract

Background: Standard pediatric cardiology examinations and echocardiography fail to discover when the cardiomyopathy will occur in patient with Duchenne muscular dystrophy (DMD). Noninvasive markers are needed to fill this gap.
Material and methods: This cohort study included a total number of 30 children (21 children (70%) with DMD and 9 (30%) healthy children. Blood samples were used for biochemical (level of creatine kinase, creatine kinase-MB, lactate dehydrogenase) and miRNA (presence of miR133a 3p, miR133b 3p, miR206 3p, miR208a 3p, miR208b 3p) analysis. All patients underwent partial conventional echocardiography ECOCG and Speckle Tracking.
Results: The children in the working group presented compared to healthy children: FCC values increased by 15 (71%) vs 2 (22%), high levels of CK, CK-MB, LDH, which is characteristic for the disease and reflects its stage. Also, there is a decrease in systolic function indicators in the working group: mean FE 59 ± 3.8 %, and GLS: -16.2 ± 3.1%. MiRNA analyses confirmed the presence of miR133a 3p, miR133b 3p, miR206 3p, miR208a 3p, miR208b 3p in both working and control group.
Conclusions: For the first time in the Republic of Moldova, we developed and adapted protocols for RNA extraction from human blood, performing screening of specific miRNA in the serum of patients with DMD and healthy children. Also, altered LV strain notwithstanding a normal or mildly modified LVEF represents an essential viewpoint for prospective pediatric drug trials in DMD-related cardiomyopathy prevention.
Key words: Duchenne muscular dystrophy, miRNA, cardiomyopathy, heart failure, qRT-PCR.

Cite this article
Rodoman I, Dorif A, Palii I, Sacara V. First manifestation and evolution of early left ventricular dysfunction in children with Duchenne muscular dystrophy. *Mold Med J.* 2021;64(5):62-69. https://doi.org/10.52418/moldovan-med-j.64-5.21.12.

Introduction

Duchenne muscular dystrophy (DMD) is the most frequent and severe form of progressive muscular dystrophy, with an incidence rate of 6.11 per 100000 population according to the data of the National Center for Reproductive Health and Medical Genetics of the Republic of Moldova [1, 2]. DMD is a myogenic disorder due to mutations in the dystrophin gene on the Xp21.1 chromosome [3]. Mutations in this gene cause the absence of dystrophin – a component of the cell membrane complex of skeletal, cardiac, and smooth muscle cells and is one of the links between the cytoskeleton and the extracellular matrix [4].

According to literature data, Duchenne myodystrophy has a rapid evolution, with severe disability, approximately at 10-15 years of age [5, 6]. In addition, DMD is the second most common lethal human genetic disease globally [7].

Cardiac complications occur in more than 90% of patients with DMD, with 20% of them dying. The fibrotic region of cardiomyocytes will gradually stretch, become thinner, and lose contractility, resulting in dilated cardiomyopathy [8].

Studies show that dilated cardiomyopathy is the leading cause of death, so it remains a severe problem that affects survival and requires timely behavior management [5, 9, 10]. According to the 2017 TREAT-NMD report, 12% of registered patients (study cohort – 5345 subjects) developed cardiomyopathy [11].

In the case of DMD, cardiomyopathy is usually asymptomatic due to the compensatory mechanisms, which leads to a later manifestation of the symptoms characteristic of heart failure (HF). Therefore, in general, any representation of HF in this group of patients is masked by the severity of myopathic syndrome [12].

So, involvement of the heart in the pathological process in Duchenne myodystrophy has been actively studied since the eighties of the twentieth century [13], the level of early detection of cardiovascular disorders and the prevention of severe complications in patients with hereditary progressive muscular dystrophies remains very low, and the cause of death in most forms of the disease is the involvement of the heart muscle in the pathological process [14, 15].

Studies in recent years, aimed at the treatment and support of people with DMD, seek a high-performance treat-

ment [16]. Current clinical and preclinical therapies are currently based on exon skipping therapies for patients with deletions or duplications of DMD gene exons. Also, there is now a long-term study with Ataluren [17].

So, the prevention of cardiomyopathy holds as one of the most challenging clinical research issues in children with DMD. At the moment, pediatric cardiology examinations, first and foremost echocardiography, fail to discover when and to what level the cardiomyopathy will occur. In addition, most studies have struggled to prove drugs' efficacy on DMD cardiomyopathy incipience and progression rate using conventional echocardiographic measures. The drugs include ACE inhibitors, b-blockers, and corticosteroids. In the modern era, pediatric DMD drug trials require reliable and noninvasive cardiac biomarkers. Unfortunately, currently available tools to evaluate cardiac function may not be precise (blood tests), challenging to perform in pediatric patients (cardiovascular magnetic resonance imaging), or too invasive (myocardial biopsy).

The above exacerbates the need to promote researches related to the early diagnosis of heart problems in DMD to determine the treatment tactics, prevent the development of severe cardiovascular disorders, improve quality, and prolong patients' lives [17].

MicroRNAs implicated in the development of the myopathic process and cardiomyopathy

Non-coding RNAs (ncRNAs), such as miRNAs and long non-coding RNAs, are key regulators of post-transcriptional gene expression and represent promising therapeutic targets and biomarkers for several human diseases, including DMD. In some studies, a role for ncRNA has been suggested in the pathogenesis of muscular dystrophies, although it is still incompletely understood. Long and short non-coding RNAs are differentially expressed in DMD and have a mechanism of action through targeted mRNAs. A subgroup of miRNAs, the so-called myomiRs (miR-133, miR-206, miR-208), have increased values in the serum of patients with DMD and animal models with dystrophin deficiency. Interestingly, myomiRs could be used as biomarkers, as their levels can be corrected after dystrophin restoration in dystrophic mice. Additional scientific evidence demonstrates that nRNAs also play a role in dystrophin expression. Thus, their modulation could represent a potential therapeutic strategy to increase dystrophin levels in combination with other gene therapies [18].

Abnormal serum expression of different miRNAs has been associated with oncological, neurodegenerative, cardiovascular, metabolic, and hereditary diseases, suggesting a potential role as a minimally invasive biomarker [19]. MiRNAs selectively involved in muscle pathways and related to muscle dystrophies have been termed dystromies or myomiRs. These include miR-1, miR-133a, miR-133b, miR-31 and miR-206 [20].

MiR-1 and miR-133a are expressed from the exact transcription in skeletal muscle but have different functions [21]. For example, miR-1 promotes myogenesis and terminal differentiation, acting on HDAC4 and connexin-43, while miR-133 enhances myoblast proliferation [22].

The same ncRNA also encodes miR-206 and miR-133b. MiR-206 is specific for skeletal muscle, especially oxidative fibers, and is expressed in the proliferation of myoblasts under negative regulation of TGF-β and myostatin and positive for myogenin [23-25].

The dystrophin gene is the largest in the human genome, containing 79 exons, and comprises 2.6 million base pairs of the genomic sequence, accounting for about 1.5% of the entire X chromosome. Its mutations lead to errors in protein-coding dystrophin.

Defects or absence of dystrophin protein in cardiomyocytes results in DCM by a similar pathway described. Specifically, recent studies have suggested that the absence or mutation of dystrophin disrupts the function of membrane ion channels, especially sarcolemmal stretch-activated channels, which respond to mechanical stress [26-29]. When cardiomyocytes with deficient or mutant dystrophin stretch during ventricular filling, the activated stretching channels do not open properly, increasing calcium influx. High intracellular calcium activates calcium-induced calpain, a group of proteases that will degrade troponin I and compromise cardiomyocyte contraction. The destruction of the Calpain-mediated membrane protein allows more calcium to enter. Finally, chronic calcium overload leads to cardiomyocyte death [28, 29]. Cardiomyocyte death initiates an inflammatory cascade during which macrophages migrate to remove damaged cells and debris [26-29]. After recruiting macrophages, fibroblasts invade the damaged area and form scar tissue or fibrosis in the heart. Fibrotic tissue is very inflexible compared to normal heart tissue and thus restricts the efficiency of myocardial contraction. Fibrosis begins in the LV wall in DMD and the right ventricular wall in BMD, moving epicardium to endocardium. It spreads progressively over most of the outer half of the ventricular wall. This pattern of fibrosis is unique to dystrophinopathy. The fibrotic region will gradually stretch, become thinner, lose contractility and result in DCM. Dilation of the heart increases left ventricular volume, decreases systolic function, and often leads to mitral valve insufficiency, resulting in decreased cardiac output and hemodynamic decompensation. The cardiac phenotype in each DMD or BMD patient results from the particular type of patient with dystrophin gene mutation; however, the relationship between genotype and phenotype remains elusive [26, 27, 29].

According to recent studies, miRNAs can alter cardiac differentiation: proliferation, maturation, and pathological remodeling responses to stress, injury, and abnormal regulatory expression [26, 28, 29]. Several miRNA matrices have been reported in human cardiac tissue, and several have addressed plasma miRNA profiles in HF [30-32].

Tijsen et al. suggested that miR-423-5p was a diagnostic marker for HF [28]. Others have disclosed classes of miRNAs in the detection of HF. In addition, many studies have identified miRNAs (miR-1, -133, -499, and -208) as signifi-

cantly elevated in acute myocardial infarction (AMI), and a series of meta-analyses were performed to verify the role of miRNAs in the AMI [33]. However, although numerous reports have been published, the impact of miRNA in CI management is still being discussed.

In the study Noviskas R. [33] miR-1, miR-133, miR-145, miR-208, and miR-499 were identified as significant diagnostic and/or prognostic markers in different stages of cardiovascular disease progression. Next miRNAs regulate endothelial function and angiogenesis (miR-1, miR-133), vascular smooth muscle cell differentiation (miR-133, miR-145), communication between vascular smooth muscle and endothelial cell for plaque stabilization (miR-145), and regulates endothelial apoptosis and CMNV (miR-1, miR-133, miR-499), differentiates cardiac myocytes (miR-1, miR-133, miR-145, miR-208, miR-499) and represses cardiac hypertrophy (miR-133).

Speckle Tracking Echocardiography

Among the new echocardiography techniques, speckle-tracking echocardiographic (STE) imaging, or two-dimensional (2D) strain, has emerged as an interesting noninvasive functional biomarker in pediatric cardiology and has also recently been considered in the early detection of DMD cardiomyopathy [34]. STE imaging evaluates myocardial function with a dynamic regionalized analysis of the overall ventricular contraction. This technique measures localized myocardial movements of natural acoustic markers, also called speckles. STE imaging supposedly allows a rapid, precise, and objective segmental and global myocardial function assessment in three longitudinal, radial, and circumferential strain [35]. In addition, several pediatric studies have pointed out the ability of STE analysis to detect preclinical myocardial dysfunction, such as after anthracycline chemotherapy, dilated cardiomyopathy [36], septic shock, ischemic heart disease, and heart transplantation.

Similarly, recent studies with small cohorts or retrospective data suggested that STE strain was altered before the onset of DMD-related cardiomyopathy in animal models and children with DMD [34, 37].

Material and methods

The research results were obtained in 2019-2021 in a prospective cohort study which took place in the Institute of Mother and Child Care, after enrolling 30 subjects: 21 boys diagnosed with DMD recruited from the Register of Neuromuscular Diseases of the Human Molecular Genetics Laboratory and evaluated in the Cardiology Department of the Pediatrics Department of the Institute of Mother and Child and 9 healthy patients recruited from the Republic of Moldova performed routine analyses within the Specialized Integrated Consultative Department of the Mother and Child Institute.

The study was conducted according to the principles of the Helsinki Declaration (Code of Ethics of the Global Medical Association, amended at the 64th General Assembly of the Global Medical Association, Fortaleza, Brazil, in October 2013) for experiments involving humans. All participants in this study expressed informed consent, respecting confidentiality. The Research Ethics Committee of *Nicolae Testemitanu* University of Medicine and Pharmacy issued a favorable opinion within the doctoral scientific project.

The children in the study were assessed according to an individualized examination protocol. Clinical examination was performed with evaluating the following anthropometric parameters: mass, waist, body mass index, hemodynamics (pulse, blood pressure), and paraclinical investigations.

Blood samples and serums taken in the morning by puncture from the ulnar vein after 12 hours of fasting, without exercise before collecting from each subject, were isolated for biochemical and miRNA analysis. Serum samples were used to determine the level of creatine kinase, creatine kinase-MB, lactate dehydrogenase. Increased values of creatine kinase level will be considered > 308 U / l, creatine kinase-MB> 24 U / l, lactate dehydrogenase for age category 0-1 year > 600 U / l, 2-14 years > 300 U / l,> 15 years> 225 U / l.

The blood samples for miRNA investigation were stored at -80°C until analysis. Then, the presence of miR133a 3p, miR133b 3p, miR206 3p, miR208a 3p, miR208b 3p was appreciated.

Molecular methods of nucleic acid analysis took place in 3 steps: total RNA purification using two different kits, control 18S rRNA presence by RT-qPCR method, and the last one with an appreciation of specific miRNA presence or absence.

All patients underwent partial echocardiography, echocardiographic measurements with the assessment of systolic function (ejection fraction and global longitudinal strain VS) and LV dimensions after the modified Simpson method [38].

The IBM SPSS Statistics program using parametric and nonparametric criteria was used for statistical processing and descriptive analysis.

Results and discussion

The DMD group included nine children aged 4-8 years, nine children aged 9-12 years, 3 children older than 12 years with a mean age of 9 ± 3.7 years, and the control group included 4, 4, and 1 child respectively but the average age 8 ± 2.5 years.

The children in the workgroup had an average BMI of 17.5 ± 3.6 kg/m^2, of which one child was impossible to measure due to immobility. On the other hand, the children in the control group showed an average BMI of 17.9 ± 2.5 kg/m^2.

In the working group, 6 children did not have the data for increased pulse rate. In 15 children, tachycardia was an indirect indicator of stage I impairment in cardiomyopathies with dystrophin deficiency [39]. Of the patients with DMD – 18 children have already started treatment with GCS, three children have refused treatment with Defal.

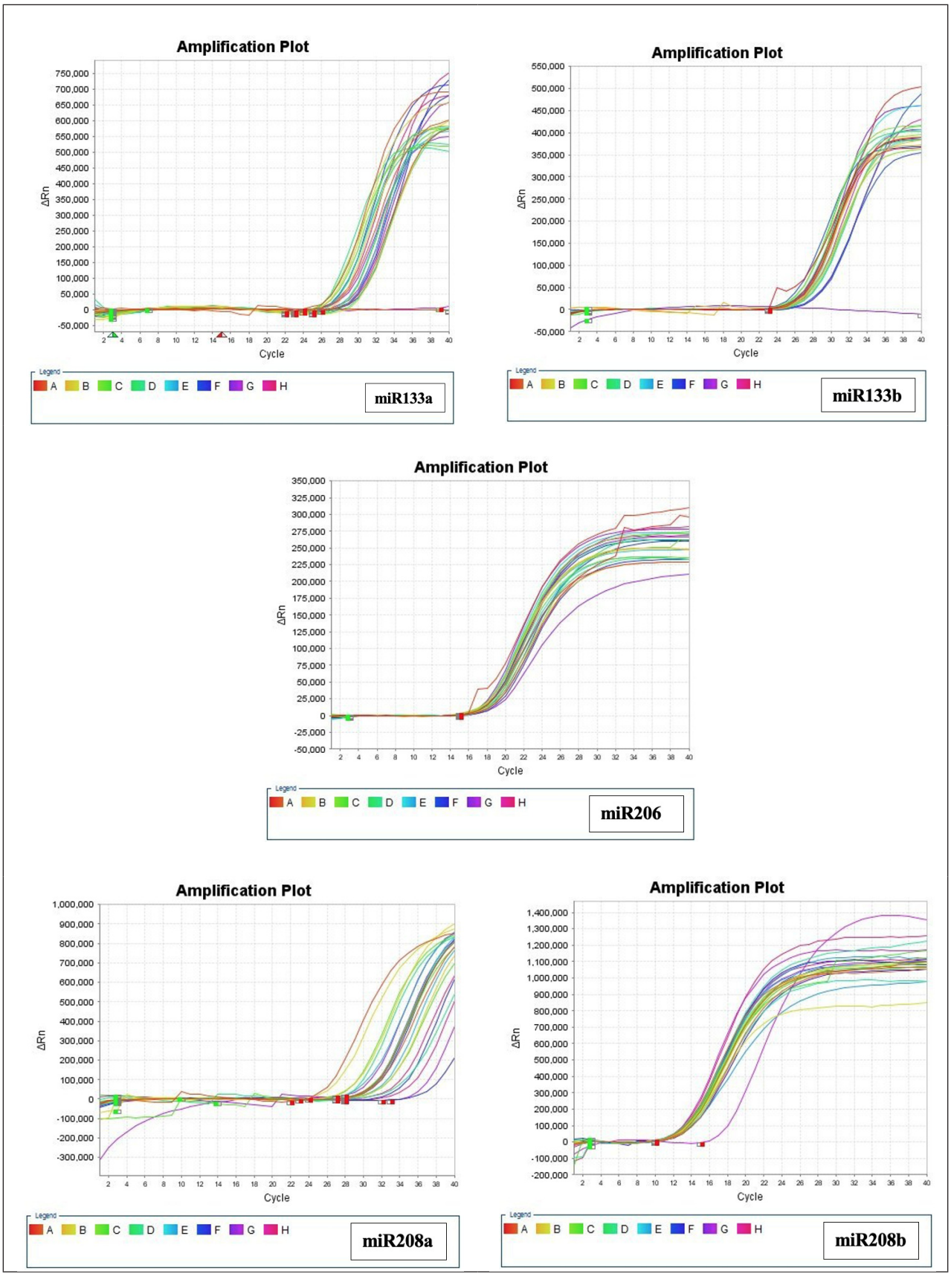

Fig. 1. MiRNA profile in children with Duchenne muscular dystrophy

Table 1. Clinical characteristics of DMD children included in the study

	Total ammount	%
Age		
4-8 years	9	42.9
9-12 years	9	42.9
> 12 years	3	14.3
Median age	9 ± 3.7	
BMI (kg / m2)	17.5 ± 3.6	
Cardiovascular symptoms		
No cardiovascular symptoms	11	52.4
Exercise dyspnea	3	14.3
Rest dyspnea	1	4.8
Palpitations	4	19.1
Weakness	3	14.3
Tachycardia	15	71.4
Muscular stage		
0 stage	2	9.5
1 stage	16	76.1
2 stages	1	4.8
3 stages	2	9.5
Glucocorticosteroids	18	85.7
Analyses		
CK, U/l	10606 ± 7489	
CK-MB, U/l	257 ± 173	
LDH, U/l	257 ± 509	
Echocardiography		
FE, %	59 ± 3.8	
GLS, %	-16.2 ± 3.1	
Mutation		
Deletion	11	52.4
Duplication	8	38.1
Point	2	9.5

The children in the working group presented different stages of the disease: stage 0 (no signs of myopathy) – 2 children, stage 1 (able to walk, impossible to run) – 16 children, stage 2 (impossible to walk) – 1 child, stage 3 (impossible to use hands) – 2 children.

In total, the children in the workgroup presented the increased values of creatine kinase levels – 10606 ± 7489 U / L, CK-MB – 257 ± 173 U / L, LDH – 257 ± 509 U / L, which is characteristic of children with DMD and reflects the stage of the disease.

Different types of mutation were reported in children diagnosed with DMD – 11 children with deletions, 8 children with duplication, and 2 children with point mutation.

The children in the working group presented data on impaired systolic heart function: mean FE 59 ± 3.8%, and SGL – 16.2 ± 3.1%.

The presence or absence of miRNA in serum was confirmed by RT and qPCR using specially developed primers for the project.

MiR133a, miR133b, miR206, miR208a, miR208b were detected in healthy children and children with DMD (fig. 1, 2).

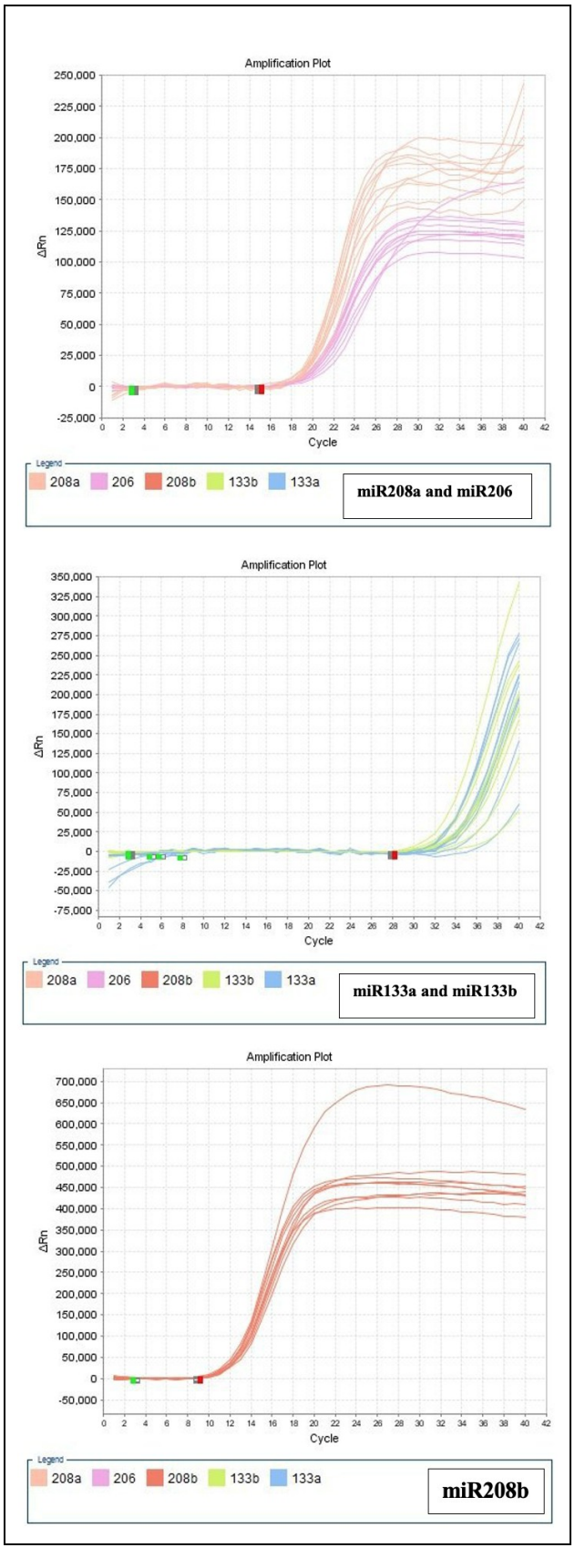

Fig. 2. MiRNA profile in healthy children

Discussion

This study was performed among children with DMD presenting no evidence of significant DMD-related cardiomyopathy and without any relevant symptoms of heart failure and with normal or subnormal systolic function on conventional echocardiography.

Global left ventricular 2D strain in boys with DMD was significantly altered with STE analysis before the onset of relevant patterns of cardiomyopathy. This study confirms recently reported preliminary results using evaluation of LV STE strain in patients with DMD. Spurney CF et al. used STE imaging to identify subclinical myocardial dysfunction, showing a decrease in circumferential and longitudinal strain, in a cohort of 33 children with DMD [34]. The retrospective study by Taqatqa A et al. analyzed circumferential and longitudinal STE strain in 19 children with DMD versus 16 control subjects and found similar results, with an even larger magnitude of difference (global longitudinal STE strain, 18.8 ± 3.0% vs 13.6 ± 5.0%; p = .001) [37].

So, our results suggest that speckle-tracking analysis in children with DMD is more accurate than conventional echocardiography to identify areas of myocardial dysfunction and, therefore, early cardiomyopathy onset. This tool could help clinicians identify early cardiac dysfunction in this specific population and begin medical therapy at an early stage of the disease.

Thus, the main result of this study, that is, altered LV strain despite a normal or mildly altered ejection fraction, represents an important perspective for future pediatric drug trials in DMD-related cardiomyopathy prevention. Indeed, reliable and noninvasive biomarkers are necessary for pediatric cardiology trials.

After analyzing several specific data, this paper reported the functions of the most important miRNAs in skeletal muscle tissue and their modification in patients with DMD. Attempts to correlate myomiR levels with clinical parameters in patients with DMD have so far produced controversial results. Several studies have reported a negative correlation between myomiRs levels and the patient's age with DMD, similar to the negative correlation observed with serum creatine kinase.

For example, data on elevated levels of ex-myomiR have been reported in younger patients with DMD (age 2-6 years); this could be explained by the fact that, over this age range, patients with DMD experience a period of average childhood growth (and a walking distance of 6 minutes) that can compensate for the degeneration of myofibers. Consequently, serum myomiR levels were significantly higher in outpatients (mean age 8.2 years) than in non-ambulatory patients with DMD (mean age 14 years), probably due to pathological progression and/or higher physical activity. Indeed, exercise in healthy individuals can also increase circulating myomiRs, and serum levels of myomiRs in *mdx* mice are increased after running exercise. In particular, the magnitude of ex-myomiR growth after acute exercise was found to be smaller than that observed for serum creatine kinase, suggesting that measuring ex-myomiR levels is less sensitive to exercise-associated variability than serum creatine kinase [40].

In our study we confirmed the presence of miR133a 3p, miR133b 3p, miR206 3p, miR208a 3p, miR208b 3p in both working and control group, and further studies are needed to check their concentration.

Overall, these studies suggest that myomiR levels are influenced by several factors that contribute to the complex pathology of dystrophin-deficient muscle, such as changes in muscle mass, physical activity, and muscle growth and/or regeneration. As a result, in elderly patients with advanced pathology, serum levels of myomiR may not be able to monitor disease progression (or response to therapy), as a decrease in their levels may indicate either improved muscle function or a reduction in muscle mass. In contrast, higher serum myomiR may suggest an increase in muscle pathology and degeneration, but may also be a consequence of muscle growth and regeneration and/or more severe physical activity, as has been observed in young patients [41, 42].

Based on the above, miRNAs promise both as a biomarker to improve the diagnosis and monitoring of DMD progression and treatment and as therapeutic targets that can be adjusted to relieve early and advanced DMD symptoms. There is a particular need for a susceptible and minimally invasive method to monitor the progression of symptoms and whether a specific treatment successfully ameliorates the outcomes.

Finally, the circumstances in which miRNAs can and should be administered therapeutically require careful analysis. The progression of DMD symptoms is not the same from one patient to another. This, combined with the fact that miRNA expression and function are tissue-specific, means that miRNAs may perform different tasks in each tissue and may not be an appropriate treatment option in all circumstances. Therefore, miRNAs with potentially opposite roles in different tissues should be further examined before considering them as therapeutic targets in treating tissue-specific symptoms observed in specific cases. Ideally, future studies will strive to combine the detection, administration, or inhibition of miRNA with methods such as exon skipping to improve the diagnosis and management of the pathology [43].

Certainly, randomized and standardized studies are needed to establish the predictive value of miRNA. Summarizing the most promising miRNAs and linking them to target genes involved in the development of cardiovascular pathology could also help in future studies [44].

Conclusions

For the first time, protocols for RNA extraction from human blood have been successfully developed and optimized methods of reverse-transcription and qualitative analysis of miRNAs using a mixture of specific primers in the Human Molecular Genetics Laboratory of the Genetic Center of Excellence. Our screening of miR133a 3p, mir133b 3p, miR206

3p, miR208a 3p, miR208b 3p in the serum of patients with DMD and healthy children will open a new perspective for the analysis variation of microRNA in the blood collected from patients with DMD and the correlation with their circulating levels and the severity of the disease. In children with DMD, global LV 2D strain was significantly decreased for longitudinal displacements before the onset of DMD-related cardiomyopathy. Moreover, children with DMD presented a significant decrease in global LV longitudinal 2D strain with age and disease stage. Altered LV strain notwithstanding a normal or mildly modified LVEF represents an essential viewpoint for prospective pediatric drug trials in DMD-related cardiomyopathy prevention. Further cohort studies need to be performed to confirm that global longitudinal LV 2D strain represents a reliable surrogate endpoint for heart failure in patients with DMD.

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IR conducted a literature review, collected the data, wrote the first manuscript, and interpreted the statistical data. IP conceptualized the idea and critically revised the manuscript. AD and VS conducted/performed the laboratory work, revised and approved the final text.

Funding
The study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The authors are independent and take responsibility for the integrity of the data and the accuracy of the data analysis.

Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 6 of October 30, 2019). Informed consent was obtained from all study participants.

Conflict of Interests
The authors have no conflict of interests to declare.

doi.org/10.52418/moldovan-med-j.64-5.21.13
UDC: 612.015+616-092.9

Erythrocytic antioxidant system in the administration of new coordination compounds thiosemicarbazide derivatives

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Manuscript received October 15, 2021; revised manuscript November 18, 2021; published online November 26, 2021

Abstract

Background: Currently, there is a growing interest in new local coordination compounds (CC), which demonstrated antitumor properties, but their influence on the erythrocyte antioxidant system has not been studied. The aim of the study: to study the effects of CC, thiosemicarbazide derivatives –TIA-160, CMT-67 and CMJ-33 on indices of the antioxidant system indicators in erythrocytes peripheral blood *in vivo* experiments.
Material and methods: The action of CC on superoxide dismutase, catalase, total antioxidant capacity was evaluated on a group of 34 white rats, randomly divided into 4 groups: the first control group was injected subcutaneously with saline. The other groups (2 – TIA-160, 3 – CMT-67 and 4 – CMJ-33) were given subcutaneously 3 times a week for 30 days, 0.1μM / kg CC.
Results: It was established that the TIA-160 compound demonstrated the highest capacity to induce the expression of erythrocyte catalase that exceeded the control level of 1.8, which did not correlate with the enzymatic superoxide dismutase (SOD) activity. Thus, this study showed that there are differences in the mechanisms of action of thiosemicarbazone derivatives.
Conclusions: The influence of tested CC on the indices of the antioxidant system is selective. This selectivity may be the base to their strong selective antiproliferative and cytotoxic action on tumor cells, but not on healthy ones.
Key words: coordination compounds, erythrocytes, antioxidant enzymes.

Cite this article
Pantea V, Gamaniuc M, Popa V. Eritrocytic antioxidant system in the administration of new coordination compounds thiosemicarbazide derivatives. *Mold Med J.* 2021;64(5):70-75. https://doi.org/10.52418/moldovan-med-j.64-5.21.13.

Introduction

Extensive research over the past two decades has revealed the mechanism by which continuous oxidative stress can lead to chronic inflammation, which can lead to a lot of chronic diseases, including cancer, diabetes, cardiovascular, neurological and lung diseases. Oxidative stress can activate a variety of transcription factors. Activation of these transcription factors can lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules and anti-inflammatory molecules. The way that oxidative stress activates the inflammatory pathways leads to the transformation of a normal cell into tumor cells, tumor cell survival, and proliferation, chemoresistance, radioresistance, invasion, angiogenesis, and stem cell survival is the focus of this review. In general, observations to date suggest that oxidative stress, chronic inflammation and cancer are closely linked. The generation of reactive oxygen radicals in mammalian cells profoundly affects many critical cellular functions, and the absence of effective cellular detoxification mechanisms that eliminate these radicals can lead to more human diseases.

Growing evidence suggests that reactive oxygen species (ROS) in cells act as the second messenger in intracellular signaling cascades that induce and maintain the oncogenic phenotype of cancer cells. ROS are tumorigenic due to their ability to increase cell proliferation, survival, cell migration and also by inducing deoxyribonucleic acid (DNA) damage, leading to genetic lesions that initiate tumorigenicity and support subsequent tumor progression. However, it is also known that ROS can induce cell senescence and cell death and therefore function as antitumorigenic agents. Therefore, the mechanisms by which cells respond to reactive oxygen species depend on the molecular background of cells and tissues, the location of ROS production, and the concentration of individual ROS species. Carcinoma cells produce ROS at high rates *in vitro* and *in vivo,* many tumors appear persistent to oxidative stress. Thus, the finding that a diet rich in antioxidants or the elimination of ROS by antioxidant compounds prevents the development of certain types of cancer, provided the framework for further investigation of the tumorigenic actions of reactive oxygen species. This review presents current knowledge on the different roles of ROS in tumor development and progression.

Oxidative stress is defined as an imbalance between the production of free radicals and reactive metabolites, so-cal-

led oxidants or ROS and their elimination through protective mechanisms, called antioxidants. This imbalance leads to the deterioration of biomolecules and important cells, with a potential impact on the whole organism [1]. ROS are products of a normal cellular metabolism and play vital roles in stimulating signaling pathways in plant and animal cells in response to changes in intra and extracellular environmental conditions [2]. Most ROS are generated in mitochondrial respiratory chain cells [3]. During endogenous metabolic reactions, aerobic cells produce ROS, such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•) and organic peroxides as normal products of the biological reduction of molecular oxygen [4]. The transfer of electrons to molecular oxygen takes place in the respiratory chain, and the electron transport chains located in the membranes of mitochondria [5, 6]. Under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO), which can generate other reactive nitrogen species (RNS). RNS can further generate other reactive species, for example, reactive aldehydes-malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), by inducing excessive lipid peroxidation [7]. Proteins and lipids are also significant targets for oxidative attack, and modifying these molecules may increase the risk of mutagenesis [8].

The adverse biological activities of thiosemicarbazone (TSC) and Schiff base derivatives (SBD) have been widely studied in rats and other animal species, using different doses and routes of administration. However, there are few studies describing changes *in vivo* biochemical parameters that indicate the antioxidant system and oxidative stress in biological systems and morphological changes in tissues. In this study, rats were injected subcutaneously with thiosemicarbazone coordination compounds containing Schiff bases.

The aim of this study is to determine the effect of TSC coordination compounds on changes in the erythrocyte antioxidant system in rats that can be used to determine the efficacy of new indigenous preparations.

Material and methods

The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 73 of 26.04.2017).

The study included new local copper coordination CC, thiosemicarbazide derivatives – TIA-160, CMT-67 and CMJ-33, synthesized at the State University of Moldova in the Laboratory of Advanced Materials in the Biopharmaceutical and Technical Field, under the supervision of Aurelian Gulea [9].

The action of local CC on the antioxidant system was evaluated in experiments on a group of 34 white male rats of the Wistar line weighing 180 – 230 g, divided into 4 groups of 8-9 animals each. The first group – the control, consisted of 8 animals, maintained on a regular diet of vivarium and which were injected subcutaneously 3 times a week saline for 30 days.

Animals in experimental groups 2-4 were given subcutaneous CC 3 times a week for 30 days, over 30 days in the following sequence: group 2 – TIA-160 (0.1 μM / kg), lot 3 – CMT-67 (0.1 μM / kg) and lot 4 – CMJ-33 (0.1 μM / kg).

After 24 hours from the expiration of the experiments, blood was collected to evaluate the parameters of the antioxidant system. The erythrocyte mass, obtained after decanting the blood serum, was washed twice with saline.

Total antioxidant capacity (TAC) in erythrocytes was assessed using the methodology described by Pellegrini N., Ying M., Rice-Evans C., adapted to the multimodal rider with Synergy H1 Hydrid Reader plates (BioTek Instruments, USA) [10].

This method has been reported as a discoloration assay, applicable to both lipophilic and hydrophilic antioxidants. The preformed radical cation of 2,2'-azinobis- (3-ethylbenzothiazolin-6-sulfonic acid) (ABTS*+) is generated by the oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both, the antioxidant concentration and the duration of the reaction on the inhibition of radical cationic absorption have been taken into account in determining the total antioxidant capacity.

Catalase Assay Kit and superoxide dismutase activity were performed using reagent kits obtained from local firms and performed according to the attached protocol.

The statistical evaluation of the obtained data was performed with the use of the computer program StatsDirect. The arithmetic mean ± error of the mean (M ± m) was calculated. The nonparametric statistical test "U Mann-Whitney" and the significance threshold "p" ($p < 0.05$) were used to test the significant difference between the studied indices of the compared groups.

Results

The evaluation results of the antioxidant system indices: TAC with ABTS, CAT and SOD in erythrocytes when administering autochthonous CCs are presented in the statistics in table 1.

Table 1. Influence of autochthonous CCs, thiosemicarbazide derivatives, on the indices of the antioxidant system in erythrocytes TAC with ABTS

Groups of study	TAC with ABTS mM/g.Hb	Catalase, μM/s.g.Hb	SOD, unit conv /g.Hb
Contol group	38.53±1.35 (100%)	18.80±0.86 (100%)	6.72±0.42 (100%)
TIA-160 0.1 μM / kg	36.08±0.84 (94%)	**33.23±1.77*** (177%)**	7.12±0.47 (106%)
CMT-67 0.1 μM / kg	**29.09±0.68*** (75%)**	**23.69±3.23** (126%)**	**5.21±0.48** (78%)**
CMG-33 0.1 μM / kg	36.58±1.02 (95%)	**27.66±1.81*** (147%)**	6.39±0.52 (95%)

Note: * – statistically significant difference with the control group (* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$).

The animals exposed to the action of CC, thiosemicarbazone derivatives demonstrated a significant increase in erythrocyte catalase activity exceeding 1.3 – 1.8 times the control values.

It was established that the compound TIA-160 (0.1 μM/kg) showed a tendency to increase the activity of TAC and catalase by 39-51% compared to the control group, and SOD was found to be maintained within the control group.

The study reveals that SOD decreases statistically conclusively by 15% ($p<0.05$) at the administration of the compound CMT-67 (0.1 μM/kg), and the total antioxidant activity decreases by 25%, at the same time, the function of erythrocyte catalase in this case increases truthfully by 26%. After the administration of the compound CMG-33 the CAT level increases by 47%, the changes of the TAC content and of the SOD activity in this case proved to be inconclusive, these remaining within the limits of the values registered in the control group.

Discussion

In this study, was analyzed the activity of the antioxidant system in erythrocytes in laboratory animals subjected to the action of native CCs, thiosemicarbazide derivatives. TSC derivatives were in the focus of chemists and biologists due to their wide range of pharmacological effects. TSC derivatives showed highly effective antitumor properties in various types of tumors (leukemia, pancreatic cancer, breast, lung, cervical cancer, and prostate and bladder cancer). To obtain a better activity, different series of TSCs were developed by modifying the heteroaromatic system in their molecules. Thus, the antineoplastic activity became significant at the attachment of the side chain carbonyl at the α position to the ring nitrogen atom, while the attachment of the β or γ side chain to the N heterocyclic atom led to inactive antitumor agents [11].

Studies in recent years have provided increasing evidence of the fundamental importance of copper for the formation and functioning of several enzymes and proteins, such as Cu/Zn superoxide dismutase, or cytochrome C oxidase, which are involved in superoxide oxygen neutralization processes, in tissue respiration, energy metabolism and DNA synthesis. The coordinating compounds of copper have been shown to be promising antitumor therapeutic agents that act through various mechanisms [12].

Acting to protect the body against certain harmful prooxidants, in this study was evaluated a complex system of enzymatic and non-enzymatic antioxidants, which included the determination of superoxide dismutase, catalase and total antioxidant capacity.

In this study were obtained significantly low levels of TAC and SOD activity, compared to the control group, at the administration of compound CMT-67, while the values of these indices under the influence of the other compounds studied did not have any changes with statistical significance.

The animals exposed to the action of CC, thiosemicarbazone derivatives were found to have a significant increase in erythrocyte calalase activity that exceeded 1.3-1.8 times the control values, and the compound TIA-160 (0.1 μM/kg) shows the highest capacity to induce erythrocyte catalase expression. This indicates existence of an excessive synthesis of CAT after exposure to these compounds, a very important fact, established by us for the first time. On the other hand, this study showed that there are differences in the mechanisms of action of thiosemicarbazide derivatives. The significance and importance of the phenomenon of catalase induction by CC, thiosemicarbazide derivatives emerge from the wide possibilities of application of these inducers in practice. Thus, these inducers could be used as promising remedies for the treatment and prevention of renal fibrosis induced by catalase deficiency [13], or the treatment of some forms of infertility, because catalase has been detected in the oocytes of mice, where it probably plays the role of protecting the genome from oxidative damage during meiotic maturation [14].

Taking into consideration the capacity of the thiosemicarbazide derivatives so easily penetrate the blood-brain barrier, along with their stability in the bloodstream, they may be used for the development of new effective methods for early diagnosis of severe infections of the brain, such as brain tumors and their metastasis, as well as visualization of Aβ plaques of Alzheimer's disease.

Similarly, catalase could be extremely useful for the development of effective therapy of brain and neurological disorders, proceeding from the fact that the catalase activity in the brain is extremely low compared to other tissues and organs, such as the liver and kidneys. The results of some studies reveal the importance of transient receptor potential (TRP) channels as a key component of the neurological pathway of Ca^{2+} ion entry in response to the harmful action of ROS. Explorers' data suggest that catalase may act effectively by suppressing the TRP channel activated by oxidative stress, showing protective effects on neuronal mitochondrial function and neuronal survival [15]. Catalase could also be extremely useful in the future for the development of effective therapies for neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, as well as sensory pain, as the decrease in catalase activity by oxidative stress plays an important role in the etiopathogenesis of diseases mentioned above.

It has been established that catalase expression is also altered in cancer cells, which promotes cell proliferation by inducing genetic instability and activating oncogenes. Regulation of catalase expression is to be controlled primarily at transcriptional levels, although other mechanisms may be involved. In addition to transcription factors, such as Sp1 and NF-Y, transcription factors JunB and RARα are crucial regulators in breast cancer cells by recruiting proteins involved in transcriptional complexes and chromatin remodeling. Therefore, catalase may be an attractive therapeutic target in the context of cancer [16].

Under normal conditions the harmful effect of SOD is in balance with the antioxidant system. Antioxidant systems

work together, not in isolation, because there are interactions between hydrophilic and lipophilic antioxidants.

SODs were the first antioxidant enzymes characterized [17]. Three different types of SOD are expressed in cells: cytosolic copper-zinc SOD (Cu-ZnSOD), mitochondrial Mn-SOD and extracellular SOD – SOD (EC-SOD), all of which are able to disperse two O_2 – anions to H_2O_2 and molecular oxygen. Catalase is then responsible for detoxifying H_2O_2 into oxygen and water. These enzymes have specific distributions and functions. Their importance lies in protecting the tissues from the harmful action of various oxidants – oxygen free radicals [18].

The function attributed to catalase is dismutation of H_2O_2 into oxygen and water, which is very important in the defense of cells against oxidative damage by H_2O_2. Hydrogen peroxide is not only toxic by its ability to form other ROS, such as the hydroxyl radical by the Fenton reaction, but H_2O_2 can act as a second messenger, being involved in multiple physiological and pathophysiological processes [19]. In addition, catalase can also act as a peroxidase, thus contributing to the metabolism of small substrates, such as methanol, ethanol, azide, hydroperoxides and, in the case of ethanol, is able to oxidize it to acetaldehyde, contributing to its metabolism in the liver. Thus, catalase may have additional roles, such as detoxification or activation of toxic and antitumor compounds.

In general, endogenous and physiological reactive oxygen species (ROS) are generated mainly in the process of oxidative reaction of the mitochondrial respiratory chain as by-products of normal cellular metabolism [20]. ROS have a comprehensive influence on cell physiology. Moderate amounts of ROS have positive effects, which include the destruction of invasive pathogens, wound healing and repair processes [21].

It is generally accepted that the cellular maintenance of redox homeostasis is controlled by a complex network of antioxidant enzymes (superoxide dismutases and glutathione peroxidases) whose expression is under the control of fine-tuning the Keap1-Nrf2 signaling pathway [22]. However, the molecular mechanisms that regulate the expression of catalase – the oldest known and discovered antioxidant enzyme – are independent of this pathway and are not fully elucidated, which would allow a new approach to modulate the antioxidant status in cancer cells in particular, in cases where its expression decreases [23]. Although the mechanisms that control catalase expression have been partially elucidated, low catalase expression in cancer cells remains an unanswered question. Under stress, the antioxidant enzyme catalase plays a major role in detoxifying H_2O_2.

As it is known, oxidative stress is an imbalance between the production of ROS and their elimination through multiple protection mechanisms, the activation of which can lead to chronic inflammation. Oxidative stress can activate a variety of transcription factors, which lead to the differential expression of genes involved in inflammatory pathways. Moreover, the levels of secondary messengers, such as cGMP, cAMP, protein kinases and calcium ions, transcription factors such as NF-κB, AP-1, protooncogenes and some enzymes (iNOS, COX-2, proteases), cytokines and growth factors (IL-1β, IL-6, TNF-α, PDGF, VEGF, FGF-b, TGF-b, etc.) are known to be central in inflammatory processes. Thus, chronic inflammation is induced by biological, chemical and physical factors and is in turn associated with an increased risk of several types of human cancer and chronic diseases. The link between inflammation and cancer has been suggested by epidemiological and experimental data [24, 25] and confirmed by anti-inflammatory therapies that show efficacy in cancer prevention and treatment [26].

The fact that continuous irritation for long periods of time can lead to cancer has already described in the traditional Ayurvedic medical system (i.e. the science of long life), written 5000 years ago [27]. Whether this irritation is the same as what Rudolf Virchow called inflammation in the nineteenth century is uncertain. Rudolf Virchow first noted that inflammatory cells are present in tumors and that tumors occur in places of chronic inflammation [28]. This inflammation is considered a "secret killer" for diseases such as cancer. For example, inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, is associated with an increased risk of colonic adenocarcinoma [29, 30], and chronic pancreatitis is associated with an increased rate of pancreatic cancer.

The exact mechanisms by which a wound healing process turns into cancer are the subject of intensive research [31], and possible mechanisms include induction of genomic instability, changes in epigenetic events and subsequent inappropriate gene expression, increased cell proliferation, resistance in apoptosis, aggressive tumor neo-vascularization, tumor-associated basement membrane invasion, and metastasis [32]. The way of oxidative stress modulates these different stages of inflammation-induced carcinogenesis is currently the subject of in-depth research. Sources of inflammation are widespread and include microbial and viral infections, exposure to allergens, radiation and toxic chemicals, autoimmune and chronic diseases, obesity, alcohol consumption, tobacco use, and a high-calorie diet [33].

In general, the longer the inflammation persists, the higher risk of cancer. There are two stages of inflammation, acute and chronic inflammation. Acute inflammation is an initial stage of inflammation (innate immunity), which is mediated by activating the immune system. This type of inflammation persists only for a short time and is usually beneficial to the host. If the inflammation lasts for a longer period of time, the second stage of inflammation or chronic inflammation sets in and may predispose the host to various chronic diseases, including cancer. During inflammation, mast cells and leukocytes are recruited at the site of injury, leading to a "respiratory explosion" due to increased oxygen uptake and thus increased release and accumulation of ROS at the site of injury [34].

Indigenous CCs are compounds with antioxidant properties, therefore they could manifest potential anti-inflammatory, antineoplastic, antiaging, antimicrobial and

antiviral properties. Thus, they can find wide application in various pathological conditions and processes.

Further clarification and understanding of involved mechanisms will allow more precise definition of situations where local CC administration will prove beneficial. Such an investigation may also be useful for the development of new compounds with beneficial effects in the medication and prevention of multifactorial diseases.

Conclusions

The most informative biomarkers of antioxidant system functionality have been estimated and selected to assess the level of oxidative stress in erythrocytes on experimental exposure by administering native CCs to laboratory animals and which can be used to determine the efficacy of new native preparations.

The influence of tested CC on the indices of the antioxidant system is selective. This selectivity may be the basis of their strong selective antiproliferative and cytotoxic action on tumor cells, but not on healthy ones.

The elucidation of the molecular mechanisms underlying the action of CC broadens the theoretical knowledge about the biological properties of a number of chemical compounds and, at the same time, offers new possibilities to explore perspective objects in order to obtain new effective drug preparations.

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VP designed the research, reviewed statistics and interpreted the data, revised the data and drafted the manuscript; MG conceptualized the project and designed the research, drafted the manuscript; VP conducted/ perfomed the laboratory work, revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative; the authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research protocol was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 73 of 26.04.2017) and the tests have been done according to the contemporary principals in biological standardization of experiences and Declaration of Helsinki with further amendments (Somerset West Amendment, 1996).

Conflict of Interests
No competing interests were disclosed.

REVIEW ARTICLES

https://doi.org/10.52418/moldovan-med-j.64-5.21.14
UDC: 546.27:616.71/.72

The role of boron in the functioning of the osteoarticular system

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Manuscript received June 26, 2021; revised manuscript November 04, 2021; published online November 26, 2021

Abstract

Background: Boron was classified by the World Health Organization as 'possible essential elements for human health'. It can be found in different forms in the environment, has beneficial effects on bones and its deprivation can impair calcium and magnesium metabolism and bone development, their health and maintenance. Dietary boron supplementation can help prevent and manage degenerative and inflammatory bones and articular diseases, such as arthritis, osteoarthritis, rheumatoid arthritis and osteoporosis. Relevant articles were identified in PubMed, Google Scholar, ProQuest, and Scopus databases published until June 2021, describing the role of boron in the functioning of the osteoarticular system.
Conclusions: From organic and inorganic boron forms that can enter the human body, organic plant based boron compounds are highly bioavailable. In the functioning of the osteoarticular system boron acts by regulation of calcium and magnesium metabolism, enhancing the vitamin D activation process and influencing serum steroid hormone levels. A boron intake equal to or higher than 3 mg per day can help prevent/or correct arthritis, osteoporosis and osteoarthritis. Calcium fructoborate can reduce pain, joint discomfort and increase endogenous vitamin D level in patients with osteoarthritis. Boron may play a role in pathophysiology of rheumatoid arthritis and its severity and a supplementation with boron element may be useful.
Key words: boron, boron containing compounds, osteoarticular system, osteoarthritis, rheumatoid arthritis.

Cite this article
Racu M-V, Scorei IR, Pinzaru I. The role of boron in the functioning of the osteoarticular system. *Mold Med J.* 2021;64(5):76-80. https://doi.org/10.52418/moldovan-med-j.64-5.21.14.

Introduction

It is supposed that trace element boron (B) is essential for human health. There is, however, clear evidence that dietary intakes of this element are beneficial to humans. In humans, boron deprivation impairs calcium metabolism and bone health as well as brain function and energy metabolism [1].

B, which was described as a trace element until 1980, was included in the class of 'possible essential elements for human health' by the World Health Organization (WHO). There is also evidence that boron plays a role in the maintenance of healthy bones and joints. It has beneficial effects on bones, especially trabecular and alveolar ones, in ways they are independent of any other nutritional stressor [2].

Following the existing findings, boron is beneficial, if not essential, for trabecular microarchitecture that promotes bone strength through affecting osteoblast and/or osteoclast presence or activity. Boron deprivation decreased bone volume fraction, trabecular thickness and impaired alveolar bone repair [3].

Also, B plays an important role in osteogenesis, and its deficiency has been shown to adversely impact bone development and regeneration. Diet supplementation with B has repeatedly been shown to markedly reduce urinary excretion of both calcium and magnesium and to increase serum levels of estradiol and calcium absorption in peri- and postmenopausal women. This trace element also beneficially impacts vitamin-D utilization [4].

B is widely distributed in the environment combined with oxygen to form compounds called borates. They are found in soils and rocks and also, in different kinds of water. Because they are essential for plants, borates occur naturally in fruits and vegetables and elsewhere in the food chain [5]. Following a database created to assess boron concentration in different foods and beverages from the American diet, the top 2 contributors were coffee and milk, but fruits, vegetables and nuts are also rich in this trace element [6]. Thus, the main sources of boron in the diet are drinking water and food products rich in boron.

Considering the growing interest in boron as a possible essential element for human health, its daily intake and

possible nutritional sources, B influence on osteoarticular system and a lack of research about boron in Moldova, it was decided to search the information about the role of boron in the functioning of the osteoarticular system in order to do a further research about its consumption impact on the osteoarticular health of the Moldovan population.

It was necessary to find out what the PubMed, Google Scholar, ProQuest, and Scopus databases published until June 2021 using the following key terms: "boron", "boron compounds", "organic boron compounds" combined using the Boolean operator AND "osteoarticular diseases", "rheumatoid arthritis", "osteoarthritis". The reference list from the resulting publications was manually searched for any relevant trials with elimination of results not related to the article topic.

Results and discussion

The purpose of this review is to present the role of boron in the functioning of the osteoarticular system, alongside with its dietary sources and active compounds in the form of which it can be found.

Boron is considered to be a biological trace element but there is substantial and growing support for it to be classified as an essential nutrient for humans, depending on its speciation. Similar to other elements, boron doesn't act on its own but as a part of various molecules [7].

Active boron compounds and dietary sources

This trace element can be found both in an inorganic and organic form. The last one, organic boron containing compounds (BCCs), is essential for human health, being involved in the cellular metabolism. Plant based organic BCCs such as sugars and polyalcohol borate esters are very important in the human nutrition. It can be used in cell metabolism during which they are at least partially transformed into boric acid that will subsequently be eliminated as waste [7].

Boric acid and borates are inorganic BCCs found in soils and employed by plants and bacteria for the manufacture of all known B organic natural compounds. It has been known for a long time that B deficiency in soils, leads to depletion of BCCs in fruits and vegetables in the food supply, was correlated with a high incidence of arthritis [7].

There are three main sources that supply B for humans: drinking water, vegetable foods (mainly fruits and vegetables) and products daily used for personal care (soaps, lipsticks, shampoo, skin cream, gastric antacids, cosmetics, detergents, contraceptives and estrogen supplements). These sources, on average, supply around of 0.6, 1.0, and 0.5 mg B a day per person, respectively [8].

Also, since 1990, two more important sources of boron, such as supplements and nutraceuticals have appeared. They can supply between 3 and 10 mg of B per day. The essentiality of B in humans is not yet widely accepted, but the scientific information indicates that to ensure an adequate nutrition, humans require between 2 and 6 mg B per day for one person [8].

According to recently published research, people older than 40 can prevent/or correct arthritis, osteoporosis and osteoarthritis by taking B equal to or higher that 3 mg per day. In the countries following the famous healthy Mediterranean diet that includes staple foods rich in B such as grape, broccoli, garlic, tomato, pomegranate and olives combined with the consumption of drinking water with high levels of B, daily intake of this trace mineral frequently results higher than 13 mg per day [8].

On average, dried fruits, nuts and avocados contain between 1 and 4.5 mg of boron/100 g. Fresh fruits, vegetables, and honey, contains between 0.1 to 0.5 mg boron/100 g, whereas animal foods provide only 0.01 to 0.06 mg of boron/100 g. Another important source of boron is water, and the content varies according to geographic location [9].

Osteoarticular diseases in which boron may be involved

Epidemiologic evidence suggests that in the areas of the world where boron intakes usually were 1 mg or less / day, the estimated incidence of arthritis ranged from 20 to 70%. On the other hand, in areas of the world where boron intakes were usually 3 to 10 mg, the estimated incidence of arthritis ranged from 0 to 10% [10].

In the diet, many people do not consume more than one milligram of B a day, which may be a clinical concern. Soils are depleted, and certain fertilizers inhibit boron absorption into plants. Low boron levels were correlated with higher serum levels of rheumatoid factor (RF), the antibody that causes destruction of the joints in rheumatoid arthritis, and in osteoarthritis, which were correlated with disease duration and severity [11].

In the 1980s, boron was discovered to play a role in regulating mineral metabolism (such as calcium and magnesium) and enhancing the vitamin D activation process in humans. These discoveries led to the hypothesis of using boron both in the prevention of osteoporosis and in the treatment of osteoarthritis. Although, studies assessing these purposes are at preliminary stages, reports are promising [12].

It was found that boron is located almost entirely and exclusively within the mineral portion of bone. Boron is suggested to affect bone mineral by influencing serum steroid hormone levels (estrogen and testosterone) and the metabolism and utilization of calcium and other mineral elements of bone. Some studies suggest that calcium fructoborate, when administered orally, is effective in reducing discomfort and stiffness associated with osteoarthritis disorders, and bone loss [13].

Calcium fructoborate is sugar-borate ester which is a complex of calcium, fructose, and boron found naturally in fresh and dried fruits, vegetables and herbs, and wine [14].

A growing number of researches indicate that the calcium fructoborate significantly reduces serum levels of the C-reactive protein in humans, suggesting that it may contribute to bone health by controlling the inflammation associated with loss of bone mineral density [14].

In one study which was performed on 53 postmenopaus-

al women aged 55–60 living in Turkey, where the subjects are naturally exposed to high (≥1 mg/L) or low (< 1 mg/L) boron concentration in drinking water, it was detected that serum concentration of osteocalcin, a sensitive and specific indicator of osteoblast function, together with dietary boron may play a role in bone metabolism. According to this study, daily boron intake of 1 mg/L and more through drinking water may affect bone metabolism in postmenopausal women positively [15].

Chronic and granulomatous inflammation (including that associated with osteoarticular diseases) was decreased in a rat model through the combination of boron with dexamethasone [16].

Inadequate boron intake is involved in inflammatory processes, including joint swelling, restricted movement, as well as body temperature, antibody production, blood hemostasis, serine protease (which is linked to platelet aggregation), activity of lipoxygenase (an enzyme that helps control inflammation), and metabolism of leukotrienes, chemical mediators of inflammation [17].

Below will be described the boron role in preventing osteoarticular diseases, such as osteoarthritis and rheumatoid arthritis. Its implication in osteoporosis will be avoided because this disease is multifactorial, and the role of boron is difficult to follow.

Boron and osteoarthritis

Osteoarthritis is a chronic disease that involves progressive destruction of articular cartilage which results in impaired joint motion, severe pain, and, ultimately, disability. This disease affects mostly elderly people [18].

Alongside with nonpharmacological interventions (weight loss, education programs, exercise, and so on) and pharmacological treatments (paracetamol, nonsteroidal anti-inflammatory drugs [NSAIDs], nutraceuticals and functional foods could provide an advantageous alternative. These nutraceuticals and functional foods that contain boron may have a beneficial effect in osteoarthritis too [18].

Joint discomfort is a common complaint in osteoarthritis which is associated with limited joint function, decreased feelings of energy and decreased quality of life. Pietrzkowski Z. et al. reported, after conducting placebo-controlled clinical study on 60 participants with self-reported knee discomfort, that supplementation with 110 mg calcium fructoborate twice per day was associated with improving knee discomfort during the 2 weeks of intake [19].

Another study has shown that boron levels, along with those of lead and zinc, were reduced in patients with osteoarthritis of the hip when those patients went for hip replacement. Also, the level of demineralization in the osteoarthritis patients is greater than in those who don't suffer from this disease [20]. So, boron key role played in the chemical make-up of bones and joints through its effects on calcium metabolism is very important fact for prevention and treatment of osteoarthritis [21].

Naza M. A. et al. released a clinical evaluation and measurement of serum boron concentration, erythrocyte sedimentation rate and total white blood cell count of 43 patients with knee OA. The results were compared with those of healthy individuals. After these, the authors concluded that serum boron levels were significantly lower in patients with knee OA, and negatively correlated with the duration and severity of the disease [22].

Another study researched the influence of calcium fructoborate (commercially marketed under the trade name FruiteX-B®) on symptoms associated with OA conditions. In this study, calcium fructoborate was tested for fourteen days at a serving of 108 mg twice a day on subjects diagnosed with minor osteoarthritis conditions of the knees by CT scan. Blood level of C-Reactive Protein in 7 out of 10 subjects was found reduced up to 37% compared to day 1 baseline levels. Also, the study showed that blood level of endogenous 1.25(OH) vitamin D was increased more than 19% compared to baseline [23].

In a double-blind study conducted in Australia, 20 patients with confirmed osteoarthritis were given a placebo or a supplement providing 6 mg of boron daily for 8 weeks; 15 patients completed the study. Of the 7 patients consuming the boron supplement, 5 reported improved subjective measures for their arthritic condition (e.g., less pain on movement), whereas only 1 of 8 patients consuming the placebo reported an improvement in their arthritic condition [24].

In two clinical cases which were presented by Hesselink J. M. and Russell A. L. in order to reveal the role of boron salts (fructoborate) in the treatment of symptoms in osteoarthritis was demonstrated the effectiveness of boron in treating the pain of osteoarthritis to a level of good control and that when taken long term it prevents exacerbations of pain [25].

Following the USA nutritional protocol for osteoarthrosis, for patients it is recommended a boron supplementation of 6 mg of B a day [26].

Boron and rheumatoid arthritis

Rheumatoid arthritis (RA) is a common, systemic autoimmune disease of unknown cause that primarily affects the peripheral joints in a symmetric pattern. It is characterized by chronic inflammation of the synovial joints which can lead to progressive joint destruction including symmetric joint swelling with stiffness, warmth, tenderness and pain and affects approximately 1-2% of the general population worldwide [27].

Trace elements may play a critical role in the onset, progress and curing of the disease. Some studies have reported reduced serum selenium, magnesium, zinc, elevated serum copper in RA patients. A cross sectional study which enrolled 107 RA patients and 214 controls found a significant low serum boron level in patients with rheumatoid arthritis. Rheumatoid factor titer was significant predictor of low serum boron level. This may suggest that boron element may play a role in pathophysiology of RA and its severity and a supplementation with boron element and diets rich in fruits, vegetables, nuts, and pulses may be useful [27].

Following the results of a double-blind randomized pla-

cebo-controlled clinical trial with 60 days supplementation period with calcium fructoborate and sodium tetraborate for 72 Iraqi patients with active rheumatoid arthritis (RA) maintained on etanercept, boron as an adjuvant has potentiated therapeutic outcomes in RA patients by improvement of the clinical scores and significantly decreases the inflammatory markers in RA patients [28].

The safety of boron supplements when used in pharmacological doses as adjuvant with etanercept in treatment of RA patients was studied and the results showed that boron supplements – calcium fructoborate and sodium tetraborate have no negative impact on erythrocyte sedimentation rate, hemoglobin, white blood cells, platelets count, hepatic and renal functions [29].

Although the role of boron in functioning of osteoarticular system has been investigated in numerous studies, at this moment more research at this subject is needed in order to recommend boron as an essential trace mineral for bones, joins and human body in general.

Following this aim, the next step is to study the link between different boron concentrations in drinking water from the different regions of the Republic of Moldova and osteoarticular morbidity (by osteoarthritis and rheumatoid arthritis) of the population.

Conclusions

1. Boron can enter the human body in an organic and inorganic form. The organic plant based boron compounds are highly bioavailable.

2. In the functioning of the osteoarticular system boron acts by regulation of mineral metabolism (such as calcium and magnesium), enhancing the vitamin D activation process and influencing serum steroid hormone levels.

3. A boron intake equal to or higher than 3 mg per day can help prevent/or correct arthritis, osteoporosis and osteoarthritis.

4. Boron, especially calcium fructoborate, can reduce pain, joint discomfort and increase endogenous vitamin D level in patients with osteoarthritis.

5. Boron may play a role in pathophysiology of rheumatoid arthritis and its severity and a supplementation with boron element may be useful.

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Authors' contribution
MVR designed the trial and drafted the first manuscript; IRS and IP interpreted the data and revised the manuscript critically. The authors revised and approved the final version of the manuscript.

Funding
This study was supported by the National Agency for Public Health of Moldova and the BioBoron Research Institute from Craiova, Romania. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
No approval was required for this study.

Conflict of Interests
The authors declare no conflict of interests.

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6. The tables and figures must be typed, consecutively numbered and followed by an explanatory text. The figures that have to emphasize a comparison or details are published in color. If colored figures are to be placed, the author must pay an additional fee of €100 per page (1-8 figures on a page).

7. The references are to be listed in order of their appearance in the text, and the appropriate numbers are to be inserted in the text in square brackets in proper places.

The list of references should contain more than 50% in Scopus or WoS, more than 80% with DOI and not more than 30% of monographs or conference abstracts.

The references must comply with the general format outlined in the Uniform Requirements for the Manuscripts Submitted to Biomedical Journals developed by the International Committee of Medical Journal Editors (www.icmje.org), chapter IV.A.9.

The references in the Cyrillic script should be transliterated into Latin script using the American Library Association and Library of Congress Romanization Tables as follows: А=A, Б=B, В=V, Г=G, Д=D, Е=E, Ё=E, Ж=ZH, З=Z, И=I, Й=I, 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=SHCH, Ъ=", Ы=Y, Ь=', Э=E, Ю=IU, Я=IA.

Immediately after the transliteration the translation of the title in English in the square brackets should follow. For example: Ivanov IV, Shchukin NF, Men'shikov VM, Ad"yunktov AM. Transplantatsiia organov i tkanei [Transplantation of organs and tissues]. Vestnik Khirurgii. 2010; 26(6):45-49. Russian.

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