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Welcome to the Moldovan Medical Journal!

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.

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ORIGINAL RESEARCHES

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Causes of excess mortality in the Republic of Moldova as compared to the European model

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Abstract

Background: Disregarding the recent rise in life expectancy in Moldova, the gap with Western countries is very high. The aim of the study is to identify the causes of death and the age groups responsible for excess mortality in Moldova in relation to the European average model in 2001-2019. Mortality beyond the European model set as a threshold was considered excessive.
Material and methods: Data were retrieved from the Human Cause-of-Death Database and the WHO mortality database. Multiple decrement life tables by cause were computed for Moldova and the model (Germany, England and Wales, Czech Republic, Poland).
Results: 27% of all deaths in males and 13% of all deaths in females under the age of 70 are excessive compared with the model. 80% of excess deaths were attributable to cardiovascular and digestive diseases (both males and females) and external causes (males). Excess deaths were mainly concentrated between the ages of 40 and 70 in men and 50 and 80 in women. Over the study period, cardiovascular diseases contributed the most to the decline in excess mortality in females, but not males. For the latter, excess mortality increased because of the cardiovascular component, completely compensating for moderate progress in external causes of death.
Conclusions: Narrowing the life expectancy gap between Moldova and Western countries should be possible through better control of the key risk factors behind the identified causes of death.
Key words: causes of death, excess deaths, life expectancy, cardiovascular component.

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Introduction

Life expectancy at birth in Moldova, despite its wide fluctuations in the 1980s and 1990s, failed to progress much in the total over the last half a century. After 55 years of evolution, male life expectancy in 2019 (66.6 years) was just one year higher than in 1965. Female life expectancy gained 5.0 years over the same period and reached 75.0 years in 2019. Most of these gains have occurred in the recent period (since 2010 in men and 2005 in women) [1]. The general stagnation in life expectancy in Moldova contrasts strongly with the progress made in western countries. The difference in life expectancy between Moldova and Japan is currently 15 years for males and 13 years for females, while between Moldova and France, it is 13 and 11 years respectively. The same long-term unfavourable trends were observed in other former Soviet Union (FSU) countries, followed by recent improvements started in the 1990s – early millennium [2-4].

Demographic experts have emphasised that the gap between FSU countries and the western world is not "generalized", but concerns a very specific range of causes of death and age groups [5]. These groups can be considered as risk groups responsible for high mortality in these countries compared to western countries. To identify them, it is useful to compare the mortality pattern in a problem country (Moldova) with that of a western country or an average model computed for a group of countries with a high life expectancy. The selected western country or model is set as the threshold and its exceedance may be considered as *excess mortality* [6]. If a problem country succeeds in reducing excess deaths from the identified causes, then it will succeed in reducing the gap in life expectancy.

The paper is aimed at identifying the age- and cause-specific components of excess mortality in Moldova compared to the European model. The research questions are as follows:

1. What are the age groups and causes of death responsible for the excess mortality in Moldova relative to the selected European model?

2. How has excess mortality by age and cause changed in Moldova since 2001?

Material and methods

Mortality data were retrieved from the Human Cause-of-Death (HCD) Database that provides access to reconstructed mortality time series with a constant classification of causes according to the 10th revision of the International Classification of Diseases (ICD-10) [7]. The mortality data were retrieved for Moldova (2001-2014), the Czech Republic (2001-2017), Poland (2001-2016), Germany (2001-2016) and England and Wales (2001-2019) according to an intermediate list of causes of death, identical for all countries. Reconstructed time series were extended with WHO mortality data until 2019 [8]. As the selected countries were seriously affected by the COVID-19 pandemic [9], 2019 was used as the last year of observation instead of 2020. Mortality data were aggregated by seven major causes of death. Ill-defined causes of death (R00-R99 under ICD-10) were redistributed proportionally. Population counts were extracted from the HCD and WHO mortality databases. For Moldova, were used the intercensal estimates [1] and since 2014 the official post-census usually resident estimates [10].

The model was computed as a simple mean of age- and cause-specific death rates for the Czech Republic, Poland, Germany and England and Wales. The mean of death rates weighted by population counts were not applied so that the experience of each country would be equally reflected in the model. Life tables and multiple decrement life tables were computed for each country and the model for 2001-2019.

Life expectancy at birth and in age x is calculated with the help of a life table for a hypothetical cohort (the radix of the life table) usually equal to 100000. In a life table, the membership in a cohort is terminated by a single attrition (exclusion) factor, i.e., a death. In a multiple decrement life table, there are two and more attrition factors, for example, a death due to different causes i (i_1, i_2, i_n) [11].

Life table deaths or table deaths (d_x) in the age group x are the function of a life table. Life table deaths due to a specific cause i $(d_{x,i})$ were computed as follows:

$$d_x \times C_{xi} \quad (1)$$

Where C_{xi} – the proportion of deaths from a cause i in the age group x in the total of deaths;

d_x – life table deaths in the age group x.

Excess deaths due to a cause i were computed as the difference between d_{xi} for Moldova and the model:

$$d_{xi}^{Moldova} - d_{xi}^{Model} \quad (2)$$

Life table deaths unlike *observed deaths* are not influenced by the population age structure and reflect the intensity of mortality in the compared populations. Table deaths by age or/and by cause computed for two populations can be safely compared. The sum of table deaths from all causes and in all ages is equal to the radix of the life table, and the differences between the sum for Moldova (100000) and the sum for the model (100000) is equal to zero. The statistical package R was used.

Results

Figure 1 illustrates life expectancy at birth in Moldova compared with that of four European countries and the model for the 2001-2019 periods. In Moldova, the indicator remained stagnant until 2005 in females and 2010 in males, followed by moderate growth. Between 2001 and 2019, life expectancy increased by 2.7 years in males (from 63.9 to 66.6) and 3.6 years in females (from 71.4 to 75.0). In 2019, Moldovan women lived 7.0 years less than in Poland or the Czech Republic and even 9.0 years less than in England and Wales. For males, the gap was varied between 7.0 years (Poland) and 13 years (England and Wales). Between 2001 and 2019, the gap between Moldova and the model increased from 9.0 to 11.0 years in males, while in females, it varied around 8.0 years.

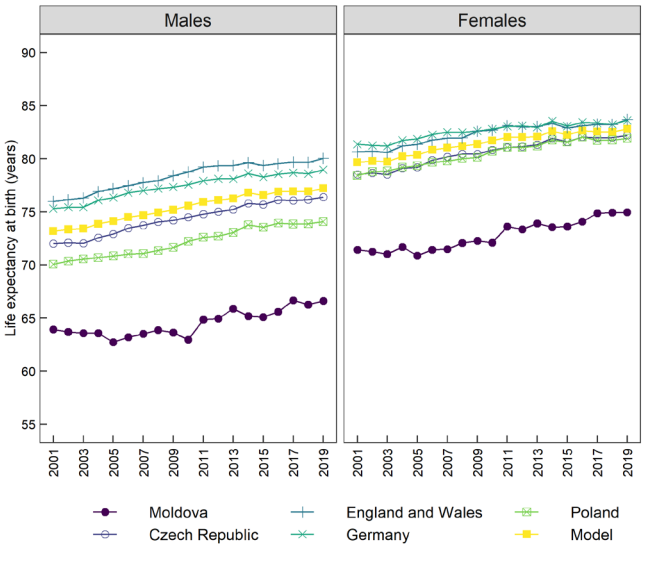

Fig. 1. Life expectancy at birth in Moldova compared to four European countries and their average model, 2001-2019, by sex

Figure 2 shows the distribution of life table deaths by age from all causes d_x in Moldova and the model. In the model, table deaths were shifted towards older age groups, especially to the last age group 85+. While in the model, in 2019, 64% of deaths in men and 80% in women were concentrated in the age group 75 years and over, in Moldova, the corresponding figures were 36% and 62%. The impact of the last age group was especially evident in females. In 2019, more than 50% of life table deaths in females were attributed to this age group in the model compared to 25% in Moldova. Table deaths in Moldova were excessive compared to the model (the difference was more than zero) mainly in the age interval between 30 and 70 years old in males and between 50 and 80 years old in females. In the model, the changes

from 2001 to 2019 were characterised by a redistribution of table death from older age groups (60+) to more advanced ages, particularly 85+. The similar dynamics, although far less pronounced, was observed among Moldovan females. At the same time, no important changes were found in Moldovan males, where the two curves in 2001 and 2019 almost overlapped.

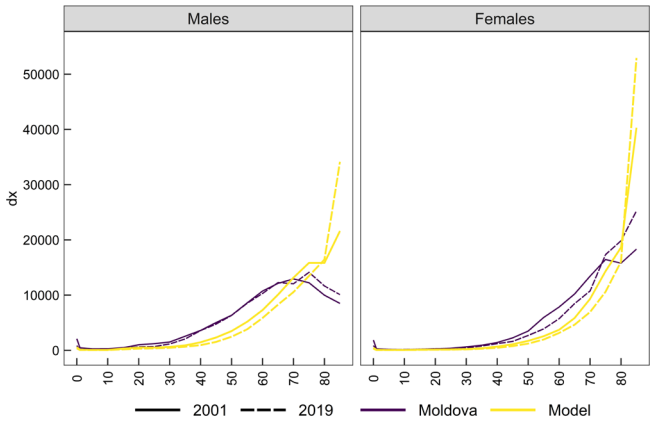

Fig. 2. Distribution of life table deaths from all causes in Moldova and the model in 2001 and 2019, by age and sex

Note: The sum of life table deaths per year is equal to 100000 (life table radix)

Table 1 illustrates the distribution of life table deaths and excess deaths $d_x^{Moldova} - d_x^{Model}$ in 2001 and 2019 by cause for the age group before 70. The data refer to the life table radix equal to 100000. The latter can be interpreted as 100000 newborns or 100000 persons dying from all causes and in all ages (hypothetical cohort). In Moldova, from 100000 male newborns, 52057 will die before 70 if age-specific death rates remain the same as in 2019 during their life (or 52%). Among females, this figure was 26813 or 27%. Between 2001 and 2019, the number of table deaths from all causes decreased insignificantly in males (8%), while in females, the reduction was 26%. In 2001 and 2019, the distribution of life table deaths by cause was characterised by the predominance of cardiovascular diseases followed by neoplasms, external causes in males and digestive diseases.

Excess deaths constituted 26686 in males and 13352 in females per 100000 deaths. It means that in Moldova, for every 100000 deaths about 27 thousand deaths in males and 13 thousand deaths in females can be considered as an excess if compared to the model. Taking into account that the radix of life table (100000) is the number of a hypothetical cohort, one can say that 27% of male newborns and 13% of female newborns in Moldova die under the age of 70 excessively compared with the model. In 2019, cardiovascular diseases accounted nearly for every second excess death (45% in males and 55% in females). Among males, the other two leading causes were digestive diseases (17%) and external causes of death (17%). Among females, excess deaths from digestive diseases contributed 26% to overall excess mortality. The impact of other causes, including neoplasms, was 10% or less. Over the study period, excess mortality increased in males by 17%, while in females, it reduced by 28%. Excess deaths decreased for all causes except for neoplasms for both sexes and cardiovascular diseases in males. In 2001, excess deaths from neoplasms were even negative, which means that under-70 mortality from this cause was lower in Moldova than in the model (the values kept negative until 2008 for both sexes).

Table 1. Life table deaths $d_{x,i}^{Moldova}$ and excess compared to the model deaths $d_{x,i}^{Moldova} - d_{x,i}^{Model}$ under 70 years old by cause and sex in Moldova per 100000 newborns, 2001 and 2019 (abs, %)

Cause of death	Life table deaths		Excess deaths	
	2001	2019	2001	2019
Males				
Infections	1983 (4%)	1093 (2%)	1661 (7%)	827 (3%)
Neoplasms	9120 (16%)	10966 (21%)	-1905 (-8%)	2620 (10%)
Cardiovascular	19623 (35%)	19039 (37%)	7963 (35%)	11964 (45%)
Respiratory	4384 (8%)	3680 (7%)	2841 (12%)	2026 (8%)
Digestive	7187 (13%)	6694 (13%)	5163 (23%)	4575 (17%)
External	10358 (13%)	7755 (15%)	6472 (28%)	4465 (17%)
Other	3674 (7%)	2829 (5)	589 (3)	210 (1%)
All causes	56328 (100%)	52057 (100%)	22782 (100%)	26686 (100%)
Females				
Infections	525 (1%)	472 (2%)	348 (2%)	323 (2%)
Neoplasms	6419 (18%)	7031 (26%)	-1197 (-6%)	708 (5%)
Circulatory	15242 (42%)	9991 (37%)	10273 (55%)	7391 (55%)
Respiratory	1456 (4%)	1020 (4%)	659 (4%)	67 (0%)
Digestive	6010 (17%)	4523 (17%)	5055 (27%)	3537 (26%)
External	3044 (8%)	1571 (6%)	2088 (11%)	696 (5%)
Other	3303 (9%)	2205 (8%)	1384 (7%)	630 (5%)
All causes	35998 (100%)	26813 (100%)	18610 (100%)	13352 (100%)

Figure 3 illustrates the distribution of life table deaths by age in males for cardiovascular diseases, digestive diseases, external causes and neoplasms. Among Moldovan males, the table deaths from cardiovascular diseases began to increase at 40 years old with the peak around age 70, followed by a decline. In the model, the increase in the corresponding curve was much smoother, with a peak at 85+. For digestive diseases, the divergence between Moldova and the model began even from 20 years old, while for external causes even from age 0. For the latter two causes, the increase in Moldovan mortality was very abrupt with a peak at age 50 years old. In the model, male mortality even from external causes was shifted to the most advanced age group; although, two classical peaks in the age groups around 20 and 50 years old were preserved. The distribution of table deaths from neoplasms among Moldovan and European males up to the age of 60 was nearly identical. However, after this age, the concentration of deaths declined sharply in Moldova but continued to grow steadily in the model.

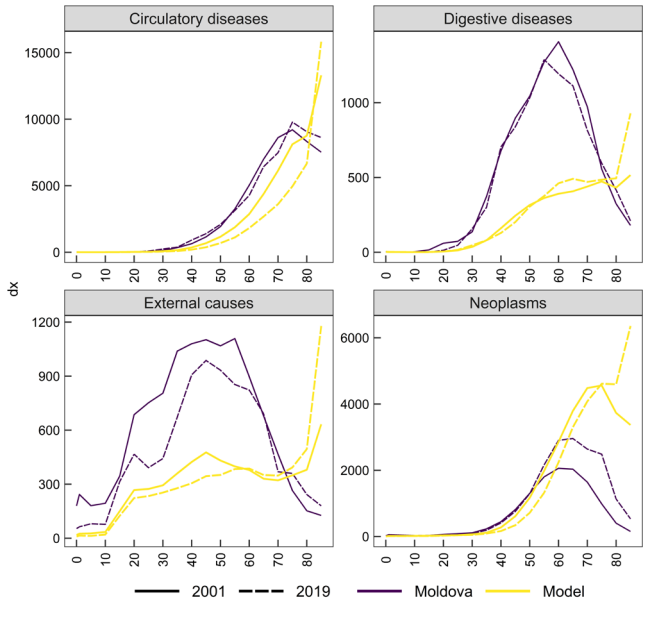

Fig. 3. Distribution of life table deaths by age from circulatory diseases, digestive diseases, external causes of death and neoplasms in Moldova and the model in 2001 and 2019, males

Table deaths from cardiovascular and digestive diseases among Moldovan males in 2001 and 2019 had the same age profile. Deaths from external causes decreased in young and middle-aged people, while table deaths from neoplasms increased after age 50 in 2019 relative to 2001, but the age pattern remained unchanged.

Discussion

This study addressed the issue of excess mortality in Moldova compared to the European model in 2001-2019. The selected model let us take into account not only the experience of the Western European countries (Germany and England and Wales) benefited from the cardiovascular revolution in the 1970s [12] but also that of the Central European countries (the Czech Republic and Poland) where the health improvements started much later [13]. Despite recent advances in population health in Moldova, the gap in life expectancy with the model widened among men and stagnated among women. Comparing the distribution of life table deaths in Moldova to the model, excess deaths by age and cause were identified. These age groups and causes of death can be considered as risk groups of excess mortality, as they are responsible for Moldova's lag behind the European model.

The distribution of life table deaths by cause reflected the cause-specific mortality profile in the country, with cardiovascular diseases and neoplasms as the two leading causes of death [14]. However, the distribution of excess deaths showed another pattern. The lion's share of excess deaths was attributable to cardiovascular diseases, digestive diseases (both sexes) and external causes of death (only in males). In other studies, the same groups of causes of death were identified as the key factors responsible for unfavourable long-term trends in Moldovan life expectancy [1, 15]. Neoplasms did not play a crucial role in the formation of excess mortality in Moldova as compared to the European model, although this pathology is the second most common cause of death. However, neoplasms have begun to contribute to excess under-70 mortality since 2008. It is highly likely that the negative impact of cancer mortality will only increase in the future, as adverse trends in Moldova stand in contrast to progress in advanced countries [16, 17].

The obtained findings showed that the recent rise in life expectancy at birth in Moldova was accompanied by a decrease in excess mortality among women, but not among men. Among females, cardiovascular component was the main contributor to this progress. The shift in the distribution of table deaths towards older ages is a good sign for sustainable growth in female life expectancy in the future. At the same time, men have advanced mainly in mortality from external factors, while the increase in cardiovascular mortality continues to widen the gap between Moldovan and European men.

Conclusions

High adult mortality from cardiovascular diseases, digestive diseases (both sexes) and external causes of death (males) played a crucial role in the formation of excess mortality in Moldova compared to the European model. Excess table deaths from these causes were mainly concentrated between the ages of 30 and 70 in men and 50 and 80 in women. Over the study period, excess deaths among women fewer than 70 years of age decreased primarily due to cardiovascular diseases. Among men, excess mortality increased at the expense of the cardiovascular component, fully offsetting progress in external causes of death. Reducing the major risk factors behind the identified risk groups is expected to reduce excess mortality and hence the gap with Western countries.

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Authors' contributions

OP conceptualized the idea, conducted literature review, collected the data, interpreted the data and wrote the manuscript; GO collected the data and revised the manuscript critically. ER collected the data and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate

No approval was required for this study.

Conflict of Interests

There is no known conflict of interests to declare.

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Audit of radiology reports of patients with neoplasms performed on computed tomography

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Abstract

Background: Evaluation of the peculiarities of radiology reports of patients with neoplasms of the abdomen and pelvis to optimize clinical imaging management.
Material and methods: The study included 104 patients, divided into two groups, experimental group (L2) and control group (L1), repeatedly investigated by computed tomography in 2009-2019, a total of 440 examinations, of which 120 prospective examinations and 320 retrospective examinations, aged between 25 and 85 years, with primary tumors of the abdomen and pelvis.
Results: Four specific descriptive criteria were analyzed from radiology reports and were obtained the following results: specification of the scanning protocols in radiology reports (0% for L1 and 95.3% for L2), specifying the reason for the examination in radiology reports (100% for L1 and 26.0% for L2), use of international terminology in radiology reports (0% for L1 and 74% for L2), patient follow-up in radiology reports (88.5% for L1 and 59.8 % for L2).
Conclusions: Following the SWOT analysis of the radiology reports, has been developed a standardized model for describing CT images of patients with oncological pathologies of the abdomen and pelvis. The decision-making process was built on Strengths, eliminating Weaknesses, exploiting Opportunities and removing Threats.
Key words: computed tomography, oncology, radiology report, imaging management; follow-up.

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Introduction

Quality has become a predominant part of our lives. People are constantly looking for quality products and services. The existence of this desire for quality has led organizations around the world to consider it an essential component of any product and service. Quality is a tool of strategic differentiation to sustain competitive advantage. The quality of medical services is more difficult to define and measure than in other sectors. Distinctive features of the medical industry, such as intangibility, heterogeneity and simultaneity, make it difficult to define and measure quality. However, the quality of healthcare services depends on the service process and the interactions between doctors and patients [1, 2]. Some attributes of healthcare quality, such as timeliness, consistency, and accuracy are difficult to measure beyond a subjective assessment of the patient. Quality standards are more difficult to set in service operations. Healthcare professionals offer different services because the factors differ, such as experience, individual and personal skills [3].

Quality healthcare is a subjective, complex and multidimensional concept. Donabedian A. defined the quality of healthcare as the application of medical science and technology in a manner that maximizes its health benefits without adequately increasing the risk [4]. He distinguishes three components of quality: technical quality, interpersonal quality and facilities. Technical quality refers to the efficiency of health care. Interpersonal quality refers to the degree of satisfaction of the patient's needs and preferences. Facilities include environmental factors and the good organization of the provision of medical services [5]. Relating these notions to the topic of this study, the quality of the radiology report depends on: the quality of the medical equipment used to examine the patient, patient's loyalty for cooperation and ensuring optimal management conditions for each patient in the Radiology Department.

Material and methods

To study the quality of the radiology report, patients with oncologic pathology of the abdomen and pelvis were examined. In order to achieve the purpose and objectives of the research, a controlled clinical diagnostic study was planned. The required number of patients for research was calculated using the following formula:

$$n = \frac{1}{(1-f)} \times \frac{2(Z_\alpha + Z_\beta)^2 xP(1-P)}{(P_o - P_1)^2}$$

where:

Po = According to the bibliographic data, the success of detecting the diagnosis of abdominal cancer by applying the traditional imaging algorithm is on average 63% (P_o = 0.63);

P_1 = In the research group the patients with abdominal cancer that will be investigated by the modified imaging algorithm, the success of the detection is assumed to be in 90% of cases (P_1 = 0.90);

$P = (P_0 + P_1) / 2 = 0.77$;

Zα – tabular value. When the statistical significance is 95.0%, then the coefficient Zα = 1.96;

Zβ – tabular value. When the statistical power of the comparison is 90.0%, then the coefficient Zβ = 1.28;

f = Proportion of subjects waiting to drop out of the study for reasons other than the investigated effect;

q = 1 /(1-f), f = 10.0% (0.1).

By entering the data in the formula were obtained 52 patients.

Criteria for inclusion in research:

- Adult patients (> 18 years) with abdominal neoplasms who have signed the informed consent to participate in the study;
- Patients with abdominal neoplasms in stages I, II, III in the preoperative phase, postoperative phase and undergoing chemotherapy.

Exclusion criteria from the research:

- The patient's desire to leave the study;
- Uncooperative patients;
- Patients included in other ongoing clinical trials;
- Patients with abdominal neoplasms undergoing symptomatic treatment (stage IV).

In the absence of a standard scan or description of patients with oncological pathologies of the abdomen and pelvis, it was considered necessary to report the data of the experimental group (L2) to a control group (L1), with subsequent comparison of the study results and useful practical recommendations of the descendants of the experimental group. Thus, two groups were created, 52 patients in each group, a total of 104 patients. The similarity of the groups being ensured in terms of age, sex, degree of disease activity, imaging parameters, the characteristics were evenly distributed; the batches were differentiated only from a geographical point of view, being considered alternatively, depending on the interest of the analysis, as experimental group (L2) and control group (L1).

The L1 research study included a number of 52 patients, repeatedly investigated by CT during 2009-2018, a total of 313 examinations, all of which were retrospective examinations. The average number of CT examinations recorded for a patient at the level of that group was 6.0 units +\- 0.58 units with a minimum value of 1.0 units and a maximum value of 20.0 units. The structure of the group according to sex was predominantly male (27 men, representing 51.9%), women – 25, representing 48.1%. The average age recorded in the group was 65.8 +\- 1.32 with a minimum of 39 years and a maximum of 85 years, with primary tumors of the esophagus, stomach, duodenum, pancreas, colon, rectum, cervical canal, ovaries, prostate, bladder, urethra.

The L2 research study included a number of 52 patients, repeatedly investigated by CT during 2013-2019, a total of 127 examinations, of which 120 prospective examinations and 7 retrospective examinations. The average number of CT examinations registered for a patient at the level of the respective group was 2.4 units +\-0.17 units with a minimum value of 1.0 units and a maximum value of 7.0 units. The structure of the group according to sex was predominantly female (30 women, which represents 57.7%), men – 22, which represents 42.3%. The average age recorded in the group was 54.9 +\- 1.53 with a minimum of 25 years and a maximum of 77 years, with primary tumors of the stomach, liver, pancreas, adrenal glands, kidneys, bladder, rectum, uterus, ovaries, prostate, colon, rectum. The patients of the study were examined during the years 2009-2019, however it is known that patients must be followed up for a long enough time to highlight the desired results.

The data collection source was the PACS (Picture Archiving and Communication Systems) and RIS (Radiological Information System) of two medical institutions, which meet the requirements of the study, in conditions of full confidentiality.

Statistical data processing was performed using the Microsoft Excel, Microsoft Office, IBM SPSS Statistics V22.0 software package, dedicated to epidemiological studies.

The studies were carried out in accordance with the Helsinki Declaration of 1975, as revised in 2000 and with the approval of the Ethics Committee. All patients signed informed consent forms at the time of examination.

Results

In addition to professional experience, continuous medical development, interdepartmental communication, which directly contributes to the development of a qualitative radiology report, the radiologist examining a patient with an oncologic pathology must take into account specific descriptive criteria, such as:

- Specifying the scanning protocols in the radiology report, in order to determine the quality of the radiology procedure;
- Specifying the reason of the examination and anamnesis of the disease in the radiology report, which increases the degree of attention of the radiologist;
- the use of international terminology, especially as regards the interpretation of the response to therapy;
- Comparison of current radiological images with previous radiological images (if the patient is not on initial assessment) – follow-up.

All these specific descriptive criteria were analyzed in the research groups and the following results have been obtained:

Specification of scanning protocols in the radiology report

In the radiology reports of the patients of the L1 research group, the specification of the scanning protocols was not indicated in 100% of the 313 examinations.

The specification of the scanning protocols in the radiology reports of 52 patients from the L2 research group was performed in 121 examinations out of 127 existing ones, which represents 95.3%, but in 6 cases it was not indicated, which represents 4.7% from the total examinations of the L2 research group.

Comparing the frequency distributions using Pearson's X2 (chi-square) test the value of X2 calculated (411328) was obtained at the degree of freedom GL = 1 and $p < 0.001$. The calculated X2 value is much higher than the values found in table X2 for any of the three assumed risks (5%, 1%, 0.1%), so the difference between the two frequency distributions is statistically significant, denoting that within the sample there are different conditions that have determined this difference.

Specifying the reason of the examination in the radiology report

The reason for the examination in the radiology reports of the 52 patients from the L1 research group was mentioned in 100% of the 313 examinations, including oncologic follow-up care – in 229 examinations among the existing ones, which represent 73.2%, postsurgical restaging – in 37 examinations, which represent 0.6%, pre-interventional staging – in 22 examinations, which represent 7.0%, staging – in 19 examinations, which represent 0.6%, restaging – in 4 examinations, which represent 1.3%, initial assessment – in 2 examinations, which represent 0.6% of the total examinations of the L1 research group (fig. 1).

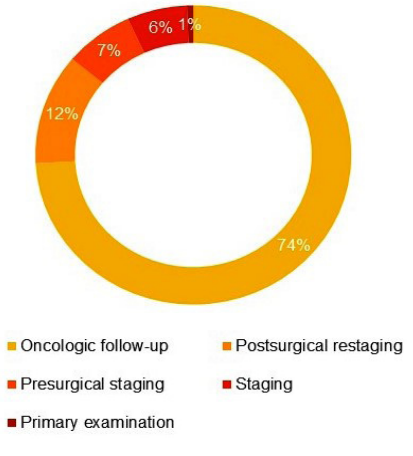

Fig. 1. Specifying the cause of the exam in L1
Source: prepared by the author

The reason sor the examination in the radiology reports of 52 patients in the L2 research group was mentioned in 33 examinations out of the existing 127, which represent 26.0%, but in 94 cases it was not indicated, which represent 74.0% of the total examinations of the L2 research group (fig. 2).

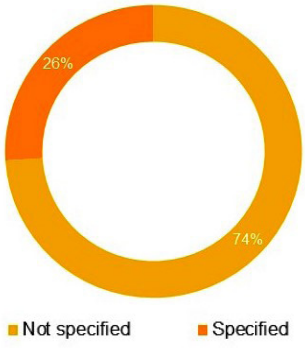

Fig. 2. Specifying the cause of the exam in L2
Source: prepared by the author

Comparing the frequency distributions using Pearson's X2 (chi-square) test the calculated value of X2 (440000) was obtained at the degree of freedom GL = 7 and $p < 0.001$, suggesting a statistically significant link (HS, 99.9% confidence).

Use of international terminology in the radiology report

In the radiology reports of the patients of the L1 research group, the use of international terminology was not noticed in 100% of the 313 examinations (fig. 3).

The use of international terminology in the radiology reports of 52 patients in the L2 research group was performed in 94 examinations out of the existing 127, which represent 74%, but in 33 cases it was not indicated, which represent 26.0% of the total examinations in L2 research group (fig. 4).

Comparing the frequency distributions using Pearson's X2 (chi-square) test the calculated value of X2 (294608) was obtained at the degree of freedom GL = 1 and $p < 0.001$, suggesting a statistically significant link (HS, 99.9% confidence).

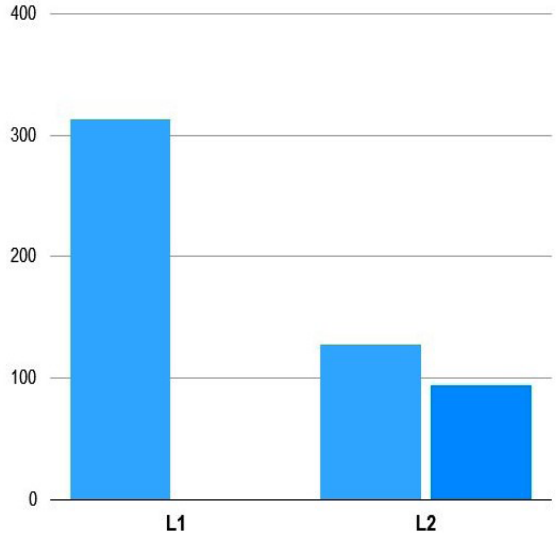

Fig. 3. Use of IT in L1 radiology reports
Source: prepared by the author

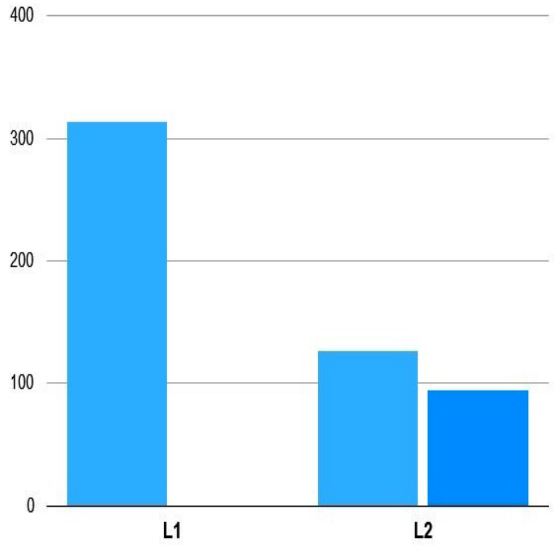

Fig. 4. Use of IT in L2 radiology reports
Source: prepared by the author

Specifying the follow-up in the radiology report

In the L1 research group, 52 patients were examined, a total of 313 examinations, investigations in which the imaging data were compared with the previous examinations – 277 examinations, which represent 88.5%, investigations in which the imaging data were not compared with the previous examinations (128165) – 36 examinations, which represent 11.5% of the total examinations of the L1 research group (fig. 5).

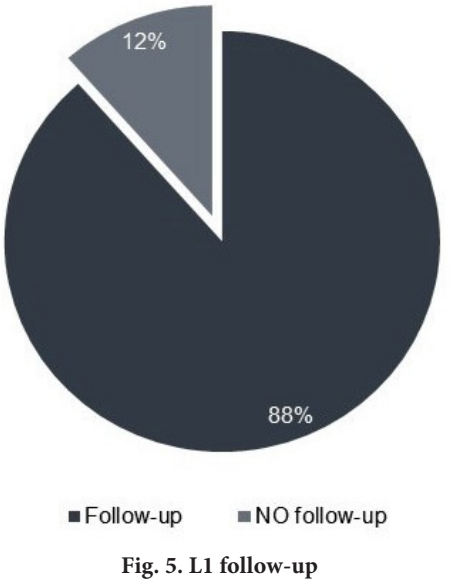

Fig. 5. L1 follow-up
Source: prepared by the author

In the L2 research group, 52 patients were examined, a total of 127 examinations, of which 46 initial assessments, representing 36.2%, investigations in which the imaging data were compared with previous examinations – 76 examinations, representing 59.8 %, investigations in which the imaging data were not compared with the previous examinations – 5 examinations, which represent 3.9% of the total examinations of the L2 research group (fig. 6).

Comparing the frequency distributions using Pearson's X2 (chi square) test was obtained the value of X2 calculated (128165) at the degree of freedom GL = 2 and $p < 0.001$. The calculated X2 value is much higher than the values found in table X2 for any of the three assumed risks (5%, 1%, 0.1%), so the difference between the two frequency distributions is statistically significant, denoting that within the sample there are different conditions that have determined this difference.

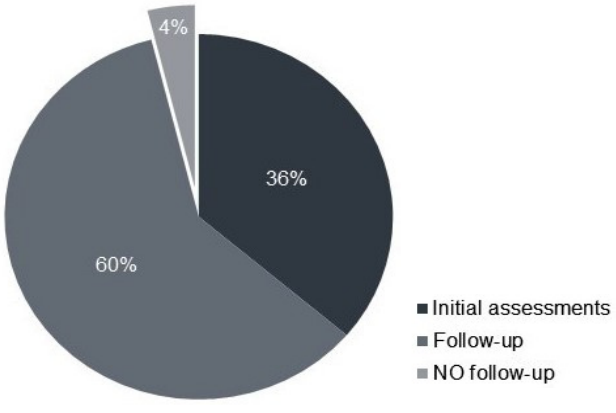

Fig. 6. L2 follow-up
Source: prepared by the author

Discussion

Interpreting the specific oncology data from the analyzed radiology reports, a significant difference is found in the specification of scanning protocols in the radiology report and the use of international terminology in the radiology report, because in the research group L1 the reported proportion of these two characteristics was 0% (tab. 1), so it requires their urgent implementation in the description routine to meet the needs of clinicians who continue to manage these patients.

On the other hand, regarding the specification of the examination reason in the radiology report, there is a 100% rate in the L1 research group, compared to merely 26% of the L2 research group answering this question (tab. 1). So improvements are needed for the L2 research group.

Regarding patient's follow-up, statistical data show that both research groups need to be improved, more obviously group L2, which interprets the radiological differences compared to previous examinations only as 59.8% versus 88.5% for group L1 research (tab. 1). Follow-up care in the radiology report is extremely important, because the tactics of subsequent treatment to which the patient will be subjected depend on this aspect. A qualitative follow-up demonstrates the role of the radiologist, but also enhances the interdepartmental relationship in the management of the pathology.

Table 1. The difference between the specific oncology data in the radiological reports of L1 and L2 groups

Specific oncological data in the radiology report	L1 group	L2 group	Comments
Specification of scanning protocols in the radiology report	0 %	95.3 %	Requires implementation for L1 group
Specifying the cause of the examination in the radiology report	100 %	26 %	Needs improvement for L2 group
Use of international terminology in the radiology report	0 %	74 %	Requires implementation for L1 group
Oncological follow-up	88.5 %	59.8 %	Needs improvement for both groups, more obviously for L2

SWOT analysis of the radiology reports

After interpreting the collected statistical data several non-conformities have been identified, but also very well-marked elements in the examined radiology reports, so it was decided to systematize the information through a SWOT analysis (tab. 2), which originates from a research conducted between 1960 and 1970 at the Stanford Research Institute in the USA. The acronym SWOT is derived from "Strengths, Weaknesses, Opportunities, Threats". SWOT analysis also has its limitations, because categorizing certain aspects as strengths, weaknesses, opportunities and threats could be very subjective due to a high degree of uncertainty. Following the SWOT analysis of the radiology reports, there was developed a standardized model for describing CT images of patients with oncological pathologies of the abdomen and pelvis. The decision-making process was built on Strengths, eliminating Weaknesses, exploiting Opportunities and removing Threats.

Improved radiology report – standardized model

Consequently, standardized radiology reports of patients with oncological pathologies of the abdomen and pelvis examined by computed tomography should contain the following mandatory fields:

- Passport data (name and surname, age, date of examination); received radiation dose data, such as DLP; the examined region (abdomen or abdomen + pelvis); use of contrast agent (type, dose, method of administration); indications for examination; history of the disease (date, year, name of surgery, specific post-intervention therapies).
- Description of each organ in the scan field: liver (positioning, right/left lobe size, contour, structure, parenchyma density in pre- and postcontrast sequences); characteristic of intrahepatic bile ducts; gallbladder (size, contour, structure, wall thickness); diameter of the portal vein, abdominal aorta, *inferior vena cava* and the splenic vein; pancreas (size of the head, body, tail, contour, structure, characteristics of the pancreatic duct); spleen (size with the calculation of the splenic index, contour, structure); adrenal glands (shape, size); kidneys (size, location of each kidney, structure of the pelvicalyceal system).
- Evaluation of intra-abdominal lymph nodes, bone changes in the thoracolumbar segment of the spine in the scanning field are reported. The presence or absence of free intraperitoneal fluid is determined. The imaging sections of the lungs and soft tissues in the scanning field, with the respective specifications, must be followed.

Table 2. SWOT analysis of radiological reports of patients with oncological pathologies of the abdomen and pelvis

Strengths • Access to advanced technologies, such as high-performance CT equipment, which allow detailed viewing of the studied structures; • Integrated PACS and RIS PACS information system, and modern post-processing programs; • DLP is indicated in the radiological report for the possibility of calculating the cumulative dose.	Weaknesses • Lack of an action guide in the radiological examination of cancer patients; • Lack of a standardized oncological scanning algorithm; • Not all radiological reports indicate the patient's medical history, indications for CT examination; • Selective interpretation of follow-up; • Therapy response is not specified according to international terminology; • The conclusions are a summary of the medical report.
Opportunities • Implementation of the «oncological assessment» as a result of the study; • Growing demand for abdominal+pelvic CT examinations as a result of the study outcomes; • Access to technological innovation Mint Medical, artificial intelligence software that contributes to consolidation and specialization in oncology.	Threats • Lack of a National Cancer Register to compare the authenticity of specific oncological data; • Incomplete access to the oncological history of the examined patients (medical records, CD holder of previous examinations, postoperative extracts) secondary to their conscious or unconscious non-cooperation; • Limited experience of medical staff with oncological pathology.

- For women – specify how many days the menstrual cycle lasts. Describe each organ separately, the uterus (position, size, contour, diameter of the cervical canal); ovaries (contour, size); the characteristic of the Douglas space with the specification of the presence/absence of the liquid in the projection; bladder characteristics (contour, structure, wall dimensions).
- For men – characteristics of the prostate (size, contour, structure, signs of capsular extension); seminal vesicles (size, shape, structure); bladder (contour, structure, wall dimensions).
- The condition of the perirectal adipose tissue is also evaluated in the ischiorectal fossa as well as the regional lymph nodes. Bone changes are reported in the lumbosacral segment of the spine.
- If a tumor is identified in the scanning field, it is characterized in detail as follows – the exact topographic location, indicating all adjacent structures in the three anatomical planes, specifying the compression/infiltration of adjacent structures; the presence/absence of perilesional edema and its side effects; three-dimensional diameters (transverse, anteroposterior and craniocaudal); shape, contour, structure.
- In examinations that use contrast material, the post-contrast sequences are characterized with the detailed description of the structural, dimensional shape changes of the described tumor. For multiple lesions it is necessary to indicate their exact number at the time of scanning.
- In conclusion, the pathologies described in the text of the radiology report should be listed with the recommendation to be correlated with the clinical, anamnestic and laboratory data and the consultation of the specialist doctor for a complete, complex diagnosis and its subsequent oncological management.
- Compare the current X-ray images with the previous X-ray images, specifying the treatment response, if appropriate, in accordance with the international terminology.
- At the end – the signature and initials of the radiologist.

Conclusions

The abdominal and pelvic CT examination with contrast is the investigation of choice for tumor identification, local metastases, distant spread [6], staging and follow-up of these patients. Thus, the qualitative interpretation of X-ray images with detailed delimitation of the primary tumor, in order to determine its resection capacity, and assessing the presence of metastatic spread that would change the surgical approach, or the mandate of non-surgical treatment – are the direct tasks of the radiologist. This information defines applicable therapeutic strategies and provides a guide to the patient's prognosis.

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Authors' contributions
NS conceptualized the idea, conducted literature review, wrote the manuscript; IC revised and approved the final text.

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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 49 of June 05, 2015). Informed consent was obtained from all study participants.

Conflict of interests
There is no conflict of interests.

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Visual acuity disturbances following brain injury in school-aged children

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Abstract

Background: Visual disturbances may result in a long-term complication after mild traumatic brain injury (mTBI) in children. These problems may affect both near work and reading, and thus affect activities of daily life and the child's return to school activity. The purpose of the study was to assess the visual acuity disturbances and refractive status in children with persisting symptoms after mild traumatic brain injury.
Material and methods: Forty-eight patients with persisting symptoms after mild traumatic brain injury and anomalies of visual acuity were included. Visual symptoms and refractive status were assessed during the eye examination.
Results: Thus, in the mTBI group the visual acuity for the right eye was of 0.09-0.5 in 83.7% (40 patients), in 16.3% (8 patients) – right eye 0.6-0.8, comparing to the control group, where 62% patients had the visual acuity ranged almost in 1.0, just 14% (7 patients) ranged 0.09-0.5 and in 24% (12 patients) – 0.6-0.8. The visual acuity for the left eye in the research group was of 0.09-0.5 in 89.8% (43 patients), in 10.2% (5 patients) – for the left eye was 0.6-0.8, comparing to the control group, where 66% patients had the visual acuity ranged almost in 1.0, just 24% (12 patients) it ranged 0.09-0.5 and in 14% (5 patients) – 0.6-0.8.
Conclusions: Visual acuity (VA) is affected primary after head trauma although it has big chances to get better with a vision therapy in a time period ranged between 3 and 6 months after the trauma. In most of the cases, we speak of a blurred vison in the near work and relative unclear perception at far. Autorefraction data usually will reveal a slight hyperopia with a possible astigmatic component ranged between 1D to 3D, and in 4.1%-8.2% of cases a slight myopia referring to the spherical compound and 18.4%-32.7% astigmatic compound, also ranged between 1D and 3D.
Key words: visual acuity, brain injury, children.

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Introduction

Traumatic brain injury is one of the most common causes of neurological morbidity, and is more often encountered in childhood and adolescence than at any other time of life [1-3]. Concussions in young people are usually diagnosed in about 90% of all traumatic brain injuries [4]. One in five children will experience a concussion by the age of 10 years [5]. As more frequent are referred falls (51%) and sports-related activities (25%) [5, 6]. The highest rates of sports-related concussion are reported in males aged 10–19 years, although young females are also involved [7, 8]. As speaking of the reported rate of concussion, contact football has the highest incidence, although all sports-related activities entail some risk [9].

Concussion is defined as a form of mild-traumatic brain injury that occurs because of a direct impact to the head or impact to the body that causes transmission of forces to the head and brain [10].

The pediatric brain has different mechanical and compositional properties (e.g. increased water content, decreased myelin, increased transition of acceleration-deceleration forces due to decreased neck strength). This increases the possibility for brain tissue displacement and shear injury [11, 12]. These properties can amplify the complicated neurometabolic cascade that comes after a concussion injury, resulting in increased vulnerability of the immature brain to secondary insults (e.g. second-impact syndrome) and more prolonged recovery [13-15]. As for the future, the prefrontal cortex, the region responsible for executive function, is particularly vulnerable to injury in adolescence [15, 16].

The visual consequences affect the input of visual information: it may prevent the patient from having clear and single vision at all distances. Poor visual impulse will undoubtedly affect visual information processing. Given the diffuse axonal injury often occurring with mTBI, damage to neurological pathways involving vision will influence negatively on the speed, accuracy, and sustaining ability to process and integrate visual information within a multisensory context [17].

A patient with mTBI presents with a constellation of general dysfunctions [18]. This is not surprising as referred to the global nature of the 2-phase brain insult that is

typical in mTBI [19]. In the first or primary phase, the immediate, biomechanically based response is installed and typical coup-contrecoup injury. This initially involves the cranial area and underlying brain tissue in the region of the direct external force (i.e., the coup). Then, due to the differential deceleration/acceleration inertial forces between the rigid cranium and the 2.5-pound jellylike brain mass, there is injury to the opposite brain pole region (i. e., the contrecoup). In addition, there are concurrent rotational, translational, and screw movements of the brain within the cranium, thus causing more brain contusion and damage (e.g., stretching), especially to the white matter fiber tracts, a key problem in mTBI. In addition, there is concomitant flexing and twisting of the highly susceptible midbrain region, especially in children, with this being a primary oculomotor control area. This primary phase is then followed by the secondary phase of the brain injury occurring from days to months afterward, with it being of a biochemically-based nature. It results in a network of events at the cellular level, thus producing cell damage and death, and related toxic events, to the brain and its environment. The degree of cellular insult during this secondary phase is crucial for the patient's recovery; the more the damage, the poorer the recovery [20]. Together, the comprehensive and global effects of the primary and secondary injury phases will produce abnormalities in the sensory, motor, perceptual, cognitive, attentional, behavioral, pharmacologic, somatic, and linguistic domains in many patients with TBI [18].

Speaking of primary patient care, more precisely visual function assessment, it is necessary to refer to the conceptual model of vision care in mTBI [18]. It has been developed as a pyramid scheme, being structured for an organized approach to the patient concerning vision assessment. Further, it will be necessary to clearly focus on the basic tier of this pyramid that states for basic vision examination, including basic refractive status, the general binocular/oculomotor status, and the ocular and general health status.

After mTBI, there can be found either increased myopia or new/increased hyperopia in a patient, which on first blush seems to be contradictory. This comes quite difficult to be explained as both of them may be presented. That is why it is important to try on building up models that would be able to explain the presence of both.

At the beginning, it was mentioned that the middle brain is the most sensible area for mTBI in children. It is known that the third cranial nerve (oculomotor nerve) contains parasympathetic nerve fibers that regulate the iris and lens of the eye. Its origin is in the Edinger-Westphal nucleus of the midbrain, afterwards preganglionic axons travel to the orbit and synapse on the ciliary ganglion. The ciliary ganglion contains two types of postganglionic neurons: one innervates smooth muscle of the iris and is responsible for pupillary constriction, and the other innervates ciliary muscle and controls the curvature of the lens [21]. The affirmation would be whether the stretching and twisting of this area would induce a prevalence of hyperopia in children after mTBI. The latter can be explained by an abnormally functioning parasympathetic system. Thus, the ability to increase accommodation to compensate for any residual, uncorrected hyperopia is compromised, and hence the latent hyperopia becomes manifest, perhaps with intermittent blur reflecting the ability to compensate only partially [18].

On the other hand, increased myopia can be explained by an abnormally functioning sympathetic system, common in mTBI, so that the pharmacologic control system of the crystalline lens cannot reduce "relax" accommodation fully and sufficiently with distant gaze, and thus increased myopia and blur become manifest. Sympathetic preganglionic neurons originate in the lateral horns of the 12 thoracic and the first 2 or 3 lumbar segments of the spinal cord [21]. Moreover, here comes the paradigm, since the spinal cord comes less many involved during mTBI why than should we confront with myopia in this case.

Traumatic myopia is a clinical entity that may be seen following ocular blunt trauma and is characterized with a usual range of -1.00 to -6.00 diopters (D) in the injured eye, or sometimes in both eyes. It is sudden onset and mostly transient, recovering within a few weeks after the trauma, although some cases may stand for a longer period. Possible causes that may lead to this condition are as follows: spasm of the ciliary body, increased crystalline lens effective power secondary to its forward shift, ciliochoroidal effusion causing forward displacement of the crystalline lens-iris diaphragm, axial thickening of the natural lens, and other sources of choroidal [22].

As to previous anatomy innervation peculiarities of the ciliary body, they found out that the ciliary body is also known to receive sympathetic innervation via long ciliary nerves [21]. And this would explain the possibility to confront with myopia after head injury.

Although increased accommodation appears to be uncommon in brain lesions, accommodative paresis is not. It has been reported in Wilson disease, encephalitis, and left parietal infarct or hematoma. Among patients with lesions of the dorsal midbrain, accommodative paresis may change with accommodative spasm. This suggests a linkage of the mechanisms involved in excess and deficient accommodation while brain stem damage is present. For example, some lesions may interfere with inhibition, while others interfere with activation of the accommodative portion of the parasympathetic (Edinger-Westphal) sub nucleus of the third cranial nerve. Accommodative spasm tends to occur in young individuals, because they have such strong accommodative reserve [23].

The mechanism of the accommodative spasm is uncertain. In cat models, accommodation is directed by a pathway from the lateral supra Sylvian cortex bilaterally to the ocular motor nuclei. Stimulation of this area also produces convergence and miosis, but accommodation may be selectively activated. Experimental accommodative spasm has not been demonstrated [23].

Material and methods

Forty-eight patients were referred to the Department of Emergency Unit of the State Mother and Child Health Care Institute, Chisinau, Moldova due to persisting visual symptoms after mild traumatic brain injury. The patients were examined for visual dysfunction primary in the first 72 hours after the trauma occurred.

As mTBI appears unpredictable, most of the patients were hospitalized in the first 6 h – 87.8% (43 patients), 40 children – 81.7% have been hospitalized more than 7 days, making possible a more complex examination. Visual acuity was measured in 48 traumatic brain injury patients. All studies used a Snellen chart/card or comparable metric to assess visual acuity. The measures noted a clear decreased visual acuity in the initial acute phase for both eyes after trauma (fig. 3-4).

The cycloplegic refraction is being evaluated individually after head trauma as mentioned by different authors. Hughes F.E. et al. mentioned that two drops of 1% w/v atropine sulphate administered into the patient's right eye provided immediate relief of the patient's visual symptoms in a 34-year-old female who developed sudden onset blurred distance vision after a rear impact car crash, having previously been emmetropic [24]. On the other hand another group of authors used in their clinical trial cycloplegic refraction evaluated with one drop of tropicamide 1% which was instilled every five minutes three times, and auto refraction was repeated 30 minutes after the last drop [22]. In addition, cycloplegic refraction performed by using cyclopentolate of 1% in a trial of 117 children with bilateral nasolacrimal duct obstruction has been reported in the review literature [25]. Due to the fact that, specific cycloplegic refraction used in neurological compromised patients has not been found in the review literature, or authors didn't mention a clear propensity for it, as for example in a trial of children with cortical impairment [26], it was decided to use the method of tropicamide 1% already used in this research.

As to the eligibility criteria the patients were divided into two groups. The first, research group, included patients that had undergone a mTBI in the last 72h and were hospitalized at the Mother and Child Health Care Institute. The patients were selected as reviewed the medical cards that demonstrated no visual disturbances before and no other chronic systemic pathology. The second group of patients was selected at the out-patient department of the Mother and Child Health Care Institute that presented with visual pathology including only refractive status disturbances with no other organic visual pathology. In addition, as reviewed, the medical cards demonstrated no visual disturbances before and no other chronic systemic pathology that may induce errors of objective examination.

Results

As to the age of the patients, teenagers boys were the most affected, age ranged between 15-18 years (45%), 11-14 years (25%) and school children age ranged 7-10 years (29%), (table 1-2). It can be outlined that most of the mTBI occur in teenagers followed by school children, while the children at the age from 11-14 years were less referred as being affected (x2= 3.412a, gl=2, p<0.01).

Table 1. Trial groups devided by sex

Sex	Research group		Control group	
	Patients	%	Patients	%
Boys	34	70.8	22	45
Girls	14	29.1	26	54

Table 2. Trial groups devided by age

Age	Research group		Control group	
	Patients	%	Patients	%
7-10 years	14	29.1	22	45.8
11-14 years	12	25	11	22.9
15-18 years	22	45.8	15	31.2

Referring to the type of trauma it may be observed that mTBI occurred mostly, being divided as localized trauma lesion in 40.8%, localized lymphatic lesions in 16.3%, cranium deformities in 1.3%, clear concussion in 16.3% and associated epidural hemorrhages in 10.2% of patients. The patients hospitalized with concussion were later re-evaluated and determined to have mTBI as diagnose. The natures of trauma were classified as following: falling from heights in 55.1%, vehicle accidents in 30.6%, falling objects in 8.2% and sport related in 6.1% of patients. For the patients involved in vehicle accidents the ophthalmologic examination was conducted later as the general status of the child was compromised. Speaking deficiency was determined in 36.7% (18 patients), while 63.3% (31 patients) – presented a clear, but delayed speech. A peripheral nervous system examination revealed an average disturbance in 40.8% (20 patients), while for 59.2% (29 patients) no problems have been determined. Pathologic reflexes were present in 38.8% (19 patients), (fig. 1).

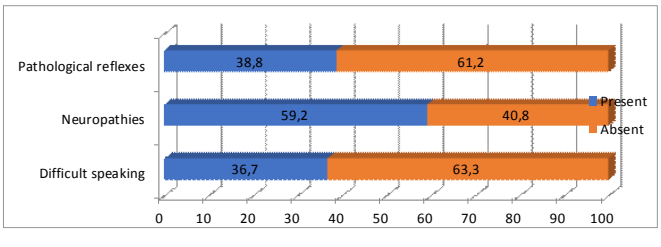

Fig. 1. Neurological findings

Cranial nerve examination, oculomotor (III), trochlear (IV) and abducens (VI), that are involved in eye motion and stability in 1/3 (15 patients) revealed late photoreaction and anisocoria. Mostly the changes were determined in the group of patients that underwent intracerebral hematoma evacuation. Ocular motility was decreased in most of the axes, with a lack of motion in case of patients presenting hematoma of the periorbital tissue.

Examination of general motility revealed a peripheral

paresis in 6.1% (3 patients), 57.1% (28 patients) had a complete peripheral motion, while in 18 patients (36.7%) it was not possible to evaluate.

Examination of the vestibular function underwent 25 patients since in the rest of the patients it was not possible to perform due to the unclear general state. Thus, positive results were determined in 79.2% (19 patients), in 8.3% (2 patients) – unstable results, in 12.5% (3 patients) – unstable results from left to right (fig. 2).

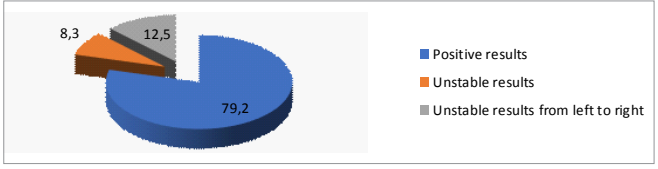

Fig. 2. Vestibular function examination

Thus, in the mTBI patients the VA for the right eye was 0.09-0.5 in 83.3% (40 patients), in 16.7% (8 patients) – AV OD 0.6-0.8, comparing to the control group, 60.4% (29 patients) had the VA ranged 0.9-1.0, just in 25% (12 patients) VA ranged 0.09-0.5 and in 14.6% (7 patients) VA was established between 0.6-0.8 (x2= 46.929a, gl=2, p<0.001) (fig. 3).

For the left eye were received the following results. Thus, in the mTBI patients the VA for the left eye was of 0.09-0.5 in 89.6% (43 patients), in 10.4% (5 patients) – VA for the left eye was 0.6-0.8, comparing to the control group, 62.5% (30 patients) had the VA ranged almost 1.0, just in 27.1% (13 patients) VA ranged 0.09-0.5 and in 10.4% (5 patients) – VA was established between 0.6-0.8 (x2= 51.281a, gl=2, p<0.001), (fig. 3).

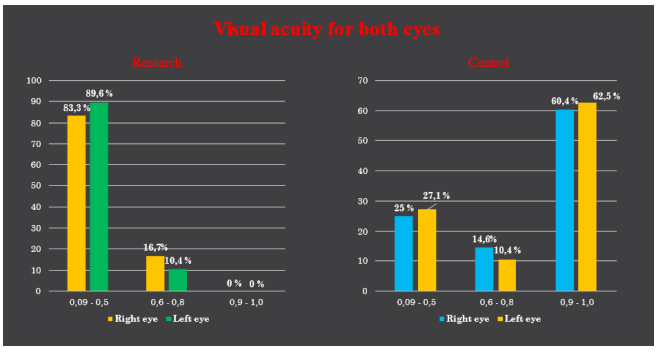

Fig. 3. Visual acuity following mTBI in children (research versus control) (%)

While examining patients in 4-6 months after the mTBI occurred were received the following numbers: VA for the right eye was 0.09-0.5 in 4.2% (2 patients), in 6.2% (3 patients) – AV OD 0.6-0.8, and 0.9-1.0 in 89.6% (43 patients) comparing to the control group, where 50% (24 patients) had the VA ranged 0.9-1.0, just in 25% (12 patients) VA ranged 0.09-0.5 and in 25% (12 patients) – 0.6-0.8 (x2= 46.929a, gl=2, p<0.001), (fig. 4).

For the left eye, were received the following results. VA was 0.09-0.5 in 4.2% (2 patients), in 8.3% (4 patients) – AV OD 0.6-0.8, and 0.9-1.0 in 87.5% (42 patients) comparing to the control group, 58.3% (28 patients) had the VA which ranged 0.9-1.0, just in 33.3% (16 patients) VA ranged 0.09-0.5 and in 8.3% (4 patients) – 0.6-0.8 (x2= 51.281a, gl=2, p<0.001), (fig. 4).

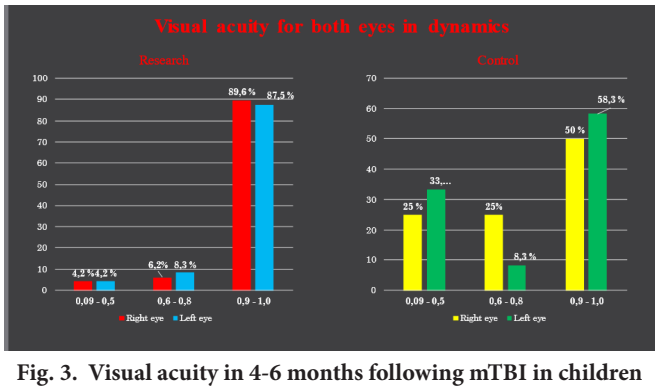

Fig. 3. Visual acuity in 4-6 months following mTBI in children (research versus control) (%)

Therefore, it ia easy to notice that mTBI patients present a clear visual deficiency occurrence as compared to the control group of patients.

Thus, after the measurements it may be concluded that 93.75% (45 patients), of the mTBI patients present hyperopic values for the right eye, comparing to the control group of hyperopic patients 70.8% (34 patients). In 6.25% (3 patients) of mTBI group was determined myopic values for the right eye comparing to 29.2% (14 patients) of the control group (x^2=9.523^a, gl=2, P <0.001). For the left eye there were the following hyperopic data in 95.8% (46 patients) for the mTBI group, compared to 66.7% (32 patients) – control group. For the left eye were received the following numbers concerning myopia – 33.3% (16 patients) in the control group, and 4.2% (2 patients) in the mTBI group (x^2= 15.682^a, gl=2, p <0.001).

For the astigmatic compound were received hyperopic values mostly. For the right eye there were the following values: hyperopic data in 68.75% (33 patients) in the mTBI group, and 31.25% – myopic data. As for the control group there were 56.25% of hyperopia and in 43.75% of patients – myopia (x^2= 0.924^a, gl=1, p <0.001), (tab. 3).

Table 3. Refraction status

Eye	Refraction (sph diopters)	Research		Control		x^2	gl	p
		Patients	%	Patients	%			
Right	0.00 till +3.00	45	93.75	34	70.8	9.523^a	2	<0.001
	0.00 till -3.00	3	6.25	14	29.2			
Left	0.00 till +3.00	46	95.8	32	66.7	15.682^a	2	<0.001
	0.00 till -3.00	2	4.2	16	33.3			

For the left eye were received the following values of hyperopic data: in 83.33% (40 patients) in the mTBI group, and 16.67% – myopic data, as for the control group – 56.25% hyperopia and in 43.75% of patients – myopia (x^2= 11.578[a], gl=2, p <0.001), (tab. 4).

Patients were re-evaluated after a period of time scheduled between 4-6 months after the trauma. The examination concerned refraction status for both groups.

The repeated measurements for refraction status revealed the following numbers: 75% (36 patients) of the mTBI patients present hyperopic values for the right eye, comparing to the control group of hyperopic patients – 45.8% (22 patients). In 25% (12 patients) of mTBI group were been determined myopic values for the right eye comparing to 54.2% (26 patients) of the control group (x^2=9.523[a], gl=2, p <0.01). For the left eye were obtained the following hyperopic values – 70.8% (34 patients) for the mTBI group, compared to 66.7% (32 patients) – control group. For the left eye were received the following myopic values – 33.3% (16 patients) in the control group, and 29.2% (14 patients) in the mTBI group (x^2= 15.682[a], gl=2, p <0.001), (tab. 5).

For the astigmatic compound were received hyperopic values mostly, for the right eye – hyperopic data 64.6% (31 patients) in the mTBI group, and 35.4%– myopic values. As for the control group there were 70.8% of hyperopia and in 29.2% of patients – myopia (x^2= 0.924[a], gl=1, p<0.05), (tab. 6).

For the left eye were received the following values of hyperopic data in 75% (36 patients) in the mTBI group, and 25%– myopic data. As for the control group there were 73% of hyperopia and in 27% of patients – myopia (x^2= 11.578[a], gl=2, p <0.05), (tab. 6).

Discussion

Visual dysfunction is a common occurrence after head trauma. Various aspects of vision are common to be affected as a consequence of a head trauma with many patients exhibiting multiple visual defects. These include aspects of primary vision, such as visual acuity which although not affected in all patients, can be a persistent deficit in some. The pediatric population is a quite difficult class of patients. The examination in this group may be affected along with the general status of the patient also the age inducing a non-cooperation patient, unable to clearly name the pictures or letters on the chart. The inability of children to self-assess and report symptoms after a brain injury can lead to the misdiagnosis of visual disturbance and a poor prognosis, and early diagnosis and proper treatments are keys to a better prognosis. Thus, early ophthalmologic examinations should be compulsory for children with head and face injuries.

As far as investigating the visual acuity loss in children the primary goal was to establish whether changes that appear may be considered permanent and important to be treated by vision therapy or glasses prescription. For that, it was essential to focus on the patient's primary vision concerns (inability/difficulty to read, draw, combine puzzle figures) and objective refractive data in order to reveal induced myopias /hyperopia by TBI. As it all comes from anatomical trails, the task was to explain whether a cause of the resulted myopia may be referred to the possible damaged pathways after a trauma. The afferent pathways that are coming from each optic nerve will eventually emerge into

Table 4. Refraction status

Eye	Refraction (cyl diopters)	Research group		Control		x^2	gl	p_1
		No abs	%	No abs	%			
Right	0.00 till +3.00	33	68.75	27	56.25	0.924[a]	1	<0.001
	0.00 till -3.00	15	31.25	21	43.75			
Left	0.00 till +3.00	40	83.33	27	56.25	11.578[a]	2	<0.001
	0.00 till -3.00	8	16.67	21	43.75			

Table 5. Refraction status in 4-6 months after mTBI

Eye	Refraction (sph diopters)	Research		Control		x^2	gl	p_1
		Patients	%	Patients	%			
Right	0.00 till +3.00	36	75	22	45.8	9.523[a]	2	<0.01
	0.00 till -3.00	12	25	26	54.2			
Left	0.00 till +3.00	34	70.8	32	66.7	15.682[a]	2	<0.001
	0.00 till -3.00	14	29.2	16	33.3			

Table 6. Refraction status in 4-6 months after mTBI

Eye	Refraction (cyl diopters)	Research		Control		x^2	gl	P_1
		Patients	%	Patients	%			
Right	0.00 till +3.00	31	64.6	34	70.8	9.523[a]	2	<0.05
	0.00 till -3.00	17	35.4	14	29.2			
Left	0.00 till +3.00	36	75	35	73	15.682[a]	2	<0.05
	0.00 till -3.00	12	25	13	27			

the visual cortex back to the occipital lobe. On the other hand, there are the efferent fibers that come from the frontal eye fields and synapse near the Edinger-Westphal nuclei. Anatomically the last ones are located in the immediate neighborhood for the oculomotor nuclei, and that is why even a mild trauma in this region could cause a lesion of the Edinger-Westphal nuclei [24]. The type of trauma can be also important in determining the kind of consequences one may face. For instance, whiplash type trauma has been reported on causing decreased accommodation, muscle paresis and even maculopathy [27, 28]. But some others declare unique cases of accommodation spam also present in this kind of trauma [24], thus making possible a different ophthalmologic outcome after mTBI. Data that reveal accommodative disfunction have been also reported by several other authors and this may involve accommodative insufficiency, accommodative infacility, or accommodative spasms that can cause a pseudo-myopia [29]. Most of the authors outline that in order to have an objective assessment of this issue an assessment of accommodative amplitudes, accommodative accuracy and accommodative facility should be done [30]. The role of the ophthalmologist in this case is very important because it should be the first one that starts a visual rehabilitation procedure. And this may involve prescribing glasses for reading or practicing rehabilitation visual exercises Management of accommodative disorders may include reading glasses with increased plus at near, or vision rehabilitation exercises [31, 32].

As to children, authors outline that in case of non-presbyopia patients, vision exercises are usually recommended as the first line treatment and may include accommodative lenses apply as well as accommodative push-up techniques. There is some evidence that 87-100% of patients with accommodative dysfunctions may show good results after with vision therapy [32].

Special mechanism that would define change in the refractive error has not been determined, although this group of patients may become more sensitive to small prescription changes or uncorrected refractive errors [33]. Special attention should be given to latent or uncorrected hyperopic patients, who may become symptomatic following a TBI, some of researchers declare [34, 35].

A Low-Concentration Atropine for Myopia Progression (LAMP) Study has revealed that 0.05, 0.025, and 0.01% atropine could prevent the progression of myopia [36]; although, there has not been any guideline for atropine concentration for accommodative spasm. Some of authors prescribed 1% atropine once a day and spectacle of +1.0 in both eyes to control the accommodation of patient with near reflex spasm [37]. While administered 1% atropine twice a day for 1 week with punctual occlusion has been reported to relax the accommodation of a patient with the spasm of near reflex [35, 38].

By analyzing the data, it may be outlined that in children there is a quite evident increase of hyperopia after head trauma, fact that may explain why most of the children complain of dizziness while reading, writing or even playing small toys. This may be for certain connected to convergence insufficiency that comes quite often after mTBI in children. The reason a hyperopia would be diagnosed in a child after TBI would be definitely connected with the altered function of the parasympathetic system and the impossibility to increase accommodation thus the latent hyperopia would become manifest. Moreover, this is one of the explanations found in resent studies although not referring strictly to children: the ability to increase accommodation to compensate for any residual, uncorrected hyperopia is compromised (e.g., slowed, delayed, ill-sustained), and hence the latent hyperopia becomes manifest, perhaps with intermittent blur reflecting the ability to compensate only partially [18]. There are small data that refer to visual acuity alteration in children and some of the researches claim that the clinical findings in some of the patients can be marginal and would not necessarily prompt spectacle treatment in healthy subjects [39]. Despite this, the spectacles may appear to provide a subjective relief. This appears to confirm with previous clinical observations that patients acquired brain injury may be hypersensitive to even low degrees of refractive error and binocular functional anomalies [40].

Received findings suggest that visual-vestibular processing deficits are present sub acutely following mild traumatic brain injury. Brain injury occurs frequently in children mostly affecting teenagers and early school children. It is true that little is known about the vision effects that may occur and the time prognosed for them to resolve. Although it may be assumed that brain plasticity in younger population is keener, there is still less information regarding time period and gravity of visual disturbances that may occur. As undergoing the basic ophthalmologic examination, it may be concluded that this study revealed that ocular manifestation almost all the time occurs in head trauma in children. The severity evaluation of these changes is compromised quite often since children become unable to co-operate due to the general state or the psychological status after the trauma (anxiety, marked phobias). Visual acuity is affected primary after head trauma although it has big chances to get resolved with a vision therapy in time period ranged between 4 and 6 months after the trauma. In most of the cases, we speak of a blurred vision in the near work and relative unclear perception at far. Autorefraction data usually will reveal a slight hyperopia with a possible astigmatic component ranged between 1D to 3D, and in a few cases a slight myopia also ranged between 1d and 3D. The reason this occurs can be explained by a latened activation of both sympathetic and parasympathetic systemic inducing ciliary process, local changes and misalignment of lens due to its increased/decreased curvature after trauma. Fewer patients require glasses correction since their return to school is delayed due to neurological status. Although it may be considered prescription for the near work optic correction. As for the future, new research data may have an important educational impact for these children and their parents, as well as for school personnel; for example, the development of return-to-learn school criteria.

Conclusions

1. Visual acuity disturbance can be commonly experienced in children after mTBI being ranged below 0.5 as referred to the Snellen chart in up to 83.3%-89.6% cases in the first 24-72 hours. However, it can be considered as being a transient situation since it becomes improved with no particular therapy in about 4-6 months after head trauma in 89.6%-87.5% in up to 0.8-1.0 as referred to the Snellen chart.

2. Exacerbated hyperopia is mostly encountered in children after head injury in the acute phase ranging from 93.75%-95.8% for the spherical compound as low as +3.00D and 68.75%-83.3% for the astigmatic compound. This issue can be explained by an accomodative disfunction since most of the patients complained of difficulties while reading and near work blurred perception. As going through time in 4-6 months after mTBI it may be outlined that hyperopia persists in almost 70.8%-75% for the spherical compound as low as +3.00D and 64.6%-75% for the astigmatic compound.

3. Induced myopia can be less determined in children after head injury in the acute phase ranging from 4.2%-6.25% for the spherical compound as low as -3.00D and 16.67%-31.25% for the astigmatic compound. As going through re-evaluation in 4-6 months after mTBI there are data that myopia persists in almost 25%-29.2% for the spherical compound as low as -3.00D and 25%-35.4% for the astigmatic compound. The entity of post mTBI myopia is still discussed between hypothesis of ciliochoroidal effusion, change of the iris-lens diaphragm or accomodation spasm.

4. Refraction state after mTBI in children should be re-evaluated since it has a passing character. Glasses prescription ahould be done carefully being related to the objective disturbance a child may have at near work or visual perception in the far.

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VV conceptualized the project and drafted the first manuscript. EB interpreted the data. JB critically revised the manuscript. All authors revised and approved the final version of the manuscript.

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Enthesopathy as early manifestation in psoriatic arthritis

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Abstract

Background: Despite the progress made in the study of psoriasis and psoriatic arthritis, their early diagnosis and treatment for practicing physicians continue to be a difficult problem.
Material and methods: 100 people were examined, including 70 patients with psoriatic arthritis aged between 18 and 60 years (23 men and 47 women), admitted to the rheumatology and arthrology departments of the *Timofei Mosneaga* Republican Clinical Hospital 2019-2022 (Favorable opinion of the Committee for Research Ethics, No 21 of 21.12.2019). The control group included 30 people with rheumatoid arthritis.
Results: Ultrasound signs of damage to the joint structures were detected, such as synovitis (p=0.26), cartilage changes (p=0.433), enthesopathy (p=0.980) and tenosynovitis, statistically significant differences (p=0.800). Magnetic resonance imaging determined that fluid was the predominant symptom in frequency (n=13, 92.86%), including in the small joints of the hands (n=1, 100%) and feet (n=2, 100%).
Conclusions: In large joints, the proliferation of the synovial membrane was detected in 51.67% of the joints and had predominantly high echogenicity. At small joints, synovial proliferation with predominantly low echogenicity occurred only in 6.1% of the joints.
Key words: psoriatic arthritis, enthesopathy, ultrasound.

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Introduction

Psoriatic arthritis (PsA) is a progressive chronic systemic disease associated with skin psoriasis, the social significance of which is determined by, first of all, an increase in debilitating forms of joint damage and an increase in mortality in patients with PsA compared to population mortality [1-4].

At the current level of development of rheumatology, there is a management in the treatment of PsA associated with the introduction of effective but also expensive antirheumatic drugs, slowing down the progression of joint destruction and the development of functional disorders (anti-TNF-alpha drugs) [5, 6]. Therefore, early diagnosis aimed at identifying morphological changes in bone structures and soft tissues of the joint, determines the effectiveness of conservative therapy and improves long-term prognosis [7-9].

Despite the progress made in the study of psoriasis and PsA, their early diagnosis and treatment for practicing physicians continue to be a difficult problem. The extreme variability of the clinical picture of PsA, various interactions between its various syndromes, the similarity with other inflammatory diseases of the joints and spine, as well as the absence of mandatory and pathognomonic symptoms create great difficulties in the correct and timely diagnosis of this disease [2, 10-12]. Objective difficulties in diagnosing PsA also include the lack of guidelines, where at the modern level the problems of radiological diagnosis of the given pathology would be presented. All this leads to an extension of the diagnostic process and late administration of appropriate treatment.

Radiological research methods come first in the diagnosis of joint diseases and include all imaging methods such as radiography, ultrasonography, magnetic resonance imaging (MRI) [3, 6, 9]. Despite the fact that radiography continues to be the main method of diagnosis in osteo-articular diseases, it does not allow to assess the condition of those structures of the joint, the impairment of which is of particular clinical importance in early diagnosis [13-15].

Analysis of data from the literature has confirmed the high diagnostic potential of modern diagnostic methods such as radiography, ultrasonography and MRI, in psoriatic arthritis [16, 17]. However, reports on the possibilities of

ultrasound and MRI in the diagnosis of PsA are mainly presented in the literature under a non-systematized character, and there are only isolated reports about the possibilities of MRI in the diagnosis of PsA of a nature S [10, 11, 18]. As for ultrasounds in arthrology, they are mainly represented by the description of rheumatoid arthritis (RA) and osteoarthritis and to a lesser extent PsA [19, 21].

Differential diagnosis of psoriatic and rheumatoid arthritis is quite difficult, belonging to a group of diseases whose morphological basis are proliferative changes in the synovial membrane [22]. In the specialized literature, there are unitary references to the problem of differential diagnosis of these two nosological units using MRI and joint ultrasound [23].

Despite the obvious success of ultrasound diagnosis, a number of diagnostic problems still remain unopened and require further study. Therefore, the ultrasound symptoms of joint damage in patients with PsA have not been definitively systematized. The available sources do not provide data on the relationship between the severity of ultrasound symptoms and clinical and laboratory indications. Accordingly, there is no data on the importance of ultrasound in evaluating the activity of the inflammatory process in PsA, which plays an essential role in monitoring treatment.

In addition, the problem of differential diagnosis of RA and PsA being diseases with a common morphological substrate in the form of synovial membrane proliferation is practically not revealed in the specialized literature.

The purpose of the study is to substantiate the use of ultrasound examination of patients with psoriatic arthritis to assess the work of the pathological process.

Material and methods

100 people were examined, including 70 patients with PsA aged between 18 and 60 years, of which 23 men and 47 women who were undergoing treatment, admitted to the rheumatology and arthrology departments of the *Timofei Moşneaga* Republican Clinical Hospital or treated in outpatient settings in the period 2018-2022 (Favorable opinion of the Committee for Research Ethics at no.21 of 21.12.2019). The comparison group included 30 people with RA.

The patient was considered included in the study after signing the informed consent form. The diagnosis of PsA was established according to the CASPAR diagnostic criteria (2006) [3-5]. Of the studied patients, patients with polyarticular variant (n=28; 40.0%) and mono-, oligoarthritis (n=29; 41.4%) were detected with the same frequency. In 18.6% (n = 13) of the observations, damage to the distal joints of the hands and plants was detected. The predominance were patients with a minimum degree of activity (n=31; 44.3%) and average (n=24; 34.3%), and patients with a maximum degree of activity (n=12; 17.1%) and patients in remission (n=3; 4.3%) were with lower frequency.

The local activity of inflammation in the joints of interest was evaluated according to the activity index of synovitis, thus taking into account clinical manifestations, such as hyperthermia, inflammation and pain.

The analysis of the clinical material took into account the nature of psoriasis and the stage of its development, the prevalence, type, as well as the age of the disease. Skin manifestations were detected in 69 patients studied with PsA (98.57%).

Peripheral blood parameters were studied in patients with PsA: in 36 people (51.4%) with an average and maximum degree of activity, there was an increase in peripheral blood indicators reflecting inflammatory activity. In the study of biochemical parameters, hypoalbuminemia, an increase of C-reactive protein, seromucoids, was observed.

All patients (n=70; 100.0%) had paired knee joints (n=140; 4.3%), ankle joints (n=46; 1.4%), radiocarpal joints (n=22; 0.7%) and 54 (77.1%) – small hand and plant joints (n=3 024; 93.6%). A total of 3.232 joints were examined.

In order to increase the possibilities of Power Doppler imaging in PsA, the vascularization of the synovial membrane in those joints where its thickening or proliferation was detected was studied. Thus, 296 joints were examined.

Magnetic resonance imaging was used as a reference method and was performed in 15 patients on the "Exselart Vantage" device (Toshiba, Japan) with a magnetic field strength of 1.5 T. In total, 14 knee joints, plant joints (n = 28) and hands (11 = 14) were examined, as a result, 56 joints. Joint examination was performed in previous, sagittal and axial projections in T1, T2 and FSat mode.

The assessment of the degree of synovial vascularization was based on the maximum number of color locus in the area of interest, the size of which depends on the studied area. Thus, in the study of the synovial membrane in the upper inversion of the knee joint, the estimated area area was 5-7 cm^2, in the study of the wrist and ankle joints – 3-4 cm^2, small joints of the hands and feet — 1-2 cm^2.

The comparison group consisted of 30 patients with RA aged 27 to 63 years (average age 45±12.3 years) with a duration of the disease from 6 months to 32 years (average duration 12±5.4 years). Patients in the main group and in the comparison group were comparable in age and duration of the disease. All patients underwent ultrasound of the knee joints and the joints of the hands and feet (n=2320). The analysis of the obtained results was carried out using standard statistical methods (Spearman correlation analysis, Mann-Whitney criterion, X^2 criterion, Fisher criterion). The differences were considered significant at $p<0.05$.

Results

The most common changes in the joints in patients with PsA were an increase in the amount of intra-articular fluid and the proliferation of the synovial membrane. The appearance of fluid in the joints occurred in the overwhelming number of patients (n = 63.90%) and only in 10% (n = 7) of the observations there was no liquid. In total,

fluid was detected in 293 out of 3.232 joints (9.1%). Among the knee joints in which there was an increase in the amount of intraarticular fluid (n = 79; 100%), in 48.8% (n = 37) of the observations were recorded joints with a small amount of fluid (gradation 1). In a smaller number, the amount of liquid corresponding to grade 2 (n = 24; 30.4%) and grade 3 (n = 18; 20.8%) was observed. In the radiocarpal joints, the maximum thickness of the liquid in the joints was 6 mm, in the ankle joints – 8 mm. The maximum thickness of the fluid in small joints was 2 mm. In this study, homogeneous effusion into the joint cavity prevailed (n=201; 68.6%). The heterogeneity of the structure (n=92; 31.4%) was due to the appearance of partitions, suspensions or hyperecogene solid inclusions against the background of unechogenic contents.

Synovial proliferation was detected in 296 (9.16%) joints. In most observations (n = 286; 96.6%) diffuse thickening of the synovial membrane was determined. The echogenicity of the synovial membrane was different: in predominant quantity – low (n = 200; 67.6%), in the smaller amount - increased (n= 96; 32.4%). Cartilage thickening was detected in 4 joints (2.9%). In 58 (97.1%) cases, there was a thinning of the cartilage, which in a number of observations was accompanied by a change in the contours in the form of unevenness and blur, and in a number of cases – a change in the normal ecostructure. In some patients with thinning of cartilage (22%), an increase in its echogenicity was observed.

A number of authors believe that the main target in PsA is not the synovial membrane, but bone tissue and inflamed enthesis [7-9]. Therefore, special attention was paid to the search of bone erosions and to assess the condition of the tendon-ligament apparatus. The ultrasound image of ligament and tendon changes included enthesopathy of the patellar ligament and the quadriceps tendon of the femoral and tenosynovitis.

In the enthesopathies of the patellar tendons and tendons of the quadriceps, in all cases thickening of the ligament at the site of attachment, loss of the typical stratified structure and the appearance of small hyperechogenic inclusions were observed. Changes in the bone in enthesopathy were manifested in the form of bumps of the cortical layer.

In some patients (n = 7; 10%), there was a significant decrease in the echogenicity of the patellar ligament at the site of bone insertion, also with the loss of the typical structure. These were patients with marked activity of the disease, and the visible clinical picture was considered as an enthesis.

Tenosynovitis of the flexors of the fingers, flexors and extensors of the hands was detected in 30 localizations. In most observations (n = 28; 93.3%), the appearance of fluid in the synovial sheath of the tendon was combined with the preservation of the normal echostructure of the tendon itself. And only in 2 cases (6.7%) there was a thickening of the tendon with loss of the typical echostructure of the tendon.

According to ultrasound data, bone erosions were detected only in 2 people (1.4%) in the ends of the metacarpal bones, while on radiography and magnetic resonance imaging, the frequency of severity of this symptom was higher. However, data from the literature indicate a higher sensitivity of ultrasound in detecting erosions compared to classical radiography [1, 4, 8].

The lesion of the small joints of the hands and feet was characterized primarily by diffuse proliferation of the synovial membrane, mainly with low echogenicity (p =0.0001), which in 92% of cases is accompanied by a homogeneous effusion (p = 0.005). Changes in the ligament apparatus in all observations are represented by tenosynovitis. From the data in the literature it is known that the low echogenicity of the synovial membrane is due to its edema against the background of active inflammation, and this pattern was reflected in the clinical picture of the lesion of the small joints of the hands and plants in this study. It remains unclear the frequent detection of the synovial membrane, mainly with high echogenicity, in the knee joints, independent of the activity of the disease. Perhaps this fact is due to the earlier fibrosis of the synovial membrane in this localization.

Magnetic resonance imaging was the second method of investigation in the complex diagnosis of PsA and was used as a reference method. In the study group, fluid was the predominant symptom in frequency (n = 13, 92.86%), including in the small joints of the hands (n = 1, 100%) and legs (n = 2, 100%). Synovial proliferation was the second most common sign of damage to the knee joint (n = 10; 71.43%) and was detected in 3.6% of the foot joints and 7.1% of the joints of the hands.

In the present study, erosions were detected in 3 joints and localized in the condyles of the femoral and tibial bones and in the ends of the metatarsal bones II, III on all surfaces. The changes in cartilage consisted of its thinning and structural changes and were observed in 28.57% of cases in the observed contingent (n = 4). In one observation, fragmentation of cartilage occurred, in the other, changes in the type of crack were revealed, which fall within the manifestations of chondromalacia.

As MRI was chosen as the reference method for correctly evaluating the diagnostic efficacy of ultrasonography in detecting existing changes, MRI results obtained in 15 patients in 56 joints were compared with ultrasound data of the same patients (tab. 1).

Table 1. Comparison of signs, viewed at USG and MRI, in 16 patients

Symptom	Number of joints with detected changes	
	USG PD	MRI
Liquid	16 (28.6%)	16 (28.6%)
Proliferation of synovial membrane	11 (19.6%)	12 (24.1%)
Cartilage modification	4 (7.1%)	4 (7.1%)
Bone erosions	2 (3.5%)	4 (7.1%)
Osteophytes	7 (12.5%)	7 (12.5%)
Degenerative changes in tendons	6 (42.9%)	7 (50%)
Tenosynovitis	3 (5.4%)	3 (5.4%)

In these studies, MRI data have generally been consistent with the results of ultrasound in the diagnosis of the

presence of intraarticular fluid, synovial proliferation, cartilage changes, osteophytes, as well as tenosynovitis and enthesopathy.

The greatest diagnostic efficacy of ultrasound was achieved in detecting fluids, cartilage changes, osteophytes and tenosynovitis, at which sensitivity and specificity were 100%. These high rates were due to the exclusion of the possibility of diagnostic errors, given the sufficiently clear ultrasonic visualization of these joint structures. Slightly lower diagnostic efficiency was achieved in the detection of synovial proliferation and enthesopathy, in which the sensitivity was 91.7% and 85.7%, specificity – 100%, diagnostic accuracy – 95.6%, respectively, 92.9%. The lowest efficiency was achieved in identifying marginal bone erosion; sensitivity was 50%, specificity – 100%, accuracy of diagnosis – 75%.

In order to assess the possibilities of energy Doppler mapping in the reflection of PsA activity, the results of ultrasound studies and clinical and laboratory study data were compared. Indicators were studied that reflect the general activity of the disease (ESR and number of leukocytes) and indicators that directly reflect the presence and activity of the inflammatory process in the studied joint: pain, inflammation and hyperemia, first of all, it was necessary to identify the severity of the main ultrasound symptoms of synovitis in the knee and small joints of the hands and feet, depending on the clinical manifestations of inflammation. At the same time, for the knee joints, the thickness and degree of synovial vascularization and the amount of fluid were taken into account, for small joints — the fact of the presence of thickened synovial, its vascularization and the presence of fluid (tab. 2 and 3).

Table 2. Expression of ultrasound symptoms of synovitis depending on the clinical activity of inflammation in the knee joints

Ultrasonographic sign	Group No 1 (n=46) low activity	Group No 2 (n=12) average activity	Group No 3 (n=10) high activity
The thickness of the synovial membrane grade 1 grade 2 grade 3	44 (95.7%) 2 (4.3%) -	1 (8.3%) 10 (83.4%) 1 (8.3%)	- - 10 (100%)
Degree of vascularization grade 0 grade 1 grade 2 grade 3	26 (56.5%) 20 (43.5%) 0 0	0 0 12 (100%) 0	0 0 0 10 (100%)
Quantity of liquid grade 0 grade 1 grade 2 grade 3	24 (52.2%) 22 (47.8%) 0 0	0 0 10 (83.3%) 2 (16.7%)	0 0 2 (20%) 8 (80%)
Cartilage changes thickeners thinning change strokes modification of the echostructure	0 40 (87.0%) 30 (65.2%) 30 (65.2%)	0 12 (100%) 8 (66.7%) 8 (66.7%)	4 (40%) 6 (60%) 10 (100%) 10 (100%)
Decrease in the echogenicity of the ligament in the place of fixation (enthesitis)	0	0	7 (70%)

Table 3. Expression of ultrasound symptoms of synovitis depending on the clinical activity of inflammation in small joints

Ultrasonographic sign	Group No 1 (n=84) low activity	Group No 2 (n=10) average activity	Group No 3 (n=90) high activity
Thickening of the synovial membrane: up to 3 mm more than 3 mm	84 (100%) 0	2 (20%) 8 (80%)	0 90 (100%)
Degree of vascularization: grade 0 grade 1 grade 2 grade 3	70 (83.3%) 14 (16.7%) 0 0	0 0 8 (80%) 2 (20%)	0 0 3 (3.3%) 87 (96.7%)
Presence of liquid	76 (90.5%)	10 (100%)	90 (100%)
The appearance of fluid in the tendon sheath (tenosynovitis)	0	2 (20%)	36 (40%)
Defect in the cortical layer (bone erosion)	0	0	2 (2.2%)

Over the course of the study, ultrasonography and clinical and laboratory activity data were compared for all joints as a whole. The results of correlation analysis indicate a positive correlation between the severity of ultrasound symptoms of synovitis and the level of clinical and laboratory indicators of inflammation. At the same time, the ultrasound symptom, which correlates the most with the level of local activity, is the degree of vascularization of the synovial membrane, which appeared both in the large joints (r = 0.508) and in the small ones (r = 0.500). The strongest correlation is observed between the amount of fluid (r = 0.401) and the degree of vascularization of the synovial membrane in the knee (r = 0.508), small joints (r = 0.500) and the level of ESR and leukocytosis. A weaker correlation is observed between the level of laboratory parameters and the thickness of the synovial membrane (r = 0.383).

When analysing the frequency of occurrence of ultrasound signs, depending on the duration of the disease, statistically significant differences were found only in the

frequency of occurrence of marginal bone growths, which were significantly more often determined in the group of patients with a duration of the disease of more than 10 years ($p = 0.04$). For other ultrasound signs of damage to the joint structures, such as synovitis ($p = 0.26$), changes in cartilage ($p = 0.433$), enthesopathy ($p = 0.980$) and tenosynovitis, statistically significant differences ($p = 0.800$) depending on the occurrence were not detected. The possibility of using modern radiographic diagnostic methods in monitoring the treatment of arthritis is widely discussed in modern literature [5, 9, 17]. The possibilities of Dopplerography have been studied in terms of evaluating the dynamics of the treatment of patients with PsA. The study was conducted in 10 patients with early diagnosed PsA. Ultrasound was performed at the time of launch of treatment and 1 month after taking nonsteroidal anti-inflammatory drugs and DMARD therapy with methotrexate. 13 knee joints and 9 metatarsophalangeal joints (22 joints in total) were examined. Before the start of treatment, vascularization was detected in all joints, and joints with the degree of vascularization 2 and 3 prevailed. Against the background of treatment was determined the number of joints in which blood flow was not recorded or a low degree of vascularization (grade 1).

When comparing the vascularization indices in the groups before and during the treatment, a significant decrease in the degree of vascularization of the synovial membrane in the joints of the knee ($p = 0.018$) and of the small ones ($p = 0.002$) was observed against the background of therapy with non-steroidal anti-inflammatories and methotrexate. Simultaneously with this, there was a decrease in indicators of laboratory activity of arthritis. To evaluate the changes in these indicators, the non-parametric criterion of Wilcoxon's paired comparison with materiality level ($p<0.05$) was used. As a result of the comparison, statistically significant differences were obtained for ESR ($p=0.012$) and leukocytes ($p=0.005$) before and during treatment.

Comparison of ultrasound data and magnetic resonance imaging demonstrated the comparability of methods for detecting intra-articular inflammatory fluid, synovial proliferation, cartilage changes, detection of tenosynovitis and enthesopathies.

Ultrasound examination of the joints in patients with PsA, including the determination of the amount of intra-articular inflammatory fluid, the thickness of the synovial membrane and the degree of its vascularization, the assessment of the state of the tendon-ligament apparatus, allows to assess the activity of the inflammatory process. An essential role in determining the activity is played by the evaluation of the degree of vascularization, as indicated by a positive correlation between local inflammatory activity and synovial vascularization ($r = 0.591$).

The use of ultrasound to assess the dynamics of treatment was justified and was characterized by a decrease in the degree of vascularization of the synovial membrane in combination with a decrease in the amount of intra-articular inflammatory fluid and the thickness of the synovial membrane.

Based on the systematization of the ultrasound image, certain diagnostic differential visual signs of joint changes in psoriatic and rheumatoid arthritis were obtained, namely the predominance of ligament and tendon enthesopathies of the knee joint in PsA.

Discussion

Ultrasound examination was the main method in the complex diagnosis of PsA. The results of the study demonstrated that in patients with PsA damage to all anatomical structures of the joint with polymorphism of the ultrasound model is detected.

During the ultrasound, some differences were found in the visual image depending on the location of the changes. Thus, the lesions of the knee joint are characterized by a high frequency of heterogeneous occurrence of overflow into the joint cavity, proliferation of the synovial membrane of a diffuse or focal nature, high echogenicity of the synovial membrane and its poorly expressed vascularization, changes in the tendon-ligament apparatus in the form of enthesopathies.

The introduction into practice of Doppler energy mapping, according to a significant number of authors [3-8], provides fundamental diagnostic capabilities. The possibilities of Dopplerography of the synovial membrane in rheumatoid arthritis are described in detail [19, 21-23]. Reports on the study of synovial vascularization in PsA are still isolated. In the studies, a high sensitivity of ultrasound was reported in detecting signs of synovitis and tenosynovitis in lesions of the joints of the hands and feet in PsA, however, the data of energetic Doppler drifting did not have correlations with clinical and laboratory signs of inflammatory activity.

The activity of synovitis plays a central role in the formation of the clinical picture of joint diseases [2, 7, 15]. How actively it evolves also depends on the choice of treatment and the prognosis of the disease. In practice, it is very important not only to establish that the patient has synovitis, but also to have an objective tool to assess his activity and dynamics of the process under the influence of the treatment that is prescribed to the patient. The use of quantitative research methods in medicine can significantly increase the reliability of assessing the patient's condition. Sources in the literature indicate the ability of ultrasonography to assess the activity of inflammation in the knee joint in RA based on the positive correlations between clinical and laboratory activity, arthritis and the symptoms detected during ultrasound, namely between the thickness and degree of vascularization of the synovial membrane, the amount of intra-articular fluid [12, 13, 16-19].

At the same time, was analyzed the severity of the changes of other joint structures whose involvement is of clinical importance, namely the presence/absence of bone erosions, the condition of cartilage and tendon-ligament apparatus.

PsA and RA are similar in morphology and clinical course

of disease. In order to study the ultrasound possibilities in identifying the differences of these two diseases, in 30 patients with RA (comparison group) ultrasound of the knee joints and small joints of the hands and feet (2.320 joints) was performed. The results of the ultrasound were compared with the data of clinical and laboratory activity. When comparing the frequency of occurrence of distinctive signs at ultrasonography of damage to the knee and small joints, depending on the nosological affiliation, the following results were obtained. In the group of patients with PsA, enthesopathy and enthesitis of their own patellar ligaments and tendons of the femoral quadriceps were detected significantly more often ($p = 0.007$). In the study of small joints in patients of the PsA group, inflammatory fluid was detected more often than in patients in the comparison group ($p = 0.009$). For patients with RA, a feature of the visual picture was the more frequent detection of proliferative changes in the synovial membrane in both the knee joints ($p = 0.03$) and in the small ones ($p = 0.001$), compared to PsA. Statistically there were no significant differences in the frequency of detection of inflammatory fluid in the knee joints, tenosynovitis, the nature of joint effusion and changes in cartilage structure in patients with PsA and RA.

Analysis depending on the lasting changes in the disease detected at USG has shown that marginal bone growths are equally often detected in a group of patients with the duration of the disease more than 10 years, regardless of nosology.

Thus, the study carried out showed the effectiveness of the ultrasonographic method in detecting morphological changes in joints in patients with PsA, determining the activity and evaluating the results of treatment.

Conclusions

1. Ultrasound is a highly informative method in detecting a wide range of morphological changes in the joints of patients with PsA. The highest index of sensitivity appeared when inflammatory fluid, cartilage changes, osteophytes and tenosynovitis were detected. Less sensitivity was achieved in the detection of synovial membrane proliferation, enthesopathy, the slightest sensitivity was observed in the visualization of marginal bone erosions. At the same time, the indicators of specificity were equally high.

2. In large joints, the proliferation of the synovial membrane was detected in 51.67% of the joints and had predominantly high echogenicity, as well as accompanied by intra-articular overflow in all observations. In small joints, synovial proliferation with predominantly low echogenicity occurred only in 6.1% of the joints, due to their rarer lesion, and was combined with an increase in intra-articular fluid in 92% of cases. Tendon-ligament injury in PsA included enthesopathy in the knee joints, tenosynovitis in the ankle, radiocarpal joints, and in small joints of hands and plants.

3. Ultrasound criteria for the validity of PsA are: the degree of severity of synovitis, as well as the presence of tenosynovitis and enthesitis. The strongest correlation was obtained between the activity of inflammation and vascularization of the synovial membrane ($r=0.591$) and tenosynovitis ($r=0.547$), as well as between the levels of ESR and leukocytes, the amount of inflammatory fluid ($r=0.401$) and the degree of vascularization of the synovial membrane ($r=0.508$).

4. An indicator of the positive dynamics of PsA treatment with DMARD drugs is the reduction of synovial membrane vascularization in combination with a decrease in the amount of inflammatory intra-articular fluid and the thickness of the synovial membrane, visualized at Power Doppler mapping.

5. The significant differences between PsA and RA are the presence of enthesopathies of the ligaments proper of the patellar tendon and the quadriceps tendon of the femoral and the predominance of intra-articular overflow in small joints compared to the predominance of the frequency of proliferation in small and knee joints in patients with RA.

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Endoscopic findings in patients with gastroesophageal reflux disease referred to antireflux laparoscopic surgery

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Abstract

Background: Gastroesophageal reflux disease (GERD) is nowadays a highly prevalent, chronic condition, with 10% to 30% of Western populations affected by weekly symptoms. The patient who does not respond to the empiric antisecretory treatment, with alarming symptoms, or referred to surgery should undergo an esophagogastroduodenoscopy (EGD).
Material and methods: This was a retrospective and descriptive study of patients with GERD admitted for antireflux laparoscopic surgery from 2012 to 2019. All endoscopic data were analyzed with the following variables: age, gender, reflux esophagitis and its severity, esophageal ulcers and strictures, Shatzky's ring, Barrett's esophagus (BE), incompetence of the esogastric junction; hiatal hernia.
Results: A total of 152 patients were included in the study. The age of the patients ranged from 19 to 76 years, averaging 52 years. Among them, 97 (63.8%) were women and 55 (35.38%) men. A wide variety of endoscopic features has been found: non-erosive GERD (6.57%); reflux esophagitis (Savary-Miller) – I (21.05%), II (44.07%), III (23.68%); esophageal ulcer (1.31%), BE (1.97%). The majority of patients present axial hiatal hernia (92.76%) corresponding to Hill grade IV incompetence of the flap valve. Hill grade III was present in 4.6% of cases, grade II – 2.63%.
Conclusions: The patients with GERD may have a wide range of endoscopic features (from normal to esophagitis, hiatal hernia, strictures and EB). Considering the multitude of data provided by endoscopic examination in patients with GERD, it can be certainly stated that EGD is one of the most important investigations in these patients, and is mandatory in those selected for surgical treatment.
Key words: gastroesophageal reflux disease, endoscopic findings.

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Introduction

Gastroesophageal reflux disease (GERD) is one of the most common gastrointestinal diseases of the western world, with increasing morbidity and treatment costs over the last two decades [1, 2]. In a systematic review, El Serag et al. estimated the prevalence of GERD to be 18.1–27.8% in North America, 8.8–25.9% in Europe, and 2.5–7.8% in East Asia. However, because of the common use of over-the-counter GERD drugs, the true incidence of the disease is probably underestimated [3].

The economic impact of GERD is significant with direct costs of almost $ 10 billion, and indirect costs related to declining productivity – $75 billion [1]. Its impact on quality of life is no less devastating, especially in the case of untreated, refractory, or complicated gastroesophageal reflux (erosive esophagitis, esophageal stricture, aspiration, asthma, Barrett's esophagus, esophageal adenocarcinoma).

The standard therapy for GERD is conservative, which includes the use of proton pump inhibitors (PPIs) and H2-blockers, antacids/alginates, prokinetics, etc. [4]. However, their frequent failure, the recent highlighting of side effects, and the high costs associated with long-term PPI therapy have led to the increasing role of surgical treatment [5]. Surgical treatment of GERD is effective and long-lasting, being the only one capable of restoring the eso-gastric barrier (anti-reflux anatomical-physiological mechanisms). At the same time, the application of the laparoscopic method in anti-reflux surgery has led to a decrease in perioperative morbidity, length of hospital stay, and costs compared to open interventions [6].

GERD is diagnosed in routine clinical practice based on typical clinical symptoms and treated empirically with a proton pump inhibitor (PPI) trial unless a patient has alarming symptoms, which include dysphagia, anemia, weight loss, hematemesis, and odynophagia [7-8]. The patient who does not respond to the empiric PPI trial or those with alarming symptoms should undergo an esophagogastroduodenoscopy (EGD) to evaluate for complications like Barrett's esophagus (BE), esophagitis, peptic esophageal ulcer, or esophageal cancer [9]. Some of the complications, like squamous cell dysplasia, Barrett's esophagus with dysplasia, and

early adenocarcinoma, can be missed with regular EGD due to subtle changes in the mucosa [10]. Advanced diagnostic endoscopic techniques like high-resolution, high-magnification endoscopy, confocal laser endo-microscopy, wireless capsule endoscopy, autofluorescence imaging, narrow-band imaging, and chromoendoscopy have been developed to improve the accuracy of the endoscopic diagnosis.

EGD is one of the mandatory investigations that need to be performed in patients with GERD expected for laparoscopic fundoplication (LF). Commonly, the endoscopic examination is performed for the diagnosis and management of GERD, with typical reflux symptoms (24%) and dysphagia (20%) being the commonest indications [11]. The indications for endoscopy in GERD, proposed by the American Society for Gastrointestinal Endoscopy [12] and finally, established at the Lyon GERD consensus meeting in November 2017 [9], are following:

- Persistent or progressive GERD symptoms despite appropriate medical therapy;
- Atypical GERD symptoms;
- Evaluation of patients with suspected extraesophageal manifestations of GERD;
- Alarm symptoms;
- Dysphagia or odynophagia;
- Involuntary weight loss, evidence of gastrointestinal bleeding, or anemia;
- Finding of a mass, stricture, or ulcer on imaging studies;
- Screening for BE in selected patients (as clinically indicated);
- Evaluation of patients before and with recurrent symptoms after endoscopic or surgical antireflux procedures.

In this context, the present study had the purpose of describing and analyzing the endoscopic features of the patients with GERD referred to LF.

Material and methods

This was a retrospective and descriptive study of 152 patients with GERD admitted for LF to *Gheorghe Paladi* Municipal Hospital, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova, 2012-2019.

The preoperative endoscopic examination was performed on an outpatient basis, in different medical institutions, by different specialists. At the same time, in most cases, the preoperative endoscopy was repeated in the institution. Upper digestive endoscopy was performed according to the standard method, with a Pentax 2790K video endoscope (Pentax, Japan), with typical anesthesia (10% lidocaine spray), without sedation. The investigation was performed in the morning, after 6-8 hours of hunger (on an empty stomach). The esophagus was carefully evaluated and all endoscopic images were recorded and stored in a computer database. All patients had been receiving PPIs at standard doses for at least 1 month at the time of endoscopy. However, the other medications of patients could not be noted because of the study's retrospective design.

Data on preoperative endoscopic examination of patients were evaluated, and the following variables were analyzed: the presence and severity of reflux esophagitis, esophageal ulcer, Barrett's esophagus, Shatzky's ring, esophageal strictures; appreciation of the degree (Hill classification) of the incompetence of the esogastric junction; assessment of the presence of hiatal hernia with its type, size and degree.

The descriptive analysis was performed according to the nature of the variables: continuous variables were expressed as means and medians with standard deviation, while categorical variables were summarized as frequencies and percentages.

Results and discussion

One hundred fifty-two patients, who presented to our clinic with typical and atypical reflux symptoms, selected for LF and underwent EGD, were included in the study. The age of the patients ranged from 19 to 76 years, averaging 52 years. There were 39 (25.65%) patients over the age of 60 years. Of the 152 patients included in the study, 97 (63.8%) were women and 55 (35.38%) men. Women were the majority in all age groups after the age of 30, with a maximum female/male ratio (2.75/1) in the 61-70 age groups.

Impairment of esophageal clearance functions prolongs the reflux contact with the esophageal mucosa, thus increasing the degree of injury, which can be documented during endoscopy. Therefore, patients with GERD may have a wide range of endoscopic manifestations (from normal to esophagitis and EB). All endoscopic manifestations in patients with GERD in this study are shown in table 1.

The presence of mucosal damage and positive endoscopic findings are not a prerequisite for the diagnosis of GERD. GERD can accurately be diagnosed by history of classical symptoms of heartburn and/or regurgitation and a positive response to antisecretory therapy [13]. Almost 2/3 of patients with GERD have a non-erosive disease and a normal endoscopy [1]. In this study, the rate of patients with non-erosive GERD is much lower – 6.57%. This is explained by the fact that these patients respond quite well to PPI therapy, rarely develop complications of GERD, and thus less often require laparoscopic anti-reflux surgery.

Most patients undergoing LF showed endoscopic data of erosive esophagitis in the distal esophageal mucosa (fig. 1). The severity of esophagitis was assessed according to the modified Savary-Miller classification (1988) [14-15], commonly used in Europe:

Grade I – Single or isolated erosive lesion(s) affecting only one longitudinal fold;

Grade II – Multiple erosive lesions, noncircumferential, affecting more than one longitudinal fold, with or without confluence;

Grade III – Circumferential erosive lesions;

Grade IV – Chronic lesions: ulcer(s), stricture(s) and/or short esophagus. Alone or associated with lesions of grades I-III;

Grade V – Columnar epithelium (Barret's esophagus) in continuity with the Z line, noncircular, star-shaped, or circumferential. Alone or associated with lesions of grades I-IV.

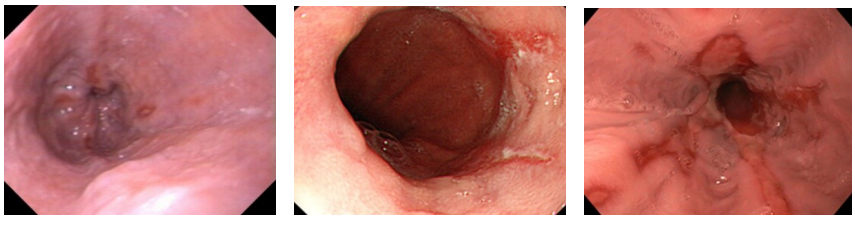
The presence of esophagitis is 90–95% specific but not sensitive for the diagnosis of GERD [16]. In the given study, 2/3 of patients presented a severe degree of esophagitis (II-III), refractory to medical treatment that served as an indication for LF. In 4 cases (2.63%), esophagitis was accompanied by the presence of the Shatzky ring (fig. 2), but without stricture of the esophagus.

It is important to note that in all descibed cases the healing of erosive esophagitis occurs 6 weeks postoperatively after LF. More severe complications of GERD referred to Savary-Miller grade IV-V, such as esophageal peptic ulcers and BE were rarer endoscopic manifestations, constituting only 3.28% – 5 cases (fig. 2). Both cases of peptic esophageal ulcer were associated with upper gastrointestinal bleeding and required endoscopic hemostasis. Following the fundoplication, the absence of symptoms and the healing of ulcers were found.

BE is a metaplastic change of the esophageal lining from the normal squamous to specialized columnar epithelium caused by chronic acid damage. Approximately 10% of patients with chronic heartburn symptoms have BE [11]. In all 3 cases of BE from this study, it was a non-dysplastic form of columnar metaplasia, associated clinically with typical GERD symptoms. According to literature data, most (90%) patients with BE have a nondysplastic disease and a very low rate of progression to esophageal adenocarcinoma at a rate of 0.3 to 0.4 per patient-year [17]. The role of LF in patients with BE remains uncertain at this time. However, numerous studies reported excellent results in patients with GERD and BE, with 95% of subjects reporting persistent symptomatic improvement after LF [18].

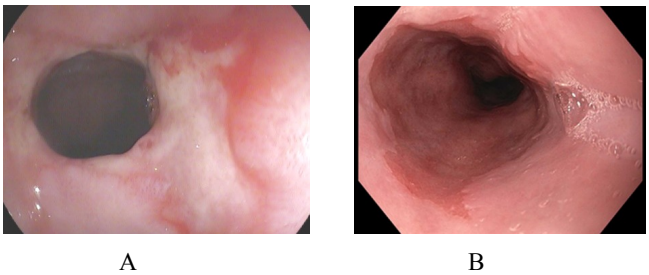
Table 1. Endoscopic findings of patients with GERD referred to LF

Endoscopic manifestations	Number of cases
Non-erosive GERD	6.57% (10)
Reflux esophagitis (modified Savary-Miller)	90.78% (138)
I	21.05% (32)
II	44.07% (67 – 2 with Shatzky ring)
III	23.68% (36 – 2 with Shatzky ring)
IV (complicated – strictures, ulcers), peptic esophageal ulcer	1.31% (2)
V – Barret's esophagus	1.97% (3)
Incompetence of esogastric junction (Hill classification)	
Hill Grade I	0
Hill Grade II	2.63% (4)
Hill Grade III	4.60 (7)
Hill Grade IV (hiatal hernia)	92.76% (141)
Hiatal hernia	
Absent	7.23% (11)
< 2cm	24.34% (37)
2-5cm	60.52 % (92)
>5cm	7.89 % (12)



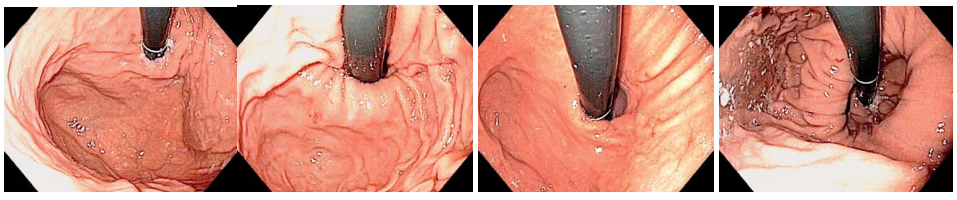
I II III

Fig. 1. Severity of erosive esophagitis (Savary-Miller)



A B

Fig. 2. A – Esophageal peptic ulcer; B – Barret's esophagus



I II III IV

Fig. 3. Hill classification

Another important endoscopic aspect is the appreciation of the competence of esogastric junction (flap valve), according to Hill's classification [19] (fig. 3):

Grade I: a prominent fold of tissue along the lesser curvature next to the endoscope.

Grade II: the fold is less prominent and there are periods of opening and rapid closing around the endoscope.

Grade III: the fold is not prominent and the endoscope is not tightly gripped by the tissue.

Grade IV: there is no fold, and the lumen of the esophagus is open, often allowing the squamous epithelium to be viewed from below. An axial hiatal hernia is always present.

Studies have shown an association between higher Hill grades and the frequency of GERD [20, 21]. Higher Hill grades are also associated with lower LES pressure [20], increased prevalence of hiatal hernia [20], and can predict poor response to proton pump inhibitor treatment [22]. The Hill classification has been proven to be reproducible and provides useful information when evaluating patients with suspected GERD who are undergoing endoscopy [20].

In the same context, EGD allows to assess the presence of hiatal hernia, its type and size. Lord and coauthor. demonstrated not only that the severity of GERD correlates with the functional and anatomical qualities of the gastroesophageal barrier against reflux, with the presence of HH and that a defective SEI is significantly more common in patients with erosive esophagitis or BE [23]. At the same time, LF which resolves the hernia and increases the pressure of the SEI, offers good or excellent results in the same way, regardless of the presence of inflammatory lesions of the mucosa and the severity of GERD [23].

The high rate of Hill grade IV (92.76%) – axial hiatal hernia, followed by Hill grade III (4.6%) – incompetent flap valve, is explained by the necessity of LF to patients with more pronounced impairment of the competence of the esogastric junction (flap valve), the surgery being the only one capable of restoring the eso-gastric barrier (anti-reflux anatomical-physiological mechanisms).

For subsequent laparoscopic antireflux surgery, it is of major importance to assess during EGD the type of hiatal hernia (axial, paraesophageal, mixed), its size, correlation with adjacent anatomical structures (fixed/free), suspicion of a short esophagus (congenital/acquired). These data have a primary role both in the diagnostic plan and in the assessment of some operative peculiarities, such as difficulties in mobilizing the herniated stomach in the mediastinum, the use of synthetic mesh to reinforce crura, the need for a Collis gastroplasty, etc.

It should be noted that EGD is irreplaceable in the post-operative assessment of the neo-valve (Nissen, Toupet). All patients in the present study were examined endoscopically at 4-6 weeks postoperatively, a regaining competence of the esogastric junction being confirmed (similar to Hill grade I).

Conclusions

The patients with GERD may have a wide range of endoscopic features (from normal to esophagitis, hiatal hernia and EB). Considering the multitude of data provided by endoscopic examination in patients with GERD, it can be certainly stated that EGD is one of the most important investigations in these patients, and is mandatory in those selected for surgical treatment.

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AS was responsible for performing of EGD and recording of the endoscopic images; SC interpreted the data and performed the analytical part of the work, drafted the first manuscript; EG conceptualized the project, designed the research and revised the manuscript critically.

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Comparative analysis of imaging investigations in the diagnosis of retroperitoneal tumors

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Abstract

Background: Primary retroperitoneal tumors (PRT) are a group of extremely heterogeneous soft tissue tumors that grow in the retroperitoneal space and have no organic affiliation. Tumors usually become symptomatically late and cause secondary symptoms or become palpable once they have become significantly large. Preoperative diagnosis of PRT is essential for assessing subsequent treatment tactics, planning the approach and volume of surgery, by detecting the structural component, relationships with adjacent anatomical structures and the degree of invasion.
Material and methods: Complex prospective and retrospective analysis of clinical, anamnestic and imaging data of 118 patients with primary and non-primary retroperitoneal tumors, investigated and treated at the Institute of Oncology of the Republic of Moldova during 2015-2020.
Results: To determine the primary PRT diagnosis for the clinician, it is primordial to rule out the organic or secondary nature of the tumor. Thus, the patients were examined by: abdominal USG – 118 patients (100%), abdominal and small pelvic contrast-enhanced CT – 118 patients (100%), MRI – 3 patients (2.5%), videoesogastroduodenoscopy – 32 patients (27.1%), videocolonoscopy – 31 patients (26.3%), irigography – 4 patients (3.4%), urography – 29 patients (24.6%).
Conclusions: Contrast-enhanced CT provides more accurate data than USG. The image obtained at the USG examination is flat, therefore the dimensional measurements of the tumor are not always performed on the longest tumor axis, especially in the case of polylobulated or giant tumors, while the tomographic examination techniques allow the three-dimensional reconstruction of the tumor with more accurate assessment of the tumor size.
Key words: primary retroperitoneal tumors, diagnostic, sonography, computerized tomography.

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Introduction

Primary retroperitoneal tumors (PRT) are a commonly used term which defines a group of tumors developed from mesenchymal, neuronal, or vestigial tissue in the retroperitoneal space. They develop themselves in the space bounded by the posterior parietal peritoneum and the endoabdominal fascia, including extraorganic structures of fatty, connective, fascial, vascular, nervous tissues, muscle bundles, vessels and lymph nodes or embryonic vestiges, more frequently derived from urogenital tract [1-5]. Analyzing the data available in the literature, PRT are tumors with an incidence constituting 0.01-1.0% of all neoplasms of the human body [6-9]. In about 8% of cases they are malignant [10].

Preoperative diagnosis, especially in the case of small tumors, is a challenge, does not present a specific clinical picture and is frequently detected incidentally. The symptoms become clinically important as a result of compression, displacement or invasion of adjacent organs, being determined by the specific properties of the developmental sites. Clinical syndromes occur depending on the affected organelles and vital structures [11-12].

The most common symptom of TRP is pain, which can be localized in the tumor area or diffuse, radiating throughout the abdominal cavity, loins or lower limbs. Abdominal pain or low back pain, according to the literature, occurs in 44–75% of patients later diagnosed with PRT. The nature of the pain may be permanent or intermittent, acute or insidious. Despite the fact that the pain manifests itself depending on the location of the tumor, it is difficult to determine its exact location. The main mechanism of pain is the direct compression by the tumor of the retroperitoneal or intraperitoneal anatomical structures.

Dyspeptic syndrome, with complex gastrointestinal symptoms, is often known in these patients. Meteorism and abdominal distension may occur in 4-35% of patients, and in some cases, intestinal occlusion may develop through compression or extrinsic invasion of the intestines.

Cachexia, weight loss, general weakness, and fatigue occur in 40–50% of patients with advanced retroperitoneal

malignancies, compared with only 3% of those with early-stage disease.

Pelvis-invading retroperitoneal tumors can compress or can grow in the sacral or lumbar plexus, causing back pain with irradiation in the lower limbs uni- or bilaterally. The edema of the lower limbs appears after compression of the pelvic veins and lymph ducts.

Fever, which is present in about 10% of patients with PRT, is due to necrosis and destruction of tumor tissue or urinary tract infections as a result of urostasis.

Occasionally, retroperitoneal tumors are found on physical or instrumental examination of patients due bloating, early satiety, and abdominal discomfort. Only about 30.0% of patients with retroperitoneal tumors initially have asymptomatic abdominal mass, most of which are addressed when the tumor becomes palpable and is characterized by "secondary" symptoms. Abdominal tumor can be detected on physical examination in more than 90%. Considering that the frequent clinical picture is secondary, the diagnosis of PRT begins with laboratory analyzes and methods of paraclinical investigations useful for the differential diagnosis with a potential pathology of the manifest clinical organ.

The aim of the study. Evaluation of the usefulness of imaging tools: the degree of informativeness and accuracy for preoperative diagnosis of PRT.

Material and methods

Complex prospective and retrospective analysis of clinical, anamnestic and imaging data of 118 patients with primary and non-primary retroperitoneal tumors investigated and treated at the Institute of Oncology of the Republic of Moldova, during 2015-2020. The group of patients with PRT included in the study consisted of 48 women (57.1% (95% CI 46.5, 67.3)) and 36 men (42.9% (95% CI 32.7, 53.5)). The mean age of patients with PRT in the research group is 57 years (σ = 12.0), Me = 59, Q1 = 51, Q3 = 67.

Results

To determine the primary PRT diagnosis for the clinician, it is primordial to rule out the organic or secondary nature of the tumor. Thus, the patients were examined by: abdominal USG – 118 patients (100%), abdominal and small pelvic contrast-enhanced CT – 118 patients (100%), MRI – 3 patients (2.5%), videoesogastroduodenoscopy – 32 patients (27.1%), videocolonoscopy – 31 patients (26.3%), irigography – 4 patients (3.4%), urography – 29 patients (24.6%).

Ultrasonography (USG) of the peritoneal cavity and retroperitoneal space is a first intention, cheap and highly informative imaging method. The main intention in applying this diagnostic method is to establish the presence of supracentimetric tumors. USG's ability to assess the origin and benign or malignant nature of retroperitoneal tumors is limited. The sensitivity of USG, according to the data presented in the literature is 84.6%. USG provides indirect information that may be useful in assessing the retroperitoneal location of the neoplastic process. The use of the DOPPLER regime may highlight the presence of feeding vessels. The malignancy of the tumor formation has several criteria: irregular character, mixed structure and abundant vascularity.

The method of investigation CT with intravenous contrast or CT angiography offers a detailed and appropriate image of the PRT and the relationship it has with the anatomical structures or adjacent organs. This method of investigation provides sufficient information that may suggest the benign or malignant nature of the tumor, having a key role in planning the treatment strategy. [13]. Although it offers the possibility of a complex differential diagnosis and has a high degree of certainty in diagnosing a retroperitoneal tumor, CT examination is not able to differentiate between the 50 histological subtypes of retroperitoneal sarcoma [14].

Ultrasound examination and intravenous contrast CT of the abdominal cavity and retroperitoneal space were the main imaging methods applied in the study, in order to provide the necessary information for planning the surgery, used in all 118 patients (100%) included in the study and have provided information on: the size of the tumor, the location, the uni- or multicentric structure of the tumor, the character of the edges and the relationship of proximity with adjacent organs.

Both diagnostic investigations, USG and intravenous contrast CT, provide feasible data regarding TRP dimensions, but CT provides dimensional information with greater accuracy. The partial correlation between the dimensions of the operating part and the dimensions estimated at USG is 0.540 (95% CI 0.295, 0.737, p <0.001), which represents a high positive correlation, as well as in the case of the dimensional values offered by CT 0.789 (95% CI 0.693, m0.873, p <0.001), there is a significant positive correlation. Since the value of p = 0.001, this means that in both cases the partial correlations between the actual size of the tumor and the size provided by the diagnostic tests performed, USG and CT with intravenous contrast are statistically significant.

Regarding the determination of the uni- or multicentric character of the tumor, the CT showed an integrative value of sensitivity and specificity of 0.733 (95% CI 0.527, 0.939, p <0.001), while the USG – a value of 0.644 (95% CI 0.415, 0.873, p <0.001). Comparing the average quality of the USG vs CT model in detecting multifocal tumors, CT is more valuable (fig 1).

Contrast-enhanced CT, as a diagnostic test to identify the character of tumor margins, showed the integrative value of sensitivity and specificity of 0.617 (95% CI 0.490, 0.745, p <0.001), which indicates that contrast-enhanced CT has a significantly better ability compared to an occasional diagnostic test. The USG-based method also showed a higher absolute value of AUC 0.720 (95% CI 0.601, 0.838, p <0.001, interpretations being similar to CT), the average quality of the USG model being higher compared to the CT with intravenous contrast (fig. 2).

The assessment of the anatomical site is essential for

scheduling the type and volume of surgery. The diagnostic test used to determine the intra- or retroperitoneal location of the tumor based on intravenous contrast CT showed the integrative value of sensitivity and specificity at 0.620 (95% CI 0.496, 0.743, p <0.001), while the test based on USG demonstrated the same integrative sensitivity and specificity values of 0.641 (95% CI 0.541, 0.740, p <0.001), indicating that both CT and USG indicate good diagnostic accuracy. Analyzing the average quality of diagnostic models, USG is insignificantly higher compared to CT (fig. 3).

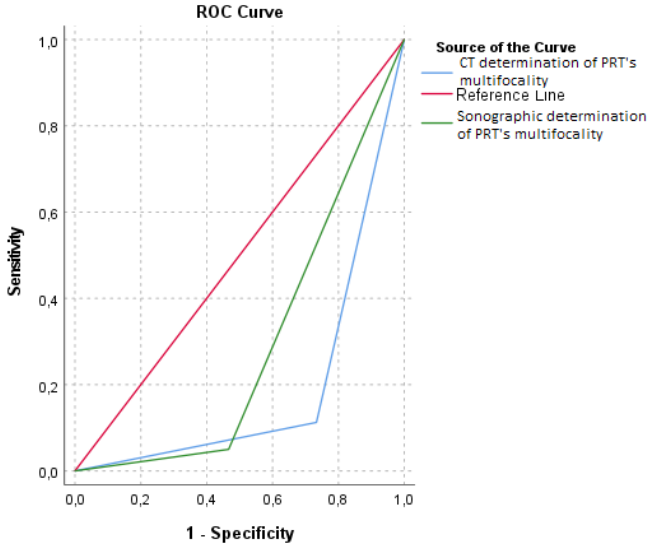

Fig.1. ROC curve for USG and CT accuracy in determining the uni- or multicenter character of the tumor

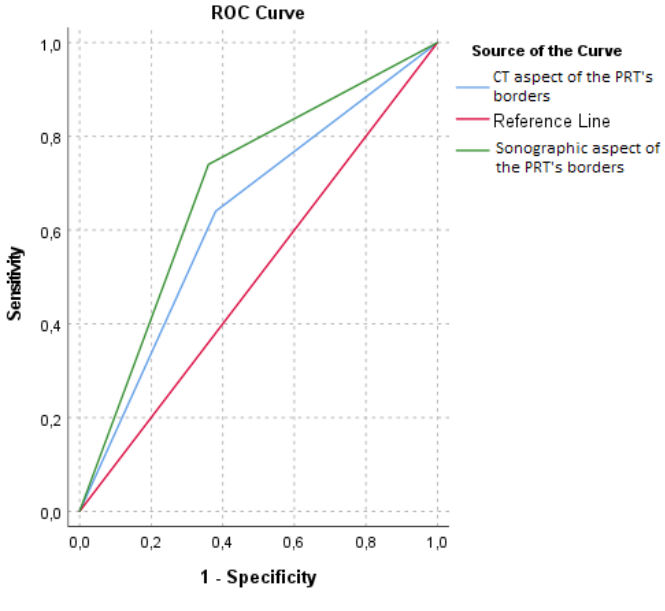

Fig. 2. ROC curve for USG and CT accuracy in determining the character of tumor margins

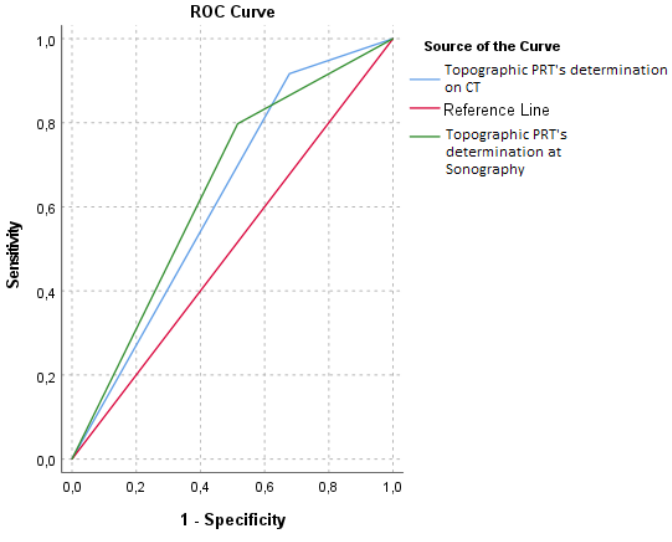

Fig. 3. ROC curve for USG and CT accuracy in determining the intra- or retroperitoneal location of the tumor

Conclusions

The symptomatic and imaging aspect of retroperitoneal tumors is not specific. The important diagnostic challenges are: the precise location of the tumor, the exact assessment of the tumor extension and invasion, the qualitative identification of the tumor cell origin. CT and USG can provide important information about the anatomy of the retroperitoneal space, so tumors can be located accurately. The retroperitoneal tumor can be assessed by determining the specific nature of the tumor (cystic, solid, or cystic-solid), appearance (round, oval, irregular, or lobed), size, relationship with neighboring organs, tissue structure, and tumor margins (clear or blurred). Contrast-enhanced CT and simple USG or DOPPLER regimen, in most cases can rule out the organic origin of the tumor, and in some cases can determine the type of growth and invasive behavior of the tumor. Although the exact structural component of the tumor cannot be determined, the diagnosis of a retroperitoneal tumor is tentative. Imaging data allow surgeons to plan surgery, select an optimal surgical approach, and evaluate the effectiveness and possible recurrence in the postoperative period. Based on the partial correlation calculated between the estimated dimensions at CT 0.789 (95% CI 0.693, 0.873, p <0.001), USG 0.540 (95% CI 0.295, 0.737, p <0.001) and the actual dimensions of tumors assessed intraoperatively or postoperatively, there was made the conclusion that CT provides more accurate data than USG. The image obtained at the USG examination is flat, therefore the dimensional measurements of the tumor are not always performed on the longest tumor axis, especially in the case of polylobulated or giant tumors, while the tomographic examination techniques allow the three-dimensional reconstruction of the tumor with more accurate assessment of the tumor size.

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VS interpreted the data and performed the analytical part of the work, drafted the first version of the manuscript; CS-C and NG conceptualized the project, designed the research and revised the manuscript critically.

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Features of hemostasis in patients with non-ST-elevation myocardial infarction

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Abstract

Background: Coronary thrombosis is the key pathogenic mechanism of acute heart attack, including non-ST segment elevation (NSTEMI). Given that, the detection of reliable markers of hemostasis disorders is important in the process of optimizing the diagnosis of NSTEMI.
Material and methods: The study was conducted on 54 patients with NSTEMI (average age 69.7±1.5 years). In 60% of cases, 3-vessel disease was noted; 56% of patients had ejection fraction >50%, and Killip class I of heart failure was revealed in 78% of patients. With the help of the STA-Liatest (France) equipment, the blood tests determined the following hemostasis markers: fibrin monomers (FM), thrombotic complex activity of factors II, VII and X. Additional markers like Procoag, the coagulation indicator dependent on circulating phospholipids or SPA, D-dimers, as well as factors C, S and antithrombin III were appreciated. The values of these markers determined by the same method in 20 healthy persons (control group) were used as normal values.
Results: Circulating level of FM on admission was increased twice, while the values of Procoag and SPA were significantly decreased by 35.3% compared to the control. Factors C, S and antithrombin III were 54-80% of the control value range, and D-dimers were within the permissible values. In the acute phase of the heart attack, a deterioration of hemostasis indicators was noted, excepting the D-dimers. The levels of FM determined 24 and 72 hours after revascularization were consistently increased (up to 3.8 times) compared to the control, while Procoag and SPA decreased by 54-57%. Further reduction of factors C, S and antithrombin III accounted for 42-54% of normal indicators. After 5 days, an improvement in hemostasis markers was observed, but a significant difference still remained comparing to the control group.
Conclusions: The hemostasis particularities discovered in patients with NSTEMI indicate the features of an activated prothrombotic status, and FM could be an important diagnostic marker of NSTEMI, due to its most significant deviation from the normal value (>100%). It can reliably reflect the thrombin level, which triggers the last enzymatic phase of thrombus formation.
Key words: NSTEMI, hemostasis disorders.

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Introduction

Impairment of hemostasis manifested by increased prothrombotic activity is common in vascular accidents (acute myocardial infarction and cerebrovascular accident) which show the highest cardiovascular mortality rate [1]. The formation of thrombus in the lumen of arteries and arterioles is classically confirmed pathophysiologically as an imbalance between the coagulation and anticoagulant systems in combination with the failure of the fibrinolysis system, which leads to a decrease in arterial blood flow and triggers the ischemic cell necrosis. From pathogenic point of view, the prothrombotic phenomenon can be initiated and sustained by endothelial damage and inflammation, platelet activation and oxidative stress, blood rheological disorders and genetic polymorphism correlating with the expression of hemostasis regulation factors, etc. [2].

Given the certain relationship between coronary atherosclerosis and acute myocardial infarction with ST-segment elevation (STEMI) or without (NSTEMI), intracoronary thrombosis is, according to the Guidelines of the European Society of Cardiology, the determining cause of the infarction, leading to total occlusion of the coronary artery in STEMI or subtotal in NSTEMI [3]. In NSTEMI, thrombosis of the subendocardial arteries (arterioles <200 μm in diameter) can lead to the development of subendocardial myocardial infarction even when the subepicardial artery (culprit artery) is less than 75% occluded. The severity of thrombotic conditions and dysfunction of the coronary microcirculatory system correlates with the prognosis of NSTEMI and explains the risk of infarction even when an average-to-minimal gradient of transmural coronary perfusion is maintained. However, in the European Society of Cardiology Guidelines for the management of STEMI, hemostasis parameters are not included in the diagnostic and prognostic biomarker algorithm, and the long-term periprocedural and postinfarction treatment (up to 12 months) is based on antithrombotic drugs (anticoagulant and/or antiplatelet therapy).

Hemostasis represents a complex and multi-hierarchical system of homeostasis, and prothrombotic events derive from intrinsic and/or extrinsic triggered cascade of reactions

resulting in the formation of respective tenases linked to the endothelial injury and activation of oxidative stress, inflammatory response and blood rheological disorders.

Thus, the assessment of key indicators of hemostasis can strengthen important landmarks for the pathophysiological detection of prothrombotic preconditioning and the prediction of the risk of NSTEMI as well as the identification of diagnostic and prognostic predictors.

In this aspect, the aim of the study was: comprehensive assessment of hemostasis markers in patients with NSTEMI. The main objectives of the study:

1. Evaluation of the functional feasibility of the main components of hemostasis (coagulation, anticoagulation, fibrinolysis) in order to identify the inherent pathogenic mechanisms, which is an argument in favor of the pathogenetic targeted treatment.

2. Evaluation of hemostasis indicators not only at admission, but also during different periods of the acute phase of MI without ST segment elevation (24 hours, 72 hours, 5 days), which has the greatest impact on the survival of a patient undergoing angioplasty.

Material and methods

The study was conducted on a group of 54 patients with NSTEMI who underwent primary coronary angioplasty in the interventional cardiology laboratory within the institutional project "*Evaluation of instrumental and biochemical markers in the management of patients with acute myocardial infarction without ST segment elevation, as well as in the assessment of the degree of coronary microvascular damage*". The general characteristics of the patients included in the study are presented in table 1. The indices detected during coronary angiogram are presented in table 2.

Table 1. Clinico-demographic indicators of patien

Indices	N	%	M±m
Age, years			69.7±1.5
Men	35	65	
Women	19	35	
Hypertension	50	92.6	
Dyslipidemia	46	86	
Diabetes	18	34	
Smoking	19	36	
Atrial fibrillation	13	24	
History of stroke	8	15	
Heart failure (HF), Killip Class I	42	78	
Killip Class II	10	19	
Killip Class III	2	3	
Ejection fraction (EF) >50%	30	56	
EF 40-49%	11	21	
EF <40%	5	10	
EF ≤ 35%	8	13	
Glomerular filtration rate, ml/min			60.7±3.26
GRACE score			129±6.4
TIMI score			4.75±0.2

Table 2. Indicators of coronary artery injury in patients with NSTEMI

Number of injured coronary arteries (stenosis >50%)			
1-VD	2-VD	3-VD	LMS
11 (21%)	9 (17%)	32 (60%)	2 (4%)
culprit coronary artery			
LAD 30 (56%)		LCX 16 (30%)	RCA 8 (15%)

The main blood values of hemostasis in all NSTEMI patients were assessed using the STA-Liatest laboratory equipment (France) at admission and 24h, 72h and 5 days after revascularization.

Regarding coagulant activity:

- Factor-dependent coagulation index (II, VII and X) or prothrombotic complex activity index (ICP), estimated in seconds (sec).
- Circulating procoagulant phospholipids (IFP) coagulation index, estimated in sec.
- Fibrin monomers (FM) as an indicator of plasma thrombin activity, estimated in mg/ml.
- Regarding anticoagulant activity:
- Protein C, estimated as % from acceptable normal reference value (4 mg/L).
- Protein S, estimated as % from acceptable normal reference value (35 mg/L).
- Antithrombin III (AT III), estimated in % from accepted reference value (< 250 ng/ mL).
- Regarding fibrinolytic activity:
- D-dimers, estimated in mg/ml.

All the above indices of hemostasis were also appreciated in 20 apparently healthy people (control group), using the same laboratory equipment.

The group of patients with NSTEMI assessed for hemostasis did not include people with raised procoagulant status due to autoimmune diseases (systemic lupus, rheumatoid arthritis), deep vein thrombosis, pregnancy, oral hormonal drugs, contraceptives, cancer, etc. All patients with NSTEMI who underwent coronary angioplasty received pre- and post-procedural therapy for up to 12 months including antiplatelet agents and, if necessary, anticoagulants in accordance with the Guidelines for STEMI and PCI [3, 4].

In order to exclude antiphospholipid syndrome, which has a convincing prothrombotic effect, the value of antiphospholipid antibody (lupus anticoagulant) was determined using the Dilute Russell Viper Venom Time (DRVVT) blood test [5]. There was no significant difference between the values of the DRVVT parameter between groups (tab. 3).

Table 3. Value of the DRVVT index (sec)

The control lot M±SD	NSTEMI patient cohort M±SD	P
39.6 ± 4.8	41.7 ± 6.5	>0.05

Evaluation of the laboratory analysis did not reveal any indicators that could affect hemostasis in patients with

NSTEMI, the hematocrit level being at 38.6±1.7%, and the platelet count 246±14 x 103/μl. The degree of inflammatory response upon admission was determined by the level of highly sensitive protein C (hsCRP) in the blood serum, equal to an average of 11.4 mg/l, and the average value of ESR (erythrocyte sedimentation rate) of 22±3 mm/hour.

Statistical processing of the obtained digital material included the determination of the mean (M) and standard deviation (SD). When comparing indicators between groups (control and patients with NSTEMI), the discrepancy was considered significant at $p<0.05$.

Results

The admission values of hemostasis indices are presented in table 4.

Table 4. Hemostasis parameters of patients with NSTEMI before revascularization

Parameters and their reference ranges according to the method	Control	NSTEMI	P vs control
ICP, sec (60-80)	72.3±9.1	46.8±6.6	<0.01
IFP, sec (70-130)	83.6±9.4	61.5±7.2	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	9.5±1.3	<0.001
Protein C, % (70-130)	85.4±10	59.4±7.6	<0.001
Protein S, % (60-170)	88.1±9.9	47.7±6.4	<0.001
Antithrombin III, % (80-120)	92.6±11	73.4±6.3	<0.05
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.41±0.08	<0.001

Analysis of the results indicates an accentuated prothrombotic pattern at the admission of patients with NSTEMI, given a significant decrease in the values of ICP and IFP by 35.3% and 26.4%, respectively, compared to the control value. The reduction in clotting time, dependent on the prothrombin complex and phospholipids, was accompanied by a two-fold increase in the level of circulating fibrin monomers (9.5±1.3 vs. 4.7±0.8 mg/ml), which reflects a high level of thrombin. In this context, it should be noted that the explored anticoagulant system indices are imposed by significantly reduced admission values. Thus, the value of protein C and protein S was estimated to be below the control value by 30.5% and 45.9%, respectively. The antithrombin III value was significantly reduced by 19.7% (74.4±6.3 vs 92.6±11%).

The blood concentration of D-dimers fell within the reference range of the imminent index of the used laboratory method, but significantly rose above the value of the control group by 42% (0.41±0.08 vs 0.29±0.05 mg/ml).

Therefore, the impairment of the functional capacity of the anticoagulant system is in an intelligible relationship with the procoagulant augmentation.

Following 24 hours from the moment of revascularization of patients with NSTEMI, the procoagulant activity becomes more pronounced (tab. 5).

Table 5. Hemostasis parameters of NSTEMI patients 24 hours after myocardial revascularization

Parameters and their reference ranges according to the method	Control lot	NSTEMI	Deviations vs admission	P vs control
ICP, sec (60-80)	72.3±9.1	34.2±5.3	-26.9%*	<0.001
IFP, sec (70-130)	83.6±9.4	50.4±6.5	-18.1%*	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	12.6±1.8	+33%*	<0.001
Protein C, % (70-130)	85.4±10	51.7±6.5	-13%	<0.001
Protein S, % (60-170)	88.1±9.9	41.4±5.9	-13.2%	<0.001
Antithrombin III, % (80-120)	92.6±11	59.7±6.1	-18.7%*	<0.05
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.44±0.07	+8%	<0.001

Note: * – significant discrepancy vs admission level.

At the distance of 24 hours after revascularization, there was a significant decrease in ICP and FPI values compared to the admission level by 26.9% and 18.1%, respectively. The result of this decline was the increase and the rebound of the indices compared to the control value, which became equal to 52.7% and 39.7%, respectively. On the background of the reduction of the coagulation time dependent on the prothrombotic complex, the blood content increased by 33%, a fact that determined an incremental gap of the index compared to the control by 2.68 fold.

Although, after 24h, the procoagulant activity was in a notable rise, the value of the anticoagulant proteins C and S decreased insignificantly with an average rate of 13%. However, it is worth mentioning the significant depreciation of AT III by 18.7%.

The circulating level of D-dimers increased insignificantly (by 8%) and remained at the maximum level of the index references accepted by the estimation method.

The changes in hemostasis indices at 72 hours after revascularization are important, when the inflammatory response reaches its peak intensity, taking into account the maximum expression of M1 pro- inflammatory macrophages (tab. 6).

Table 6. Hemostasis parameters of patients with NSTEMI, 72 hours after myocardial revascularization

Parameters and their reference ranges according to the method	Control lot	NSTEMI	Deviations vs admission	P vs control
ICP, sec (60-80)	72.3±9.1	31.3±4.1	-33.1%*	<0.001
IFP, sec (70-130)	83.6±9.4	37.8±5.3	-38.5%*	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	17.9±2.1	+89%*	<0.001
Protein C, % (70-130)	85.4±10	50.2±6.3	-15.5%*	<0.001
Protein S, % (60-170)	88.1±9.9	40.1±5.1	-15.4%*	<0.001
Antithrombin III, % (80-120)	92.6±11	53.8±5.9	-26.7%*	<0.001
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.50±0.06	+22%*	<0.001

Note: * – significant discrepancy vs admission level.

This post-infarction period excels with the maximum reduction of indices reflecting procoagulant activity compared to the admission level: ICP by 33.1% and, in particular, IFP by 38.5%. It is important to emphasize that the coagulation time dependent on the procoagulant phospholipids, decreased from the imminent 24h index, more than the ICP rebound: 25 vs 8.4%. The circulating level of MF increased by 42% and exceeded the control value by 89%.

Another important feature of hemostasis dynamics in patients with NSTEMI at 72h after angioplasty compared to the values at 24h is the insignificant decline in the level of proteins C and S, which was within the limits of 2.9-3.1%. AT III decreased during this period by 9.9% and reached an underlying level compared to the control at the rate of 26.7%.

The average blood content of D-dimers increased by 22% compared to the admission level, but does not exceed the index reference range accepted by the estimation method.

At a distance of 5 days after revascularization, the analysis of the obtained results indicates an attenuation of the prothrombotic activity (tab. 7).

Table 7. Hemostasis parameters of NSTEMI patients 5 days after myocardial revascularization

Parameters and their reference ranges according to the method	Control lot	NSTEMI	Deviations vs admission	P vs control
ICP, sec (60-80)	72.3±9.1	40.5±5.8	-13.4%	<0.001
IFP, sec (70-130)	83.6±9.4	51.4±6.7	-16.4%*	<0.001
MF, mg/ml (0.1-6.0)	4.7±0.8	12.0±1.7	+27%*	<0.001
Protein C, % (70-130)	85.4±10	52.7±6.1	-11.3%	<0.001
Protein S, % (60-170)	88.1±9.9	42.4±5.3	-10.5%	<0.001
Antithrombin III, % (80-120)	92.6±11	56.4±6.2	-23.2%*	<0.001
D-dimers, mg/ml (0-0.5)	0.29±0.05	0.44±0.08	+8%	<0.001

Note: * – significant discrepancy vs admission level.

Thus, the main indices reflecting the procoagulant activity, ICP and IFP, demonstrate an increase of 30% and 36%, respectively, compared to their minimum level recorded after 72 hours. However, their values remain below the admission level by 13.4-16.4%. It should be noted, that in relation to the control indices, ICP and IFP have a significant rebound of 44% and 38.5%, respectively.

The blood content of fibrin monomers decreased to an average rate similar to the decline of ICP and IFP, by 33% from their maximum level during the 72-hour period, but remained significantly higher than the index admission value by 27%.

Protein C and protein S values were insignificantly elevated at 72 hours to 5 days, approaching admission levels with a small difference, but the discrepancy versus control remained consistent at rates between 38.3% and 52%.

The increase in antithrombin III was 5%, and the difference from admission and control remained significant at 23.2% and 39.1%, respectively.

The blood concentration of D-dimers decreased by 8% compared to the maximum level attested at the distance of 72 hours and reached the admission level, consequently maintaining within the range of normal references accepted by the method.

Discussion

The study revealed important changes in hemostasis in patients with NSTEMI, and can strengthen the conclusive pathophysiological landmarks regarding its evolution. On the other hand, the revealed changes can serve as diagnosis predictor, prognosis and optimization of the patient's post-infarction treatment. Hemostasis parameters were determined in 54 patients with NSTEMI using laboratory equipment STA-Liatest (France) at admission, as well as in the acute phase of myocardial infarction. In order to detect the mechanisms which endanger hemostasis, there were evaluated procoagulant markers (ICP, IFP and FM), anticoagulant markers (protein C, protein S and AT III), and D-dimers as a marker of both fibrinolytic enzyme activity and presence of intravascular thrombi, degraded by proteolysis. It should be noted that the group of patients with NSTEMI did not include people with prothrombotic preconditioning, such as deep vein thrombosis, autoimmune diseases (e.g. systemic lupus, rheumatoid arthritis), pregnancy, cancer, long post-traumatic immobilization and/or post- surgery, acute inflammatory process, administration of anticoagulant remedies, affecting blood rheology.

Estimating the connection between the early key events of the post-infarction evolution and the character of hemostasis, it is important to elucidate its contribution as a NSTEMI trigger on the background of subendocardial arteriole thrombosis. Meanwhile, it states the remodeling of the myocardium, starting already in the first hours after the onset of necrosis and involving prothrombotic factors, such as endothelial damage due to angioplasty, activation of oxidative stress and inflammation. In this aspect, hemostasis indices in the post-infarction period were determined at the interval of 24 hours (maximum infiltration of the necrotic area with neutrophils), 72 hours (the period of maximum expression of M1 macrophages) and 5 days, the period of macrophage repolarization (macrophage differentiation M1 in anti-inflammatory macrophages, M2).

The obtained results showed that, upon admission, the coagulation time dependent on the prothrombotic complex (II, VII, X), ICP and dependent on circulating procoagulant phospholipids, IFP was significantly reduced by up to 35.3%. Given the increased blood coagulation in patients with NSTEMI, a double raise in the circulating level of fibrin monomers was also observed. Importantly, the value of ICP and FM is indispensable for thrombin content, which results from the proteolytic cleavage of prothrombin under the action of tenases (intrinsic and extrinsic) and thrombokinase. Once formed, thrombin cleaves plasma fibrinogen into fibrin monomers, stating its direct relation to thrombin concentration [6-8]. Although both tenases finally converge

in a common path of thrombin formation under the action of factors Va and Xa, in atherosclerosis and coronary injuries, extrinsic tenase (tissue factor + factor VIIa) may have a notable role regarding prothrombotic activity, given the endothelial injury. Tissue factor is also a trigger of the inflammatory response, which in turn may increase coronary endothelial injury, resulting in excessive growth factor release.

It is noteworthy that at 24h and 72h from the onset of NSTEMI, marked by neutrophil infiltration and M1 macrophage activation, respectively, the inflammatory response is maximum, and the 33.1% decrease in ICP during these intervals in this study was logically associated with an increase in MF levels in blood up to 89%. Namely, in the 72-hour postinfarction period, recognized as the peak of the inflammatory reaction, the ICP value was minimal, and the MF value was maximal. Thus, there is a close pathophysiological relationship between ICP and FM, and changes in these parameters in patients with non-ST elevation MI, similar to those observed in this study, reflect the activation of the coagulation cascade in both ways and can be diagnostic predictors of NSTEMI.

In addition, the change in the IFP index is in clear contiguity with the change in ICP and correlates with FM. The circulating procoagulant phospholipid-dependent clotting time reduced on admission in NSTEMI patients decreases exponentially at 24 hours and further at 72 hours. Circulating procoagulant phospholipids represent a factor facilitating the formation of tenases by assembling clotting factors in the presence of phosphatidylserine expressed by activated platelets, apoptotic bodies during apoptosis of endotheliocytes, or endothelial exosomes appreciated rather like extracellular vesicles. The formation and activation of external tenase, which is also reflected in the ICP index, occurs much faster in the presence of negatively charged phospholipids [9, 10]. Elevation of circulating procoagulant phospholipids is a direct repercussion of the endothelial injury potentiated by the inflammatory response and oxidative stress in the period between 24-72 hours. In particular, during this period, IFP was observed to decrease by up to 38.5% compared to the admission value, and compared to the control index, the rebound was 54.8%. Similar to the ICP, the decline in the value of IFP index correlated with the increase in the quantitative level of fibrin monomers. Moreover, at a distance of 5 days after revascularization, during the period of attenuation of the inflammatory response caused by expression of M2 anti-inflammatory macrophages, their improvement was noted at similar levels.

The increase of FM blood levels in patients with NSTEMI has been noted in other studies, and an increase similar to the one in this study is reported by M. Arthamin et al. (2019) who applied the ROC curve to demonstrate that at the marker value of 8.2 mg/ml, its specificity and sensitivity towards NSTEMI is 87-90%, which is close to the value of 18 ng/L obtained for troponins (85-91%) [11].

In the present research, the average circulating level of FM at admission was 9.5 mg/ml, which establishes the predictive value of the marker regarding the diagnosis of NSTEMI. Moreover, it is intelligible to assume that FM can show an increasing dynamic up to the moment of the necrotic injury of cardiomyocytes manifested by troponins elevation, also stated by other authors [12-14]. At this point, it is conceptually and practically important to note that, in patients with NSTEMI, the circulating level of FM correlates directly with the level of troponin elevation above the 99th percentile, as well as with the risk of MACE at a distance of 6-12 months.

Thus, there is no doubt that the assessment of the factors that have a definite contribution to the fibrinogen cleavage process under the proteolytic action of thrombin has not only diagnostic and prognostic significance, but also a predicting role of NSTEMI development. The presented data provide important evidence in this context, given the association between FM and activated tenases (ICP index), circulating procoagulant phospholipases (IFP index), and increase of the inflammatory response.

Therefore, these 3 measures of hemostasis (FM, ICP and IFP) demonstrate a consistent pathophysiological association with procoagulant activation in NSTEMI patients and can be accepted as a panel of hemostasis markers with predictive value for diagnosis and prognosis. At the same time, the degree of decrease in ICP and IFP, corresponding to the FM increase, can serve as a marker for the extent of coronary arteries thrombosis, but also a predictor of the degree of cell damage, cell apoptosis, inflammation and oxidative stress. Efficient strategies for correcting these prothrombotic conditions can also be designated under this umbrella. These postulates are supported by literature data reported by other authors which demonstrated that procoagulant activity in patients with NSTEMI is closely related to the circulating level of micro-particles expressing phospholipids (derived from endotheliocytes and platelets), a fact that suggests the diagnostic feasibility of IFP estimated in this study [15, 16]. In this context, it is important to approach phosphatidylserine blockade as a tool to mitigate procoagulant activity in patients with NSTEMI, primarily in the acute phase of myocardial infarction or to prevent the development of NSTEMI in those at imminent risk.

It is also important to mention that in patients with acute coronary syndrome the level of microparticles expressing procoagulant phospholipids does not increase on the background of a moderate inflammatory response, according to the serum value of the proinflammatory markers [17]. So, it is plausible to admit that the transition of unstable angina to NSTEMI is facilitated by the activation of the procoagulant status against the background of increased inflammation and the elevation of circulating procoagulant phospholipids. The complement system activated by C-reactive protein through the alternative pathway may be involved in procoagulant activation, given the ability of the C5a component to activate CD88 receptors expressed on platelets, leukocytes and endotheliocytes, resulting in the expression of phosphotadylserine. The determination of C5a is presumptively imposed by the feasibility of predicting prothrombotic activation, and the modulation of com-

plement activity may be a tool to prevent the prothrombotic process in patients with NSTEMI.

In this study, the circulating level of hsCRP is increased, which is in agreement with the recent data report by K. Pluta et al. (2022) according to whom, on the ground of inflammation, the activation of the procoagulant system is imposed by the formation of aggregates from leukocytes and platelets (aggregation factors being the GPIIb/IIIa receptors, PSLG-1 and SD-40 ligands, as well as fibrinogen), constituting a trigger for ACS, including NSTEMI [18]. Thus, the attenuation of inflammation can also contribute to the attenuation of procoagulant activity.

Considering the trigger role of thrombin in thrombus formation, an objective of this study was to estimate the components of the anticoagulant system, which basically have the natural mission of preventing or attenuating thrombin, such as: antithrombin III, proteins C and S, and thrombomodulin. Although their role in triggering and exacerbating prothrombotic events is well argued, there are currently very few reports on the nature of their changes in patients with NSTEMI.

The obtained results indicate a significant reduction in the admission value of proteins C and S between 30 and 46%, as well as AT III by up to 20%. There are opinions, according to which the reduction of protein C below the level of 60-70% of the normal value indicates a hereditary cause of deficiency of this important anticoagulant factor [19]. The protein C regression up to 46% established in this study could plausibly be determined by 2 causes: (1) exaggerated consumption of the factor against the background of excess demand from the activated procoagulant status and/or (2) its poor synthesis in the liver.

This functional incompetence of the anticoagulant system may clearly be a mechanism for compromising the regulation of the thrombin cascade. Moreover, the impairment of these 3 anticoagulant factors at 24 and 72 hours after revascularization was directly associated with the modification of ICP, IFP and FM indices. The anticoagulant activity of factor C and cofactor S, vitamin K-dependent glycoproteins synthesized in the liver, is determined by the selective inhibition of procoagulant factors Va and VIIIa, involved in the activation of factor X and the respective formation of thrombin [20, 21]. Coronary endothelial injury accompanied by the inflammatory process decreases the expression of endothelial receptors of the annexin-V family involved in the activation of anticoagulant factor C.

Antithrombin III, synthesized in the liver, is part of the family of serum protease inhibitors, capable of inhibiting thrombin and factor Xa (the effect on factors IXa and XIa is weaker). The inhibitory activity of AT III and, respectively, the anticoagulant activity, increases, on average, by 1000 fold when it binds with heparin.

Therefore, proteins C and S, as well as AT III can be included in the panel of hemostasis markers together with ICP, IFP and FM that have predictive value for prothrombotic activity in patients with NSTEMI, in the context of detecting the pathogenetic mechanisms of the hemostasis disorder, as well as the justification of pathogenetic treatment.

The estimation of D-dimers in patients with NSTEMI did not reveal a notable change in the marker of fibrinolytic activity. Its circulating level was within the limits of the normal references of the explored method STA-Liatest and although it increased during the first 72 hours from the moment of angioplasty, it did not exceed the average value of 5 mg/ml. The presence and circulating level of D-dimers in the blood indicates the phenomenon of enzymatic degradation of the fibrin thrombus under the action of plasmin, the blood content being correlative in particular with the severity of venous thromboembolic events.

Remarkably, that J. Kim et al. (2018) found a mean concentration of D-dimers in patients with NSTEMI at the value of 5 mg/L [22], a fact that corresponds conclusively with the received data. So, it is plausible to admit that D-dimers remain, however, a specific marker of deep vein thrombosis and a predictor of the risk of pulmonary thromboembolism. However, some reports suggest that D-dimer levels are lower in NSTEMI patients than expected in STEMI patients, and these findings are understandable taking into account the difference in thrombus volume [23].

At the same time, F. Biccire et al. (2021) support the role of D-dimers as a predictor of residual thrombotic risk as well as of major cardiovascular complications in ACS patients undergoing angioplasty [24]. The blood content of D-dimers depends not only on the expression and activity of plasmin, but also on the level of some fibrinolysis inhibitors of such as alpha2-antiplasmin, lipoprotein-a, complement C3a, thrombin-triggered inhibitor of fibrinolysis, etc., while the real predictive value of D-dimers regarding hemostasis endangerment in NSTEMI, remains to be proven more consistently [25].

Attempts to explain the reduced fibrinolysis in patients with NSTEMI manifested by the absence of a notable increase in D-dimers convincingly appeal to the inhibitory role of thrombin [12, 26], which according to the changes in the explored indices (i.e., ICP and FM) is conclusively high. Thrombin induces the activation of procarboxypeptidase in the liver, which cleaves the residues carboxy-terminals of the lysine in the composition of fibrin, necessary for the functional exercise of the tissue plasminogen activator to convert the precursor into plasmin, the enzyme that ensures the fibrinolysis of the fibrin thrombus and, respectively, the increase of D-dimers. Remarkable, in this context, are the results obtained by E. Shantsila et al. (2012), demonstrating increased levels of active carboxypeptidase (also defined as thrombin-derived fibrinolysis inhibitor) in NSTEMI patients, even more significantly compared to the marker attested in STEMI patients [27]. Thus, thrombin is a double-edged sword, which on the one hand triggers fibrinogen cleavage as a decisive procoagulant process, and on the other hand inhibits the formation of plasmin to the detriment of fibrinolysis, followed by the increase of D-dimers. Indices of hemostasis, such as ICP, IFP and FM, become of a greater importance, through their estimated

value of thrombin, predictive value of prothrombitic activity, the risk of developing NSTEMI, as well as markers of diagnostic value. In addition, the pathogenetic significance of monocytes in prothrombotic activation and the risk of developing NSTEMI is currently under debate, given their ability to release extrinsic tenase trigger tissue factor, thrombin promoter and, therefore, thrombin-derived fibrinolysis inhibitor, and the fibrin thrombus formed in this way is, according to some reports, much more resistant to the action of heparin and enoxaparin [28].

So, hemostasis modifications in patients with acute coronary syndrome remain a current topic of cardiology, and from the clear finding of the role of coronary thrombus in the genesis of STEMI and NSTEMI derives several research directions regarding the assessment of: (1) predictors of prothrombosis and risk of infarction in combination with various cardiovascular risk factors; (2) diagnostic and prognostic predictors; 3) risk predictors of MACE (major cardiovascular events) in the postinfarction period; 4) efficacy of hemostasis correction therapy in relation to postinfarction clinical and functional evolution of patients with NSTEMI.

Conclusions

1. Changes in hemostasis indices estimated in patients with NSTEMI excel by significantly reducing the value of ICP, IFC and increasing more than twice fold the FM, which indicates the activation of procoagulants, as well as a decrease of up to 46% in the circulating level of markers of the anticoagulant system (proteins C, S and antithrombin III) compared to normal values.

2. The plasma content of soluble fibrin monomers was imposed in this study by the most important deviation from the normal value (>100%), compared to other markers, it directly reflects the level of thrombin that triggers the last enzymatic phase of fibrinogen cleavage and can thus be prioritized as a diagnostic predictor of prothrombotic activation and NSTEMI.

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MP, LC and IP conceptualized the project and drafted the first manuscript. VI, MM, IIP and TD interpreted the data. VC critically revised the manuscript. All authors revised and approved the final version of the manuscript.

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Overview on possible causes of COVID-19

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Abstract

Background: The infection with the new coronavirus SARS-CoV-2 has caused a large number of cases of disease and death worldwide. Identifying the source of COVID-19 is an important issue though still unresolved. The analysis of the literature on highlighting possible sources of the SARS-CoV-2 virus was carried out.

Conclusions: The COVID-19 pandemic is occurring on the underlying imminent global ecological catastrophe as a result of the anthropogenic activity. Therefore, it can be stated that *Homo Sapiens* in the context of the interaction with the biosphere is a maladaptive species. According to the literature, the species' adaptive responses to environmental changes are due to endogenous retroviruses. The latter act as evolutionary factors. Possible pandemic COVID-19 is not a separate epidemic process caused by the penetration of a new virus into human populations, but rather is one of the manifestations of a more complex natural phenomenon – an evolutionary process under the guise of an infectious one. In terms of evolution, COVID-19 plays the role of a biosphere factor that seeks to help a relatively new species to adapt to the general conditions of survival in a symbiotic relationship with other living organisms.

Key words: COVID-19, lateral gene transfer, viruses, microbiota.

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"People usually blame fate, circumstances, other people for their own misfortunes, but not the main culprit of their misfortunes – themselves" - Plato.

Introduction

More than two years after the first documented cases in Wuhan, the origin of SARS-CoV-2 has not yet been established. In-depth researches do not indicate that Chiroptera is a direct zoonotic agent. At the same time, genomic analysis showed that SARS-CoV-2 has many specific characteristics not found in other Sarbecoviruses [1]. According to Boni et al., SARS-CoV-2 is not a recombinant of any sarbecovirus detected so far [2].

According to researchers, human activities can trigger various interactions between animal species and their viruses, sometimes causing the emergence of new viral pathogens with unknown pathogenic properties [1]. They, in turn, could quickly adapt to a new host organism (human) and acquire a stable intraspecific contagion.

Examples of this scenario are SARS-CoV and perhaps SARS-CoV-2.

The unique mechanism of CoV replication provides high-frequency genetic recombination in their RNA and subsequent mutations, thus enabling an extreme adaptability to new hosts and ecological niches [3].

Xiao K. et al., suggested that stray dogs in Hubei province may be natural reservoirs of the SARS-CoV-2 precursor and that high levels of antiviral protein in their intestinal tissue could trigger the development of a potential antecedent virus [4].

The above listed reflections of the authors, complemented by literature research, may outline a credible hypothesis for the origin of SARS-CoV-2. For a clearer understanding and explanation of possible plausible events, a bibliographic foray into some properties of microorganisms and particularly of viruses is necessary to be carried out.

Typically, the history of humankind communication with viruses is portrayed as a permanent conflict. This pattern is as follows – the emergence of infection, its generalization and, ultimately, its control or eradication. All stages are associated with fear, suffering and death. This picture shows the darkest virus image.

Retroviruses and retroelements found in the genome of modern humans have their own evolutionary history that began in the Mesozoic era (according to the chronology,

not less than 100 million years ago) [5]. Almost half of the human genome is made up of various transposable elements as HIV-like structures, collectively referred as retroelements [6].

Examples of different virus hypostasis refer to how the Arc neuron gene was obtained (retrotransposons, which are the ancestors of retroviruses). According to scientists, the Arc is necessary for synaptic plasticity viz. the ability of nerve cells to form and strengthen new nerve connections [7].

With a seemingly very simple structure, the virus contains vast genetic information. The discrepancy between the amount of information and the insignificant molecular weight of RNA and viral DNA is explained by special mechanisms that allow replicating to the same genetic sequence in different ways [8].

Virus persistence in a cell is possible due to a specific property called lysogeny [9]. Lysogeny (from the Greek *lysis* means decomposition, degradation and *geneia* – origin, creation) is a genetically determined ability of bacteria to lysis with the release of a bacteriophage several generations after the direct infection with it. Lysogenic state is associated with the presence of a potentially infectious structure of bacteria in cells, namely a prophage. Thus, the virus that has entered the cell does not betray its presence. Therefore, an infected cell is similar outwardly to a normal cell.

As a result of lysogenization, some properties of the bacterial cell can change (the so-called lysogenic conversion) that allows the bacteria to acquire new genetic information [10].

The authors reported that a viral infection is not only an infectious process, but also ultimately acts as an important evolutionary trigger [11, 12]. The researchers noticed that when the virus-induced lytic cycle develops, the host chromosome degrades. Subsequently, the bacterial chromosome fragments of the corresponding size are packed into phage bodies and, strictly according to the canonical rules of viral transfer (adsorption on receptors, injection of DNA contained in the cell body), are introduced into other cells, thus causing their transformation. At the same time, in a cell with formed bodies containing host's DNA fragments, the viral genome is also replicated and packed to a certain extent, thus causing it to multiply. Therefore, most viral bodies perform completely different functions, such as being a packing material for cellular genes without viral admixture.

Gene transfer for species perpetuation has also been reported between viruses. The symbiosis between mimivirus and the tiny Sputnik virophage is an example of that. Both infect amoeba, however, the virophage cannot reproduce in the absence of mimivirus [13]. Of the 13 virophage genes, 3 are similar to mimivirus and mammavirus genes, which can be introduced into the virophage genome during particle formation. It can be assumed that the Sputnik virus could transfer genes between viruses similarly to how bacteriophages transfer genes between bacteria [14].

The above-mentioned evolutionary changes are possible due to a unique property of microorganisms called *lateral gene transfer* (LGT). This phenomenon is a process in which an organism transfers genetic material to a non-descendant organism [15, 16], unlike vertical gene transfer, when an organism receives its genetic material from an ancestor [17]. Lateral gene transfer is the main mechanism in the evolution, maintenance and transmission of virulence [18]. The researcher Peter Gogarten points to horizontal (lateral) gene transfer as a "new paradigm for Biology" [19].

The long-term survival of the host bacteria is actually necessary for the phage (virus) [20]. The best way is to make sure that their hosts adapt to the rapidly changing environments and challenges. Some cases have reported that phages remove antibiotic resistance genes from neighboring cells competing for host bacteria in a process called autotransduction [21]. According to the researchers, viruses play an extremely important role in microbial evolution, thus creating an extensive biological network, which connects all the genomes in the bacterial universe [20].

Recent in-depth researches show that viruses can communicate with each other. Scientists noticed that during a host cell infection, temperate phages infecting *Bacillus subtilis* release a signaling peptide that shape the lysis-lysogeny decision in subsequent infections [22]. Thus, the phages produce new virions and then lyse their host when the signal concentration is low, but favor the latent infection when the signal concentration is high by lysogenizing the host cell. They found via a mathematical model, that a communication strategy in which phages use a lytic cycle at the onset of an outbreak (when susceptible host cells are abundant), shifting to a lysogenic cycle later (when the susceptible cells become scarce) is more appropriate than a strategy in which cells lysogenized with constant probability.

According to Stanciugelu I. et al., communication is the perfected result of thinking, the ascending process from the specific sphere to the abstract one [23]. According to Vladutescu S., communication is a structure in itself, through which we think about reality [24].

How is this possible that a non-living organism thinks? Perhaps, there is a mathematical model that allows this possibility and namely, the theory of integrated information, developed in 2004 by the Italian scientist Giulio Tononi [25]. He considers consciousness as the ratio between the quantity and quality of information, which is determined by a special measurement unit – φ (phi). The idea is that there is an upward series of transition states between the completely unconscious matter (0 - φ) and the conscious human brain (maximum - φ). Any object capable of receiving, processing, and generating information has a minimum level of φ, including inanimate objects, such as tonometer or LED, since they can convert blood pressure and light into data. Consciousness is the highest level of data processing. The scientist G. Tononi called this phenomenon integration. Integrated information is something that qualitatively surpasses the simple amount of collected data: not just a set of individual characteristics of an object, such as the yellow light, round shape and heat, but rather a full image of a lighted lamp consisting of all these characteristics.

To test whether inanimate objects can adapt and develop

experience, G. Tononi and his colleagues designed a virtual model [26]. The subjects were "animated" units with basic artificial intelligence. Each received randomly generated instructions for the body parts, being placed in a virtual maze. From time to time, researchers selected and copied animations that showed the best coordination. The next generation inherited the same code from the "parents". Its size did not change, but random digital "mutations" were introduced to strengthen, weaken, or supplement the connections between the "brain" and "limbs". Therefore, over 60000 generations, natural selection increased the efficiency of passing the maze in animates from 6% to 95%.

In this sense, the virus is far superior to many inanimate objects, since it carries (genetic) information itself. Viruses are more likely to increase the φ – level as viral generations succeed much faster. For example, the picornavirus genome is amplified by up to 50000 copies of each host cell in its active phase [27, 28], thus, in the active phase, hepatitis A viruses are also found in blood, reaching a concentration of up to 10^5 virus particles per milliliter [29].

There is another condition to integrate information at the level of consciousness, which requires a complex system. Similar to a computer program compressed by a file archiver, a virus does not need the entire control algorithm of the host cell. A short viral code is quite enough to make the entire operating system of the cell work properly for the virus itself.

According to recent experiments, while integrated in the organism, viruses can share and control the metabolic processes of the cell and the whole organism [30]. According to Raftery et al., and Delgado-Rizo et al., several viruses, including Hantaan virus (HTNV), H1N1 influenza virus (IV), human immunodeficiency virus (HIV-1) and the respiratory syncytial virus (RSV) directly stimulates immunocompetent cells to release neutrophil extracellular traps (NETs) [31, 32]. Therefore, in a complex system (like our body) viruses can use additional options to increase information processing which, according to G. Tononi's model, is assumed as intelligent life.

Viral interference with a macroorganism

Scientists describe amazing examples of the various impacts of viruses on the survival of host microorganisms [33]. The studies on the interaction of phytoplankton showed that marine viruses are able to change the way algae cells receive nutrients from the environment, while concomitantly harming and destroying the algae hosts. The viral genome often encodes genes derived from their host. These genes may allow the virus to manipulate the host's metabolism to improve its adaptive properties. Thus, a host-derived ammonium transporter has been identified in the genome of the phytoplankton virus infecting the small green algae *Ostreococcus tauri*.

This gene is transcribed during infection and keeps algae growing when cultured with ammonium as the only nitrogen source. Viral infection also changes the way the host cells absorb the nitrogen compound, allowing the host to access various sources of nitrogen. This is important since nitrogen availability often limits phytoplankton growth. Collectively, these data show that a virus can acquire genes encoding nutrient transporters from a host genome and that viral gene expression can alter the nutrient uptake behavior of host cells. These results show how viruses affect the physiology and ecology of phytoplankton, influence marine nutrient cycles and act as vectors for horizontal (lateral) gene transfer.

The researchers have reported a similar phenomenon [34]. The study of the complete 668 kilobase genome of a mimivirid infecting the green algae *Tetraselmis* (Chlorodendrophyceae) described viral genes that have never been observed before. These genes are represented by the enzymes for mannitol metabolism viz. *mannitol 1-phosphate dehydrogenase*, the saccharide-degrading enzyme, *alpha-galactosidase* and the key fermentation genes – *pyruvate formate-lyase.* These genes indicate complex mechanisms by which viruses can manipulate the host metabolism.

Another recent study revealed genes-encoding enzymes that play a potential role in photosynthesis, various substrate transport processes, light-activated proton pumps, and retinal pigments [35]. This diversity of virus-encoded genes, which play a role in energy production and nutrient supply, points to a large-scale impact of viruses on ecosystem dynamics.

Microbiota – the "second genome" of a macroorganism

One of the largest microbial communities, accounting for about 10^{14} microorganisms of more than 500 species from nine bacterial divisions, which inhabit the human gastrointestinal tract, include *Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Fusobacteria, Proteobacteria, Spirochaeates, Verrucomicrobia* [36-38]. The enormous bacterial density and physical protection create an ideal environment for horizontal gene transfer [39, 40]. The environmental physical and chemical conditions allow natural transformation, conjugation and transduction. According to the researchers, the importance of natural transformation in this environment is underestimated [39, 40].

According to the scientists, viruses, plasmids, conjugative transposons and integrons play an essential role in adaptation, virulence maintenance and antibiotic resistance of the human microbiome due to the mechanism of lateral gene transfer (LGT) [41-43].

The researchers provide living evidence of the viral involvement in protecting the integrity of the microbiota [44]. When analyzing the intestinal microflora, it was observed that bacteria are exposed to stress conditions under antibiotic administration (ciprofloxacin and ampicillin), generating a SOS-inducing signal. In response to this signal, the concentration of antibiotic resistance genes in phage particles increases. Subsequent bacteriophage infection allows transduction of resistant genes to intestinal bacteria. Thus, phages maintain the functional integrity of the intestinal microflora. The following study reflects the enormous value of lateral gene transfer (LGT) between microorganisms for adaptation and maintenance of the

functionality of the human microbiota in various conditions [45]. Thus, the use of unique dietary components will also facilitate the selection of appropriate additional genes. For example, the gene for the enzyme porphyrinase appears to have been successfully transferred from the *Bacteroides marin* to *Bacteroides plebeius* in the microbiota of the Japanese population. This enzyme helps digest seaweed, a common element in the Japanese diet [46].

Gut microbes are significantly influenced by many factors such as host genetics, lifestyle (urbanization and global mobility), medical interventions (antibiotic use, vaccination and hygiene), and overall health [47]. Moreover, food is delivered by modern intensive farming systems, characterized by an extensive use of herbicides, insecticides, fungicides, fumigants, desiccants, crop agents, antimicrobials, growth regulators and many other chemical substances. Similarly, the modern human microbiota is affected by genetically engineered microorganisms, plants and animals, as well as new nutrients, new food technologies, engineered microbial delivery systems, and various food additives [48].

In this context, it is worth mentioning the researchers, who could point to particularly high loads on the microbiota of modern humans [49]. Thus, the study showed that lateral gene transfer (LGT) is 25 times more intense among human-associated bacteria than among non-human isolates. Ecology is the most important factor that drives a global network of gene exchange.

Some researchers stated that human microbiota is a reservoir of a diverse and a dense mass of species, as well as multiple antibiotic resistance genes with functional systems for horizontal gene transfer [50]. The ability of new pathogens to develop in this environment is exceptional.

Amoeba – "single cell engineer"

In the context of the interaction of living beings, the lateral transfer of genes and, finally, the mystery of the emergence of new microbial properties, adjusting to the dynamic environmental conditions, amoeba is worth mentioning as a supercomplex organism, and considered as "*a single cell engineer*".

Amoebas are phagocytic protists, which can be considered wild macrophages [51]. Some amoeba have the largest genome size currently known on Earth. For example, dubia amoeba has a genome of 670000 Mb (200 times larger than human at 2900 Mb) and has the largest known genome of any living organism [52].

Amoebas are very sensitive to environmental changes, including increased pollution, climate changes and conditions of water bodies [53]. According to the researchers, the morphological and functional characteristics of the tested amoeba, due to its pronounced susceptibility, can reflect important information about the functioning of ecosystems [54, 55]. Amoebas phagocytize any inert particles larger than 0.5 μm [56]. A phagocytic amoeba can simultaneously contain various bacteria, fungi and viruses without harming them. Additionally, there are evidences on lateral gene transfer between amoebas and their hosts [57]. For example, *Marseillevirus*, a giant virus, has recently been identified in amoebas. Its analysis showed the chimeric nature of its genome with genes derived not only from mimiviruses, but also from archaea and eukaryotic bacteria [58]. A similar phenomenon is also observed in intracellular amoeba symbionts, such as *Legionella drancourtii*, containing an amoebic-derived sterol reductase [59, 60].

The important role of amoebas in the spread of *Legionella spp*. among people was assumed by T. Rowbotham as early as 1980 [61]. The scientist admitted that human infection occurs not by direct inhalation of free bacteria, but rather by inhalation of vesicles or amoebae containing *Legionella spp.* Later studies confirmed that free-living amoebae are needed to multiply Legionella in water biofilms, although bacteria can survive dormant in amoeba-free biofilms [62]. This phenomenon may explain the onset of the disease after an increased exposure of people to aerosol water due to the use of new devices such as air conditioning systems, spas, showers, etc. [63].

The genus *Entamoeba* (order *Amoebida*, family *Endamoebidae*) lives in the human gastrointestinal tract. Some of them are commensal, others are of uncertain pathogenicity, and *D. fragilis* and *E. histolytica* are confirmed human pathogens [64].

The researchers believe that protists constantly generate new species with a chimeric repertoire, which can subsequently be viable after adapting to environmental conditions and may occupy a specific niche [65].

Results and discussion

Amazing observations of the symbiosis of algae with viruses in the marine phytoplankton ecosystem were made at the beginning of the article. The described phenomena show eloquently that the microbial world is able to finely perceive the fluctuations of the environment and even the metabolic deficiencies of the host organism. Under hostile environmental conditions, through the unique mechanism of lateral gene transfer (LGT) microorganisms correct the host's exchange of substances, ensuring its adaptation to new conditions and ultimately to its survival.

According to researchers, microorganisms are the "invisible majority" living on Earth that plays a critical role in human and animal health, agriculture, world food network, and industry [66]. Although invisible to the naked eye, the microbial abundance (about 10^{30} of bacteria and archaea) and diversity underlie a healthy global ecosystem, which actually provide support for the biosphere life [67]. At the same time, only 1% to 10% of microbes have been classified, cultured in the laboratory, and further studied [68].

The oldest known traces of bacterial colonies (filamentous cyanobacteria) are 3.7 billion years old. Retroviruses found in the genome of modern humans existed even before the emergence of the mammalian class [5].

According to V. Kordyum, viruses as infectious agents that cause pathological processes are a small part of some general principles in nature [70]. These general principles

are non-cellular information transmitters. Thus, viruses are considered primarily as factors for genetic material transferring. A viral epidemic twice mobilizes an explosive transmission of information, viz. at first, by damaging the cell and transmitting the information contained in it, and secondly, by acting environmentally friendly. An "instant" epidemic will shortly cover the entire population (or even several populations) at once, providing an explosive information transfer to the entire population instantaneously. Thus, what we perceive as a virus-induced disease is nothing but our perception of a side effect of the main event, which is a minor and by no means the main manifestation of the basic and universal process, vital for the whole biosphere.

The article continued on the example of another ecosystem, perhaps one of the most specific and complex on Earth – the human ecosystem. On the one hand, the system involves the host or the human being that is the "crown of creation" endowed with higher consciousness, and microbiota, on the other hand, whose bacterial cells exceed the number of human eukaryotic cells in a ratio of ten to one [71]. Similarly, the genes encoded by the gut microbiome outnumber the human genome by 100 to 1 [72].

The complexity and specificity of the human ecosystem is evidenced by the fact that lateral gene transfer (LGT) is 25 times more intense among human-associated bacteria than among non-human isolates [49].

Historically, it is believed that the phylogenetic line associated with the origin of modern humans (*Homo sapiens*), separated from other hominids 6-7 million years ago (in the Miocene) [73, 74].

Thus, if the above data are true, then *Homo sapiens,* as a separate factor and being still a component or a product of the biosphere, is a relatively recent link of the balanced world ecosystem for over billions of years. At the same time, the 2005 Millennium Ecosystem Assessment concluded that changes in ecosystems due to human activity occurring in the last 50 years have been faster occurring than at any time in history [75]. Human activity disrupts both the structure and functions of ecosystems and natural biodiversity. These disturbances reduce the abundance of some organisms, increase the populations of others, alter the interactions between organisms, and alter the interactions between organisms and their physical and chemical environments.

According to experts, the rapidly mutating viruses as well as the occurrence and recurrence of epidemics will continue and intensify increasingly [76].

Summing up the observations of scientists outlined above, it could be mentioned that our global ecosystem has evolved over approximately 4 billion years. An ecosystem that includes a huge variety of unicellular and multicellular organisms. A microcosm is characterized by a surprising plasticity of adaptive mechanisms to the environment. Despite all the latest technical advances, our environment has little been studied [68]. Over the past 50 years, the Earth biosphere has been subjected to more unprecedented challenges than throughout the whole history, resulting in the loss of many species and habitats, brought to the edge of a planetary catastrophe as a result of human activities. As a product of the biosphere, the human being is actually a very "young" member among the various surrounding organisms. In the current situation (considering the evidence listed above), it can be stated that *Homo Sapiens,* in terms of interaction with the biosphere, is a maladaptive species.

According to some scientists, the adaptive reactions of the species to environmental changes are due to endogenous retroviruses [6]. The latter ones develop the genome of the host species by evolving and producing new descendants of their own, as well as due to the genetic changes via the formation of new exons from introns and/or an increase in the number of genes undergoing alternative splicing. Due to the abundance of genetic material created by endogenous retroelements and under the pressure of natural selection, the species become more complex, thus, adapting to the environment and ultimately surviving. The original species, which have become maladaptive to changing environmental conditions, gradually disappear.

At this point, it is appropriate to develop the above data in terms of the self-similarity principle. This concept assumes that an object is exactly or approximately similar to a part of itself. Self-similarity can be widely found in nature as well. Examples include the blood and lung vascular system, cauliflower or broccoli, crystals, mountain ranges, lightning bolts, river networks, etc. [77].

As previously reported, the possibility of the impact of terrestrial environment through microorganisms on the species is truly astonishing. An example worth mentioning is the relatively simple ecosystem, such as phytoplankton, which can survive under harmful conditions, being influenced by virus. Another different ecosystem or the humans are also constantly exposed to the environment. The environment is represented by the biosphere, which has evolved over billions of years, being on the verge of catastrophe for the last 5 decades due to human activity – a relatively recent species.

The human microbiome always interacts with the global pangenoma (the sum of all genes in the biosphere) through environmental exposure and lateral gene transfer (LGT) [78.] Thus, why it cannot be assumed that the adaptive deficiencies of the human beings, as well as of the other ecosystems cannot be perceived by the microbial consortium that inhabits it. The community is actually a biosphere continuum. The microbiota can potentially acquire any gene from the external environment through lateral gene transfer [79]. This assumption has been supported by a number of scientists, who observed that bacteria can perceive and react to signals from the host microorganism [80-83].

The microbial high-density and biofilm communities of the human microbiota are a good environment for gene exchange and the emergence of microorganisms with novel properties [50]. Moreover, the presence of "single-cell engineers" sensitive to environmental fluctuations, such as amoebae, are likely to generate chimeric forms of viruses.

Thus, it is quite interesting to note some COVID-19 fingerprints. According to the scientists, the SARS-CoV-2 genome contains a unique insert – the Y674QTQTNSPRRAR685 motif, homologous to the neurotoxins of highly venomous snakes of the genera *Ophiophagus* (cobra) and *Bungarus*, as well as the neurotoxin-like sequences from three RABV strains [84]. On the other hand, the study of 236379 medical records of patients diagnosed with COVID-19 showed a development of neurological or psychiatric disorders over the next 6 months following the disease in 33.62% of cases [85]. Therefore, a logical question arises, like what long-term consequences may possibly occur in case of an eventual endogenization in the human genome of viruses with such properties.

According to the bibliographic data, human life on Earth can face the most terrible diseases that can be imagined, due to the man-made environmental disasters [76]. The repeated waves of the COVID-19 pandemic compel us to abandon the idea of human superiority over other species, and rather care for and protect the Earth's ecosystem, plant and animal diversity for a sustainable future on this planet.

Conclusions

Perhaps the COVID-19 pandemic has not emerged as a separate epidemic process, caused by a new virus penetration into the human population, but rather a manifestation of a more complex natural phenomenon – an evolutionary process disguised as an infectious one. From an evolutionary perspective, COVID-19 plays the role of a biospheric factor, trying to help a relatively new species adapt to general conditions for symbiotic survival with other living organisms. The COVID-19 pandemic may be somewhat an exam for non-adaptive species and for our existence as human individuals.

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

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Low-grade systemic inflammation in subclinical hypothyroidism

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Abstract

Background: Hypothyroidism is the deficiency in the production of thyroid hormones to meet the requirements of peripheral tissues. Subclinical hypothyroidism (SCH) is defined biochemically as normal serum free thyroxin concentration in the presence of an elevated serum thyroid-stimulating hormone (TSH) concentration. SCH may be associated with low-grade systemic inflammation (increased high sensitivity-C reactive protein (hs-CRP), one possible explanation may be that TSH in adipocytes promotes the release of interleukin-6 (IL-6). Studies have confirmed inflammatory biomarkers like hs-CRP and IL-6 to be a predictor of cardiovascular (CV) events. However, the treatment of SCH remains subject to debate.

Conclusions: It is increasingly evident that SCH has prognostic values and crucial clinical effects, which leads to the view of SCH not being a compensated biochemical change *sensu strictu*. Even a modest but consistent fluctuation in the circulating thyroid hormone levels can create a response from the human heart. Well-timed treatment should be considered as a precaution to avoid the unfavourable CV diseases. The inflammatory biomarkers, namely CRP and IL-6 are exceptionally robust markers of cardiovascular risk. Thus, using these biomarkers may be helpful in assessing the cardiovascular risk in patients with subclinical hypothyroidism.

Key words: subclinical hypothyroidism, low-grade inflammation, levothyroxine treatment.

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Introduction

Hypothyroidism is the deficiency in the production of thyroid hormones to meet the requirements of peripheral tissues. Subclinical hypothyroidism (SCH) is defined biochemically as normal serum thyroxin (T4) concentration in the presence of an elevated serum thyroid-stimulating hormone (TSH) concentration. The incidence of SCH reaches 10-12% in women, 2-5% in men, and after the age of 70, it varies between 15 and 20% [1-3]. In recent years, the attitude of specialists towards the problem of SCH, especially regarding the diagnosis and management of patients has been revised [4]. SCH is recognized as an independent medical condition that requires increased attention.

SCH may be associated with low-grade systemic inflammation (increased C-reactive protein (CRP) and thereby, an increased cardiovascular (CV) risk. However, the treatment of SCH remains subject to debate.

The major bibliographic sources were collected from the PubMed online database. Key search terms were "subclinical hypothyroidism", "inflammation biomarkers" and "levothyroxine treatment". All English-language research articles or translations published on the topic were reviewed. Full-length English articles are only included in this review.

Subclinical hypothyroidism

Hypothyroidism is a frequent endocrine disease that, if left untreated, can have major health consequences, including cardiovascular disease – coronary heart disease and heart failure [5]. The definition of subclinical hypothyroidism is principally biochemical. TSH concentrations above the standard range and free thyroxine concentrations below the reference range are considered overt or clinical primary hypothyroidism. In primary hypothyroidism, an increase in the production of TSH via a feedback mechanism is present to balance the low thyroid hormones level [6].

TSH values above the reference range and free thyroxine concentrations within the normal range constitute mild or subclinical hypothyroidism [7], which is widely seen as a symptom of early thyroid failure. SCH is mild in nature and the patients are usually asymptomatic. The only abnormal hormone level is an increased TSH [8]. The most frequent cause of hypothyroidism is Hashimoto thyroiditis or autoimmune thyroiditis, which involves self-attack of the thyroid gland through inflammation and damage [6, 9]. Other causes include thyroid surgery in the past, radiation to the front of the neck, drugs like lithium and amiodarone [10]. The prevalence of SCH is about 1-2% among the general population and this value increases in iodine-

deficient areas [11]. The prevalence is up to 20% in older individuals (> 65years) [12].

SCH can be divided in 2 forms, mild and severe [13]. In the mild form, the upper limit of TSH is 9.9 mIU/L, while in the more severe form TSH values are above 10 mIU/L. Approximately 80-90% of patients fall in the mild form of SCH [14]. Annually 2-5% of cases of untreated SCH progress to overt hypothyroidism [15]. SCH patients with positive thyroid peroxidase antibodies (TPO) show a high tendency to convert to overt hypothyroidism (two-fold increased risk) [16].

The treatment of SCH with levothyroxine (LT4) is recommended to begin under any of the following circumstances by the American Thyroid Association (ATA) and American Association of Clinical Endocrinology (AACE) [17]:

– TSH >= 10 mU/L.
– TSH between 7 and 9.9 mU/L in younger persons and only with symptoms of hypothyroidism in patients > 65 years.
– TSH above upper limit of normal to 6.9 mU/L in patients < 65 years and symptoms of hypothyroidism. High TPO antibody titer may also be an argument for treatment [18].

In SCH the LT4 therapy is started at a dose of 25-50 mcg and then up-titrated as required, keeping the goal of normalizing TSH.

Some authors suggest that patients with ultrasonographic findings that show chronic thyroiditis even in the absence of TPO should receive levothyroxine therapy [19]. Even though a large number of studies have shown that levothyroxine therapy is helpful in treating patients with SCH, there is a lack of evidence from randomized controlled trials. Hence, it is still ambiguous whether to treat a patient with SCH LT4 therapy or not [20].

Hypothyroidism is the least defined known risk factor of cardiovascular disease. Subclinical hypothyroidism and overt hypothyroidism show almost the same cardiovascular consequences [9]. Even though the entire pathophysiological mechanisms are unknown, the most known causes include insulin resistance, hypertension, disturbed lipid balance, and endothelial dysfunction [12, 21].

SCH is associated with hypercholesterolemia, atherosclerosis [22] and increased carotid intima-media thickness [5]. SCH is also associated with elevated levels of C-reactive protein, homocysteine, TNF-α, MPO-Myeloperoxidases, IL-6, and other inflammatory markers [14], so that over time untreated SCH can contribute to heart disease [22]. There is a higher risk of CV disease especially in younger population (aged < 65years) compared to the older ones [23]. Kvetny J. et al. have shown that SCH is an independent predictor of cardiovascular disease in patients younger than 50 years [24]. An important factor in CV disorders in hypothyroidism is the change in peripheral vascular resistance and elasticity of the arterial wall [25]. The researchers established a close correlation between TSH levels and the thickness of the intima media, a correlation that is interpreted as a link in the pathogenesis of atherosclerosis [25]. Ghasemi M. et al. found correlations between hypothyroidism and the average intimate thickness of the carotid vessels [26]. Cardiovascular diseases, being a leading cause of death globally, appropriate measures have to be taken regularly to identify and stratify individuals. Usually this stratification is done based on evaluating the risk factors [27]. A long list of CV disease risk factors has been identified. The list is still expanding with the addition of numerous novel biomarkers. This includes various biochemical markers, as well as genetic and inflammatory markers. The successful quantification of these risks is helpful in ascertaining that the patients receive proper treatment on time. Thus, the integration of novel as well as existing risk factors into the therapeutic guidelines is necessary to prevent and reduce the globally leading cause of death [27].

Low-grade inflammation

The current definition of inflammation is "a response to infections and damaged tissues that bring cells and molecules of host defence from the circulation to the sites where they are needed in order to eliminate the offending agents" [28]. Acute inflammation is characterized by the 5 cardinal signs – redness (*rubor*), heat (*calor*), pain (*dolor*), swelling (*tumor*), and loss of function (*functio laesa*).

Inflammation is an important constituent of innate immunity. Low-grade inflammation is the innate immune response to potentially harmful stimuli, such as pathogens, injury, and metabolic stress [29]. Subclinical inflammation is associated with increased risk of several metabolic diseases, such as obesity and diabetes mellitus. In addition, modest elevation in the level of acute phase protein – CRP between 3 – 10 mg/L is also associated with inflammation [29, 30].

Low-grade inflammation is also associated with cigarette smoking, sleep deprivation, lack of physical activity, hypertension, PCOS, and a large variety of unhealthy diets [29].

Table 1 shows a comparison between acute inflammation and low-grade inflammation.

Table 1. Comparison of acute and low-grade inflammation [2]

Parameter	Acute inflammation	Low-grade inflammation
Cause	Pathogens, trauma, tissue infarction	Metabolic malfunction
Mediators	Molecules and cells of the innate immune response	Molecules and cells of the innate immune response
Classic signs of inflammation	+++	None
CRP response	+++	+
Purpose	Healing and repair	Restoration of Homeostasis
Triggering mechanisms	PAMPs and DAMPs	UPR

Note: UPR – unfolded protein response, DAMP – damage-associated molecular patterns,

PAMP – pathogen-associated molecular pattern. In addition, symbols indicate magnitude.

Biomarkers of low-grade inflammation

C-reactive protein

CRP, a pentameric protein, with a discoid shape, is synthesized in the liver. It is a member of the pentraxin family, family of innate immune response proteins [14]. The monomer has 22 amino acids and a molecular mass of 25106 Da. The overall mass of the whole protein, which is made up of five monomers, is around 120000 Da [31]. The location of CRP gene is 1q23.2 [31]. CRP plays a vital role as a dynamic and sensitive marker of inflammation [32]. During acute responses to serious changes in the body like acute infection or tissue damage, a significant increase is usually noticed in a time span of 48 hours [30-33]. There could be chronic or acute rise in the levels of CRP depending on the situation. Acute rise in CRP could be a result of acute bacterial or viral infections, burns or even trauma or exercise, and the chronic rise in CRP is noticed as a result of elevated blood pressure, elevated body mass index, diabetes mellitus, cigarette smoking, cancer, or chronic bacterial or viral infections [34].

CRP is a non-specific inflammatory marker that mediates atherosclerosis [35]. Independent of other cardiovascular risk factors, studies in both men and women of different age groups have shown that there is an association between inflammatory biomarkers like CRP, IL-6 and cardiovascular events [36, 37]. Recent investigations have confirmed inflammatory biomarkers like CRP and IL-6 to be a predictor of cardiovascular events.

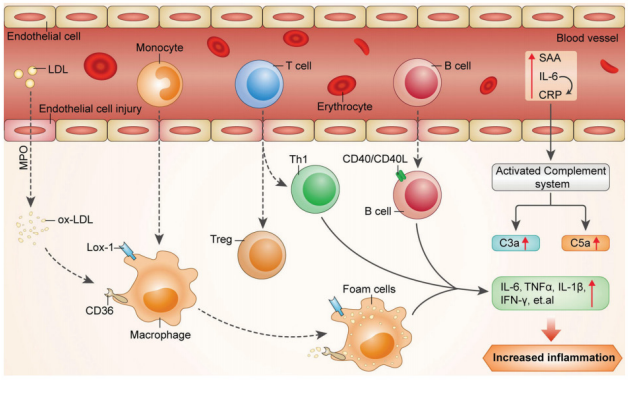

Fig. 1. Cascade of inflammation in atherosclerosis [38]

In a study done by Oemrawsingh RM. et al. it was found that the patients who undergo percutaneous coronary intervention (PCI), and with a high level of hs-CRP at the time of the procedure, have a predictive value for myocardial infarction and 10 year mortality [40]. Thus, hs-CRP being a valuable biomarker, is helpful in assessing subsequent risk in patients having PCI [40]. Even though CRP has been confirmed to be a predictor of cardiovascular events and have direct associations with cardiovascular events, hs-CRP is not a causative factor of cardiovascular diseases [14].

The standard examinations measures CRP in a range of 10-10000 mgL, while the range of high-sensitivity C-reactive protein (hs-CRP) is between 0.5 and 10 mg/L. This indicates that the trace amounts of CRP in the blood can be measured by estimating hs-CRP [39]. In comparison with other acute-phase reactants, hs-CRP have many advantages which include excellent assay precision, availability, accuracy. Due to these, hs-CRP is the analytic element of choice for CV disease risk assessment [34]. Table 2 shows the risk of CV diseases based on the hs-CRP levels [34].

Table 2. The CV disease risk based on hs-CRP level [34]

RISK	hs-CRP level (mg/L)
Low	<1
Average	1-3
High	>3

In a meta-analysis by Tellechea M, an association between CRP and hypothyroidism has been found [12]. Nevertheless, LRT in overt hypothyroidism patients showed significant effect in decreasing the level of CRP, but not in patients with SCH.

Taking into consideration that SCH may progress to overt hypothyroidism, monitoring for TSH and hs-CRP may be useful [9, 12]. Thus, these patients could benefit from early interventions [9].

IL-6

IL-6 is a circulating cytokine with pleiotropic effects (both neurodegenerative and neuroprotective properties) [41] and on inflammation, immune response, and haematopoiesis [42]. The locus of IL-6 is mapped on the chromosome 7p21. The structure comprises 212 amino acids and a core protein of ~ 20 kDa. The synthesis of acute phase proteins, such as CRP, fibrinogen, serum amyloid A and inhibition of albumin is induced by IL-6. Continuous dysregulated production of IL-6 leads to the onset of several diseases.

IL-6 is secreted by activated macrophages, lymphocytes and adipocytes and gets activated by binding to high-affinity receptor complexes [43]. The level of IL-6 is increased in circulation during conditions like obesity (a risk factor for coronary heart disease (CHD) and diabetes mellitus). In systemic inflammation, the level of IL-6 is increased, which in turn increases the level of CRP in CHD prone patients. Elevated IL-6 levels in acute coronary syndrome patients are a strong independent predictor of increased mortality.

IL-6 plays the core role in vascular inflammation. In patients with autoimmune thyroiditis, an increased level of IL-6 is noticed. Even in patients with SCH, a positive correlation is found between SCH and IL-6 [44].

IL-6 being a pro-inflammatory cytokine, increases hepatic production of CRP and thereby promoting atherogenesis [45]. The studies conducted by Turemen EE. et al. and Taddei S. et al., concluded that the presence of high amount of CRP and IL-6 is seen in patients with SCH [46, 47]. One possible explanation may be that TSH in adipocytes promotes the release of IL-6 [1].

Conclusions

It is increasingly evident that SCH has prognostic values and crucial clinical effects, which leads to the view of SCH not being a compensated biochemical change *sensu strictu* [48]. Even a modest but consistent fluctuation in the circulating thyroid hormone levels can create a response from the human heart. Well-timed treatment should be considered as a precaution to avoid the unfavourable CV diseases.

The inflammatory biomarkers, namely CRP and IL-6 are exceptionally robust markers of cardiovascular risk. Thus, using these biomarkers may be helpful in assessing the cardiovascular risk in patients with subclinical hypothyroidism.

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Authors' contributions
SV designed the study, revised the manuscript; GDK conducted literature review, collected the data, interpreted the data, wrote the manuscript. Both authors revised and approved the final version of the manuscript.

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

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The authors have no conflict of interests to declare.

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Phenotypic polymorphism in Wilson's disease – between genetics and epigenetics

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Abstract

Background: Wilson's disease is a rare, autosomal recessive genetic disorder that affects the biliary excretion of copper and its toxic accumulation in various tissues, especially the liver and brain. It is widespread throughout the world, with a high prevalence in socio-culturally isolated communities. The course of the disease and the age of onset depend on the site of mutation in the gene and the degree of functional impairment of the ATP7B protein. The presence of the compound heterozygous patient complicates the comparative genetic and clinical evaluation. Therefore, it is necessary to analyze Wilson's variants in both the homozygous and the compound-heterozygous conditions to better understand the genotype-phenotype correlations and the incomplete penetrance observed in this disorder. Outlining clear phenotype-genotype associations is difficult due to a large number of mutations and different clinical presentations, but the involvement of epigenetic factors, modifying genes, environmental and lifestyle factors could explain the differences in evolution and onset in members of the same family and not only.

Conclusions: Wilson's disease is a genetically and clinically complex disorder. Although the results of genotype-phenotype correlation studies are not well defined, and in some cases are completely contradictory, some peculiarities related to the age of onset, sex, clinical phenotype, and the evolution of the disease have been highlighted. The interaction between genetic mutations and epigenetic factors may explain the phenotypic variability, but needs further study.

Key words: Wilson's disease, ATP7B gene, mutation, genotype-phenotype correlation, epigenetic.

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Introduction

Wilson's disease (WD) is a monogenic disease with an autosomal recessive transmission, caused by a disorder of copper (Cu) metabolism. Reduced excretion of Cu leads to its accumulation in tissues, and toxic deposits induce oxidative stress, alter gene expression, inhibit directly protein activity and affect mitochondrial function, causing multiple structural and functional damage in several organs, especially the liver and brain [1, 2].

The disease manifests as a multisystemic condition with an unpredictable clinical picture, and the symptoms appear depending on the most affected organ. The severity of the disease depends on the site of mutation in the ATP7B gene and the functional capacity of the ATP7B protein [3]. WD may have an asymptomatic or silent course (no symptoms: apparently good), which makes it difficult to make a diagnosis. Due to dysfunction in many organs, clinical and laboratory features are often subtle and may mimic alternative diagnoses, and in the process of evaluating a patient with a variety of signs, symptoms, and laboratory abnormalities, a differential diagnosis with WD is necessary [4].

The definitive diagnosis can only be established by performing specific tests for WD, and using the Ferenci score (Leipzig, 2001) facilitates the process of assessing a suspected patient of WD [5]. For a patient with a defined clinical diagnosis of WD, molecular-genetic analysis is not mandatory but may provide the information needed to guide relative screening, and knowledge of mutational status may help assess genotype-phenotype correlation about demographic, clinical, and paraclinical indices, as well as in new treatment technologies, e.g. gene therapy [6].

The publications were selected from the PubMed and HINARI databases – Research4Life program, using search terms in English: *"Wilson's disease", "ATP7B gene", "ATP7B mutation", "genotype-phenotype correlation", "Wilson pathogen variant", "epigenetic in Wilson's disease", "prevalence", "phenotypic diversity"*. According to the search criteria, all full-text publications offered by these platforms were selected, and articles in English have been prioritized since 2002, although no language limits have been set. Sources also include articles published in the Republic of Moldova and Romania. After a preliminary analysis of the titles, in the final bibliography were selected original articles, narrative synthesis, meta-analyses, systematic reviews, series of cases relevant to the research topic, book chapters, which addressed epidemiological data and global distribution of

the disease, the role ATP7B gene and mutations associated with WD development, the correlation between genotype and phenotype, the involvement of genetic and epigenetic factors in the evolution of the disease. Articles on clinical and paraclinical diagnosis, current therapies, new diagnostic and treatment methods, scores, and questionnaires used in WD were excluded.

Discussion

After processing the information from the PubMed and HINARI databases, according to the search criteria, 38 relevant sources were selected, which were considered representative of the material published on the topic of this synthesis article. Publications, the content of which did not reflect the subject matter in this article, although they were chosen by the search engine, as well as the articles, which were not accessible for free viewing and through the HINARI database (*Health Internet Work Access to Research Initiative*) or available in the medical scientific library of *Nicolae Testemitanu* State University of Medicine and Pharmacy were excluded from the list.

Epidemiological data. Wilson's disease is a worldwide genetic disorder [7]. Epidemiological estimates have established significant differences between the clinical and genetic prevalence of the disease, and this is due to several factors, including genetic, epigenetic modifiers, and habits (diet, traditions, exposure to toxic environment) [4]. Incidence varies between 1: 5000 and 1: 30000, but is considered to be more common than previously thought [5, 7]. The frequency of carriers in the general population varies from 1: 90-150, which makes it one of the most common Mendelian disorders [1]. The prevalence of the disease is higher in socio-culturally isolated communities, as well as in populations where marriages between relatives are practiced (consanguinity) [7, 8]. In Europe, the clinical prevalence is estimated at 12-20: 10000, while the prevalence of the affected gene is higher at 1: 7000 [9]. The age of onset of WD varies from 2 years to 80 years, but most are diagnosed between 5-35 years [5, 7]. The frequency and distribution of ATP7B mutation in the Republic of Moldova are not known for sure.

ATP7B gene: structure and role. The ATP7B gene is located on the long arm of the 13th chromosome (13q14.3). It consists of 20 introns (non-coding nucleotide sequences) and 21 exons (coding nucleotide sequences) and has a genomic length of about 80 kb. It is mostly expressed in the liver but can be found in the kidneys, placenta, mammary glands, brain, retina, and lungs [10, 11]. The gene encodes the structure of a Copper-transporting P-type ATPase, officially called ATP7B, which carries Cu intracellularly, and mutations in the gene cause the synthesis of a defective or dysfunctional protein. Currently, ATP7B is the only gene known to cause WD [12]. The ATP7B protein, also called Wilson ATPase, is a protein with a complex multi-domain structure, with an essential role in biosynthesis and in maintaining Cu homeostasis in the body. In each functional domain of the enzyme, there are unique amino acid sequences ("motif"), with a major impact on the process of transporting Cu, and mutations in their level are associated with the development of WD [11]. The biosynthetic function is achieved by incorporating Cu into the apo-ceruloplasmin (the main substrate that receives Cu from Wilson protein), and after the acquisition of 6 Cu atoms, the "mature" ceruloplasmin is released into the systemic stream. The function of maintaining Cu homeostasis is achieved due to the ability of the protein to rapidly relocate from the Golgi complex to the biliary pole of the hepatocyte, in response to changes in the level of Cu in the cell, to eliminate excess metal in the bile [2].

Transmission and inheritance. WD is a monogenic disorder with autosomal recessive transmission according to the Mendelian distribution. This means that to develop the disease it is necessary to inherit 2 affected genes, from each parent one (recessive homozygous), and the one who inherits only one mutated gene becomes a healthy carrier or simple heterozygous, respectively he will not develop the disease, but will pass on the gene mutation to his children [9]. Compound heterozygotes are people who have a different mutation on each chromosome, while recessive homozygotes have the same type of mutation on both chromosomes. Thus, the disease is found in recessive homozygotes and compound heterozygotes, the latter being the most common of WD [12]. It is extremely important to make the differential diagnosis between compound homozygotes/heterozygotes and simple heterozygotes (healthy carriers), especially at the asymptomatic stage, because for patients in the first group it is recommended to initiate chelating agent therapy as early as possible to prevent while healthy carriers will not develop WD and do not require the administration of specific preparations, which could cause potentially serious side effects [13].

Despite Mendelian transmission of the disease, some deviations have been observed. It is unusual for autosomal recessive mutations to occur in several consecutive generations, but in WD this is due to the high frequency of healthy carriers [9, 10]. Thus, Poujois A. and Woimant F. [9], Chang I.J. and Hahn S.H. [10] reported cases of "pseudo-dominant" inheritance, which led to the screening of the entire family. Given that WD is a treatable disorder, it is insufficient to inform only about the possible risk of genetic disease in the family, so a complex family screening is recommended. In the first stage, the siblings are checked, because their risk of having two mutations is 25%. The second stage will examine the descendants (0.5% risk), then the parents, uncles, aunts, and grandchildren [9].

In a significant number of clinically diagnosed cases of WD, the mutation of the ATP7B gene failed to be detected, and this highlighted the genetic heterogeneity of this condition, but also suggested the presence of paradoxical mutational mechanisms. Thus, Coffey A.J. and colleagues [14] in the genetic study of patients with WD in the United Kingdom identified patients with atypical patterns of inheritance that may cause WD, such as single-parent segmental isodisomy.

The model results when both homologous chromosomes are from the same parent and represent a rare accidental error in the process of chromosomal segregation during meiosis [14]. This is important for clinical practice and genetic counseling, as genotyping of asymptomatic parents or obtaining complete sequencing of the ATP7B gene will be considered to confirm the pathogenic variant [12].

Pathogenicity of mutations. Mutations can occur anywhere in the gene – exons, splicing site (intron/exon boundary zones), introns, untranslated regions 5 '(UTR5) and 3' (UTR3), and promoter. The most common mutations are located in the 8th exon (p.R778L) and the 14th exon (p.H1069Q). However, there have been reports of people diagnosed with WD based on clinical and biochemical tests, but no mutations were detected. Thus, the involvement of another gene that could cause BW has been suggested, but no other gene has been identified so far [12]. The most common mutation is missense substitution type, other variants are frameshift, nonsense substitution, and splicing type. In general, the frameshift and missense mutation is associated with a more severe phenotype [2]. Rare mutations are also reported, such as regulatory type, deletion of an entire exon, the presence of 3 concomitant pathogenic variants, and monogenic dysomia [14].

Using guidelines from the American College of Medical Genetics and Genome (ACMG) and the Molecular Pathology Association (AMP), was created a clinically relevant electronic source for WD that includes genetic variants of the ATP7B gene reported through February 2019, manually selected from the literature and 6 official international databases associated with WD. The source is available at http://clingen.igib.res.in/WilsonGen/. Therefore, the database formed includes 3662 genetic variants of ATP7B, which can be found in different places of the gene; of which 1458 proved to be unique. The unique variants were classified according to several principles: the type of mutation (substitution, insertion, deletion, deletion/insertion), the functional consequences (frameshift, Stop loss, Stop gain, Start loss, introns, and splicing site), and according to the ACMG guide (pathogenic variant, probably pathogenic, benign, probably benign, of unknown significance) [15].

Mutations in unique amino acid sequences, located in different areas of the ATP7B protein, cause WD. His1069Q – is the best-known mutation, with an allele frequency in the general population ranging from 10-40%, and in the European population being 30-70% [16]. It occurs in the N-domain SEHPL sequence by substituting histidine → glutamic acid at position 1069 of the ATP7B polypeptide chain, resulting in a misfolding of the protein in this domain, with abnormal phosphorylation in the P-domain and a reduced affinity for ATP, a thermally unstable and aberrantly localized protein, not in the Golgi complex, but the reticulum endoplasmic [10, 12]. Other pathogenic variants of this sequence are: E1064A (affecting the protein by the inability to bind the molecule to ATP), G1099, G1101, and I1102 [17].

Mutation in the DKTGT sequence does not allow the relocation of the protein to the site of biliary excretion of Cu, respectively with its intrahepatic accumulation; mutation in the TGEA sequence – inhibits the recovery of cytosol protein in the Golgi complex, when Cu levels are reduced; CPC mutation – C985T – leads to complete loss of Cu transport activity. The protein segment 37SFAFDNVGYEG45 (located just before the MBD1 – metal-binding domain) interacts with the N domain and directs the ATP7B protein to the apical region of the hepatocytes to ensure biliary excretion of Cu as its concentration increases and the N41S mutation in this segment changes intracellular protein behavior, causing bladder trafficking even when Cu is not high, also affects the targeting of the canalicular surface [18]. Missense mutations in MBD5 and MBD6 are associated with WD, and the change in serine → alanine, an amino acid located between MBD3 and MBD4, induces redistribution of ATP7B protein to peripheral endo/lysosomes [19]. The presence of the mutation in the MBDs linker causes WD; for example, in the MBD1-MBD2 linker – R136W substitution; in the MBD3-MBD4 linker – G333> R mutation; in the MBD4-MBD5 linker – A486S mutation; in the segment connecting MBD6-TM1 – a group of mutations H639Y, L641S, D642H and pMet645Arg (the most common pathogenic variant of missense type in Spain 27%) [7]. Mutations that occur near to N-terminus can lead to a complete blockade of protein synthesis, which is associated with severe WD evolution [18]. LLL mutations associated with WD have not been detected, however, several mutations that cause WD to lead to fragmentation of the C-terminal region containing LLL, and this is likely to affect the recognition of ATP7B by adaptive proteins. Consequently, the mutant ATP7B protein remains peripherally blocked, and its recovery in the trans-Golgi network is suppressed [19]. Missense T1434M or Q1399Rfs mutations in the C-terminal 1450DKWSL1454 sequence are also associated with WD [20].

Patients with WD present with genetic heterogeneity in different races and geographical regions. For example, the H1069Q mutation of ATP7B is more prevalent in patients of European origin, such as those in Italy, Romania, and Sweden, while the R778L mutation is more common in East Asia, but neither of these mutations has been reported in India, where another 17 new mutations have been identified. Theoretically, a full spectrum of mutations would be useful in establishing a pre-symptomatic diagnosis for patients with BW; however, due to the mutational variability and heterozygosity of the ATP7B gene, it is difficult to use mutation as a diagnostic tool [21].

According to the data published by Hlistun V., Sacara V. and colleagues [22], a clinical-molecular evaluation of 120 patients with WD in the Republic of Moldova (40 patients genetically examined in Germany, and 80 – at the Genetic Center of the Republic of Moldova), 18 different mutations were identified, of which 4 new variants, the most common pathogenic variants being p.H1069Q (exon 14) and p.G1314D (exon 20), and in 13 patients evaluated in Germany no mutation was determined.

Epigenetic mechanisms. It is established that the accumulation of Cu in patients with WD is due to mutation in the ATP7B gene encoding the ATP7B protein. However, it is suggested that the presence of other factors, such as epigenetic mechanisms – which act at the interface between the genome and environmental factors and modify genes – could be involved in altering Wilson's gene expression and clinical variety and disease progression. In addition, environmental factors, such as diet, lifestyle, and exposure to toxins, both in utero and postnatal, can affect gene expression through epigenetic mechanisms [23].

Epigenetic mechanisms include reversible changes in DNA or histones, thereby affecting gene expression without altering the DNA sequence. Gene expression is regulated at various levels, including the chromatin remodeling stage, which is composed of DNA, histones, and non-histone proteins. Chromatin remodeling is initiated with DNA methylation, by the addition of methyl groups (H_3C) to DNA from methyl donors –betaine, choline, folate, and methionine. Many methyl donors and other structures involved in the metabolism of methyl donors are essential nutrients, respectively, that must be obtained from the food. DNA methylation requires a sufficient supply of methyl donors, such as methionine, and the changes that underlie its metabolism can affect DNA methylation and, ultimately, disease progression [24].

Major mechanisms involving histone changes, such as acetylation, methylation, phosphorylation, sumoylation, ubiquitination, and ribosylation, indirectly affect gene expression. These are complex cellular mechanisms that induce changes in protein function, chromatin conformation, and gene expression; they can also stimulate protein inactivation/degradation and initiate cellular apoptosis. Another third mechanism is the action of non-coding RNAs. MicroRNAs increase the degradation of messenger RNA required in the process of gene transcription and protein synthesis, thus affecting gene expression by inducing "silence". Long RNAs influence gene expression by interacting with chromatin-modifying proteins, regulating their translation process and stability. However, these mechanisms are to be studied in WD [25].Of particular interest is the interaction between mitochondria and epigenome. Mitochondria are more susceptible to damage by excess Cu compared to other cell compartments, and in patients with WD, their morphology is altered in both early and late stages. It inhibits key enzymes in mitochondria, thus disrupting the production of metabolites derived from mitochondria that are involved in epigenetic regulation. Mitochondria are also a major production site for reactive oxygen species, which in normal concentrations act as signals, but increasing intracellular concentrations can induce oxidation of DNA, lipids, and mitochondrial proteins, degrading mitochondria and causing posttranslational changes (conformation, activity, location) of cellular proteins [23, 25].

Modifying genes are genes that affect the expression of another gene located in another locus or the phenotypic expression of another gene. Such genes are known to influence the evolution of WD. Thus, the role of the genes APOE (apolipoprotein E), COOMD1 (copper metabolism domain-containing 1), ATOX1 (human homolog antioxidant 1 copper chaperone), and HFE (hemochromatosis) has not been confirmed in large studies. [49] Additional research is also needed to demonstrate the involvement of ATPase copper transporting alpha (ATPase) genes, DMT1 (divalent metal transporter 1), PNPLA3 (patatin-like phospholipase domain-containing 3 genes), PRNP (Prion Protein), XIAP (X-linked inhibitor of apoptosis protein), ESD (esterase D), INO80 (INO80 complex subunit) and MTHFR (5, 10-methylenetetrahydrofolate reductase) in the pathogenesis of WD [23].

Epigenetic changes that are caused by environmental factors (e.g., diet, exercise, stress, and toxins) can exacerbate the changes induced by excess Cu, thereby altering the clinical picture and disease progression, and improving these factors provides an opportunity to mitigate the toxic effect of Cu in patients with WD. Kieffer D. and Medici V. [24] proposed the multi-hit hypothesis by which Cu and lifestyle can change the phenotype in WD. In the case of a mother pregnant with a homozygous fetus with an ATP7B mutation, the first hit is caused by exposure to the maternal toxic environment, namely the diet high in fructose, sedentary lifestyle, social stress, and contact with various toxins. The second hit follows after the birth of the child, due to the pathogenic mutation that determines the accumulation of Cu in the organs with the appearance of mitochondrial dysfunction and the alteration of the epigenetic mechanisms of regulation of gene expression. Subsequent hits are caused by exposure to own toxic environmental factors, such as a diet high in fructose and lipids, sedentary lifestyle, social stress, and contact with various toxins. These factors aggravate pre-existing mitochondrial lesions and worsen the epigenetic mechanisms of gene expression regulation. Finally, the disease begins early and/or presents with a severe evolution of WD. However, these suggestions need to be studied and confirmed for their role in WD progression [24].

The close interrelationship between Cu accumulation, methionine metabolism, mitochondrial function, and gene expression regulation, with strong evidence from animal model studies, indicates that epigenetic phenomena most likely contribute to phenotypic diversity in WD, and their analysis is essential for understanding multiple factors interacting in metabolic and complex liver disease – including WD [25].

Microbiome and Wilson's disease. The human intestinal microbiota has a wide range of unique genes encoding different proteins, which are not present in humans, thus greatly expanding the genetic potential of the host; can produce metabolites capable of altering DNA expression, thus exerting epigenetic regulation of human gene expression; changes in various diseases, both liver and neurological; it changes rapidly in response to food and medicine. Lichtmannegger J. and colleagues [25], explored, in animal models, the efficacy of a microbiota-derived peptide,

methanobactin, which has a high affinity for Cu. Short-term treatment with methanobactin regressed mitochondrial and liver damage in treated ATP7B-deficient rats compared to untreated ones [25]. This area of research is largely unexplored but may offer new treatment options, especially for liver damage, as the liver is the first organ to come into contact with metabolites derived from intestinal microorganisms [26].

Gender differences and Wilson's disease. Few studies have investigated the effects of Cu accumulation and sex differences in WD pathogenesis. In a study by Ferenci P. [27], more than 1000 patients with WD, it was reported that sex and age are modifiers of clinical presentation. Acute hepatic impairment occurs more frequently in young women, and the neurological picture predominates in men; while neurological manifestations in children are rare. Data confirmed in previous studies. Litwin T. and colleagues [28], in their research that included 627 patients with WD in Poland, showed that the neuropsychiatric phenotype predominates in men compared to women. Hepatic phenotype occurs more frequently in women, and neurological manifestations develop later than in men. Thus, it has been suggested that the differences are due to the protective effects of estrogen on neurons, which delay the onset of neurological damage, differences in iron metabolism, and occupational factors. However, no conclusive studies have been performed to confirm these claims.

Identification of the phenotype-genotype correlation. WD is characterized by a high mutational variety and an unpredictable clinical picture, and despite the response to treatment and autosomal recessive transmission, the mechanism of phenotypic diversity is currently unknown. Several studies have attempted to find a correlation between genotype and clinical phenotype, but recent studies in a large number of patients have not identified any association [29]. However, some research suggests a possible relationship between the age of onset or type of clinical presentation and a specific genotype [8].

One of the strongest genotype-phenotype correlations in WD is that mutations that cause loss of ATP7B protein function, especially early codon stop mutations (with the synthesis of an abnormally short and unstable protein) and those in functionally important regions, are associated with severe evolution, early-onset, and a predominantly hepatic phenotype, while mutations that partially retain Cu transport function present with a milder evolution. Point mutations in less important regions of the gene are associated with late-onset and a predominant neurological or psychiatric phenotype [12].

Kalita J. and colleagues [30] observed that most patients with the R778L mutation present with a hepatic phenotype and an earlier onset of the disease, while patients with the H1069Q substitution present with a neurological phenotype and a later onset. Kayser-Fleischer rings are more common at the time of diagnosis in H1069Q homozygous patients than in compound heterozygous patients.

A study conducted by Cocos R. and colleagues [8], of 2 large families in the mountainous region of Romania, with 50 members – 7 affected by WD, reported a high intra-familial concordance of patients with WD, with predictability higher for neurological presentation with the same set of clinical features at the time of diagnosis and identical age at the onset of the disease; such data being observed by other researchers. Also, the coexistence of H1069Q and frameshift p.M769H-fs substitution mutations in compound heterozygous patients was associated with a lower age at onset, coinciding with previous reports of the age of onset for homozygous/heterozygous patients with p. H1069Q. A family study by Chabik G. and colleagues [31] found that although the type of clinical presentation varies, siblings present with the same phenotype or age at the onset of the disease, while research by Czlonkowska A. and colleagues [32], did not reveal any genotype-phenotype correlation in homozygous/heterozygous siblings composed of the same mutation or monozygotic twins, the studies suggesting the involvement of genetic and environmental factors that could influence the phenotype.

Mihaylova V. and colleagues [33], in their research including 123 patients with WD from Bulgaria, observed a lack of correlation in patients with p.H1069Q homozygous/heterozygous compound mutation or patients without this mutation with neurological phenotype. The homozygous p.H1069Q mutation was associated with the more frequent hepatic phenotype and the significantly rarer presence of the Kayser-Fleischer ring than with the compound heterozygous p.H1069Q mutation and other mutations. In contrast, patients with the p.R616Q mutation, either homozygous or compound heterozygous, had significantly more frequent neurological manifestations and a higher level of ceruloplasmin than in homozygotes with p.H1069Q and patients without p.H1069Q; in all subjects with the p.R616Q mutation, the Kayser - Fleischer ring was detected.

Usta J. and colleagues [16], evaluated a Lebanese family of 76 members, in which inbreeding is present, in 9 members diagnosed with WD. All 9 patients had the mutation c.2299insC, 5 were homozygous and 4 were heterozygous compounds with p.Ala1003Thr. The study reported a correlation between c.2299insC homozygous patients with liver disease and c.2299insC/p.Ala1003Thr compound heterozygous patients with neurological disease.

The results of a Chinese study by Cheng N. and colleagues [34], which included 1222 patients with WD, showed that the pArg778Leu mutation correlated with an onset at a younger age and with lower levels of ceruloplasmin and serum Cu. Other variants of pArg919Gly and pThr935Met were associated with higher levels of ceruloplasmin, the pArg919Gly variant being correlated with neurological symptoms, while pThr935Met with combined neurological and visceral manifestations. The greater the impact of the mutation on the structure and function of the ATP7B protein, the earlier the onset of the disease and the lower the level of serum ceruloplasmin.

A report by Takeshita Y. and colleagues [35], of 2 unrelated families in Japan, in which family members with WD had similar mutations in the ATP7B gene, found that all members of the same family had different phenotypes and the onset of the disease at different ages. Thus, it was suggested that there may be a difference in allelic dominance (in the identified mutations), as all patients in both families were found to be compound heterozygotes. This was also confirmed by Sapuppo A. and colleagues [36], in the case of 2 sisters with WD, heterozygous compounds with similar genotype (c.3207C-> A (p.H1069Q) / c.3904-2A-> G), which presented with different phenotypes both at the beginning and during the disease, being presumed several factors that would change the evolution of the disease (initiation of therapy in the asymptomatic phase, lifestyle differences, possible intervention of modifying genes of Cu metabolism). Yahata S. and colleagues [37] examined 11 Japanese families, including 23 brothers diagnosed with WD, and reported that in 5 families the disease had the same phenotype, while in the other 6 families with a different phenotype.

Research conducted in India by Santosh S. et al. [38], investigated the impact of 4 different mutations on members of 4 unrelated families (>1 member affected by WD), patients were evaluated to identify correlations between genotype (mutation type, homozygous/heterozygous compound) and phenotype (clinical presentation, age of onset of disease/ age of diagnosis, serum ceruloplasmin level and urinary Cu within 24 h). The results of the research evoked a strong genotype-phenotype concordance between members of the same family, except for 2 minor phenotypic differences between members of 2 families, this being explained by the evolution of the long-term untreated disease, which could be a confusing factor in establishing correlations.

Thus, genetic polymorphism, unique pathogenic variants, atypical mutational mechanisms, the presence of compound heterozygotes, rarity of the disease, clinical variety, epigenetic factors, as well as socio-demographic characteristics (sex, race, and age) complicate the process of associating a genetic variant with a certain phenotype. Efforts to identify some genotype-phenotype interrelationships have shown conflicting results, as the mechanisms of WD heterogeneity are not fully elucidated, especially due to the unpredictable effects of mutations on the structure and functionality of the ATP7B protein [12].

Conclusions

Wilson's disease is a complex disease manifested by mutational heterogeneity and a polymorphic clinical picture. The results of genotype-phenotype correlation studies are not well defined, and in some cases are completely contradictory. The interaction between genetic mutations and epigenetic factors may explain the phenotypic variability. Determining the mechanisms of varied clinical presentation in WD is a challenge for contemporary researchers, and understanding the biochemical, genetic, and physiological processes at the micro-/macromolecular level, as well as at the macro-organism level, could highlight additional avenues in elucidating the genotype-phenotype interrelationship conflict and identify potential therapeutic targets.

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