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ORIGINAL RESEARCHES

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Clinical and serological characteristics of early systemic lupus erythematosus

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Abstract

Background: Early diagnosis in patients with systemic lupus erythematosus (SLE), which is based on the knowledge of the variability of the initial disease manifestation, followed by prompt initiation of basic therapy is essential for a favorable prognosis in these patients. Thus, the determination of early manifestations of the disease in patients with lupus was the main objective of this study.
Material and methods: This present descriptive study included 68 patients with early SLE – the disease duration being of up to 2 years after the diagnosis. The evaluation of the characteristics of the disease was performed by a questionnaire developed by this study, which included the clinical and paraclinical examination. Statistical data processing was performed via Excel program.
Results: The analysis of the results on early manifestations of the disease revealed the high frequency of joint involvement in 64.7%, photosensitivity and malar rash – in 58.82% and 32.35%, respectively, and oral ulcers and alopecia were found in about 1/4 cases. The signs detected, but omitted from the criteria with increased occurrence were represented by fatigue in 42.64% of cases, fever – 29.41%, myalgia and Raynaud's syndrome in 20.58% of patients. It should be noted that the first lupus-associated manifestations were noticed 1-4 years prior to diagnosis.
Conclusions: Top early manifestations in patients enrolled in the current study included arthralgia, photosensitivity and fatigue. These symptoms were followed by malar rash and fever.
Key words: early systemic lupus erythematosus.

Cite this article
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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a wide spectrum of clinical and immunological abnormalities of unknown etiology, which develops on the base of imperfect genetically determined immunoregulatory processes, associated with the overproduction of autoantibodies. Genetic, environmental and sociodemographic factors play important roles in the pathogenesis and expression of this disease. This multiplicity of etiological factors could explain the variability of disease manifestations observed, not only between individuals, but also between ethnic groups [1, 2]. SLE is a pathology that affects people of different ages, races, origins, gender; mostly women of childbearing age are affected, in 83-97% of cases. The systematic literature review (Rees, 2017) of the global incidence of SLE reported the highest estimated incidence and prevalence of SLE which were found in North America (23.2/100 000 person-year and 241/100 000 people, respectively). The lowest incidence of SLE was reported in Africa and Ukraine (0.3/100 000 person-year), and the lowest prevalence was found in Northern Australia (zero cases in a sample of 847 people) [3].

Over the last few decades, SLE has changed its expression, which was reflected in the revision of the disease classification criteria. The first SLE classification criteria were developed by the American College of Rheumatology in 1971 by the Cohen A.S. Working Group, and were subsequently revised in 1982 (E. Tan et al.). In the light of the new findings , that is the presence and association of antiphospholipid antibodies in patients with SLE, the 1982 criteria were revised and new criteria were approved in 1997 (Hochberg M.C.). The latest criteria from 2012 – SLICC (Systemic Lupus International Collaborating Clinic) have been extended due to skin manifestations and strengthening of immunological indices by the complement fractions C3, C4 [4].

Systemic lupus erythematosus, however, remains an ac-

tual research domain over the last years, especially the early stage of the disease. Over the last decade, SLE has changed due to improved classification criteria and, last but not least, due to the early use and administration of aggressive treatment [5]. The clinical onset depends on several risk factors, including gender, age, ethnicity, geographic area, etc. Specific and non-specific clinical events, which occur during the inception and evolution of SLE, have a high variability. Thus, the vector of the research in the field is being directed for a few years at studying the clinical and immunological manifestations of onset of SLE, as well as in the first years of the disease [6-9]. This trend can be explained by the attempt of researchers to further improve the criteria for classifying the disease, increasing the sensitivity and specificity of these criteria, to reduce the time of diagnosis of the disease from the onset of the first symptom associated with lupus erythematozus to clinical diagnosis.

The evolution of SLE is characterized by the complexity and uncertainty of the disease diagnosis, which can lead to considerable delays between the initial manifestations of the disease, establishing a diagnosis and initiating appropriate medical treatment. Delayed or lack of treatment may increase the likelihood of organic damage due to high disease activity. Thus, in earlier diagnosed patients, the inflammatory disease can be treated earlier and organic damage could be minimized [10].

Thus, the necessary time for diagnosis of the disease expresses the first principle in the proper management of the disease, while the reduction of the diagnosis period reveals the importance of early therapeutic intervention.

Based on the aforementioned, this research study was oriented towards determining the manifestations of early SLE, which is the most important factor for a prompt establishment of the disease diagnosis. The purpose of the study was to evaluate early manifestations of the disease in patients with SLE within the study group.

Material and methods

A cross-sectional study was conducted at the Department of Internal Medicine within *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova, and at the Rheumatology Department of the Institute of Cardiology. The patients were enrolled from June 2016 to May 2019, according to the accepted approval of the Research Ethics Committee (No 66 of 16.06.2016). The research included 68 patients who were selected according to SLICC classification criteria for systemic lupus erythematosus, validated in 2012. The SLICC 2012 classification criteria consist of 11 points, which form two compartments: clinical and immunological. The diagnosis is established if at least four criteria are included, one clinical and one immunological, except for renal damage confirmed by renal biopsy associated with an immunological criterion. The research included subjects over 18 years old, who signed an informed consent for study participation. Exclusion criteria included other confirmed rheumatologic diseases as well as patient's refusal. The clinical and demographic data, as well as information about disease characteristics were collected according to a file prepared within the present study. The questionnaire included information on clinical and demographic data – gender, place of residence, marital status, age at onset and duration of the disease. The special investigation was performed in order to highlight the symptoms and signs at the onset of the disease, which represented the criteria for the established diagnosis. The model used within the present study was based on literature analysis, and presented as early signs of lupus, which was completed together with the subject. Moreover, every patient was asked about the time of symptom onset, before and after the diagnosis of SLE was established.

The obtained results were analyzed via the Microsoft Office Excel program. The structure and dynamics of the researched phenomena were examined using statistical methods with the assessment of arithmetic means (M), standard deviations (SD) and confidence interval (CI). The statistical comparison of the data and the determination of the significance test allowed the assessment of the differences between the mean and percentage values. The differences between the mean values of the studied parameters were estimated using the t-Student criterion.

Results

A performed cross-sectional study included 68 consecutive patients with early SLE, admitted to the Rheumatology Department at the Institute of Cardiology, the duration of the disease lasted up to 2 years after being diagnosed.The demographic data of the study lot is presented in the table 1.

Table 1

Demographic indices in the investigated sample (No = 68)

Parameters	Patients, No	%
Gender:		
Women	65	95.59
Men	3	4.41
Place of residence:		
Rural	36	52.94
Urban	32	47.06
Marital status:		
Married	36	52.94
Divorced	14	20.59
Widower	4	5.88
Bachelor	14	20.59

The data presented in the table 1 revealed the predominance of women (95.59%) in the study group, with a female to male ratio of 22:1. According to the place of residence, patients from rural areas evidenced their light preponderance in the space. After the segregation of the patients' marital status we attested that at the time of research 36 (52.94%) subjects lived with families being married, 20.59% of cases were divorced or not married yet, and 5.88% of the subjects included in the study were widows/widowers.

Further reseasch assessed the age characteristics at the time of research and at the onset of the disease in patients included in the study, as well as the mean duration of the disease and the time from the first symptoms to diagnosis.

Table 2

Characteristics of variables in the research group

Parameters	Mean value±SD	Min V Max V
Mean age at the time of the research, years	39.6±15.0	20-73
Mean age at the time of the disease onset, years	38.47±14.88	20-67
Mean duration of the disease, months	12.42±8.70	0.5-24
Time from the disease onset to the SLE diagnosis confirmation, months	7.08±8.22	1-47
SLICC cumulative criteria number, abs.nr.	7.32±2.06	4-12

The data presented in the table 2, concluded that patients with early SLE had a mean age of 39.6 years, ranging between 20 and 73 years, while the mean age at the disease onset was 38.47 years. Concerning the duration of the disease, it varied from 1 to 24 months, as the definition of the early SLE, the mean duration was 12.42 months. The time from the first symptoms, claimed by the patient, until the confirmation of the SLE diagnosis varied from 1 to 47 months, with the mean time of the disease diagnosis of 7 months. The cumulative number of SLICC based on 2012 classification criteria at study entry was in average 7.3, with the highest number of 12 criteria.

In order to analyze and describe the study group, the time from the onset of the first symptoms to the referral to the doctor and later to diagnosis confirmation of systemic lupus erythematosus was identified.

Table 3

Quantification of the disease diagnosis term

Parameters	Mean value±SD	Min V Max V
Time from the disease onset to referral to healthcare (months)	4.81±6.57	0.25-37
Time from referral to healthcare to diagnosis confirmation (months)	2.27±2.1	0.75-10

Patients referred to the doctor on average at 4.81 months, with one or more symptoms that were later related to systemic lupus erythematosus (tab. 3). The shortest time was one week, the earliest manifestations being fever and edema. Patients with clinical signs, such as malar rash, arthritis and serositis referred to the doctor one month after the symptoms appeared. In addition to these manifestations, many patients experienced a marked fatigue at the time of disease onset, which they thought was not a reason for referring to healthcare. Patients who had photosensitivity, malar rash and joint pain as first manifestations referred to medical healthcare starting from 0.25 to 37 months. Thus, regarding the time of diagnosis confirmation from the first medical referral, the mean time of diagnosis established was 2.27 months, the shortest term being 3 weeks.

The complexity of the autoimmune process and the difficulty of diagnosing early SLE, due to the insufficiency of diagnostic criteria, can lead to considerable delays between the onset of the disease and the time of diagnosis. In fact, patients from the study group developed specific manifestations of lupus at the onset of the disease, stated in the disease classification criteria, as well as pathological changes omitted from the classification criteria, which however deserve special attention in case of early diagnosis of the disease. Further, the study identified the early signs of the disease by calculating their frequency in the study subjects (tab. 4).

Table 4

Clinical signs in patients before referral to primary care

SLE signs	Patients No=68		
	No	%	95% CI
Malar rash	22	32.35	0.22-0.44
Fotosensitivity	40	58.82	0.46-0.69
Maculopapular rash	4	5.88	0.02-0.14
Discoid rash	2	2.94	0.008-0.01
Oral/nasal ulcers	18	26.47	0.17-0.38
Difuse alopecia (non-cicatriceal)	18	26.47	0.17-0.38
Arthritis/arthralgia	44	64.70	0.52-0.75
Serositis: Pleuritis Pericarditis	6 2	8.82 2.94	0.04-0.17 0.008-0.01
Lupus nephritis (nephrotic/nephritic syndrom)	4	5.88	0.02-0.14
CNS involvement: Polineuropathy	4	5.88	0.02-0.14
Headache	8	11.76	0.06-0.21
Depression	10	14.70	0.008-0.25
Hemolitic anemia	1	1.47	0.02-0.07
Fever	20	29.41	0.19-0.41
Fatigue	29	42.64	0.31-0.54
Weight loss	10	14.70	0.08-0.25
Limphadenopathy	9	13.23	0.7-0.23
Myalgia	14	20.58	0.12-0.31
Livedo reticularis	10	14.70	0.08-0.25
Sjogren syndrom	8	11.76	0.06-0.21
Raynaud syndrom	14	20.58	0.12-0.31
Vascular thrombosis	2	2.94	0.008-0.1
Spontaneous pregrancy loss, n-65	3	4.62	0.001-0.12

The material presented in table 4 resume the early signs that can be associated with lupus. Therefore, the clinical picture from the onset of the disease until the doctor's assessment was determined in 64.70% of cases related to joint involvement according to the classification criteria. It should

be noted that joint pain without synovitis or morning stiffness, was most commonly reported, accounting for 80.88% of patients.

The skin involvement manifested by photosensitivity and malar rash was registered in 58.82% and 32.35% of cases, respectively; followed by oral and / or nasal ulcers and diffuse non-scarring alopecia in 26.47% of cases. Maculopapular and discoidal rash were rare skin manifestations in the early period and were reported in 5.88% and 2.94% of patients. The characteristic SLE signs, however not occurring at the onset of the disease were serous pleurisy and pericarditis (8.82% and 2.94%, respectively). Regarding renal impairment, it was manifested by nephrotic or nephritic syndrome and was present in 5.88% of cases. The involvement of the nervous system, which is a part of the SLICC classification criteria, was characterized by polyneuropathy, in 5.88% of cases. Hemolytic anemia, which is an early manifestation of lupus, has been rarely reported, only in one patient viz. 1.74% of cases.

Carefully analysis identified signs related to systemic lupus erythematosus in the early stages of the disease, though not included in the 2012 SLICC classification criteria. Fatigue, one of the most exhausting symptoms in patients with SLE was reported in 29 (42.64%) subjects from our study lot. Another important constitutional sign was fever in the absence of infection, estimated in 20 (29.41%) cases. Weight loss of more than 5-10% of body weight in the last 6 months or a decrease of more than 5 kg in the last month, in the absence of other causes, as an early symptom of the disease, was reported in 14.70% of cases. Another early manifestation-myalgia, was reported as pain or muscle weakness in the absence of obvious causes in 14 (20.58%) patients. The presence of lymphadenopathy was characterized by the increase in size of more than 5 cm of the lymph nodes in the cervical, axillary or inguinal areas, in the absence of infectious or malignant process, being detected in 9 (13.23%) cases. Neurological manifestations, such as depression and headache, which are not included in classification criteria of the disease, were reported in 10 (14.70%) and eight (11.76%) subjects, respectively. The involvement of peripheral vessels at the onset, manifested by Raynaud's Syndrome and / or reticular livedo, was found in 14 – 20.58% and 10 – 14.70% of patients, respectively. Venous thrombosis was an early manifestation of the disease in 2.94% of cases. Sjogren's syndrome, one of the early signs of the disease, was present in 8 (11.76%) patients as the first symptom of the disease. Spontaneous abortion, which is an important manifestation at the onset of the disease among young patients, occurred in 3 patients out of 65 (4.62%).

Following the idea of early signs, this present study separated the top most common manifestations of systemic lupus erythematosus (fig. 1).

The first signs attributed to lupus, at the time of referral corresponded to the 2012 SLICC classification criteria, as well as included the constitutional ones, which were not provided by these criteria. In fact, as shown in figure above, the top three early manifestations were arthralgia, photosensitivity and fatigue, while the top five variables were followed by malar rash and fever.

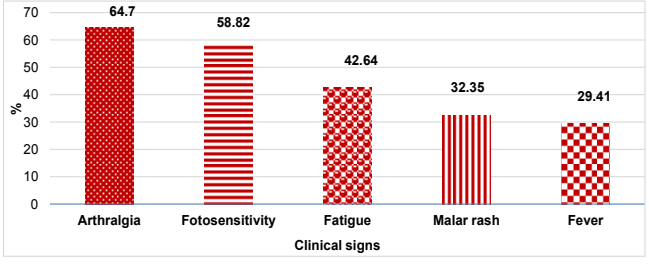

Fig. 1. Top early manifestations of SLE

Hypothetically, the study was oriented towards the clinical signs preceding the diagnosis of SLE and their chronological stratification. The earliest SLE-associated clinical manifestations were recorded 1-3 and even 4-5 years before the diagnosis of the disease. From the first year until the established diagnosis, 68 patients presented 188 criteria, each patient had 2.64 clinical signs, 2 years before patients had 104 (1.52), 3 years before – 88 (1.29) signs and 4-5 years before the diagnosis was established 39 (0.57) criteria were recorded for patients with early SLE. A year prior to establishing the diagnosis was characterized by presence of at least 3-4 signs of lupus in the same patient, which led to the motivation of their immunological research. Thus, transient arthralgia, seasonal photosensitivity and episodic fever were the 4-5 year preceding signs of the diagnosis; at the same time, 3 years before, malarial rash, weight loss and leukopenia up to 4.0 x 10^9 occurred. Two years before the diagnosis, the patients' signs were characterized by installation of serositis, oral ulcers and thrombocytopenia, increased ESR, anemia and false positive MRS. The year preceding the diagnosis was characterized by installation of several signs, including the laboratory ones.

Moreover, the supplementation of clinical variables with laboratory research could have accelerated the establishment of the diagnosis of lupus, which could have been established at least 2 years before, based on clinical picture and in compliance with four diagnostic criteria.

Furthermore, the study examined the laboratory indices by analyzing the hematological parameters at the time of the research in the context of the disease classification criteria. Thus, the most frequent hematological manifestation in early disease was leucopenia in 29.41% of cases, followed by anemia in 20.59% of subjects. Thrombocytopenia and lymphopenia were found in 19.12% and 16.18% of cases, respectively.

The immunological criteria analysis distinguished that the most common were the antinuclear antibodies (ANA) (92.65% of cases) and anti-dsDNA (91.17%). Another immunological criterion in early lupus patients was the increased incidence of low titers of complement fractions C3 and C4, identified in 58.82%. The presence of antiphospholipid antibodies was characterized by a higher frequency of lupus anticoagulant – 17.64%, followed by anti-CL antibodies and anti-β2GP1 antibodies, found in only 5.88% and

2.94% of cases, respectively. As regarding the anti-Smith antibodies and the Coombs test, these were present in 11.76% and 14.70% of patients, respectively.

Consequently, the most common paraclinical manifestations, including the immunological ones, in the early period of the disease were the anti-dsDNA (91.17%), ANA (79.41%), low titer of complement fractions (C3, C4) (58.82%), as well as leukopenia (29.41%).

Discussion

The present study, described the frequency and characteristics of the major SLE clinical manifestations and the time of the disease diagnosis in patients from the Republic of Moldova. The important fact is that the time between the onset of symptoms and established diagnosis in last decades has been shortened [2, 5, 11], and yet suggests that it is not short enough and more efforts should be made to establish the diagnosis of SLE even faster [12]. For patients diagnosed with SLE before 1980, the mean time between the onset and established diagnosis was 59 months, which subsequently decreased to 28 months for patients diagnosed between 1980 and 1989, and to 20 months for patients diagnosed between 1990 and 2010 [11, 13]. ANA testing credited differences in the diagnosis delay before 1980th and after 1980th. Some authors suggest that the average time to disease diagnoses after the 2000s has been reduced to 9 months [14]. The present study data showed that the average time for disease diagnosis was 7.08 months. These results could be explained by improving the diagnosis, by providing a wider use of immunological criteria, which are more extensive and accessible to perform, as well as medical assistance provided by the highly qualified doctors and dissemination of information through reports presented at conferences and working groups or multidisciplinary and continuing medical education. The main cause of the shortest diagnosis, lasting up till one month prior to primary healthcare was the addressability of the patient presenting such symptoms like fever, edema, malar rash and arthritis. The longest period requiring medical referral from the symptom onset lasted 37 months, and the patients experienced their first manifestations as photosensitivity, malarial rash or non-swollen joint pain. Thus, as regarding the time of diagnosis confirmation from the time referral to primary care, the mean time of diagnosis established was at 2.27 months, the shortest term being 3 weeks, which was confirmed by paraclinical and immunological tests that require time to perform. Moreover, based on the data obtained, the period of diagnosis of the disease in recent years has improved due to the high addressability of patients in the first 3 months after the disease. The review analysis of the literature from the last decades reported that the time to establish the diagnosis varies depending on the cohort performed, the countries involved in the research and the methods of patients' selection. At the same time, the results of research in the field do not present similar data regarding the time of diagnosis of the disease, thus, the topic of the early diagnosis of the disease remains in force [6-9].

The importance of recognizing the initial manifestations of the disease is indisputable and is explained by the researchers' attempt to further improve the disease classification criteria, with increasing their sensitivity and specificity, in order to reduce the time of diagnosis confirmation from the onset of the first SLE-related symptom to complete clinical diagnosis. However, the disease diagnosis is currently difficult to establish due to both symptom variety and nature of acute to insidious symptom onset. Moreover, the signs of the disease may be non-specific and characteristic of several medical conditions, which may lead to a delayed diagnosis [5-7, 14].

In order to compare the data the present study examined some of the important researches on early SLE. Data published by Pons Estel B.A. et al. (2004) presented the study findings of the Latin American Lupus Study Group research (GLADEL), which assessed the manifestations at the onset of the disease in the research groups according to race: Whites, Mestizo and African-Latin Americans [15]. Thus, according to the study findings, the top manifestations at onset in the total group of patients with joint involvement were recorded in 67.3% of cases, fever – 28.6%, photosensitivity – 24.5%, malarial rash – in 23.6 % and alopecia – in 20.3% of cases, and total skin disorders – in 46.3% of cases. It should be mentioned that the top five manifestations are followed by weight loss, nasal / oral ulcers, Raynaud's syndrome and hematological manifestations, which were present in more than 10% of cases. Due to the difference in race of the total research group, the study aimed to compare the frequency of onset manifestations with the cumulative signs found within the group of White people. Therefore, the most common symptoms were arthralgia and / or arthritis – 93.5%, skin manifestations – 89.5% and fever – 60.2%, which is similar to the data from this study, while hematological manifestations – 68.2%, alopecia – 55.0% and renal impairment – 43.6% were detected more frequently in this cohort. To note, the frequency of the top manifestations in that cohort described was much higher than the data presented in our study.

The most recent data on early lupus research were published in 2018 by M. Mosca [6]. In this study, researchers evaluated the manifestations of the disease at the time of diagnosis compared with the manifestations of the diseases that mimic lupus (Sjogren's syndrome, antiphospholipid syndrome primary, mixed connective tissue disease, systemic sclerosis, rheumatoid arthritis, thyroiditis and autoimmune hepatitis). Thus, the most common clinical manifestations, according to the researchers and appropriate to our data were arthritis (57.6%) and alopecia (30.6%), while photosensitivity (31.6%) and malar rash (49.6%), which were also in the top, in this research cohort showed a higher frequency. The important signs of the disease highlighted by this cohort study and by M. Mosca were those not included in the classification 2012 SLICC criteria. The frequency of fever (34.5%) and Raynaud's syndrome (22.1%) in these studies was compliant, whereas the fatigue (28.3%), livedo

reticularis (3.1%) and Sjogren's syndrome (3.9%) prevailed within this study. Regarding fatigue, which was one of the most important signs of this research paper, it was included in the list of the early lupus symptoms only in the study conducted by M. Mosca, while in other important studies it was omitted [6-9, 15-17].

The study of the laboratory abnormalities, namely hematological manifestations showed the highest frequency in the early period of leukopenia, which ranged from 5.1% in the GLADEL study to 54% in the Europe Inception cohort, but also for lymphopenia, noted in these studies in 5.9% and 45%, respectively, our data being intermediate, consisting 29.41% and 16.18%, respectively. Regarding thrombocytopenia, it was found in only 5.2% of cases in GLADEL and 21% of cases from Europe Inception studies, respectively, which is more appropriate to our results [9, 15]. Thus, as it could be seen the frequency of hematological changes in the early period of lupus varies, however, the presence of leukopenia and lymphopenia requires greater attention. Immunological criteria data highlighted a very high frequency of ANA found in most of studies, similar to our study data. It should be noted that the LUMINA study reported only one third of the patients with positive ANA [2]. Partial examination of each immunological marker revealed the presence of Anti-DNA in 71.7% of cases in the study conducted by Mosca and 78% of cases each in the studies conducted by Rees and Sebastiani, while this criterion was more frequenty encountered in our study (91%) [6, 7, 9]. The frequency of antiphospholipid antibodies did not vary significantly and was found in 18.1% and 22% of patients in the Early SLE and Europe Inception cohort [7, 9], which corresponds to our data. Another immunological marker, which is part of the 1992 ACR criteria, Ac anti-Smith, was determined in 10% of cases in the Euro-Lupus cohort [16], comparable with our data, thus, a higher frequency was noted in the Early SLE and Europe Inception studies, 30.2% and 54% respectively [6, 9]. Complement analysis as an immunological criterion for diagnosing the disease was introduced only in 2012, thus the previous studies did not determine it. Only the Early SLE study determined the frequency of this marker in patients with early lupus and established its presence in 73.4% of cases while 58.8% of our patients fulfilled this criterion [6].

Thus, this present research work might assume that the frequency of clinical and paraclinical manifestations in the early period of the disease largely varies depending on the study performed, the inclusion criteria and the study conducting approach. Disease-specific manifestations, such as arthritis / arthralgia, malar rash, photosensitivity and lupus nephritis, as well as nonspecific ones – fever, fatigue and Raynaud's phenomenon, as well as hematological and immunological changes show a higher frequency in early disease among patients with systemic lupus erythematosus and requires a more detailed assessment.

Conclusions

This present study results indicate that top early manifestations in SLE patients included in the study were the arthralgia/arthritis, photosensitivity and fatigue, followed by malar rash and fever. The mean time of the diagnosis confirmation from the first symptom onset that can be referred to lupus was 7.08 months.

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Authors' contribution
MG drafted the first manuscript, VS and VSB acquired and interpreted the data, MG, LMN and MM designed the trial, MM and GC revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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The authors have no conflict of interests to declare.

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Management of elderly patients with chronic myeloproliferative hemopathies

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Abstract

Background: Chronic myeloproliferative hemopathies (CMPH) as a whole are the most common chronic leukemias in the elderly in the structure of morbidity by hematological malignancies with primary bone marrow involvement, being characterized in the advanced stages by a severe, recurrent evolution and unfavorable prognosis, with negative socio-economic impact.
Material and methods: A clinical, analytical, and descriptive study was carried out along with the narrative review of the international literature on the subject. The study enrolled 91 elderly patients with different phases of chronic myeloid leukemia (CML), primary myelofibrosis (PMF) and polycythemia vera (PV), who were followed up and treated at the Institute of Oncology in the period of 1995–2020. According to the impact score, 25 relevant primary sources were identified and selected having a scientific, reproducible and transparent approach to the relevant subject, followed by data extraction and analysis.
Results: The overall one- and 5-year survival in patients aged greater than or equal to 60 years old treated with tyrosine kinase inhibitors (TKIs) was 97.6 and 79%, being lower as compared with the same indices in the totality of CML. In elderly PV patients the overall 5-and 10-year survival made up 93.5% and 76.4%, being lesser than registered in all patients with PV. As reported in the recent references, a significant rate of patients with CMPH underwent reduced working hours, discontinued employment, and medical disability: PMF – 38%, 35%, 33%, and PV – 33%, 28%, and 15%, respectively.
Conclusions: The long-term treatment results in elderly patients with CMPH fail compared to those in the CMPH totality, due to the development of age-related diseases and vascular accidents caused by leuko- and thrombocytosis.
Key words: myeloproliferative hemopathies, myeloid leukemia, myelofibrosis, polycythemia vera, elderly patients.

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Introduction

Chronic myeloproliferative hemopathies (CMPH) as a whole are the most common chronic leukemias in the elderlies in the structure of morbidity by hematological malignancies with primary bone marrow involvement, being characterized in the advanced stages by a severe, recurrent evolution and unfavorable prognosis, with negative socio-economic impact [1-5, 7]. Chronic myeloid leukemia (CML), primary myelofibrosis (PMF) and polycythemia vera (PV) are considered the most common CMPH. CML is a clonal neoplastic pathology of the hematopoietic system, which results from the malignant transformation of the pluripotent stem cell, while maintaining the ability of differentiation into all cell lines [6-9]. CML morbidity increases with age, with a maximum incidence between 35 and 65 years (median age – 53 years), that indicates the predominant involvement of the workable population. CML morbidity varies between 1.0-2.0 cases per 100000 of population. The clinico-evolutional and hematologic patterns of CML comprise splenomegaly, myeloid hyperplasia of the bone marrow, hypercatabolic symptoms and progression to the acute leukemia in the majority of cases. PMF represents a chronic myeloproliferative neoplasm, which derives from the clonal myeloid prolifetaion as a result of malignant transformation of stem cell. The disease is manifested by splenomegaly, bone marrow fibrosis, anemia, extramedullary hematopoiesis, tendency to cachexia and blastic transformation. According to the majority of references, the incidence of PMF constitutes 0.7-1 case per 100000 of population [1, 3, 4]. In 67% of cases PMF is diagnosed in persons over 54 years old. PV is a clonal trilineage proliferation of the malignized hematopoietic stem cell, being characterized by blood hyperviscosity and increased risk of thromboses. The estimated incidence of PV per 100000 of population ranges from 0.4 to 2.8 cases in Europe and from 0.8 to 1.3 cases in the USA. The reported age median encompasses 65-70 years. The bone marrow is hypercellular and exhibits hyperplasia of myeloid, erythroid, and megakaryocyte lineages. Erithrocyte formation is predominantly increased. The symptoms and signs of PV can be attributed in large part to the expanded total blood volume and to the slowing blood flow as a result

of increased blood viscosity. The latent thrombogenic status occurs. Arterial hypertension commonly develops. PV and PMF are considered orphan diseases in the USA because they affect less than 200000 people regardless of the observation period [10]. Marketology researches have shown that in 2003 the prevalence per 100000 population of PV was 22 and PMF – 19. The development and increased prevalence of CMPH in the elderlies correlates with the demographic aging process in the USA and the European Union [11], in which the rate of the population over 80 years old will triple with predictability between 2011 and 2060.

Patients across all CMPH experience a marked disease burden in terms of symptoms and negative effects on quality of life, productivity, and daily living activities. It is important to have an updated and appropriate understanding of these burdens from a financial standpoint in order to improve the health and life quality of patients with CMPH. Predominantly late diagnosis, increased degree of disability, morbidity and mortality indices in the age categories greater than or equal to 60 years [2, 11] identify CMPH as an actual problem of public health and clinical hematology.

Material and methods

A clinical, analytical and descriptive study was carried out along with a narrative review of the international literature regarding this subject. The study enrolled 91 elderly patients with different phases of chronic myeloid leukemia (CML), primary myelofibrosis (PMF) and polycythemia vera (PV), who were followed up and treated at the PMSI Institute of Oncology in the period of 1995 – 2020. The following research methods were used: epidemiological, descriptive, comparative, clinical-analytical, and cohort statistics [12]. The type of CMPH was identified according to the Revised 2017 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues [13-15]. The diagnosis was confirmed via histopathological, cytological, cytogenetic and molecular examinations of the bone marrow and peripheral blood [1, 5, 6, 8, 9, 15, 16]. The quantitative real-time PCR was used to detemine the expression of the BCR-ABL chimeric gene p210 and p190 transcripts while proceeding CML diagnosis. Five transcription products (b2a2, b3a2, b2a3, b3a3 and e1a2) were analyzed by using the quantitative PCR test [8]. The quantitative detection of JAK2 V617F mutation served as a major criterion in the diagnostically unasserted cases of PV and PMF. CML patients underwent TKI single-agent chemotherapy. The first-line treatment of PMF and PV patients included a single-agent conventional chemotherapy with busulfan and hydroxycarbamide. The research data collection was carried out by analyzing information provided by the international scientific sources and official statistics related to the above mentioned nosological entities. More than 50 reference bibliographic sources have been studied. According to the impact score, 25 relevant primary sources were identified and selected with a scientific, reproducible and transparent approach regarding this relevant subject, followed by data extraction and analysis. To minimize the error, a copy of the data extraction sheet was initially obtained, listing all the elements that should be extracted from the primary studies. When doing the qualitative research, a narrative synthesis of data has been performed.

Results and discussion

Thirty-four (37.3%) patients with PMF, 26 (28.6%) – with CML and 31 (34.1%) – with PV were diagnosed in the elderly age groups and followed up by our study. The prefibrotic stage of PMF was confirmed in 15 (44.1%) cases, fibrotic stage – in 21 (55.9%). The diagnosis of CML was made in 24 (92.3%) patients in chronic phase and in 2 (7.7%) patients in accelerated phase. In all cases PV was diagnosed in the erythremic stage: II A – in 27 (87.1%) patients, IIB – in 4 (12.9%). The age group of 60-69 years was more numerous in CML (22 cases, or 84.6%), accounting for 25 (80.6%) cases in PV and 25 (73.5%) cases in PMF. The duration of the disease from the time of onset of the initial clinical symptoms to diagnosis ranged in PMF between 1.4-7 months (on average – 3.7±0.63 months), in CML between 1.5-12 months (on average – 2.1±0.37 months) and in PV between 1-7 months (on average – 3.8±0.54 months). The clinical onset and addressability of patients with CML and PMF did not differ significantly, most patients (over 90%) being consulted by a family doctor because of the presenting complaints such as fatigue, heaviness and then pain in the left hypochondrium or in the left hemiabdomen. Most patients with PV (21 persons or 67.7%) went to the territorial healthcare institutions for medical assistance, complaining of steady hypertension or so-called "astheno-vegetative" syndrome. In 3 (9.7%) cases the diagnosis of PV was made, following a treatment for myocardial infarction within the cardiology wards. Two (6.5%) patients diagnosed with ischemic stroke were hospitalized at the neurology wards for emergency medical care; the diagnosis of PV was further confirmed.

According to the references updates, the mean life span of CML patients under the conventional chemotherapy ranges between 4-5 years, exceeding 10 years in 30% of them. Although the allogeneic hematopoietic stem cell transplantation is considered in many instances as the most efficient treatment option, with a potential of complete recovery, especially in cases refractory to TKIs, it remains currently inapplicable in the elderly CML patients [9, 16, 17]. Regardless the age, recombinant interferon α (IFN α-2b) preserves its role as a valid treatment option in chronic phase of CML, committing to the achievement of complete hematologic response in 81% of cases [18]. Under the treatment with IFN α-2b the major cytogenetic response may be obtained in 40% and complete cytogenetic response in 25% of CML patients. The overall 5-year survival of patients treated with IFN α-2b is rated at 57%, being superior to that one in patients managed with conventional chemotherapy (42%). This present study showed no significant difference in the rate of complete clinical-hematological (92.3% vs

92.8%) and complete molecular response (23.1% vs 24.7%) under TKIs medication between elderly patients and total number of CML patients. The overall one- and 5-year survival in elderly patients treated with TKI was 97.6% and 79%, being comparable with the respective parameters in all CML patients (98.5% and 87%, correspondingly). IFN α-2b was used in rare cases of resistance to conventional chemotherapy and TKIs, showing a partial response.

A combination of chemotherapy and phlebotomies was used in all 31 patients with PV that led to a clinical-hematological remission. The response duration ranged from 3 to 9 months (on average – 5.8 months). The disease relapsed in all cases of plethoric syndrome and thrombocytosis, which required the resumption of induction chemotherapy with busulfan and hydroxycarbamide, followed by regaining remissions. Fatal cases associated with the treatment and thromboembolic complications did not occur. The over one-year overall survival in elderly patients constituted 100%, over 5 years – 93.5% and over 10 years – 76.4%, thus exhibiting a lower rate than those registered in all PV patients (over one year – 100%, 5 years – 98.6%, 10 years – 85.9%). Although the relapse rate was lower in patients treated with busulfan as compared to those managed with hydroxycarbamide, there was no significant difference in the overall survival of the elderly patients undergoing chemotherapy with these antineoplastic agents.

The recent literature sources consider single-agent chemotherapy with hydroxycarbamide as the first-line treatment option in the elderly with PMF, associated with splenomegaly and thrombocytosis. Thalidomide or lenalidomide in combination with prednisolone, danazol may be administered in cases with marked symptoms, especially in those with splenomegaly and anemia. The patients with intermediary-2 risk, especially those with prognostically unfavorable mutations ASXL1, SRSF2 and aged ≥ 65 years old, should be administered treatment with JAK kinase inhibitors (ruxolitinib, etc.). In most studies the mean survival rate is estimated to 3.5-5 years, ranging from 1 year in some patients to even decades in others. According to the international literature data, the survival rate in PMF (on average – 5.9 years) is still lower compared to the same indicator in other Ph-negative CMPH like PV (on average – 13.5 years) and ET (on average – 19.8 years) [19]. This study reported on the rate of clinical -hematological responses (73.5%) and survival rate under busulfan and hydroxycarbamide treatment in patients with PMF, which were also lower than in PV and CML. The 5-year overall survival of elderly PMF patients, constituted 67% and proved to be lower, if compared to the mean 5.9-year survival in all PMF patients, thus showing similar data as reported by other relevant studies. [20-22]. Only three PMF patients, diagnosed in 2001, 2007 and 2009 respectively, are being followed up. Generally, this suggested that new therapies may be recommended in CMPH at any age without absolute contraindications. An individual precise therapy should be mandatorily considered for every patient [17, 23]. In PMF and PV cases, refractory or intolerant to hydroxycarbamide, COMFORT and RESPONSE studies showed the rate of 41.9-62% of disease control under the treatment with JAK inhibitors, as compared with the best available therapy (0-19%). For these reasons, an accurate definition of diagnosis and prognostication is required. Precision in CMPH definition and prognostication is decisively useful for a customized therapeutic approach [17].

In order to assess the financial burden of CMPH on public health, the narrative review of the international experience was performed. A study on financial burden of CMPH on patients was carried out in the USA in 2014 [24]. The subjects who were diagnosed before 2013, aged between 16-65 old at the time of diagnosis were eligible for this analysis (PMF – 85, PV – 172). Almost all patients (99%) had health insurance, primarily group commercial insurance through an employer (PMF – 46%, PV – 53%) and Medicare (PMF – 40%, PV – 34%). The mean 2013 household incomes of patients with PMF and PV were similar to each other ($79800, and $80200, respectively) and slightly higher than the total 2013 USA mean household income of $75839. A significant rate of patients in each CMPH group reported that their disease led to reduced work hours, discontinued employment, and medical disability: PMF – 38%, 35%, 33%, and PV – 33%, 28%, and 15%, respectively. Patients' demographic features, such as age and health insurance status, were similar among patients who reported CMPH-associated effects on employment and patients who did not report these within each CMPH case. In each CMPH group, the mean percentage household income loss in patients with reduced work hours, discontinued employment, and medical disability were: PMF – 16%, 18%, 28%, and PV – 15%, 24%, 17%, respectively, compared with patients who did not experience any effects of their CMPH on employment. Discontinued employment and medical disability, especially in elderly patients, tended to have a greater negative impact compared with reduced work hours across CMPH [24, 25].

Conclusions

The long-term treatment results in elderly patients with CMPH fail compared to those of overall CMPH cases, because of the development of age-related diseases and vascular accidents due to leuko- and thrombocytosis. The slow onset, gradual increase of hemoglobin, erythrocyte count and blood viscosity along with inappropriate oncologic vigilance of primary care doctors may lead to the occurrence of thrombotic and vascular complications in the elderly PV patients. The targeted treatment with TKIs remains a therapeutic option of choice for CML patients aged over 60 years old. In the elderly PV patients, no significant difference was revealed in short- and long-term outcomes of chemotherapy with busulfan and hydroxycarbamide in combination with phlebotomy, thus providing better overall results than those used in PMF patients. The review of the literature shows that the patients with CMPH, especially the elderly ones, may suffer a considerable unfavorable impact of their employment status, which in turn may be associ-

ated with a reduced annual household income. Prevention or backtracking discrete aspects of CMPH, that negatively impact individual productiveness, may be considered as an important factor in the management of these diseases.

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Authors' contribution
VM conceptualized the study, designed the research and drafted the first manuscript; VS conducted the laboratory work and revised the manuscript critically; LC conducted the management work and revised the manuscript critically; LM collected and interpreted the data, and revised the manuscript critically; DC collected the data. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
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Modern industrial pharmaceutical forms used in dermatological practice in the Republic of Moldova

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Abstract

Background: Fighting skin diseases is a strict medical, as well as socio-economic issue for the Republic of Moldova, since they may have an aesthetic and psychological impact. The author of this scientific article presented an analysis of the pharmaceutical preparations and their forms used in the treatment of dermatological diseases based on those recorded in the State Nomenclature of the Republic of Moldova according to Anatomical Therapeutic Chemical classification, dose, division, manufacturing country and industrial pharmaceutical company, producer price, and terms of validity.
Material and methods: This research paper is based on the literature and 400 clinical documents, State Nomenclature of the Republic of Moldova, the result analysis of dermatological drug manufacturers and producers, the shelf life declared by the manufacturer, etc.
Results: From a total of 10924 preparations registered in the Republic of Moldova on 14.04.2017 – 526 were preparations used in dermatological practice. The top of pharmaceutical forms used in dermatological practice and registered in the Republic of Moldova were the ointments – 274 (52.09%) and solutions for external use – 162 (30.80%). Of the 27 producing countries, the most pharmaceutical preparations, registered in the Republic of Moldova, 212 preparations (40.30%) were of domestic origin, which also had the lowest price. The leader was *Farmaprim SRL* with 46 registered preparations. The shelf life of most dermatological preparations – 505 (96%), registered in the Republic of Moldova was at least 24 months – a parameter-guarantor of quality.
Conclusions: The share of preparations used in dermatological practice in the Republic of Moldova was 4.82%, of which pharmaceutical external forms accounted for over 80%. Most preparations registered and used in the dermatological practice in the Republic of Moldova were of local origin, which are qualitative, as well as the most accessible.
Key words: pharmaceutical forms, dermatology diseases, preparations, dose, accessibility.

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Introduction

Statistical data show that skin pathology is quite common in the world, with an estimated incidence of 250 million cases per year. Skin diseases and infections in this segment can severely affect both the vital prognosis, as, for example, in the case of autoimmune pemphigus, and the functional prognosis, by affecting the quality of life reported in many dermatoses [1, 2]. The idea that "common things happen frequently" is a true finding in dermatology – a huge chapter, embracing more than 2000 different conditions. It is estimated that approximately 70% of a dermatologist's work is caused by only nine types of skin disorders: malignant skin tumours, acne, atopic dermatitis, psoriasis, viral warts, infectious skin diseases, benign tumours and vascular lesions, leg ulcers, contact dermatitis and other eczemas. In the United States, almost half of all dermatologist visits are for one of these three diagnoses: acne, warts, and skin tumours. Whatever the cause, skin diseases should not be ignored, as they can worsen and have a negative impact on the quality of life [3, 4].

In general, the literature reveals that skin diseases include a wide range of causal factors, among which we can notice fungal, viral, bacterial, parasitic infections, etc. Likewise, quite frequently in the etiopathogenesis of skin diseases are involved practically all types of immuno-allergic reactions, which lead to the appearance of allergic dermatoses, autoimmune dermatoses or immune-mediated dermatoses. Finally yet importantly, skin disorders are caused by other extracutaneous pathological diseases, being often attested as complications [3].

Most of them, skin diseases are diagnosed by a careful examination of the, skin to which is added information related to the patient›s medical history. Some skin conditions are more difficult to diagnose, as they resemble other skin diseases, but not only. For example, rosacea requires further investigation and clinical examination by a dermatologist, so as not to be confused with acne in adolescents [5]. Even skin biopsy or tissue culture may be required to accurately diagnose and differentiate skin diseases [6].

Among the most serious skin diseases, some authors note toxic bullous necrolysis, Stevens-Johnson syndrome, pemphigus vulgaris, acne, malignant tumours, skin lym-

phomas, etc., a multitude of diseases and pathological conditions, which involve the use an equally varied arsenal of active substances and pharmaceutical forms in their treatment, according to the ATC classification in the D – code Dermatological preparations.

The purpose of this study is to assess the pharmaceutical forms and preparations used in the specific treatment of skin diseases based on bibliographic analysis and practice of those registered in the State Nomenclature of the Republic of Moldova, according to ATC classification, dose, division, country and industrial enterprise, shelf life, cost, etc.

Material and methods

The data materials were collected from specialized literature, clinics (400 documents) and the State Nomenclature of the Republic of Moldova, as well as based on the results of a systemic analysis of producers and production in this segment of drugs, prices for manufactured drugs, the shelf life declared by the manufacturer, etc. The methodology of scientific research was developed based on the publications of local authors [7, 8], using the methods of analytics, descriptive statistics, comparison, price analysis, etc.

Results

In the Republic of Moldova, 526 preparations are used in the treatment of dermatological diseases according to the ATC classification [9], with code – D Dermatological preparations, which also includes 11 subcodes, namely:

Table 1

Dermatological preparations registered in the Republic of Moldova, according to the ATC classification

Sub-code	The destination of the preparations	Quantity of preparations registered in the Republic of Moldova
D01	Antifungals for dermatological use	98
D02	Emollients and protectors	10
D03	Preparations for the treatment of wounds and ulcers	31
D04	Antipruritic, includes antihistamines and local anesthesia	7
D05	Antipsoriatic	6
D06	Antibiotics and chemotherapeutics for dermatological usage	66
D07	Corticosteroids, dermatological preparations	93
D08	Antiseptics and disinfectants	160
D09	Medicinal dressings	0
D10	Acne preparations	27
D11	Other dermatological preparations	28

Note. These 11 ATC subcodes include another 40 subcodes.

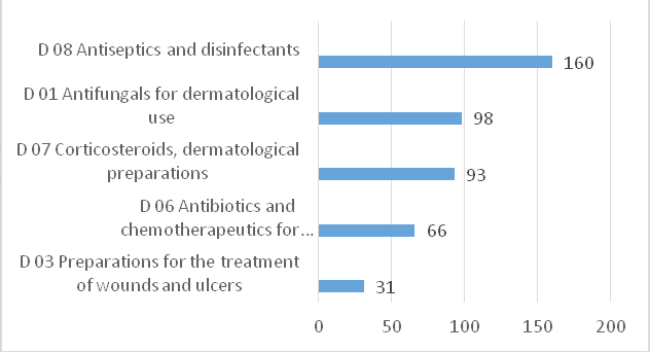

Fig. 1. Preparation used in dermatological practice, registered in the Republic of Moldova, according to ATC

On 14.04.2017, 18 pharmaceutical registered preparations used in dermatological practice were exhibited on the pharmaceutical market of the Republic of Moldova, among which: ointments – 274 (incl. nasal ointment – 1, ophthalmic ointment – 1, creams – 104, vaginal creams – 2, gels – 31, liniments – 8, pastes – 3); cutaneous solutions – 162 (incl. solutions – 7, alcoholic solutions – 5, cutaneous alcoholic solutions – 8, oropharyngeal solutions – 3, cutaneous solutions – 141, oily solutions – 5, shampoos – 10); tablets – 16; sprays – 19 (incl. skin spray, sol. – 16, skin spray, susp. – 2, buccopharyngeal / cutaneous / vaginal spray, sol. – 1); powders – 11 (incl. powder + solvent / skin solution – 1, skin powder – 7, powder / cutaneous sol.– 3, powder / injectable sol. – 2), etc. [10, 11].

The distribution of preparations and their pharmaceutical forms used in dermatological practice accounts for 4.82%, of which ointments – 52.09%, skin solutions – 30.80%, tablets – 3.04%, spray – 3.61%, powders – 2.09%, capsules – 1.52%, herbal products – 0.57%, injectable solutions – 0.38%, suppositories – 0.19% and others (fig. 2).

It is necessary to mention the specific group of herbal products used in the treatment of dermatological diseases, registered in the Republic of Moldova:

1. Elecasol, herbal combination, vegetable product / tea, 1.5 g N20, Ukraine;
2. Elecasol, vegetable product / tea, 75g N1, Ukraine;
3. Aerial parts of dentition, Bidens tripartite, fragmented plant product, 50g N1, Ukraine.

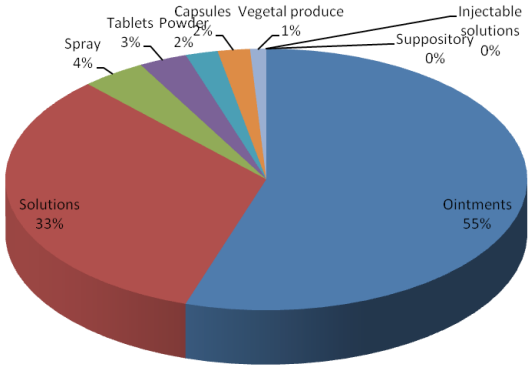

Fig. 2. The distribution of pharmaceutical forms, used in dermatological practice in the Republic of Moldova

As it is shown in figure 2 the ointments and skin solutions are among the top pharmaceutical forms used in dermatological practice and registered in the Republic of Moldova. [11].

Dermatological preparations were assessed according to the manufacturing country, shown in figure 3. Of the 27 drug-producing countries, registered in the Republic of Moldova regarding preparations and pharmaceutical forms used in dermatological practice, the Republic of Moldova is on the first place with 212 registered pharmaceutical forms (40.30%), followed by the Ukraine – 45 preparations (8.55%) and Switzerland – 33 preparations (6.27%). Other countries (in alphabetical order): Austria – 12 (2.28%), Belgium – 7 (1.33%), Bosnia and Herzegovina – 4 (0.76%), Bulgaria – 6 (1.14%), Czech Republic – 3, Croatia – 1, Egypt – 1, Estonia – 1, France – 2, Georgia – 1, Germany – 24 preparations (4.56%), India – 16 (3.04%), Italy – 2, Latvia – 3, Great Britain – 20 preparations (3.80%), Netherlands – 15, Poland – 14, the Republic of Belarus – 13, Romania – 26 preparations (4.94%), Russia – 32 (6.08%), Serbia – 1, Slovenia – 6 (1.14%), Turkey – 14, Hungary – 12 preparations (2.28%) [10, 11].

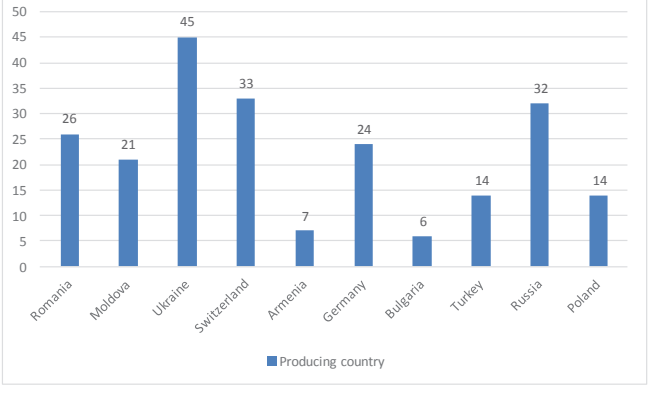

Fig. 3. Top 10 producing countries with the largest number of dermatological preparations and their pharmaceutical forms, registered in the Republic of Moldova

The list of drug manufacturing companies used in dermatological practice and registered in the Republic of Moldova, iz as following: *Farmaprim SRL*, the Republic of Moldova ranks first with 46 preparations, followed by *Beta SRL*, Moldova –33 preparations, I.M. *RNP Pharmaceuticals SRL*, Moldova – 24, *Universal-Farm SRL*, Moldova – 21, *Bayer Consumer Care AG*, Germany – 20, *Farmacia Cojusna SRL*, Moldova – 20, I.M. *Farmaco SA*, Moldova – 20, *Nijfarm SA*, Russia – 19, *Astellas Pharma Europe B.V.*, Netherlands – 15 (fig. 4) [10, 11].

One of the decisive factors in ensuring the competitiveness of medicines is their quality. An important parameter of the quality of pharmaceutical forms is considered the "shelf life", determined by experimental methods, such as the «accelerated aging» method or other methods [12].

As it is shown in figure 5, 237 preparations and their pharmaceutical forms were declared by the manufacturers

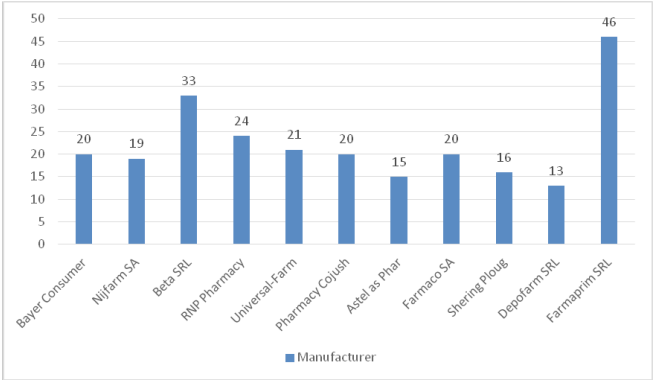

Fig. 4. Top 10 dermatological drug manufacturers with the highest number of drug preparations, registered in the Republic of Moldova

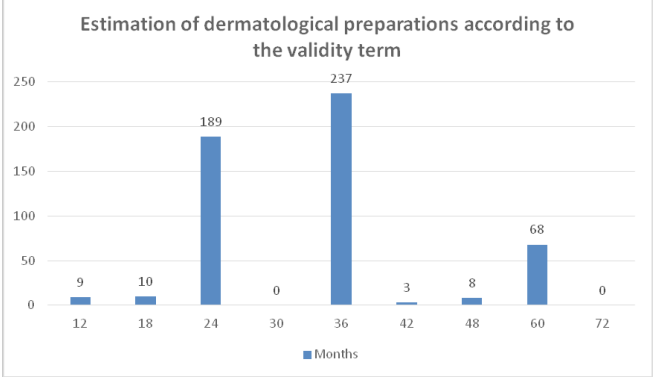

Fig. 5. Shelf life as a parameter of the quality of preparations used in dermatological practice, registered in the Republic of Moldova

as having a 36-month shelf life of (e.g. Triderm® ointment, 0.5 mg + 10 mg + 1 mg/g, 15 g N1, D07CC01, Betamethasonum + Clotrimazolum + Gentamicinum, *Schering-Plow Central East AG* (prod.: Schering-Plough Farma Lda, Portugal; Schering-Plow Labo NV, Belgium); 189 preparations and their pharmaceutical forms were declared to have a 24-month shelf life (e.g. skin spray, Mycofin® sol., 10 mg g, 30 ml N1, D01AE15, Terbinafinum, *Nobel Ilac Sanayii ve Ticaret A.S.* (prod.: Nobel Ilac Sanayii and Ticaret AŞ, Turkey); 68 preparations and their pharmaceutical forms – 60-month shelf life (e.g. skin spray, Acerbine sol. 2.15 g + 0.15 g + 0.04 g / 100 g, 80 ml N1, D03AX, Acidum malicum + Acidum benzoicum + Acidum salicylicum, Pharmazeutische *Fabrik Montavit Ges.mbH*, Austria); 2 preparations and their pharmaceutical forms do not have a declared shelf life (e.g. Polividon-iodine skin alcohol solution, 10%, 100ml No1, D08AG02, Povidoni iodidum, *Beta SRL*, the Republic of Moldova) [10, 11].

A particularly important index that describes the accessibility of the population to an adequate and effective treatment (and the "cost-effectiveness" index) is the price of medicines declared by the manufacturer. Thus, in the chapter of "price declared by the manufacturer", dated on 14.04.2017, the highest declared prices for preparations and their pharmaceutical forms, used in dermatological practice

and registered in the Republic of Moldova (fig. 6) were as follows:

1. Gelatin capsules Roaccutane® 20mg, N10x3, D10BA01, Isotretinoinum, *F. Hoffmann-La Roche Ltd* (prod.: *Catalent Germany Eberbach GmbH*, Germany), Switzerland. Shelf life – 36 months, Manufacturer's price (ex works) MDL – 310.38, EUR – 14.06.
2. Skin solution Betadine®, 10%, 1000 ml, N1, D08AG02, Povidoni iodidum, *Egis Pharmaceuticals PLC*, Hungary. Shelf life – 36 months, Manufacturer's price (ex works) MDL –258.83, USD – 12.84.
3. Lyophilized / injectable sol., Chymotrypsin 10 mg, N10, D03BA, Chymotrypsinum, *Samson-Med SRL*, Russia. Shelf life – 36 months. Manufacturer price (ex works) MDL – 222.24, USD – 11.20.
4. Tablets Terbisil®, 250 mg, N14x2, D01BA02, Terbinafinum, *Gedeon Richter PLC*, Hungary. Shelf life – 60 months, Manufacturer's price (ex works) MDL – 208.58, EUR – 9.50, etc.

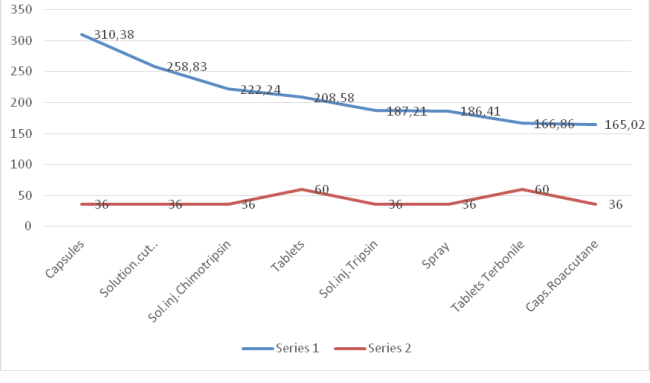

Fig. 6. The assessment of the manufacturer's price on preparations used in dermatological practice, registered in the Republic of Moldova

5. Lyophilized / crystalline sol., Tripsin injection, 10mg, N10, D03BA01, Trypsinum, *Samson-Med SRL*, Russia. Shelf life – 36 months, Manufacturer's price (ex works) MDL – 187.21, USD – 9.50.
6. Skin spray, sol., Pilfud®, 50 mg / ml, 60 ml N1, D11AX01, Minoxidilum, *Bosnalijek*, Pharmaceutical and Chemical Industry JSC, Bosnia and Herzegovina. Shelf life – 36 months, Manufacturer's price (ex works) MDL – 186.41, EUR – 8.35.
7. Tablets Terbonile, 250 mg, N14x2, D01BA02, Terbinafinum, *Bilim Pharmaceuticals AS*, Turkey. Shelf life – 60 months, Manufacturer's price (ex works) MDL – 166.86, USD – 8.44.
8. Caps., Roaccutane® 10mg, N10x3, D10BA01, Isotretinoinum, *F.Hoffmann-La Roche Ltd* (prod.: *Catalent Germany Eberbach GmbH*, Germany); Switzerland, Shelf life – 36 months, Manufacturer's price (ex works) MDL – 165.02, EUR – 7.76.
9. Skin spray, sol., Pilfud® 2%, 60ml N1, D11AX01, Minoxidilum, *Bosnalijek*, Pharmaceutical and Chemical Industry JSC, Bosnia and Herzegovina. Shelf life – 36 months. Manufacturer's price (ex works) MDL – 164.84, EUR – 7.50.
10. Gel Skinoren® 150 mg / g, 50 g N1, D10AX03, Acidum azelaicum, *Bayer AG* (prod.: *Bayer Health Care Manufacturing S.R.L.*, Italy), Germany. Shelf life – 36 months, Manufacturer's price (ex works) MDL – 157.14, EUR – 7.15.

The lowest price was attested at 3% Boric acid-RNP sol., 10 ml N1, D08AD, Acid boric, *RNP Pharmaceuticals SRL*, the Republic of Moldova. Shelf life – 24 months, Manufacturer's price (ex works) MDL – 3.68, USD – 0.18 [10, 11].

Discussion

1. The fight against skin diseases is a persistent medical and socio-economical problem for the Republic of Moldova, as these can have a strong psychological and aesthetic impact on people who constantly struggle for improving self-image. Untreated dermatological skin diseases might leave deep marks, which are often difficult to reverse, thus causing emotional and physical discomfort among population.

2. According to the ATC classification, out of the total of 10924 preparations registered in the Republic of Moldova on 14.04.2017, 526 are used in dermatological practice. The most registered preparations refer to the subcode D08 Antiseptics and disinfectants – 160 preparations, the least ones refer to subcode – D05 Antipsoriasis – 6 preparations. Among the subcodes, most of the registered preparations belong to the subcode D08AX – 68 preparations, D08A – 21 et al. The subcode D09 Drug dressings do not contain any preparations.

3. According to the data from the State Nomenclature of the Republic of Moldova, the distribution of preparations used in dermatological practice makes up 4.82%, which are present in various pharmaceutical forms: ointments – 52.09% (274), solutions for external use – 30.80 % (162), sprays – 3.61% (19), tablets – 3.04% (16), powders – 2.09% (11), capsules – 1.52% (8), plant products – 0.57% (3), injections – 0.38% (2), and suppositories – 0.19% (1).

4. Of the 27 dermatological drug manufacturing countries, most preparations were registered in the Republic of Moldova including: the *Republic of Moldova* – 212 preparations (40.30%), the *Ukraine* – 45 preparations (8.55%), *Switzerland* – 33 preparations (6.27%), *Russia* – 32 (6.08%), *Romania* – 26 preparations (4.94%) etc.

5. The list of dermatological drug manufacturing companies, registered in the Republic of Moldova includs: *Farmaprim SRL*, Republic of Moldova – 46 preparations, *Beta SRL*, Republic of Moldova – 33 preparations, *RNP Pharmaceuticals* SRL, IM, Republic of Moldova – 24, *Universal-Farm SRL*, Republic of Moldova – 21, *Bayer Consumer Care AG*, Germany – 20, etc.

6. The maximum shelf life of 60 months is stated for 68 pharmaceutical preparations and forms. The shortest shelf life – 12 months is stated for 9 pharmaceutical forms.

7. A particularly important index of the accessibility of

the population to adequate and effective treatment is the price of the medicine declared by the manufacturer. Thus, as regarding to the "manufacturer's price" for the pharmaceutical forms used in dermatological practice, the lowest price was registered for 3% Boric acid – RNP, 10 ml N1, D08AD, Acidum Boricum, *RNP Pharmaceuticals SRL*, the Republic of Moldova – (ex works) MDL – 3.68, USD – 0.18. Shelf life – 24 months. The highest price declared for preparations in this group is Roaccutane® 20mg, N10x3, D10BA01, Isotretinoinum, *F. Hoffmann-La Roche Ltd* (producer: *Catalent Germany Eberbach GmbH*, Germany), Switzerland. Shelf life – 36 months, Manufacturer's price (ex – works) MDL 310.38, EUR – 14.06.

Conclusions

The dermatological drug distribution across the Republic of Moldova was 4.82%, of which over 80% accounted for pharmaceutical forms for external use. The most registered and used dermatological drugs from the Republic of Moldova were of local origin, which are both qualitative and the most accessible ones.

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Authors' contribution

MF, AZ designed the study and performed its logistic part, drafted the first manuscript; MF, TC collected and processed the material, performed the work; AZ, VG interpreted the data, revised the manuscript. 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

No competing interests were disclosed.

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Evolutionary particulars of COVID-19 in elderly patients

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Abstract

Background: The new SARS-CoV-2 coronavirus affects a large number of people worldwide, and the elderly are particularly affected because of their vulnerability. Thus, the elderly patients and those with comorbidities have an increased risk of developing a severe disease and show an increased mortality rate. Although they may show mild symptoms of illness and low-grade fever in the early days, they may worsen clinically rapidly, requiring ongoing monitoring.
Material and methods: The prospective study was performed on a group of 96 patients (mean age 61.41 ± 3.42 years), with a predominance of men, hospitalized in the *Holy Trinity* Hospital of Chisinau who met the clinical case definition and were laboratory case-confirmed with COVID-19. Patients were clinically and paraclinically investigated according to the WHO Provisional National Clinical Protocol for COVID-19 infection reporting. The data were statistically processed by the Statistics 10 program.
Results: Of the 96 patients with COVID-19, 85 (88.54%) reported at least one had a comorbidity. The prevalence of comorbidities was the following: chronic coronary syndromes (40.1%), hypertension (39.7%), diabetes (16.04%), chronic obstructive pulmonary disease (17.3%), malignancy (13.04%), cerebrovascular disease (10.6%), chronic kidney disease (4.3%) and viral hepatitis B (1.8%). Severe cases of the disease were revealed – 58, medium severity – 38 cases. All critical cases resulting in death (7.29%) showed comorbidities with respiratory symptoms, as well as with the onset of acute respiratory failure.
Conclusions: The elderly, male gender and the presence of comorbidities in patients with COVID-19 determine the severe course of the disease and an increased mortality rate.
Key words: COVID-19, elderly, mortality.

Cite this article
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Introduction

Coronavirus (COVID-19) is a respiratory tract infection caused by a newly emerging coronavirus, which was first identified in Wuhan, China, in December 2019. Genetic sequencing of this virus suggests that it is a beta-coronavirus closely related to SARS virus [1]. The complete clinical picture is not yet well defined, as the reported symptoms range from asymptomatic and mild to severe cases, such as acute respiratory distress syndrome, septic or septic shock, and polyorganic failure, including acute kidney damage and cardiac, with a high risk of mortality in the elderly [2-5]. The elderly, especially those with comorbidities, are a group at high risk of death. In addition, a recent multivariate analysis confirmed that advanced age is the cause of sequential organic failure [1, 2].

While most people with COVID-19 develop only a mild or uncomplicated form, about 14% develop a serious illness that requires hospitalization and oxygen therapy, and 5% require hospitalization in the intensive care unit [6]. Susceptibility is associated with age, sex, and comorbidities [7, 8]. There are currently numerous studies on SARS-CoV-2 and COVID-19. This review provides a comprehensive introduction to this disease, including the structure of the genome and the SARS-CoV-2 receptor, epidemiology, clinical features, diagnosis, treatment, and prognosis of COVID-19. At present, research is needed to provide us with more information to understand this disease, to help limit the spread of the disease and to invent the vaccine and specific drugs [1].

COVID-19 infection begins as an infection of the local upper respiratory tract, which can spread to affect several organ systems with consequences that are only now understood. When it spreads, it might result in a multisystem disease associated with a high risk of death. The clinical picture accompanied by manifestations from other organs and systems of COVID-19 infection is caused by a combination of specific host defense responses with associated inflammatory activity and (micro) vascular involvement, with distinct coagulopathy and a strong propensity to develop thromboembolic complications [3, 8]. The purpose of this paper was

to evaluate the evolutionary features of SARS-VOC-2 infection in elderly patients.

Material and methods

The prospective study was performed on a group of 96 patients (mean age 61.41 ± 3.42 years), with a predominance of men (men 59.3% vs 40.7% women) hospitalized in the *Holy Trinity* Municipal Hospital who had met the clinical and laboratory case-confirmed definition of COVID-19. Patients were clinically and paraclinically investigated according to the WHO Provisional National Clinical Protocol for COVID-19 infection reporting. The data were statistically processed via the Statistics 10 program. The information was searched in PubMed, Springer, including the pages of the official websites of the European Geriatric Society, French, American and Italian National Geriatrics and Gerontology Societies, to identify scientific journals dedicated to COVID-19. Sources published between December 2019 and May 2020, in English and French, were selected, using the following keywords: "SARS CoV-2, COVID-19", "clinical characteristics of older adult CoV-2", "coronovirus impact", and "Elderly".

Results

According to the results of the studies, the elderly show similar symptoms of SARS-CoV-2 compared to the younger people. The first manifestations in geriatric patients, related to COVID-19 disease reported by Nguyenau S. et al. were the worsening of the general condition, the decreased mobility on the background of myalgias and the persistence of an overall weakness. Some clinical manifestations in the elderly, described by this group of authors may occur separately or may even be preceded by a few days the appearance of respiratory symptoms or fever [9].

Symptoms of COVID-19 initially start with fatigue, prolonged low-grade fever, myalgia, dry cough, and difficulty breathing, which then improve with early identification, initiation and administration of conservative treatment, or worsen and progress to dyspnea and productive cough. In several studies, it was found that the average time for the onset of dyspnea in different cohorts was 6 days after exposure. The most common complications that develop in COVID-19 are bilateral pneumonia that can progress to respiratory distress, sepsis and septic shock, acute kidney damage and others, such as acute heart damage (arrhythmias, heart failure, MI), coagulopathy, rhabdomyolysis, hyponatremia and acidosis. Complications are more severe than non-severe diseases [1, 8, 9].

The main underlying co-morbidities that complicate the course of COVID-19 by increasing the disease severity, use of mechanical ventilation as well as length of stay, thus exhibiting high risk of mortality include uncontrolled hypertension, diabetes, coronary heart disease, hepatitis B, cerebrovascular disease, chronic obstructive disease of the respiratory tract and other diseases, such as cancer, chronic kidney disease and immunodeficiency. Covid-19 has many clinical features similar to SARS. Although the symptoms are characterized as nonspecific, they often resemble flu even more than the common cold. Predominant symptoms include fever, cough and myalgia. Diarrhea and nausea may precede fever and respiratory symptoms. The elderly men and those with comorbidities have the highest risk of severe form (huang, chen). The milder form can resolve without medical care or can progress to pneumonia and respiratory failure that requires hospitalization. Patients can progress rapidly to respiratory distress with polyorganic dysfunction and insufficiency. Leukopenia and lymphopenia are frequently detected [1, 10, 11].

The prospective study was performed on a group of 96 patients (mean age 61.41 ± 3.42 years), hospitalized within the Holy Trinity Municipial Hospital. Of the 96 patients with COVID-19, 85 (88.54%) were reported to have at least one comorbidity. The prevalence of specific comorbidities were the following: chronic coronary syndromes (40.1%), hypertension (39.7%), diabetes (16.04%), chronic obstructive pulmonary disease (17.3%), malignancy (13.04 %), cerebrovascular disease (10.6%), chronic kidney disease (4.3%) and viral hepatitis B (1.8%) (fig. 1).

The stratification of patients according to the disease severity revealed severe cases in 58 patients (60.4%) of the studied subjects, medium severity – 38 (39.6%), whereas milder forms were not detected. Severe forms predominated in patients older than 65 years (mean age – 69.2 ± 4.42 years), compared with moderate forms (mean age – 53.6 ± 3.12 years). All the critical cases resulting in death were registered in older patients (average: 80.33 vs 55.64 years) that made up 7.29% of cases with present comorbidities, in whom the respiratory symptoms worsened within a week with the onset of insufficiency acute respiratory and rapid progression of the lung CT scan.

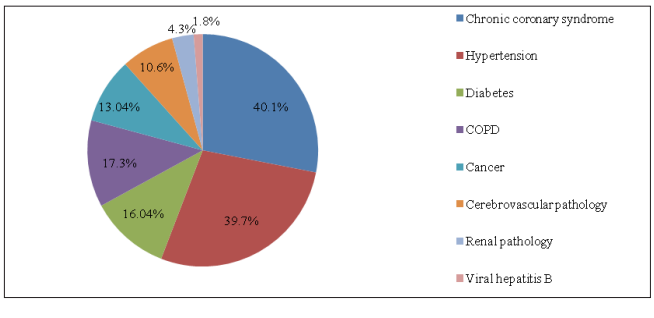

Fig. 1. Prevalence of comorbidities in patients included in the study

Independent predictors of high mortality are the elderly (age ≥70 years); the underlying co-morbidities, such as uncontrolled hypertension, diabetes and coronary heart disease, chronic obstructive pulmonary disease and malignancy; severe lymphopenia (<0.8 × 109 / L) and D-dimer (>1 μg / L) were reported [10]. Other prognostic factors are elevated C-reactive protein, HDL, ALT, serum ferritin, IL-6 and high-sensitivity cardiac troponin.

Clinical manifestations of patients infected with SARS-

CoV-2 ranged from mild nonspecific symptoms to severe pneumonia with impaired organ function. Common symptoms were fever (98.6%), cough (81.8%), fatigue (69.6%), dyspnea (55.0%), myalgia (34.8%), sputum production (56.5%) and headaches (33.9%). Sore throat, rhinorrhea, chest pain, hemoptysis, conjunctival congestion, diarrhea, nausea and vomiting were uncommon [6, 12, 13]

The elderly are more sensitive to COVID-19 and have a significantly increased risk of morbidity and mortality [11]. Infections are often atypical, sometimes confusing. The factors that contribute to this are the physiological changes of aging; multiple age-related comorbid conditions, such as cardiovascular and pulmonary pathology, diabetes and dementia, as well as associated polypragmatism. Older adults living in care facilities have the highest risk due to chronic diseases and socio-economic impact. However, for all older adults, prevention is paramount [11].

Discussion

Timely recognition of the appropriate history of exposure and prompt recognition of symptoms will help to early identify these cases and better track contacts for early isolation. This will help reduce unwanted events and prevent the further spread of the infection; it will also help reduce COVID-19-related morbidity and mortality. Due to physiological changes in aging, immune function decreases and the prevalence of multi-morbidity increases, especially in the elderly, who have a significantly increased risk of COVID-19 [1]. Older adults are more susceptible to the infection itself and are more likely to suffer from the severe form of COVID-19 as well as its complications. Age changes can also complicate the diagnosis in the elderly category with often atypically respiratory viruses present. The average duration from the onset of symptoms to death is 11.5 days in people > 70 years vs 14 days in young people [5].

Coronaviruses are important pathogens in humans and animals that can cause diseases ranging from the common cold to more severe and even fatal respiratory infections. In the last two decades, two highly pathogenic human coronaviruses, the coronavirus responsible for severe acute respiratory syndrome (SARS-Cov) and the coronavirus responsible for respiratory syndrome in the Middle East (MERS-Cov), 12 have appeared as two separate events. They induced lower respiratory tract infections as well as extrapulmonary manifestations, leading to hundreds or thousands of cases with high mortality rates of up to 50% in some populations.

The comorbidities detected in the patients included in the study were hypertension, diabetes, cardiovascular pathology, cerebrovascular disease, chronic renal disease, COPD, CVD, diabetes mellitus, malignancy, which correlated with the severity of the disease and the increased mortality rate in elderly patients [14-16]. It is noteworthy that the 70-year-olds had a shorter time interval (11.5 days) between the first symptom and death than the younger subjects (20 days), suggesting that the disease progressed faster in older adults [17].

Conclusions

The situation of the COVID-19 pandemic, the world is facing now is one of the most important geriatric emergencies in 2020. The elderly are often asymptomatic or have atypical symptoms, often against an underlying physical and cognitive impairment, which are indicators of an unfavorable prognosis. The old age, male gender and presence of comorbidities in patients with COVID-19 determine the severe course of the disease and its increased mortality.

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Authors' contribution
GS conceptualized the study, designed the research and drafted the first manuscript; AP conducted the laboratory work and revised the manuscript critically; AN conducted the management work and revised the manuscript critically; AP collected and interpreted the data, and revised the manuscript critically; AP collected the data. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The research was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 51 of June 16, 2020)

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

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Diagnosis and modern medical-surgical tactics in treatment of biliary atresia in children

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Abstract

Background: Biliary atresia (BA) is a serious pediatric condition that tends to progress to cirrhosis, liver failure, and death within a short time. It is the result of a continuous inflammatory, sclerosing, destructive process in the biliary tract and the most common indication for liver transplantation.
Material and methods: The study included 46 patients up to 1 year of age hospitalized with cholestasis syndrome in IMSP IM and C, during the years 2014-2019. The basic methods in the diagnosis of BA were the biochemical examination, FGDS, USG doppler dupplex color of the biliary system before and after the meal intake, MRI with cholangiography, dynamic hepatobiliary scintigraphy.
Results: Following the analysis of clinical and paraclinical results, surgical pathology was excluded in 25 patients, the diagnosis of BA was established in 11 cases. 6 patients with BA underwent Kasai surgical intervention , a primary liver transplant was performed in 3 cases, and 2 patients died before the surgery.
Conclusions: Portoenteroanastamosis (Kasai operation) performed as early as possible (up to 60 days postnatal) considerably increases life expectancy. The embryonic form of BA is a severe condition that is indicated for the initial liver transplant.The prognosis of untreated biliary atresia is unfavorable, leading to the death of most children in the first 2 years of life due to liver failure. In decopensated late-diagnosed cases, liver transplantation remains the only treatment option.
Key words: biliary atresia, Kasai, transplant, portoenteroanastamosis.

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Introduction

Biliary atresia (BA) is defined as a progressive inflammatory, fibrotic, destructive-sclerosing process, continuous, which is complicated by endoluminal obliteration of the bile ducts both intra and extrahepatic [1]. Complicated with destructive cholangitis, it partially or totally affects the biliary excretory system, and leads to continuous fibrosis of the biliary system, resulting in numerical reduction of the intrahepatic bile ducts with stenosis of the entire biliary excretory system, ending with biliary cirrhosis [1]. The pathology is determined by a pathogenetic, multifactorial complex that initiates inflammation, fibrosis, sclerosis and bile duct obliteration. Based on the etiopathogenetic evolution, BA is not the agenesis of the biliary system, but the continuation of the destructive processes conceived in both, embryonic and fetal periods [1, 2].

Despite the detailed etiopathogenetic argumentation, some authors currently support the theory of biliary atresia as an acquired notion. The justification for this theory is supported by statistical results that indicate that 35-40% of patients have normal meconium stool at birth, and more than 50% have colic stools at birth, and in the short postnatal period, being met periodicity of colic and acholic stools [1, 2].

The diagnostic differentiation in a short time creates difficulties and imposes an interdisciplinary activity in order to exclude neonatal hepatitis, metabolic, hematological, genetic disorders, etc., which can be easily confused with BA. The surgery on the background of the above-mentioned pathologies, considerably aggravates the child's condition and has an unfavorable prognosis [1, 3].

Histologically, it is demonstrated that in most cases the intrahepatic bile ducts are numerically diminished and the lack of the lumen is appreciated with the transformation of the bile ducts into connective tissue cords. In the specialized literature are described cases when in the fibrous cord the premature lumen of the bile ducts is appreciated. BA may be present as an isolated abnormality or in combination with other birth defects [1]. Fibrosis obliteration of the biliary system is directly proportional to the patient's age, this finding raise major problems in the diagnostic differentiation from a histological point of view, with neonatal hepatitis. Multiple classifications based on the anatomy of these fibrous biliary ducts have been carried out. It was found that most often the extrahepatic ductal system is obliterated, and

in 20% of cases the gallbladder, cystic duct, choledochus remain permeable. Children with biliary atresia are usually born at term with a weight within the norm [1, 3].

Morbidity accounts for 28-30% of all newborns with cholestasis, the incidence being from 1:8000 to 1:18000 live newborns, most often with sporadic occurrence in the absence of a positive family history [4]. In the eastern countries, such as Japan, China, Korea, Vietnam, Laos, etc., the disease is found in about 1:8000 children [5, 6]. The highest incidence was recorded in Taiwan 1:3000. More common in females, the ratio being 1.3:1 [6].

Annually in the USA there are over 400 cases of biliary atresia, mostly extrahepatic, with an incidence of 1:14000. It is found in Europe with an incidence of 1:16000 live births [6]. Annually in the Republic of Moldova 2-3 children are born with biliary atresia having an incidence for the years 2014-2019 of 1:13000. In 2014, in Moldova, according to the statistics of the Republic of Moldova, 5 children were born with biliary atresia, the given frequency being inexplicable.

Material and methods

The clinical study was carried out at *Natalia Gheorghiu* Scientific Center of Pediatric Surgery on a group of 46 patients up to 1 year of age hospitalized with cholestasis syndrome during the years 2014-2019. The basic methods in diagnosing BA were the clinical manifestations, scalocolurometry, biochemical examination, VGDS, USG doppler dupplex color of the biliary system before and after the meals, cholangiographic MRI, dynamic hepatobiliary scintigraphy, and liver biopsy.

Results

Following the analysis of clinical and paraclinical results, surgical pathology was excluded in 25 patients, the diagnosis of BA was established in 11 patients, 6 girls and 5 boys (fig. 1, 2).

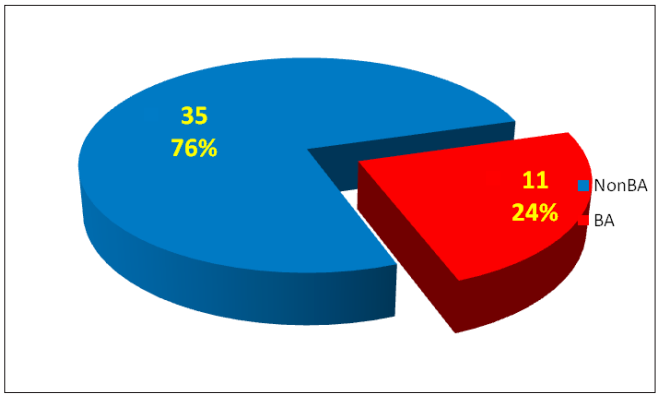

Fig. 1. Incidence of BA for patients with cholestasis

6 patients with BA underwent Kasai surgery, in 3 patients a primary liver transplant was performed, and 2 patients died before surgery.

In eight children there was a meconium stool, in 3 children postnatal there was an acholic stool. Subsequently,

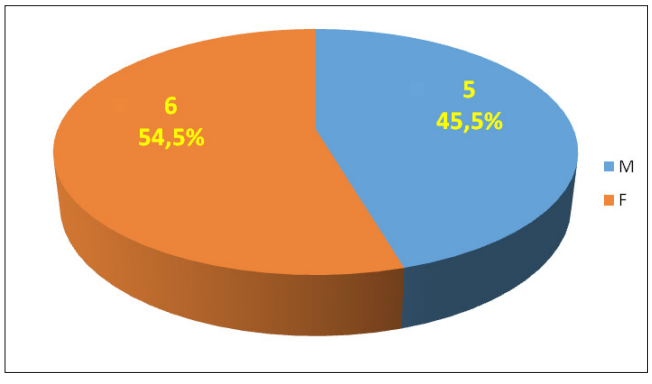

Fig. 2. Gender prevalence in children with BA

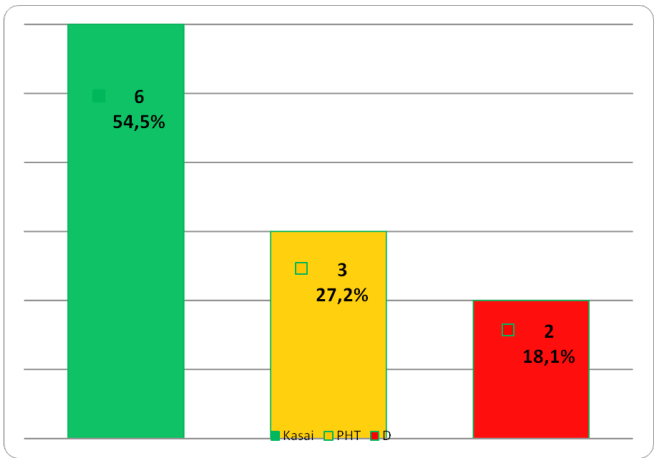

Fig. 3. Type of treatment for pacience whith BA

all children experienced symptoms no less important than acholic stools and hyperchromic urine. Eight children were born at term, one child at 34 weeks, 2 at 36 weeks. Body weight at birth ranged from 2500 g to 3800 g.

BA was established according to the following results, the primary sign for diagnosis confirmation was prolonged to cholestatic jaundice, persistent in the first weeks of life, being present in 100% of examined patients. Neonatal jaundice was reported in two children (4.3%).

The initial objective examination revealed mild hepatomegaly with dynamic liver enlargement and pronounced splenomegaly, pruritus, coagulopathy and hemorrhagic syndrome manifested by frequent petechiae and bruising. Ascites and pronounced vascular pattern on the abdominal wall was assessed in the late stage of the disease or in a general decompensated state. Palpation of the liver revealed induration with a rounded edge; splenomegaly was assessed in the case of intrahepatic portal hypertension (PH).

Scalocolurometry was performed in all patients for at least 14 days and repeated as needed. Initially, all patients had an acholic stool, in 4 patients there was a change in the yellow color for 2-3 defecations, with subsequent installation of acholic stool. In 3 patients the acholic stool was followed by the presence of black stools, which indicate an association of complications and the presence of coagulopathy.

Special attention was drawn to the laboratory findings

to the fractions of bilirubin, mainly to direct bilirubin, which in cases of BA was considerably increased, showing values from 180-420 mcmol/l, persistent after conservative treatment, and regressing only after the Kasai successful intervention. It should be noted that the prevalence of direct bilirubin is predominantly characteristic in the early stages of the pathology, whereas in case of hepatocellular involvement the increased direct bilirubin is not so obvious compared to indirect bilirubin. Moreover, sometimes indirect bilirubin may prevail, which requires careful analysis for diagnostic differentiation. In addition to increased bilirubin, elevated levels of transaminases, GGTP, increased alkaline phosphatase, lactase hydrogenase, cholesterol, amylase, betalipoprotein, phospholipids, 5 nucleotidase, and decreased indices of prothrombin, fibrinogen, disorder of the synthesis function of albumin and total proteins with an increased coagulation time were reported.

It is important to mention that in case of BA, the above-mentioned indices show a slight decrease following the infusion treatment, though these do not return to normal values, which is an important fact in the diagnostic differentiation. All patients were also tested for viral liver markers (HBs antigen, anti HBs antigen, HBcor, anti HCV, anti HBV, anti HDV), assessment of antibody levels of IgG, IgM classes to viral damage (papeloma virus, cytomegalovirus, ebstein- bar, rotavirus, type 3 RNA reovirus, human herpes virus type 6, shingles virus), alpha 1 antitrypsin (deficiency of which is indication for liver transplantation). CMV was recorded in 8 patients with BA. No other viral damage was reported.

Pre- and post-feeding abdominal ultrasound investigation was indicated in all patients. The examination was performed during pre-feeding, 4 hours after the last feeding and 40-50 min post-feeding. Gallbladder aplasia was diagnosed in 7 patients and dysfunctional gallbladder with anatomical location and extrahepatic bile ducts with a fibrous band appearance was found in 4 patients. It should be noted that intra- and extra-hepatic bile duct dilation was not recorded in any patient with BA.

Polysplenia was diagnosed in 2 children and congenital heart failure in 3 children. More information on liver parenchyma and the installation of intrahepatic PH was revealed by the doppler ultrasound examination, where 3 patients reported severe changes in the liver parenchyma-biliary cirrhosis with the installation of intrahepatic PH were observed.

Videogastroduodenoscopy (VGDS) was performed to visualize the Vater papilla, describe the duodenal mucosa, assess the bile in the duodenum, as well as to provide a differential diagnosis. The presence of bile in the upper digestive tract was not detected in all patients with BA. It should be mentioned that the duodenal jaundice mucosa can be confused with the presence of bile in the duodenum, which considerably transcends the medical-surgical tactics.

This study did not include the retrograde cholangiopancreatography, being mainly based on USG, MRI and scintigraphy that show a much lower harmful impact. We would like to mention that percutaneous cholangiography is an invasive method and it is not recommended unless dilatations of the biliary tree are present.

Cholangiographic MRI was performed in all children with BA, which allowed the study of both intra- and extrahepatic bile ducts. The examination performed in 8 children showed obvious changes of the gallbladder, of which in 2 children the intrahepatic location of the aplastic gallbladder was assessed. The virtual reconstruction of the biliary tree completed the previous examinations and played a decisive role in the differentiation of the diagnosis and the appreciation of the medical-surgical tactics. At the same time, we would like to mention that as a result of the investigation we could not assess with certainty the biliary atresia and the degree of parenchymal damage, which indicates the need to establish the diagnosis in the context of correlating to all clinical and paraclinical examinations.

Dynamic liver scintigraphy with Tc-99m isotopes (technetium 99m) was performed in a patient at whom the accumulation of the pharmaceuticals within the liver and the lack of communication of the biliary tract with the duodenum was eloquently visualized. The presence of radionuclide in the intestine confirms ductal permeability and excludes biliary atresia. Failure to visualize the tracer in the intestinal lumen more than 3-4 hours after administration, especially with phenobarbital, suggested a biliary obstruction caused by biliary atresia. At the same time it should be mentioned that in premature infants with a body weight of less than 2000 g and on a full parenteral diet, the excretion of the pharmaceutical substances in the duodenum may be absent, and does not allow us to differentiate the diagnosis. It should be mentioned that the dynamic scintigraphy of the liver is superior to contrast examinations but due to a prolonged general anesthesia (3 hours) it complicated the investigation in newborns. To improve radioactive uptake, 5 mg-kg phenobarbital should be administered daily for 5 days prior to scintigraphy.

Transcutaneous hepatic puncture under ultrasound guidance was performed preoperatively in 6 children, in all cases there was a numerical decrease of the bile ducts with structural destruction and sclerosis of the bile duct, and transformation of the bile ducts into connective tissue cords. In persistent jaundice, percutaneous liver biopsy did not allow confirmation of BA, by identifying liver fibrosis with bile duct proliferation, the presence of biliary plugs in the bile ducts in the liver triads; biliary stasis in canals and cells; edema and fibrosis in the hepatic triads; swelling, vacuolation and desquamation of the bile duct epithelium; inflammatory cellular infiltration; gigantocellular transformation of liver cells. The presence of biliary thrombi in the lumen of the interlobular bile ducts or of the primary ducts and the neoductular proliferation in the peripheral portal spaces indicate a certain occurence of BA, which helped us to make a differential diagnostic.

Following the systematization of clinical, paraclinical and histological findings, the embryonic form of BA was established in 4 children and the perinatal form was found in 7 children (fig. 4).

Two children with embryonic form died during a pre-

transplant, one child was diagnosed with BA syndromatic type (Alagille syndrome), the other 10 children presented a nonsyndromatic type (fig. 5).

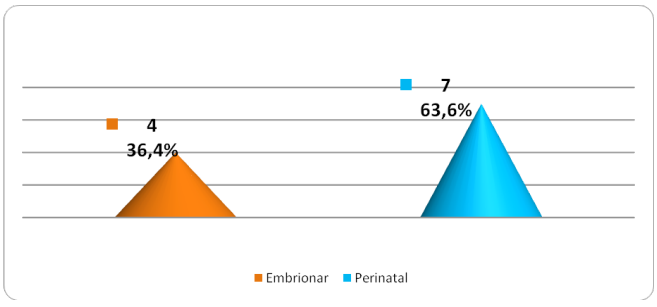

Fig. 4. Etiology prevalence for BA

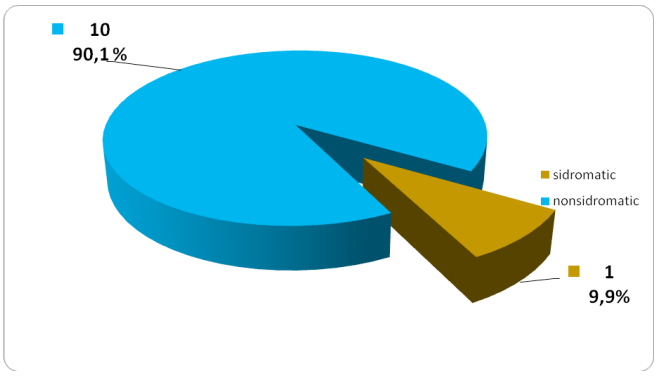

Fig. 5. Types of BA described within the study

An embryo-shaped child was initially subjected to liver transplantation and subsequently died at 6 months after transplant, and an embryonic-shaped child survived after transplant.

Two children were initially diagnosed with intracranial haemorrhage; 1 child was treated conservatively with subsequent Kasai surgery, the other child underwent surgery and initial liver transplantation.

Preoperative management. General preoperative measures included parenteral administration of fat-soluble vitamins A, D, K, E; medium chain triglycerides as a source of fat (Lipofundin, SMOFlipid, etc.), desensitizers to alleviate skin itching. Vitamin K (1-2 mg / Kg / day) was administered 3 days preoperatively. Oral feeding was ceased 12 hours preoperatively. For the preparation of the intestine = oral metronidazole, 10 mg / kg per day in two doses was administered, as well as the evacuation clyster (Carbo erbo glycerol bambini) was applied. The patients were administered broad-spectrum antibiotics preoperatively and intraoperatively.

Treatment. The medical-surgical approach was aimed to restore the bile duct, by implementing two tactics: the Kasai surgical procedure and liver transplantation.

There are currently known over 6 BA classifications .

The medical-surgical tactics was assessed according to the classification of the Japanese Society of Pediatric Surgery which includes the following variants of atresia:

Type 1 – choledochal duct atresia

Type 2 – (a, b)

- 2a – atresia of the common hepatic duct
- 2b – atresia of the choledochus, hepatic duct, cystic duct and gallbladder with preservation of anastomoses, ducts in the hepatic hilum

Type 3 – atresia of the choledochal duct, hepatic duct and bile ducts of the hepatic hilum (without preservation of the anastomoses of the ducts in the hepatic hilum), in combination with atrophy of the gallbladder, without biliary content in the lumen.

All children from our study were diagnosed with type 3 BA according to the Japanese classification. It should be mentioned that it is very difficult to assess the level of biliary atresia preoperatively, and only intraoperative cholangiography can suggest the true effect of bile ducts, which determines the type of biliodigestive anastamosis.

Portoenterostomy (Kasai surgery) was performed in 6 patients. The surgery began with an extensive laparotomy approach, selected in each case. Other concomitant malformations were initially ruled out. Subsequently, suturing and resection of the sickle ligament was performed in order to mobilize the liver and visualize the operating field. For demonstrative confirmation of the diagnosis, intraoperative cholangiography and thin-needle biopsy of the liver parenchyma, segment 6, 7, 8, and marginal biopsy were performed. Confirmation of BA by intraoperative cholangiography was followed by retrograde cholecystectomy with preparation of fibrous cords in the liver gate and preparation of ductal plaque (fig. 6).

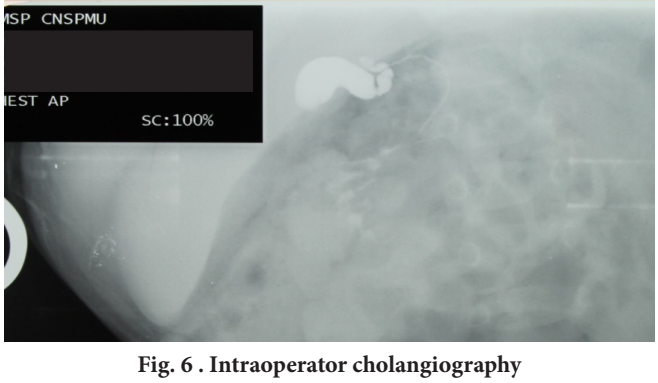

Fig. 6 . Intraoperator cholangiography

The left and right branch of the hepatic artery and the bifurcation of the portal vein served as the anatomical orientation of the ductal plaque boundaries . Safety loops were applied to the right and left hepatic arteries in order to provide a broader view of the ductal plaque and prevent bleeding in case of an operating accident (fig. 7).

The preparation of the fibrous callus was performed by applying a fixator on the fibrous tissue and two other loops (fixators) on the hepatic arteries. It is important to mention that hemostasis by electrocoagulation was never used in the preparation of the ductal plaque.

The preparation of the hepatic arteries by the fibrous tissue was performed until their branching into the hepatic tissue, which represented the reference plan for the dissection of the ductal plaque.

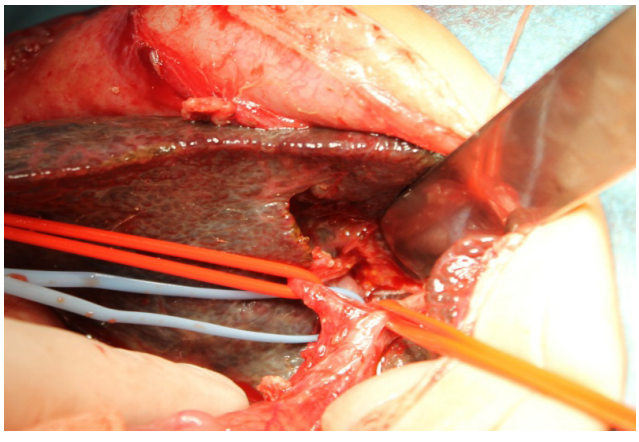

Fig. 7. Ductal plaque preparation

anterior lip was fixed with interrupted sutures with the subsequent formation of a jejunal lumen (fig. 9).

Every second suture was made in the dorsal wall of the open jejunum, ensuring the widest possible anastamosis with the complete inclusion of the sectioned fibrous surface.

Postoperatively, early feeding was encouraged due to the stimulation of bile secretion and the favorable effect on liver function. Choleretic therapy with ursodeoxycholic acid (20-30 mg / kg daily in 2 doses) was indicated throughout the postoperative year. Phenobarbital was indicated at a dose of 5-10 mg/kg daily, for 30 days, in order to provide a prolonged activation of the microsomal enzymes of the hepatocyte endoplasmic reticulum.

Nasogastric drainage was performed until the restoration of intestinal function, which commonly lasted on avera-

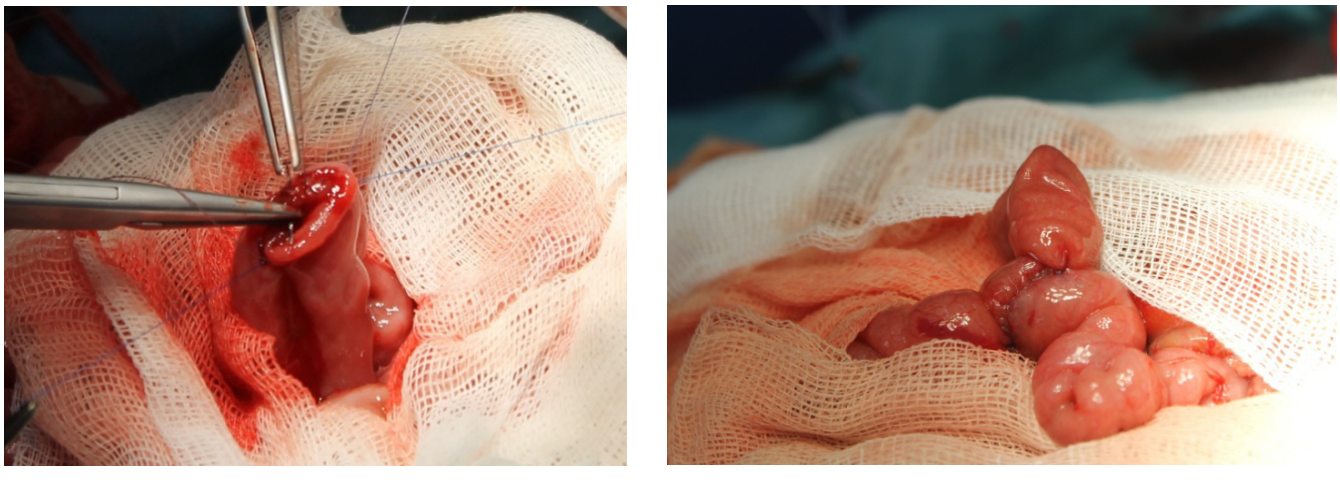

Fig. 8. Termino-lateral anastomosis formation

The success of the procedure was determined by the presence of microscopic permeability of the bile ducts. The 1st step finished by applying a buffer with 0.9% warm NaCl to the liver hilum, after an early irrigation. In 2 patients, the bile was removed with the naked eye, in which the Kasai procedure might be successfull at 2-2.5 years.

The jejunum, with its proximal section 20 cm from the Treitz ligament, was used to restore the integrity of the biliary system and digestive tract and to prepare a Y-shaped Roux loop (fig 8).

The distal edge of the jejunum was closed by an inverted ligature. Subsequently, the termino-lateral jejunostomy was performed at 40-50 cm distance from the inverted edge of the distal jejunum ensuring intestinal continuity; the Roux loop was sutured cranially with the ilium at an angle of 6-8 cm from the anastomosis, which in our view facilitates evacuation of the gall and prevented the reflux from the ilion into the Roux loop. No anti-reflux valves on the Roux loop were applied.

Portoenterostomy was initiated with the application of continuous and interrupted absorbable sutures 6-0 (prolen, PDS, polyglatil, maxon) with the application of two fixatives to the corner of the posterior lip, subsequently the posterior lip was fixed with uninterrupted suture, whereas the

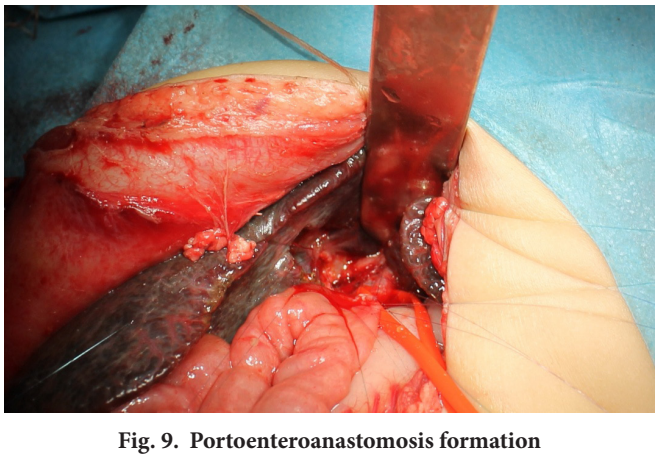

Fig. 9. Portoenteroanastomosis formation
(Posterior lip, prolen 6-0)

ge for 48 hours. Intravenous antibiotics were administered during the enteral feeding, being continued with long-term administration of oral antibiotics. Anti-inflammatory drugs were gven for 21 days postoperatively. Homeostasis monitoring, general blood analysis, coagulogram, blood biochemistry and urinalysis were performed daily until the homeostasis stabilized, then once a week.

In the postoperative period, adequate drainage of the bile ducts was obtained in 4 patients. The postoperative

period for one patient was complicated by sclerosing cholangitis and hepatic impairment. Adequate biliary drainage was not obtained in two patients, thus 3 patients required liver transplantation at 6 months, 8 months and 9 months postoperatively.

Two patients were late diagnosed – at 4.5-5 months and one patient with BA embryonic form underwent initial liver transplantation. Two patients died after liver transplantation.

Two patients who were late diagnosed and hospitalized in an extremely serious condition died preoperatively.

Discussion

Embryological development of the biliary system begins in the 4th week of the hepatic diverticulum of the anterior primitive intestine [7]. Subsequently, the differentiation of the cranial and caudal components takes place, namely: the proximal extrahepatic ducts and most of the intrahepatic biliary system develop from the cranial segment, and the gallbladder, cystic duct and choledochus from the caudal one [8].

Although bile duct atresia is often associated with other congenital abnormalities, suggesting prenatal pathogenesis, the presence of progressive inflammatory bile duct lesions in children with isolated biliary atresia suggests perinatal exposure to a harmful factor [9].

The combination of biliary atresia with other congenital anomalies is fully demonstrated, with rates ranging from 10 to 50%. The most commonly reported cardiac abnormalities, persistence of the oval foramen, persistence of the arterial duct, ventricular septal defect, splenic and gastrointestinal abnormalities have been reported [10].

Each newborn-infant with cholestatic jaundice should be considered a patient with biliary atresia until the etiology is established [11]. To date, there is no true test that would differentiate between neonatal hepatitis (intrahepatic cholestasis) and biliary atresia [12].

In the neonatal period on the 3rd day, unconjugated hyperbilirubinemia has a physiological character (physiological jaundice). 2-15% of newborns retain this feature after 2 weeks postnatal and are defined by prolonged physiological jaundice (14-24 days), and only 0.2-0.4% are present with mechanical jaundice, without liver cholestasis or other disorders that would disrupt bile flow [12, 13]. Paradoxically, initially the most important indicator in the diagnosis of biliary atresia in a child with conjugated hyperbilirubinemia is the general lack of clear symptoms and signs of hepatocellular destruction [14].

Changes in scalocolurometry after administration of the needle test. Ursodeoxycholic allows us to differentiate the diagnostic of BA from other liver pathologies complicated by cholestasis syndrome [15].

At the age of 3 weeks in children with BA, bile excretion is completely absent, which can be easily confirmed in VGDS. The resumption of biliary transit in case of a successful Kasai intervention is recorded for up to 30 days, and liver function is restored for several years [16]. The accelerated dynamics of hepatocellular destruction is complicated by biliary liver cirrhosis, intrahepatic PH, ascites, esophageal varices with upper digestive hemorrhage [17]. Malabsorption of fat-soluble vitamins contributes to the development of anemia, malnutrition, weight and psychoemotional retardation, rickets, xanthoma of the palms and knees, neuromuscular disorders [18].

Differential diagnosis. BA is a complicated and long-lasting process, due to the presence of a whole range of inflammatory, infectious, metabolic, genetic, syndromic, parasitic, specific pathologies, etc., which require a differential diagnosis [19, 20]. It is also necessary to differentiate the diagnosis with a series of very rare surgical pathologies, such as obstructive choledochal malformations, spontaneous perforation of the bile duct and hyperdense bile syndrome, congenital choledochal cyst, congenital liver fibrosis, etc. [19].

Therefore, ultrasonography and cholangiopancreatography are used, which highlight the common bile duct with a considerably dilated diameter (usually > 5 mm) or congenital choledochal cyst with considerable modification of the excretory shaft [2].

Spontaneous biliary perforation is manifested by ultrasound and by the presence of a small amount of fluid in the peritoneal cavity , as well as the presence of an echogenic mass at the transverse fissure of the liver [20].

Viscous bile syndrome usually occurs in premature infants. Ultrasound examination reveals a dilation of the bile duct, which contains hyperdense bile. In these cases, percutaneous cholangiography is performed and sometimes surgery to release the bile ducts from the obstruction [21]. The differential diagnosis is also made in various jaundice syndromes, such as: drug damage to the liver, congenital transport disorders (Crigler-Najar, Rotor, Dubin-Jonson, galactosemia, tyrosinemia, alpha-1-antitrypsin deficiency), neonatal hepatitis with giant cells, cytomegalovirus hepatitis and cystic fibrosis. Jaundice that persists during 30 days postnatally with the prevalence of conjugated bilurbine, 90% of cases are diagnosed as bile duct atresia. Jaundice due to hepatitis in the newborn is very difficult to differentiate [21, 22].

Liver biopsy, viral serology, and sweat testing are also performed for differential diagnosis. A diagnostic problem is biliary hypoplasia characteristic of Alagille syndrome, which can be misdiagnosed [22, 23].

The clinical features, such as specific facial expression, systolic murmur on the pulmonary artery (AP stenosis), spinal pathology or ophthalmic system may develop in patients with Alagille syndrome (cholestatic liver failure), but not in patients with extrahepatic bile duct atresia [23].

In biliary atresia the only solution is surgical treatment, general preoperative measures include parenteral administration of fat-soluble vitamins A, D, K, E; medium chain triglycerides as a source of fat (Lipofundin, SMOFlipid, etc.), desensitizers to alleviate skin itching [24]. Vitamin K (1-2 mg / Kg / day) is administered 3 days preoperatively. Oral feeding is stopped for 8 hours preoperatively [24].

Kasai surgery is the treatment of choice for extrahepatic biliary atresia becoming the surgical standard in extrahepatic biliary atresia, which was first performed in 1957 [25]. Kasai surgery is recommended in the first 90 days postnatal and only in extrahepatic forms of BA [26, 27].

The survival rate over 10 years after the Kasai procedure is 40-46% and continues to increase. Despite successful results worldwide, only 20-30% of patients survive until the age of 20 or more without a transplant [27].

The success of the Kasai procedure depends on the early diagnosis of BA, the application of portoenterostomy up to a maximum of 90 days postnatal, strict adherence to the surgical technique, prevention of complications and adequate postoperative monitoring [28].

Portoenterostomy should anticipate irreversible sclerosis of the intrahepatic bile ducts. Obtaining an obvious biliary flow of over 10mg of bilirubin per day can be appreciated as a success of surgical treatment [28, 29].

The rapid decrease of cholestatic syndrome with the return of biochemical indices within the norm in the first 6 months postoperatively indicates a long-term survival, while the presence of jaundice up to 1 year with maintenance of hepatocellular destruction, suggests the need for a planned liver transplantation. A successful intervention might be assessed by the appearance of the stool in 2 weeks postoperatively, at 6 months the total bilirubin does not exceed 50 mmol / l [29].

Many surgeons support the opinion of the initial liver transplant in children with biliary atresia, which would exclude the long-term risk for the evaluation of Kasai surgery. Others propose an initial liver transplant, only in those patients in whom the diagnosis of biliary atresia was established more than 120 days after birth, or in those with unfavorable histological changes. Currently, the opinion of the initial Kasai intervention is mainly supported in the surgical world, the liver transplant being an approach in cases of inefficiency of portoenterostomy [30].

The success of the treatment depends on several factors, of paramount importance being the age at which the Kasai operation was performed. Another key to success is performing the operation in the first 100 days, after which liver decompensation develops. However, it is difficult to estimate and predict treatment outcomes even in the first 40 days. Thus, the high rate of complications means that a large number of patients require a revision of the portoenterostomy [30].

The prognosis of untreated biliary atresia is unfavorable, leading to the death of most children in the first 2 years of life due to liver failure. The Kasai procedure, although not definitive in all patients, increases life expectancy considerably [31]. Survival at 5 years in patients without liver transplantation is approximately 30%. Thus, children with biliary atresia, in addition to the benefits of hepatoportoenteroanastomosis, also have a certain degree of residual liver failure, often with indications for liver transplantation. 50% of children aged 5 years with normal biochemical indications develop esophageal varices, and require endoscopic ligation,whereas 15% of them develop hemorrhages by varicose rash despite the drug and endoscopic treatment. Biliary cirrhosis subsequently occurs in 10% of patients with normalized serum bilirubin, and liver transplantation is indicated.The survival rate after Kasai surgery over 10 years in children with normal clinical-paraclinical indices does not exceed 10% [32, 33].

The success of the Kasai procedure depends on several factors: the age at which the surgery was performed, the exact dissection and transection of the liver hilum, the severity of the liver pathology, the postoperative treatment performed, and the occurrence of postoperative complications, especially cholangitis [33, 34].

The maintainance of acholic stools in the postoperative period and discontinued postoperative biliary excretion indicate an unfavorable prognosis. Death usually occurs within 1 year. The surgery performed after the age of 90 days significantly influences the unfavorable prognosis due to the progression of the sclerosing-destructive process of the bile duct and advanced hepatocellular destruction [35].

Patients who did not resist primary biliary drainage (15-30%), develop progressive liver failure, the optimal treatment being liver transplantation [36].

Conclusions

1. Biliary atresia is the most common cause of extrahepatic obstructive jaundice in newborns and is the most common indication for liver transplantation in children.

2. The embryonic form of BA is a severe condition that is indicated for the initial liver transplant. In this form, Kasai surgery is not effective and creates impediments to liver transplantation.

3. Biliary atresia is a serious pediatric condition that tends to develop into cirrhosis, liver failure and death.

4. Portoenteroanastamosis (Kasai surgery) performed as early as possible (up to 60 days postnatal) significantly increases the success of surgery.

5. The success of the Kasai procedure depends on several factors: the age at which the surgery was performed, the exact dissection and transection of the liver hilum, the severity of the liver pathology, and the occurrence of postoperative complications, especially cholangitis.

6. Liver transplantation remains the only treatment option in decopensated and late- diagnosed cases (over 120 days), as well as in those showing complications in the postoperative period resulting in liver failure.

7. The prognosis of untreated biliary atresia is unfavorable resulting in the death of most children in the first 2 years of life due to liver failure.

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Prevalence of 35delG mutation in GJB2 gene in the Moldovan population

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Abstract

Background: Guanine deletion 35delG in GJB2 exon 2 is the pathogenic mutation responsible for up to 70% of cases of congenital non-syndromic sensorineural hearing loss (NSHL) among Europeans. The early molecular diagnostic of hearing loss nature has become important while considering the cochlear implants. The purpose of this study was to establish the frequency of 35delG deletion in GJB2 gene among patients with severe NSHL and its prevalence among Moldovan residents with normal hearing.
Material and methods: 40 patients with congenital bilateral profound NSHL and 300 individuals with normal hearing were examined for deletion 35delG, by using Custom TaqMan SNP genotyping Assay.
Results: 12 (30%) patients with homozygous genotype for 35delG mutation were identified, whereas 8 patients (20%) were heterozygous. The study reported 4 (1.33%) carriers of 35delG mutation among 300 Moldovan individuals with normal hearing.
Conclusions: The present study results suggest a need for including the 35delG molecular testing into the national program of neonatal screening of hearing loss. Considerations on the genetic carrier testing should be made in genetic counseling and family planning.
Key words: GJB2, 35delG mutation, non-syndromic deafness.

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Introduction

Hearing loss (HL) or deafness is the most frequent congenital sensory impairment in humans, which is a very heterogeneous trait. Based on the World Health Organization's (WHO) data, in 2020 around 466 million people worldwide had disabling hearing loss, and 34 million of these people were children [1]. The incidence of severe to profound deafness in the first two decades of life is about 1 in 650 newborns.

Over 150 associated loci and about 80 different protein-coding genes are involved in the perception of sound, up to 1% of these causative human genes have been mapped. Mutations in these genes can lead to similar clinical manifestations of hearing impairment. Non-syndromic HL accounts for up to 70% of genetic deafness, which is almost exclusively monogenic [2].

More than half of cases of congenital autosomal recessive non-syndromic HL resulted from mutations in the DFNB1 locus. Prelingual non-progressive sensorineural forms of deafness are expressed as moderate to profound [3].

The DFNB1 (OMIM 220290) locus on chromosome 13 (13q11-q12) contains two genes, GJB2 (OMIM 121011) and GJB6 (OMIM 604418) [4]. Encoding connexin 26 (Cx26) and connexin 30 (Cx30), respectively are members of intracellular gap junction β proteins family. In the inner ear, the six monomers of Cx26 or Cx30 oligomerize to form as homo- or heteromeric connexons, which are involved in the recycling of ions K+ between hair cells and endolymph [5, 6]. This is believed to play a crucial role in efficient generation of action potentials in mechanosensory transduction of sound.

The literature review demonstrated that more than 100 pathogenic mutations in the GJB2 gene have been identified with a significant contribution of the frameshift and nonsense mutations [7, 8]. The frequency of the individual mutations associated with HL varies within ethnic groups [9]. In Caucasians, the most frequent mutation is 35delG (rs80338939) a point deletion of one of six guanines at the codon position 30-35 of the second exon. This deletion leads to shifted reading frame, creation of stop codon, and premature termination of the protein Cx26 synthesis [10, 11].

This study was aimed to establish the carrier frequency of 35delG deletion in GJB2 gene among patients with severe NSHL and among Moldovan population with normal hearing.

Material and methods

40 children with prelingual non-syndromic HL from the Republic of Moldova and Romania and 300 unrelated participants with normal hearing were included in the study. Written informed consent was signed by all the minors' parents and by healthy participants.

A total 40 hearing impaired children (aged 1-14 years) underwent audiological analysis. The degree of their hearing loss was evaluated as severe (71-90 dB) to profound (>90dB), according to the HL Classification from WHO.

All examined volunteers with normal hearing were aged between 18-29 years. Venous blood samples were taken from all participants.

Genomic DNA isolation from 100 µL of peripheral blood was performed with proteinase K and spin column purification protocol (#K0722, ThermoFisher Scientific). The purity and concentration of DNA samples were detected using a NanoDrop 2000c spectrophotometer (ThermoFisher Scientific). The average DNA concentration of each sample was adjusted to 2 ng/µL.

The targeted search for the 35delG mutation was detected using a Custom TaqMan SNP Genotyping Assay Kit (#4351379, ThermoFisher Scientific) [12]. The primers and MBG probes for allelic discrimination assay were designed using software Primer3 web version [13].

The molecular-genetic analysis was performed in a total reaction volume of 5 µL on 384-well plates. All samples analysis was performed on the QuantStudio 6 flex device (Applied Biosystems, ThermoFisher Scientific), according to manufacturer's protocol. The allelic discrimination data were analyzed using a TaqMan Genotyper Software application (v.1.3.1., Applied Biosystems, ThermoFisher Scientific). The successful genotyping call rate was <96%, the undetermined samples were automatically eliminated from allelic discrimination analysis.

Statistical analysis was performed using the Statistica v.6.0 software.

Results

The molecular screening for the pathological allele in GJB2 gene among the group of children revealed 12 (30%) patients with homozygous genotype for 35delG mutation and 8 patients (20%) with heterozygous form. Genotyping among 300 individuals with normal hearing identified 4 subjects with carrier rate of 1.33%. The distribution of allele frequencies of the rs80338939 samples analyzed is shown in fig. 1.

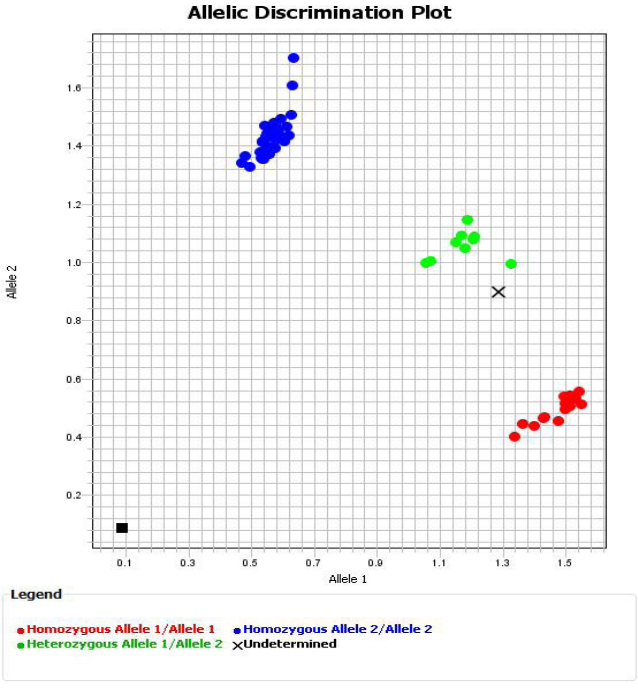

Fig. 1. Allelic Discrimination Plot showing 35delG/rs80338939 Assay

Notes: Representative plot showing performance of allelic Discrimination of 35delG/rs80338939 SNP.
Separation between the signals derived from allele 1 (VIC) and allele 2 (FAM). The red cluster represents homozygous mutants containing copies of the 35delG mutant allele. The green cluster includes individuals having one normal and mutant allele (heterozygous). The blue cluster represents samples homozygous with normal sequence for both alleles.

Discussion

The major pathogenic mutation is point deletion 35delG (rs80338939), which accounts for about 70% of recessive mutations of GJB2 associated with NSHL in populations of European origin, with a carrier frequency of 2-4% [9-11]. Other ethnic groups may have additional or different specific mutations, such as 235delC (rs80338943) in Japanese and Koreans, 167delT (rs80338942) among Ashkenazi Jews, R143W (rs80338948) in Africans [14-16]. W24X mutation has a high frequency in Indian and Roma populations [17]. Almost half of the recessive mutations are frameshift of nonsense type. They have no specific localization and can affect all domains of Cx26 protein [3, 7].

These study findings showed that the prevalence of 35delG mutation in GJB2 gene among volunteers with normal hearing was around 1.33% (4/300). This is in concordance with similar data obtained for other European populations. According to our data, the 35delG deletion was identified in the homo- or heterozygous state in 50% of the patients.

On the other hand, 20% of samples tested were found to have only one mutant allele, 50% of patients had non-carrier rate of 35delG mutation. These results could be explained by the fact that other recessive mutations were present in GJB2 gene in homozygous or compound heterozygous form. It is known that, the NSHL may also develop in compound heterozygous form of the GJB2 mutations with the second mutation in GJB6 (Cx30) gene in the DFNB1 locus [9]. In the case of compound heterozygote of two genes, the expression of these genes is probably affected which is not due to digenic inheritance, as previously assumed [2, 8].

Therefore, it is clinically important to explore other recessive disease-causing mutations in GJB2 gene and to establish a relationship between genotype and phenotype correlation [18].

Due to high frequency of worldwide hearing impairment and its public health impact, early identification and stratification of patients with HL becomes an important

issue. In addition to main directions, research should also focus on the optimization of methods for molecular diagnostics, gene expressions in choosing gene therapy of hereditary pathologies, as well as on prediction of the correct treatment strategy and the estimation of risk of disease recurrence in families.

Conclusions

Our study results support the position that 35delG mutations are a cause in the etiology of non-syndromic hearing loss in other European populations.

In addition, genetic defects that underline deafness could become a good foundation for gene therapy development, treatment strategy, and treatment success. It should be used as a tool in molecular testing to detect genetic origin of deafness in population of Moldova. Our findings suggest including the 35delG testing into the national program of neonatal hearing loss screening. Taking clinical and social impact of NSHL into consideration it is advisable to introduce carrier detection testing in genetic counseling and family planning.

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REVIEW ARTICLES

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Updates on classification and management of status epilepticus

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Abstract

Background: Status epilepticus (SE) is a major medical emergency and requires not only an emergency symptomatic treatment with antiepileptic drugs (AED) but also a rapid identification and treatment of the underlying cause. This narrative review summarizes the most important advances in SE classification and treatment. Data sources included being PubMed / Medline, and tracking references of the relevant studies, reviews and books. SE is now defined as "a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally prolonged seizures (after time point t1). It is a condition, which can have long-term consequences (after time point t2), including neuronal death, neuronal injury, and alteration of neuronal networks, depending on the type and duration of seizures." The most effective acute treatments for early SE are the intravenous benzodiazepines (lorazepam, diazepam, and clonazepam) and intramuscular midazolam. In children, oral or intranasal midazolam are useful alternatives. The intravenous antiepileptic drugs (phenytoin, valproate, levetiracetam, phenobarbital and lacosamide) are administered in confirmed SE. Treatment options in refractory SE are intravenous anesthetics; ketamine, magnesium, steroids and other drugs are used in super-refractory SE , showing variable results and outcomes.
Conclusions: Over time, major progress has been made in defining, classifying, and understanding of SE mechanisms. Despite this, the first-line drug management is ineffective in up to 40% of patients with SE. The super-refractory SE treatment is still unknown and no evidence-based data have been found yet. Thus, SE treatment strategies vary substantially from one institution to another due to the lack of data supporting a specific treatment plan.
Key words: status epilepticus, classification, guideline, antiepileptic treatment.

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Introduction

Status epilepticus (SE) is a major neurological and medical emergency that is commonly expressed by a brain injury or systemic changes that lead to cerebral hyperexcitability. The incidence accounts for 61 episodes per 100000 per year, with a total mortality of approximately 20% (range 1.9-40%) [1, 2]. The reported incidence varies considerably depending on the used definition of SE. In addition, the incidence refers to episodes of clinically apparent SE, which do not incorporate the underestimated incidence of nonconvulsive SE. Multiple publications are controversial, with a partial approach to evolution, diagnostic and management criteria. However, there has been considerable development in recent years in understanding the pathophysiology, causes, clinical features, changes in EEG, its prognosis and treatment [3, 4].

Classically, it was defined as a "situation characterized by epileptic seizures long enough or repeated at short intervals to produce a long-lasting epileptic disorder" [5]. Initially, the proposed times ranged from 60 to 30 minutes. However, in terms of the operational definitions, clinicians do not wait for diagnosis confirmation and treatment, since the SE prognosis might worsen over time [6]. This issue has led to a more detailed operational definition [7]: a generalized convulsive SE in adults and children over 5 years of age is defined as "a continuous seizure lasting ≥5 min or one or 2 seizures or even more might exhibit an incomplete recovery of consciousness between them". This time interval was in general accepted by the medical community and used to guide the emergency treatment of generalized convulsive SE. However, other forms of SE were not considered until the last definition, being proposed in 2015 by the SE Working Group of the International League against Epilepsy (ILAE) [8].

According to the new definition approved in 2015, the SE is a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally

prolonged seizures (after the time point t1) and which can have long-term consequences (after time point t2), including neuronal death, neural injury, and alteration of neural networks, depending on type and duration of seizures [3, 8]. This definition is conceptual, with two operational dimensions: the first is the duration of the seizure and time point (t1, at 5 min), above which the seizure should be considered as "continuous ictal activity". The second time point (t2, at 30 min) is the time followed by the risk of long-term consequences [3, 8].

This new definition of SE provides good guidance when the emergency treatment needs to be considered. In general, the time point t1 is the time when treatment should be started, which is within 5 minutes, for generalized tonic-clonic seizures, and over 10 minutes, for focal seizures with or without an altered consciousness. Time point t2 highlights the time when the neuronal damage occurs or self-perpetuation of alteration of neural networks starts, thus indicating that SE should be controlled as quickly as possible; 30 min, in case of generalized tonic-clonic seizures [3, 6]. The proposed time points are based on clinical trials performed on animal models, as well as on clinical researches. These data might vary, thus these specific moments should be considered as the best estimates available now. However, there are no data that have correctly defined all forms of SE , thus the study of these subtypes will allow their incorporation into the definition without changing the basic concept [6].

SE can be considered as the second most common acute neurological emergency after stroke. SE makes up 3.5% of total hospital admissions in developed countries and 11% within the developing countries [9, 10]. Nonconvulsive status epilepticus (NCSE) accounts for approximately 1/3 of all SE cases. Compared to convulsive SE, NCSE has been given less attention, is underdiagnosed and undertreated. NCSE comprises a group of syndromes that have a great diversity in terms of response to anticonvulsant drugs, from practically self-limiting variants to completely refractory forms. The etiology and clinical form of NCSE are strong predictors for the overall prognosis [11].

Status epilepticus classification

The ILAE working group also came up with a new classification that will provide a framework for clinical diagnosis, investigation, and therapeutic approaches for each patient, based on four axes. [3, 8]: (I) semiology, (II) etiology, (III) EEG correlations, and (IV) age. Semiology is thought to be the backbone of this classification. Different clinical forms of SE are differentiated based on two taxonomic criteria: the presence of motor activity and impaired consciousness falling into two major groups: SE with prominent motor symptoms, including all convulsive seizures, and SE without prominent motor symptoms that represent the underlying forms of NCSE (see Table 1).

Axis 1 (semiology) includes different forms of SE, being divided into those with prominent motor manifestations, those without prominent motor manifestations, as well as conditions not determined so far (such as acute confusional states with epileptiform patterns at EEG) [8]. Each group can be divided again, depending on the degree of the impaired consciousness, which is extremely clinically relevant. NCSE with coma is a life-threatening condition that requires urgent and consistent treatment, while NCSE without coma commonly occurs in the form of absence SE or focal status with impaired consciousness (the previous terms for these conditions were the "psychomotor status" or "partially complex epileptic status") [3, 8, 12].

Axis 2 (etiology) is divided into two groups: (i) known or symptomatic and (ii) unknown or cryptogenic. The symptomatic group can be subdivided into acute symptomatic, remote symptomatic and progressive symptomatic [3, 6]. SE frequently occurs in the context of genetic epileptic syndromes, however, there are some triggers for the status itself, such as fever, electrolyte disturbances, or other intrinsic factors [13].

Axis 3 of classification includes EEG correlations. In convulsive SE, the clinical presentation is most often clear and with unclear artifacts on EEG, thus the EEG has a low significance. The non-convulsive SE otherwise cannot be often correctly diagnosed without an EEG. In most severe cases of patients with deep coma, only an EEG can reveal the epileptiform or rhythmic discharges that lead to the diagnosis [12, 14].

Table 1

Axis 1 of the classification of SE – semiology [3, 8]

(A) with prominent motor signs
1. Convulsive SE (CSE, synonym: tonic-clonic SE)
a. Generalized convulsive
b. Focal onset evolving into bilateral convulsive SE
c. Unknown whether focal or generalized
2. Myoclonic SE (with prominent epileptic myoclonic jerks)
a. With coma
b. Without coma
3. Focal motor
a. Repeated focal motor seizures (Jacksonian)
b. *Epilepsia partialis continua* (EPC)
c. Adversive status
d. Oculoclonic status
e. Ictal paresis (i.e., focal inhibitory SE)
4. Tonic status
5. Hyperkinetic SE
(B) Without prominent motor symptoms (i.e., NCSE)
1. NCSE with coma (including so-called "subtle" SE)
2. NCSE without coma
a. Generalized
i. Typical absence status
ii. Atypical absence status
iii. Myoclonic absence status
b. Focal
i. Without impairment of consciousness (aura continua, with autonomic, sensory, visual, olfactory, gustatory, emotional/ psychic/experiential, or auditory symptoms)
ii. Aphasic status
iii. With impaired consciousness
c. Unknown whether focal or generalized
i. Autonomic SE

Table 2

Salzburg EEG criteria for NCSE [29-31]

Patients without known epileptic encephalopathy:
EDs > 2.5 Hz, or EDs ≤ 2.5 Hz or rhythmic delta/theta activity (>0.5 Hz) and one of the following: EEG and clinical improvement after intravenous AED*, or Subtle clinical ictal phenomena during the EEG patterns mentioned above, or Typical spatiotemporal evolution**
Patients with known epileptic encephalopathy:
Increase in prominence or frequency of the features mentioned above, when compared to baseline with observable change in clinical state Improvement of clinical and EEG features with intravenous AEDs

*If EEG improvement occurs without clinical improvement, or if fluctuation is without definite evolution, this should be considered possible NCSE.

** Incrementing onset (increase in voltage and change in frequency), or evolution in pattern (change in frequency >1 Hz or change in location), or decrementing termination (voltage or frequency).

EDs, epileptiform discharges (spikes, poly spikes, sharp-waves, sharp-and-slow-wave complexes); AEDs: antiepileptic drugs.

Finally, the age should be considered, given that the etiologies are different and there are some age-specific electroclinical syndromes: newborn (<30 days), early childhood (1 month to 2 years), childhood (2–12 years), adolescence-adult (12–59 years), and old age (> 60 years) [3, 6].

Status epilepticus – pathophysiology

The basic SE generating processes can be considered as failure of the normal mechanisms responsible for cessation of the seizures. Reduced inhibition and persistent excessive excitation might produce and sustain further epileptic activity. During a prolonged seizure activity, dynamic changes in gamma-aminobutyric acid A (GABAA) and N-methyl-D-aspartate (NMDA) receptors are observed, these have been termed as "receptor trafficking" [15]. During the excessive neuronal discharges, there is a gradual reduction of GABA-A receptors on the surface of the synaptic membrane with the internalization of receptors in the endocytic vesicles and its subsequent degradation. This process induces the loss of endogenous GABA-ergic inhibition mediator giving rise to sustained epileptic activity [15-17]. The loss of post-synaptic GABA-A receptors is a relevant pathophysiological factor for the onset of progressive drug resistance to drugs, such as benzodiazepines, barbiturates and propofol [18]. In contrast, in continuous epileptic activity, NMDA receptors are progressively transported to the surface of the synaptic membrane, resulting in an increase in the number of excitatory NMDA receptors in the synaptic cleft. This process facilitates neuronal excitability and SE continuity [15]. On the other hand, an increased expression of GABA receptors may be a useful target for pharmacological management in advanced stages of SE [18]. Absence SE and 3 Hz slow spike-wave discharges are induced by excessive inhibition. This form of SE does not lead to significant neuronal damage.

Brain damage in Status epilepticus

The severity of cerebral hypoxia during convulsive SE is unlikely to cause brain damage [18, 19], though it may trigger some other factors that impair the brain functioning, such as hyperthermia, hypotension, hypoglycemia, and acidosis [19]. These factors are particularly relevant after the compensatory mechanisms have failed [18, 19]. In non-human primates, prolonged seizures lead to lesions in the cortex, cerebellum and hippocampus with a pattern similar to that seen in circulatory arrest, systemic hypotension or hypoglycaemia [18]. A characteristic neuropathology that has been associated with prolonged convulsive SE is hippocampal sclerosis which consists of a loss of neurons in the dentate nucleus and pyramidal layer of the hippocampus with variable gliosis [20].

In 1880, Sommer was the first to describe in detail the hippocampal sclerosis in the brains of epileptic patients [21]. Since then, the controversy over hippocampal sclerosis is considered both a cause and a consequence of SE, which support the existing hypotheses [22]. While the relationship of prolonged seizures to hippocampal sclerosis has well been established in animal models, there is no strong evidence that SE causes hippocampal sclerosis in humans [23]. Therefore, it is likely that hippocampal sclerosis might be both the cause and the consequence of convulsive SE, showing a varied predominance in different clinical scenarios [20].

Although most of the specialized literature on the neuropathology of brain lesions in SE refers to seizures, there is evidence that non-seizures also might cause brain damage. When prolonged seizures are induced in paralyzed and artificially ventilated non-human primates, the neuronal injury is less severe, especially in cerebellum. Epileptic activity alone leads to neuronal damage and neuronal death, mainly due to excessive activation of glutamate receptors and subsequent influx of Ca^{2+} into the neuron [24]. Although the epileptic mechanisms are fully understood in animals, the additional impact over the etiology in humans is still uncertain.

Status epilepticus diagnosis

The diagnosis of SE is based on the EEG. The recording of epileptiform changes, which correspond to the motor clinical manifestations in convulsive SE is the main diagnostic criteria in this clinical form [25]. Moreover, if the diagnosis of convulsive SE is usually not difficult to confirm, then in nonconvulsive SE the EEG has a decisive role, whereas the lack of motor clinical manifestations is the main impediment in this regard. Over the years, a number of researches have been published that have tried to standardize and develop the criteria for diagnosing NCSE [26-28].

The ILAE Working Group recommends describing EEG correlations in a SE patient by using the following descriptors: paternal name, morphology, location, time-related characteristics, modulation, and effect of intervention, as well as by using the terminology recently proposed by the

American Clinical Neurophysiology Society and "Salzburg EEG criteria for NCSE" (Table 2) [14, 29-32], as a practical diagnostic guide. Thus, based on the peculiarities of EEG, the researchers proposed to develop a diagnostic decision, based on the NCSE type (electroclinical classification) and the presumed etiological factor [33]. Subsequently, a reduction in the rate of false diagnosis of NCSE was reported due to the implementation of this score [31]. Therefore , different EEG patterns in coma and the diagnosis of NCSE in these cases were difficult to differentiate for a while, however, this dilemma was solved later [14]. Conventional and quantitative methods in the diagnosis of major emergency cases have also been studied and proposed [34]. However, according to Lettinger's data [35], the so-called Salzburg criteria for diagnosing NCSE, proposed by Beniczky and his workteam [33], show a sensitivity of 75% in short recordings up to 97.7 % in long-term EEG recordings (up to 74 hours), with a specificity of 89.6% [35] and are an important tool in diagnostic and therapeutic decisions in these patients.

Status epilepticus treatment

The management of SE and its pharmacological treatment is another area of limited evidence derived from high-quality randomized controlled trials, appropriately selected to inform clinical practice. However, there has been clear progress in understanding the pathomechanisms, which have led to more effective treatment strategies [3]. The therapeutic principle "Time is the brain" might be applied not only to stroke, but also to SE, since the prognosis of SE worsens with the duration of increasing convulsive activity [36, 37]. Indeed, prompt SE confirmation and early treatment is associated with lower morbidity and mortality, fewer drugs required for inpatients, and a decreased seizure duration [38]. Fortunately, SE responds to relatively simple treatment, but when simple interventions fail, refractory SE requires a more aggressive treatment to prevent complications. However, there is a limited interest in the industry to develop new treatments to prevent refractory status. However, the latest ILAE definition has led to standard action protocols, which have been adapted to time points t1 and t2 [6].

The most recent reviews focus on SE pharmacotherapy, but the general measures of any neurological emergency are just as important (airway maintenance, oxygen therapy SaO_2 > 95%, stabilization of vital signs: blood pressure, temperature, and glycemia). Other measures include intravenous glucose and thiamine as required, emergency measurement of antiepileptic drugs, electrolytes, and magnesium, a complete haematological screening and liver and kidney function [39, 40]. In addition, it is essential to carry out a thorough search of the simultaneous etiology, because an early etiological treatment is highly important for the subsequent prognosis [3, 6, 39].

The main purpose of treatment is to immediately stop both clinical convulsive activity and electrographic ictal activity. The initial treatment strategy includes simultaneous assessment and management of the airways, respiration rate and circulation (aimed to provide intravenous access, O_2administration, and airway safety as needed), immediate treatment with AED drugs (benzodiazepines), screening for the main cause of SE, and immediate treatment of life-threatening causes of SE (e.g. meningitis, intracranial mass injury) [39, 40]. Once SE is under control and the vital signs are stable, specific diagnostic examinations should be performed. These diagnostic investigations are selected based on the patient's medical history and physical examination. Not every diagnostic test is necessary for every patient. For example, a lumbar puncture is generally necessary if there is any suspicion of central nervous system infection but may be unnecessary in suspected meningitis, especially in patients with AED noncompliance [39]. If the patient is currently being treated with antiepileptic drugs (AED), the serum AED levels should be checked, and compliance history should be obtained. A comprehensive toxicology profile should be performed if there is no clear etiology for SE. Specific toxicological testing should be carried out if history or physical examination suggests a specific toxin.

By the late 1980s there were large variations in patient stabilization procedures, laboratory measures, and the sequence of drugs in SE management [41]. In 1993, the Epilepsy Foundation of America organized a working group on SE. They published guidelines and a treatment protocol [42], which was based on the literature and expert opinions. Some of the key treatment principles included within this guide are still valid. All treatment protocols recognize a step-by-step approach to treatment with different drugs used in early SE (stage I), established SE (stage II), refractory (stage III) and super-refractory SE (stage IV) and underline the recognition and prompt treatment of persistent convulsive activity at each stage in order to reduce morbidity, mortality and long-term consequences of SE (other than t2) [39, 43]. Therefore, these guidelines have revised the traditional SE treatment paradigm to initial emergency therapy, emergency control therapy and refractory SE therapy. Patients with refractory SE who do not respond to initial therapy and super-refractory SE should be treated in highly experienced centers. All patients with SE will need initial AED emerging therapy (i.e., first line) and emergency control AED therapy (i.e., line 2), in addition to AED maintenance therapy, even if SE has been controlled immediately. According to the definition, refractory SE therapy (i.e. 3rd and 4th line) is administered for those who do not respond to the first 2 antiepileptic drugs.

If the SE is caused by a metabolic disorder (e.g. hypoglycaemia), the underlying metabolic disorder must be corrected, thus the maintenance therapy may not require. It must be considered that, although the treatment includes a series of stages, the treatment itself is an ongoing process, thus the urgent cessation of convulsive activity is the major goal applied to each stage.

Most clinical trials were conducted in the early stages of SE, which was the subject of several trials and critical evaluations in systematic reviews of meta-analyses [44-49] and included in treatment protocols or practical guidelines [39, 43, 50, 51].

Stage 1: early SE. Although several AEDs have been studied as first-line therapy for SE, the evidence and experts agree that benzodiazepines should be the drug of choice for initial treatment. Benzodiazepines can rapidly control SE in about two-thirds of patients [4, 48]. The most commonly first-line treatments are diazepam, midazolam and clonazepam (intravenous lorazepam is not marketed in our country). Although the controlled studies demonstrated the superiority of lorazepam [52, 53], a recent comparative meta-analysis of 5 clinical trials found that there is no difference in efficacy or side effects between lorazepam and intravenous diazepam [54]. Benzodiazepines exert their antiepileptic properties by increasing inhibitory neurotransmission by increasing channel opening and GABA-A receptor frequency, with subsequent increase in chlorine conductance and neuronal hyperpolarization [55, 56]. This first-line treatment should be used as early as possible before point t1 in the SE definition, which means it has a major role in the pre-hospital settings. In this context, the intravenous route may be difficult, and other routes of administration, such as intramuscular [38], intranasal or oral midazolam [57], have proven to be more practical, faster and safer alternatives. The pre-hospital recognition of SE is easy to perform in convulsive SE or that with motor involvement; however, the non-convulsive SE may be more difficult to detect and treat, thus clinical scores should be developed or devices should be used to allow faster detection [6].

At the same time, supportive treatment should be provided, as rapid administration of benzodiazepines may cause respiratory depression and hypotension. Patients who have responded to initial emergency therapy and have a complete resolution of SE should continue dosing for maintenance therapy in order to rapidly achieve the therapeutic levels of AED. Urgent control therapy is to stop SE in patients who do not respond to initial emergency therapy.

Stage 2: established SE. Approximately 40% of patients with generalized convulsive SE are refractory to benzodiazepine treatment [53, 58]. This ongoing convulsive activity is called the established SE (or stage II). In established SE, intravenous antiepileptic drugs (phenytoin, valproic acid, levetiracetam, phenobarbital and lacosamide) are the most commonly used; however, there are no classes of evidence to choose between them. This unsatisfactory condition has several consequences: first of all, most patients are provided off-label treatment.

A meta-analysis comparing the first 4 drugs resulted in higher rates of cessation of seizures with valproic acid (75.7%, 95% CI: 63.7–84.8) and phenobarbital (73.6%; 95% CI: 58.3–84.8) than with levetiracetam (68.5%; 95% CI: 56.2–78.7) or phenytoin (50.2%; 95% CI: 34.2–66.1). Based on this and the favorable tolerability profile of levetiracetam and valproic acid, the authors prefered these drugs to phenytoin / phosphenytoin in established SE [59].

In patients with known epilepsy who have been on an AED prior to admission, case-by-case intravenous bolus administration of AED was given, if available, prior to initiation of an additional agent. This may also include additional boluses to achieve higher-than-normal target concentrations of AED to achieve the desired therapeutic response (i.e., cessation of seizure activity).

Currently, a large multicenter, randomized, blinded study is being conducted, funded by the National Institute of Health (Established Status Epilepticus Trial), which compares the efficacy of phosphenytoin, valproic acid or levetiracetam in the treatment of patients with benzodiazepine-refractory SE [60]. Unless the results of this study are avai-lable, other drugs, such as lacosamide, are also widely used in established and refractory SE, which have been recently reviewed and published [61]. In any case, it is about prioritizing drugs with better tolerance, easy to administer and with few pharmacological interactions (levetiracetam and lacosamide).

It is important to use the correct doses at this stage, as one of the recognized problems for non-response is the use of subtherapeutic AED doses [62]. The recommended doses are presented in table 3.

Table 3

Different doses of drugs used in the second – and third-line-treatment

Drug	Dose
Second-line treatment (AED)	
Valproic acid	30 (20–40) mg/kg on 5–10 min
Phenobarbital	10–20 mg/kg in 15–20 min
Phenytoin	15–20 mg/kg. infusion <50 mg/min
Lacosamide	5–6 mg/kg in 10–15 min
Levetiracetam	60 (30–60) mg/kg, max.4500 mg, in 5–10 min
Third-line treatment (anaesthetics)	
Propofol	Bolus 2 mg/kg; infusion 2–10 mg/kg/h
Midazolam	Bolus 0.1–0.3 mg/kg at –4 mg/min; infusion 0.05–2 mg/kg/h
Ketamine	Bolus: 0.5–3 mg/kg; infusion 1 mg/kg/h – of 10 mg/kg/h
Thiopental	Bolus: 3–5 mg/kg in 3–5 min; repeat bolus 1–2 mg/kg; after 3 min perfusion: 3–7 mg/kg/h

AEDs – antiepileptic drugs, h – hour, kg – kilogram, mg – milligrams.

Regarding lacosamide, a weight-adjusted dose was not considered until recently [63].

Stage 3: refractory SE. 31-43% of patients with established SE seizures are not controlled with antiepileptic drugs [64-66]. In most cases, continuous EEG and / or clinical examination will determine the persistence of SE after initial AED treatment. Refractory SE is considered when two treatment lines have failed (one of which is benzodiazepines) at appropriate doses. Refractory cases are associated with mortality and therefore there is consensus in recommending the use of intravenous anesthetics (midazolam, propofol or barbiturates) as the next line of treatment to control ictal activity. However, there are no data from randomized trials to support the recommended anesthetic, thus medications should be used, based on the experience of each separate hospital.

There is no consensus on achieving optimal sedation (only ictal activity suppression, burst-suppression pattern or isoelectric pattern). Each anesthetic option has its own considerations (doses are shown in Table 3).

– Propofol may be associated with metabolic acidosis, rhabdomyolysis, renal failure and heart failure. The propofol infusion syndrome is less likely to be treated for less than 48 hours and not more than 5 mg / kg / h.

– Midazolam seems the safest drug at this stage, with the lowest rate of metabolic complications [67, 68].

– Barbiturates are frequently associated with cardiovascular complications, severe immunosuppression, and infections.

In a worldwide research study of 488 episodes of refractory SE, a continuous infusion of midazolam was the most widely used anesthetic (59%), followed by propofol (32%) and barbiturates (8%) [69]. The ongoing use of infusion AEDs often requires assisted ventilation and cardiovascular monitoring. Vasopressor agents may be needed due to hypotension and cardiopulmonary depression related to these agents. Once the sedation is discontinued, the dose is recommended to be gradually reduced over 24 hours if no ictal activity occurs, over 12 hours in case of barbiturates, as well as gradually reduced over the next 12 hours and 24 hours in case of midazolam or propofol.

Another anesthetic that has regained interest is ketamine. It has a significant antagonistic effect on N-methyl-D-aspartate glutamate receptors, which play a key role in the advanced stages of SE [70]. Ketamine is a racemic mixture that contains equal amounts of two enantiomers, (S)- and (R)-ketamine. Ketamine is metabolized by N-demethylation to produce norketamine, a non-competitive NMDA receptor antagonist that may also exhibit enantioselective pharmacological activity. (S)-ketamine has different pharmacodynamic activities and is two to three times stronger as an analgesic agent than (R)-ketamine. (S)-ketamine administered alone has a higher clearance than in the racemic mixture resulting in rapid elimination, shorter duration of action and faster recovery from anesthesia [71]. There are several series and isolated cases that report an efficiency in refractory SE of about 63%, and in particular it seems that the form (S)-ketamine has advantages in terms of better psychomotor recovery than the racemic form [72].

The problem, apart from the lack of clinical data, is that various observational studies have recently shown that the use of anesthetics has been associated with a worse prognosis of SE, in addition to the increase in hospital stay, which leads to some concern about to the safety of the use of intravenous anesthetics in the refractory SE approach [73, 74]. For this reason, an individualized approach to sedation should be applied, in case if the ictal activity can leave permanent sequelae (t2) or depending on the type of SE.

There are no data to guide the transition from continuous infusion therapy to intermittent maintenance therapy after resolution of refractory SE. The overall maintenance drugs are given in sufficient doses to maintain therapeutic concentrations during and after the continuous infusion is discontinued. Therapeutic concentrations may exceed target concentrations for several antiepileptic drugs and dosage should be individualized to control seizures and minimize side effects. The success of the maintenance regimen is predicted by many clinical features, including EEG pattern, cause of SE, concurrent systemic disease, and drug-drug interaction profiles.

The most recent AEDs that have started to be used in this refractory phase of SE are the following:

Lacosamide. The use of lacosamide is based on clinical experience. A review of all 522 SE series cases (486 adults and 36 children) has recently been published, showing an overall efficiency of 57%. The efficacy is the same for both non-convulsive SE and convulsive SE, being higher than in previously used ones [61]. Although its use was first implemented in the refractory phase, it has been updated as the first- or second-line treatment option after benzodiazepines in many healthcare centers due to its speed of action and few side effects.

Perampanel. It exerts its mechanism in the antiglutamatergic pathway, through AMPA receptors. Unlike ketamine it does not act, even at high doses on NMDA receptors, but it is an uncompetitive antagonist of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor [75]. Although the intravenous formula has not been marketed yet, its use via the nasogastric tube has been reported in limited cases of SE, with doses ranging between 4 and 32 mg [6, 64, 76]. The number of patients in these series of cases was quite small (the largest single-center experience with 12 cases), while the patients were too heterogeneous to draw valid conclusions about its efficacy.

Brivaracetam. It is available on the market as intravenous form and therefore considered potentially usable in SE. To date, only 2 series have been published with a total of 17 patients, showing a variable efficacy [77, 78].

Stage 4: Super-refractory SE. Super-refractory SE is considered when SE continues, even though the anesthetic treatment has been initiated in high doses or when it resumes within the first 24 hours after the anesthetic withdrawal. At this stage, there are no data from clinical trials that have shown effective treatment and several options are described [68, 79], some of which have been published as isolated clinical cases.

Pharmacological therapies:

- Intravenous anesthetics (thiopental / pentobarbital, midazolam, propofol, and ketamine). It is usually the first-line option administered in SE patients to restart or increase sedation again.
- Inhalation anesthetics.
- Other AEDs: topiramate, lacosamide, pregabalin, levetiracetam and brivaracetam.
- Magnesium sulphate.
- Pyridoxine.
- Immunotherapy.
- Neurosteroids.

Non-pharmacological therapies:

- Hypothermia.

- Ketogenic diet.
- Surgery.
- Electroconvulsive therapy.
- Drainage of the cerebrospinal fluid.
- Repetitive magnetic stimulation.
- Vagus nerve stimulation.
- Deep brain stimulation.

As regarding the immunotherapy, there is a growing evidence of the role of inflammation in some refractory SEs, as well as in epileptogenesis [80]. In addition, antibodies against neuronal components are more commonly described as the cause of encephalopathy with seizure and refractory and super-refractory SE [81]. In addition, final results of complementary examinations that have confirmed an autoimmune cause might take weeks in these cases and in these serious conditions, especially in newly-onset SE (NORSE, New-Onset Refractory Status Epilepticus). Thus, various studies explain that early use can be beneficial to avoid serious consequences [82, 83]. Therefore, immunotherapy, including glucocorticoids, immunoglobulins, and plasmapheresis, might be an option in various guidelines in the super-refractory phase [39].

On the other hand, glucocorticoids may have additional non-immunological effects, such as changes in the opening of the blood-brain barrier essential for the persistence of epileptic activity and may reverse GABAergic inhibition, in addition to their effects on intracranial pressure [79]. However, when testing these therapies, potential side effects, such as severe infections or metabolic disorders should be considered.

Recently, the potential use of neurosteroids (brexanolone) has been described in super-refractory phase. Despite the name, these are not anti-inflammatory treatments, but they modulate the synaptic and extrasinaptic gamma-aminobutyric type A (GABA-A) receptors (synaptic receptors are internalized in the cell during the super-refractory SE phase, that is why benzodiazepines do not respond ; they only bind to the synaptic ones) [84].

Conclusions

Major clinical advances have been made, regarding the new definition and classification of SE, thus providing the clinicians with better guidance on the time of treatment initiation, aggressiveness of treatment, and how to avoid over- or under-treatment of this condition. Furthermore, new small pharmaceutical companies are involved in the development of niche products, such as neurosteroids in super-refractory SE or new alternative routes of administration. An increased interest among physicians regarding SE has alerted them to provide an early and more appropriate treatment, as well as to determine the causes of SE in each individual patient.

Despite these progresses, there are still many issues to solve, starting with identifying the cause-oriented treatment not only to prevent the SE recurrence, but also to protect the brain from the SE impact and the development of epilepsy, as well as better distinguish the status subtypes. This might be achieved only by a better understanding of the mechanisms of various SE etiology, as well as by reducing the gap between the preclinical knowledge used in treatment of humans.

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CM and SG designed the study, conducted the laboratory work, and drafted the first manuscript. SG, CV and CM revised the manuscript and completed the final design. All the authors approved the final version of the manuscript.

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Uterine arteriovenous malformation

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Abstract

Background: Uterine arteriovenous malformations (AVM) are extremely rare entities, with less than 100 cases reported in the literature. Synonyms for AVM are arteriovenous fistula, branched aneurysm, hemangioma, pulsating angioma, and cavernous angioma. Incidence of uterine AVM according to the studies of O´Brien et al., who identified uterine AVM in 21 women based on 464 pelvic ultrasound examinations for uterine bleeding, reported an incidence of 4.5%. However, Yazawa et al. examined 959 patients prospectively and observed an incidence of 0.6% of uterine vascular malformation on ultrasound examination The etiology of uterine AVM can be congenital or acquired. The purpose of the study was to raise awareness on the existence of these injuries and understanding their risk factors, to study different treatment methods, especially conservative or minimally invasive ones, as well as methods for diagnosing of these malformations. The following key words were used as a search engine: uterine arteriovenous malformation, uterine malformation, circoid aneurysm. Only full-text articles were analyzed.
Conclusions: Uterine AVM is a rare cause of uterine bleeding. However, it is a potentially life-threatening disorder in which patients present with vaginal bleeding that may be profuse and cause hemodynamic instability.
Key words: malformations, uterine artery, venous system.

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Introduction

Uterine arteriovenous malformations (AVM) are extremely rare entities, with less than 100 cases reported in the literature. Uterine arteriovenous malformation (AVM) is defined as abnormal and nonfunctional connections between the uterine arteries and veins. The etiology of uterine AVM can be congenital or acquired; the incidence rate of acquired AVM is currently increasing [1-3]. Congenital lesions are considered to occur between the fourth and tenth week of embryogenesis as isolated, spontaneous failures of vascular development. Acquired malformations are more common and usually precede a pregnancy, being diagnosed after an uncontrolled uterine bleeding despite drug therapy [1]. Synonyms for AVM are arteriovenous fistula, branched aneurysm, hemangioma, pulsating angioma, cavernous angioma. AVM consists of proliferation of arterial and venous channels with fistula formation and a mixture of capillary-like vessels. It is difficult to distinguish between arteries and veins because secondary intimal thickening occurs in the veins due to increased intraluminal pressure [4]. Uterine AVM plays an important role in gynecologic practice due to the risk of massive bleeding that could be life threatening in some patients. In 1926, G. Dubreuil and E. Loubat described for the first time this pathology as a "branching aneurysm" [5]. Incidence of uterine AVM according to the studies of O´Brien et al. [6] who identified uterine AVM in 21 women based on 464 pelvic ultrasound examinations for uterine bleeding, reported an incidence of 4.5%. Instead, Yazawa et al. followed up 959 patients prospectively and observed an incidence of uterine vascular malformation on ultrasound of 0.6% [7]. Some authors consider that the term AVM is hyper-used once the number of examinations performed by ultrasnography or dopplerography has increased, and the lesions detected with hypervascular and/or turbulent flow are designated as unfounded "uterine vascular malformations". The term uterine AVM should be limited only to those lesions that prove a hypervascular mass, with early filling on angiography or on pathological examination of the uterus after hysterectomy. Uterine AVM can be the cause of unexplained uterine bleeding, the severity of which depends largely on their occurrence and the diameter of the hemorrhagic vessel. Commonly, the only way to stop the bleeding is surgical intervention. The described anomaly represents a direct communication between arteries and veins, formed by the internal iliac artery or its branches. These abnormalities consist of dysplastic vessels with an abnormal wall structure and usually persist lifelong, often without symptoms. All vascular abnormalities are divided into two main types: slow blood flow (capillary, venous, lymphatic) and fast blood flow (arteriovenous anastomosis), but sometimes mixed formations are found [8]. Acquired uterine arteriovenous abnormalities are most common in patients

with trophoblastic disease [9] or previous interventions on the uterus, which may be due to a previous uterine trauma (prior pelvic operation and curettage), pathologic pregnancy-related conditions and infections [10]. Although there are evidences that uterine AVM can be detected in patients under 18 years of age [11], usually this pathology is diagnosed at the age of over 30 years. According to J. Kasznica and N. Nissar arteriovenous abnormalities can also be congenital, being observed in an isolated uterine arteriovenous abnormality in a stillborn fetus [12]. Most of the described congenital arteriovenous abnormalities were diagnosed by ultrasonography in the prenatal and postnatal periods [13]. It should be noted that uterine AVMs are extremely rare in women who have not been pregnant. Apparently, pregnancy plays a role in the onset of uterine AVM [14]. Often the disease is combined with spontaneous abortion. All women of reproductive age with abnormal vaginal bleeding and a negative pregnancy test (absence of functional trophoblast tissue) may be susceptible to uterine AVM [6]. It is important to suspect uterine AVM in the differential diagnosis of unexplained vaginal bleeding, intermittent births and in women of reproductive age, postpartum or following surgical procedures in the uterus.

Acquired malformations may be due to previous uterine trauma (prior pelvic operation and curettage), pathologic pregnancy-related conditions, infections, and the treatment of gestational trophoblastic disease. Congenital AVMs are considered to arise from arrested vascular embryologic development resulting in anomalous differentiation in the capillaries and abnormal communication, between the arteries and veins. Moreover, congenital AVMs can have multiple vascular connections and may invade the surrounding structures. It is important to diagnose uterine AVM correctly and to start appropriate treatment promptly, because uterine AVM often causes life-threatening massive and persistent vaginal bleeding [15, 16].

Although the imaging characteristics of congenital and acquired AVMs may be similar, a detailed history may help to distinguish between these two. Congenital lesions are commonly presented by severe menorrhagia, which do not respond to traditional drug therapy. Traumatic uterine AVMs are often present with features suggestive of arterial bleeding. They tend to cause episodic vaginal bleeding, which is often torrential, leading to significant anemia and even shock.

Clinical manifestation

It is important to consider uterine AVMs in the differential diagnosis of unexplained, intermittent and heavy vaginal bleeding in women of reproductive age, after delivery or surgical procedures on the uterus. Although the imaging features of congenital and acquired AVMs may be similar, a thorough history may help to distinguish between the two [6].

Congenital lesions classically present with severe menorrhagia, irresponsive to conventional therapy. Acquired or traumatic AVMs are invariably associated with one of the risk factors previously mentioned. The symptoms can appear very slowly or suddenly. Vaginal bleeding occurs when the endothelial lining of the vessels in the AVM is disrupted, such as during menstruation or curettage [17].

Traumatic uterine AVMs often present with features suggestive of arterial haemorrhage. They tend to cause episodic vaginal bleeding, which is often torrential, leading to significant anaemia and even shock. It is critical under these circumstances to consider the diagnosis of uterine AVM and avoid attempts of uterine instrumentation (dilatation and curettage), which may significantly worsen the bleeding. Large AVMs may present as clinically recognizable pulsatile masses, which may aid in making the correct diagnosis [18].

Sonographic features suggestive of uterine AVM can be found in women, who present with vaginal bleeding and a positive pregnancy test. Under these circumstances, pregnancy-related conditions, such as intrauterine pregnancy, ectopic pregnancy, retained products of conception, or gestational trophoblastic disease should be considered. A combination of history of the specific pattern of vaginal bleeding, a negative pregnancy test and characteristic features on colour and spectral Doppler should be used to diagnose uterine AVM.

Investigation

Traditionally, uterine AVMs are diagnosed accidentally after hysterectomy based on histopathological evidence of arteriovenous fibers. Yet, the gold standard for diagnosing uterine AVM is pelvic angiography [15]. Findings with DSA include bilateral hypertrophy of uterine arteries that feed a tortuous, hypertrophic arterial mass with large accessory feeding vessels, and early drainage into enlarged hypertrophic veins [19]. However, angiography is rarely performed for diagnosis alone, due to its invasive nature and it is usually reserved when a patient requires surgical intervention or embolisation [20]. Gray scale ultrasound (US) can detect the presence of multiple tubular or "spongy" anechoic or hypoechoic areas within the myometrium of a normal endometrium. However, other conditions may present a similar appearance, such as retained products of conception, hemangioma, gestational trophoblastic disease, multilocular ovarian cysts, or hydrosalpinx. Thus, the use of colour and spectral Doppler US is important for obtaining more accurate information. A normal myometrial signal will show a PSV of 9–44 cm/s and RI of 0.6–0.8. In addition, uterine AVM will exhibit intense vascular and multidirectional flow (regions of juxtaposed reds and blues caused by multiple tortuous vessels of varying orientations). Spectral Doppler US will show high velocity (mean PSV: 136 cm/s), low resistance (mean RI: 0.3) flow, low pulsatility of the arterial waveform, and pulsatile high-velocity venous waveform. Differentiation between the venous and arterial waveform is often difficult, and the pelvic veins distal to the AVM may show pulsatile flow instead of the normal monophasic flow [15, 21].

Gadolinium-enhanced MRI demonstrates a hypervascular arterial-dominant flow. Similar to MRI, computed tomography (CT) may be used to determine the size, ex-

tent, vascularity, and involvement of the adjacent organs [22, 23]. In angiographs, the affected arteries appear thicker and more convoluted than the normal ones. AVMs appear as a complex tangle of vessels supplied by enlarged feeding arteries and show early venous drainage during the arterial phase [24]. Angiography, an invasive technique, allows the confirmation of the diagnosis and helps identify the leading feeder vessels where embolization may be indicated as a conservative treatment option.

Several cases of AVMs have been found during hysteroscopy, but their value is limited [25]. Uterine AVMs should be differentiated from the retained products of conception, gestational trophoblastic disease, dysfunctional uterine bleeding, subinvolution, hemangiomas, varicosities, and malignancies of the uterus, such as sarcomas. When the clinical history, ultrasonographic findings, and serum β-hCG test results are considered, AVMs can be differentiated potentially from these pathologic conditions with an arteriovenous shunt. Meanwhile, overdiagnosis of uterine AVMs should be avoided [22].

Principles of therapeutic management

The therapeutic management of uterine AVM depends on the following factors: hemodynamic status, size and location of the lesion, degree of hemorrhage, age, desire for future fertility. When a woman has severe vaginal bleeding, the basic principles of resuscitation must be followed, and stability and hemodynamic recession must be achieved and maintained. The mainstay for management of uterine AVM has been hysterectomy or the embolization of uterine arteries. However, the uterine artery embolization (UAE) remains the first choice of treatment in women at reproductive age having expectation of future fertility [26]. Whether this procedure is safe for women desiring future fertility is controversial; however, women who become pregnant after UAE are at risk of malpresentation, caesarean delivery, preterm birth, and postpartum hemorrhage.

Hysterectomy remains the last but the most appropriate treatment, especially for women with uncontrolled bleeding, who do not respond to drug therapy and for people who do not want to maintain fertility. Therapeutic options for uterine MAVs range from medical hormone therapy to uterine artery embolization or permanent hysterectomy. Internal iliac artery ligation or uterine artery ligation and/or hysterectomy were the traditional treatment options for counterproductive due to the development of a rich collateral blood supply distal to the ligature, resulting in a recurrence of hemorrhage after surgery. Advances in pelvic angiography and selective arterial embolization techniques have shown that embolization is currently the preferred therapeutic option for uterine AVM, and internal ligation of uterine AVMs in the past, and ligation of the internal iliac artery may prove that the iliac artery may have an important role in cases of failed embolization.

A minimally invasive approach through angiographic embolization of the AVM is currently the preferred treatment for uterine AVM. There are both permanent and temporary methods of embolic agents that can be used as an embolic agent. Various embolic materials can be used, including polyvinyl alcohol, histoacryl (glue), stainless steel coils, detachable balloons, and hemostatic gelatine. However, uterine artery embolization may not always be successful and multiple sessions may be required for recurrent episodes [15]. Imaging assessment of uterine AVMs, especially their vascular anatomy, is very important during the planning stages before embolization, such as Doppler ultrasonography, CT, and MRI [26-29]. Pre-procedural imaging evaluation is necessary in order to assess the presence of possible extra-uterine feeders. An example is the ovarian-uterine anastomotic connection, which is often detected when performing UAE for uterine fibroids [30, 31]. Possible complications of angiographic embolization include infection, perianal skin sloughing, uterovaginal and rectovaginal fistulas, and neurological deficit in the lower limb [6]. Fertility after UAE remains speculative. Despite advances in therapeutic techniques and embolic agents, pregnancy following successful embolization of uterine AVMs remains rare [32]. Decreased vascularization of the placenta has been proposed as being the main cause of adverse pregnancy outcomes following embolization.

This literature review was made by accessing and analyzing the MEDLINE and Hinari databases. The key words used as search engine were uterine arteriovenous malformation and uterine malformation. Only full-text articles were analyzed.

Conclusions

Uterine AVM is a rare cause of uterine bleeding. The vast majority of AVM cases resolve spontaneously or medically. The rest of the cases normally respond to conservative management options. Uterine AVMs have the potential to cause life-threatening bleeding, despite the fact that, with the availability of uterine artery embolization, hysterectomy is rarely necessary to stop the bleeding. The normal menstrual cycle and fertility are restored in the vast majority of women suffering of this condition.

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CT designed the study, drafted the first manuscript and completed the final design.

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The use of penile Doppler ultrasonography in the detection of vascular erectile dysfunction

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Abstract

Background: Erectile dysfunction is defined as the inability to get sufficient erection for a satisfactory sexual intercourse. Penile erection is a complex phenomenon that involves a coordinated interaction of psychological, hormonal, nervous, arterial, venous and sinusoidal systems. According to recent studies, on average about 10% of men aged 40–70 years have severe or complete erectile dysfunction, and about 20–40% – partial erectile dysfunction. According to the 2018 European Association of Urologists (EAU) Guide, up to 12% of the European male population up to the age of 40 has a certain degree of erectile dysfunction (from minor to severe), after 40 years, this figure exceeds 50%. The prevalence of erectile dysfunction (ED) in the Republic of Moldova is significantly higher in men over the age of 40–67.4%, compared to men up to the age of 40–21.1%. Imaging-directed Doppler ultrasound of the cavernous arteries provides a functional and quantifiable assessment of the arterial flow of the penis during a pharmacological erection. Subjects without vascular disease show arterial dilation after intracavitary injection, the vessels appearing with thin and parallel walls, homogeneous lumen and following a straight course, subjects with arteriogenic problems had thickened walls and an inhomogeneous lumen. It is also mentioned that no differences were observed between the two drugs. At the same time, to achieve maximum erection using intravenous injections with a vasodilator is a sign of the veno-occlusive cause, which is independent of both penile stiffness and tumescence. Patients with arterial insufficiency were relatively older than other patients. They also had complicated medical conditions for diabetes and hypertension.
Conclusions: Vascular etiologies are important contributors to erectile dysfunction. Arterial insufficiency is suspected with poor blood flow, while veno-occlusive dysfunction is lower in the face of adequate blood flow and poor erectilesponse.
Key words: penile vascularization, Doppler ultrasonography, erectile dysfunction.

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Introduction

Erectile dysfunction is defined as the inability to get a sufficient erection for a satisfactory sexual intercourse. Penile erection is a complex phenomenon that involves a coordinated interaction of psychological, hormonal, nervous, arterial, venous and sinusoidal systems. The etiological diagnosis of erectile dysfunction usually requires a study of penile vasculature. Arterial origin involves a high percentage of erectile dysfunction. Eco-Doppler allows the study to be performed in a fast, non-invasive and efficient way, providing information about the morphological aspects of the arteries and flow parameters. The study was aimed to assess the importance of using Doppler ultrasonography in detecting vascular problems that lead to erectile dysfunction and its use in differential diagnosis with other causes of erectile dysfunction.

The literature review was performed by analyzing articles from the databases MEDLINE, Hinari. The words used as a search engine were the following: penile vascularization, doppler ultrasonography, erectile dysfunction. Only full-text articles were analyzed.

Results

The corpora cavernosa are homogeneous and relatively hypoechoic cylindrical structures [1] lined with tunica albuginea, a thin membrane that has a thickness of approximately 2 mm when the penis is flaccid and 0.25 mm when it is erect [2]. The corpus spongiosum, a ventral, medial body that is more echoic than the corpora cavernosa, is also covered by the tunica albuginea and contains the urethra,it is more dilated and prominent in its proximal segment, known as the bulb, and in its distal segment, constituting the glans [1]. Buck's fascia is superficial to the tunica albuginea and covers all of the structures described. Venous drainage is performed by the deep and superficial dorsal veins of the penis. The dorsal arteries of the penis are located adjacent to the deep dorsal vein and a cavernous artery is located in the center of each corpus cavernosum. On color Doppler, the cavernous arteries present single-phase flow. In the flaccid penis the normal cavernous arteries show a systolic peak between 11 and 20 cm/s [3]. At the beginning of erection, the systolic and diastolic flows undergo progressive increases. When vein occlusion begins, the diastolic flow decreases

progressively, and once stiffness is established, it becomes negative [3].

Psychological factors (mental impulse) cause transmission of parasympathetic impulses to the penis. This causes relaxation of arterioles and corpora cavernosa sinusoids. As the sinusoidal spaces start filling, the corporal veno-occlusive mechanism activates, and the fibrous tunica albuginea compresses the emissary veins of the corpora, and rigid erection is achieved [3, 4].

According to recent studies, on average about 10% of men aged 40-70 years have severe or complete erectile dysfunction, and about 20–40% – partial erectile dysfunction [5, 6]. According to the 2018 European Association of Urologists (EAU) Guide, up to 12% of the European male population up to the age of 40 has a certain degree of erectile dysfunction (from minor to severe), after 40 years this figure exceeds 50%. [7]. The prevalence of erectile dysfunction (ED) in the Republic of Moldova is significantly higher in men over the age of 40–67.4%, compared to men up to the age of 40–21.1% [8].

The underlying processes of vascular erectile dysfunction are arterial insufficiency, venoocclusive disease or a combination of both, as well as the Peyronie's disease and priapism [9]. Imaging-directed Doppler ultrasound of the cavernous arteries provides a functional and quantifiable assessment of the arterial flow of the penis during a pharmacological erection. A high-frequency transducer (7.5-9.0 MHz) is used for penile Doppler examination. The patient is placed in a supine position and the penis is positioned in its anatomical position along the anterior abdominal wall. Doppler angle is set at 30-60 degrees. In case of using drugs for pharmacological erection, pre-injection and post-injection measurements are required. Pre-injection measurements: inner diameter of the cavernosal artery (normal value is 0.3-0.5 mm), baseline peak systolic velocity and end diastolic velocity. Corpora cavernosa are localized as two well-defined oval compartments with central cavernosal artery on both sides of the corpus spongiosum (urethra is in center of corpus spongiosum). Insulin syringe is used for injection under sonographic guidance. Post-injection measurements (at 5, 10, 15, 20 minutes): inner diameter of cavernosal artery (normal value is 0.6-1.0 mm), peak systolic velocity, end-diastolic velocity, visual tumescence and erection. In this sense, this method is superior to arteriography as a means of assessing arteriogenic impotence. Maximum flow rate, arterial dilation and pulsation of vessels are the most reliable ultrasonic indicators of arterial health. It is advisable to cease smoking three days prior to the examination. Medication history and cardiac status should be enquired. Aberrant arterial anatomy should be noted, as it can significantly contribute to the total blood flow to the penis. Recognition of the pathological pattern helps to choose the best treatment method [10].

Vidal Moreno in a study of 93 subjects, aged 20 to 66 years, without vascular disease: 20 healthy volunteers and 73 with psychogenic dysfunctions, who underwent a reference study and 89 – a second study after intracavitary injection (ICI) (10 papaverine and 79 PgE1) showed that subjects without vascular disease showed arterial dilation after ICI, the vessels appearing with thin and parallel walls, homogeneous lumen and following a straight course; subjects with arteriogenic problems had thickened walls and an inhomogeneous lumen. It is also mentioned that no differences were observed between the two drugs [11, 12]. At the same time, Yafi FA states that failure to achieve maximum erection using intravenous injections with a vasodilator is a sign of the veno-occlusive cause, which is independent of both penile stiffness and tumescence [13].

He ZJ, Cheng M. in a 2006 study of 527 patients who were evaluated using color Doppler ultrasonography after intracavernous injection of 20 micrograms prostaglandin E1 revealed 112 patients (26.99%) with nonvasculogenic ED, 207 patients (49.88%) with arteriogenic ED, 144 patients (34.70%) with venogenic ED, and 64 patients (15.42%) with mixed ED [13]. He also mentions that patients with arterial insufficiency were relatively older than other patients. They also had complicated medical conditions for diabetes and hypertension [14]. Golubinski and Sikorski in a similar study, using an intracavernous injection of papaverine of 40 mg analyzed the maximum systolic velocity (MSV), end diastolic velocity (EDV) and resistive index (RI). After papaverine injection, 7 patients had a normal erection and adequate waveform patterns; their mean MSV was 30.7 cm/s, EDV 4.42 cm / s and RI 0.85; five patients had no erection. Abnormal flow values showed insufficient arterial vessels in a quarter of men, venous discharge in 15% and mixed ED in 20% [15].

Ismail in a study of 21 patients up to the age of 40 described that 5 patients had normal findings, while 10 had evidence of venous discharge. Five patients had arterial insufficiency; of which 3 patients showed calcifications of the albuginea tunic, suggesting the Peyronie's disease. Interestingly, one patient showed the characteristics of a combined arterial insufficiency and venous discharge. Those with arterial insufficiency were relatively older than other patients [16].

Two important parameters must be considered for assessing the cause of erectile dysfunction. Peak systolic velocity is the best Doppler indicator of arteriogenic impotence. Its value <30 cm/sec during the examination indicates arterial dysfunction. Some people consider <25 cm/sec as definite arterial dysfunction and 25-30 cm/sec as borderline case. Less than 60% increase in cavernosal diameter after papaverine injection is also an indicator of arterial impotence. End-diastolic velocity is the best Doppler indicator of venogenic impotence. Its value >5 cm/sec indicates venous dysfunction. A good diastolic reversal virtually rules out venous insufficiency.

Conclusions

Doppler ultrasonography with intracavernous injection of papavarin and prostoglandin E1 shows promises for the accurate evaluation of patients with erectile dysfunction.

Vascular etiologies are important contributors to erectile dysfunction. Arterial insufficiency is suspected with poor blood flow and is more common for older male, while veno-occlusive dysfunction is lower in the face of adequate blood flow and poor erectile response and is more common in younger men.

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The psychotherapeutic aspect of psychic trauma in epilepsy

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Abstract

Background: The management of epilepsy can be reviewed in a multidimensional way, medical, psychotherapeutic, familial and social. In this regard, we are talking about the position given to the patient suffering from epilepsy.From a psychopathological point of view, there is a system of interactions between crisis and personality based on confusional anxieties, related to ambiguity and the feeling of crisis for the subject. The psychotherapeutic understanding of the patient suffering from epilepsy is situated in a bio-psycho-familial and social context. Whether it is the first, the second, or the next crises, they point out that there is an intrapsychic trauma.
Conclusions: System of interactions between epilepsy, the patient and the environment contributes to the creation of the framework that will provide opportunities to help the patient. Psychotherapy sessions allow the patient to reintroduce the crisis in his history. The description of psychological experiences that are associated with the evolution of epilepsy symptoms, will allow us to form an idea about the influence of psychic trauma on the clinic and the dynamics of epilepsy, which could help identify an approach to the patient's adaptation.
Key words: epileptic seizures, psychic trauma, psychotherapy.

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Introduction

The child builds his inner world by integrating his experiences and surroundings. Depending on the age of onset of the first seizure, epilepsy more or less disrupts this process [1]. If most people with epilepsy feel completely normal outside of seizures and the number of seizures can be reduced, then some patients (about a third of the total number) are severely affected by their disease. They can be affected by the repetition (high frequency) of epileptic seizures, but also by the physical, psychological and social consequences that they involve, which makes their life impossible in their normal environment [2].

Epilepsy alters parents-children and social interactions. The function of doctors is not only to cure the disease, but also to take into account all the surrounding factors (social, family, school) to facilitate the harmonious development of the sense of identity [1]. Caelius Aurélien points out that "Epilepsy takes its name from what captures both the senses and the mind" and draws consequences [3].

Before discussing the issue of epilepsy, it is worth considering how the child is psychologically constructed. Referring especially to the work of Anzieu (1995) "Le Moi Peau", it can be said that the mind derives from the body. Thus, the child builds his inner world by integrating his own experience and the experience of his environment. Epilepsy in childhood is a source of difficulty, since it is responsible for intermittent or permanent neurological dysfunction, which will disrupt the messages in the body and will disrupt the internalization of bodily sensations. The earlier the epilepsy begins, the more severe the disorders, sometimes leading to arrest or even regression of psychological development [4].

When it comes to identifying landmarks, one must consider the time of onset of epilepsy. A disorder at an early age will not have the same effects as when the disease occurs in a person who has already developed a balanced personality. The fusional relationship of the mother with the child will be paralyzed by the existence of seizures. To this, we must add that the child evolves in a system that, from the beginning, occurs only with the parents and becomes more complex as he grows up. Every day we notice the importance of parents' narcissistic satisfaction with their child when he is well and, in the mirror image, the jubilation of the child who is identifying himself with parents or other images of the entourage. These interactions, so important for the correct development of mental development, can be disrupted when epilepsy occurs [5,6].

At this time of the announcement of the diagnosis, regardless of the severity of the disease and whatever the child's age, parents ask themselves: is my child's epilepsy a source of suffering? Is it a failure? A fatality? Or is it for me,

the parent, the opportunity for an individual and emotional experience? If the parental couple is united and if the professionals who will take care of the child agree to take all the necessary time to accompany them in an authentic way, then they will be able not to fall into depression, they will be able to overcome the pain. They will be able to be with their child, what Beauchesne calls "supportive parents" [6].

In the process of the psychotherapeutic approach to epilepsy, it is essential not to talk about epilepsy, but about people with epilepsy, who are the subject of their seizures. In this sense, we are talking about the place given to the patient suffering from epilepsy to allow him to express himself and others to strive to listen to him. At the family level, it is, in fact, desirable to listen to the fears and anxieties caused by crises. The information should allow the family to avoid extreme attitudes, both excessive protection and unjustified rejection [7,8].

Psychotherapy sessions allow the patient to reintroduce the crisis in his history and the opportunity to make sense of it. It is equally dependent on another system of interactions between epilepsy, the patient and the environment. The family and social approach to epilepsy contributes to the creation of the framework that will or will not provide opportunities to help the child to mentalize and conflict with what he or she is experiencing and has so many difficulties in understanding [9-11].

Results

Epilepsy is one of those unfavorable circumstances, that is a source of suffering, of rejection; it is responsible for the difficulties encountered at school; brings prohibitions, limitations in daily life; it can finally be the cause of the child's total impasse. It is possible to oppose the care, to manipulate treatments or result in total passivity [12,13].

We also notice disorders in the psychopathological field. Most often, they are related to the anxiety generated by the disease. Symptoms of depression are very common; this changing mood can turn into the severe depression with the risk of suicide or an exacerbation of risky behavior. Hyperactivity that is often associated with epilepsy in childhood becomes a severe obstacle and deserves specific management [14, 15].

Entering adolescence is also a delicate time in the development of the child with epilepsy. The child knows that he is ill; he has learned the limits imposed by the disease, the constraints that it brings. He feels guilty that he is ill and is responsible for his parents' suffering [16, 17]. All this weight becomes unbearable in adolescence when there is a search for a new identity with the desire for autonomy and the desire to transgress the limits imposed by his usual environment. On the other hand, non-epileptic manifestations can also appear during this period, the adolescent not knowing how to "exist" in the eyes of his environment, other than with convulsions [18, 19].

The work of the psychotherapist allows to pay attention to early symptoms and to "decentralize" what is crucial in epilepsy problems. For Gilbert Diebold, a physician, psychiatrist and psychoanalyst, a member of the French League Against Epilepsy, epileptic seizures often have a precise meaning, which must be taken into account to heal the patient. The suffering of living, the presence of death, this confusion must be revealed in order to be able to express the inexpressible convulsions [20].

Lucien Mélèse releases, from his long years of psychoanalytic practice with people with epileptic seizures, a theory of critical phenomena that justifies what the clinic has brought: a possibility of dismantling the "neurological storm" machine, returning the family story and especially the genealogical one. The theory of trauma, history and genealogy constitutes the essential background of these neurological manifestations. Re-appropriation of patient's transfer during the analysis often allows the subject to develop a way out of this nightmare. Epilepsy becomes the model of the crisis (fear, flight, non-existence) for any treatment practice, especially in the psychosomatic field [21].

It is common for a child with epilepsy to experience his first seizures after the psychological trauma endured [22]. Mourning will be found in a huge number of observations. In the process of working with the child who is experiencing epileptic seizures we need to consider three key points: the subjective experience of convulsions by the child; their representative value of unconscious phenomena; their special psychosomatic quality. Since the epileptic seizure occurs in a state of unconsciousness, it is possible to think that it is driven by unconscious factors [23].

As Ferenczi described the return to motor behavior and a resolution of consciousness, in which the individual finds his prenatal state and his postnatal emotion in a regressive movement, the phenomenon referring to "birth trauma". Ferenczi offered us a psychoanalytic interpretation of epilepsy, based on the hypothesis of a regression to embryonic state; therefore, he postulates the existence of a memory persistence of this extremely passive degree, although he does not relate it to death or to the death instinct. Under this high patronage, it is justified to formulate the idea that the loss of consciousness, which is one of the major manifestations of many clinical forms of epilepsy, refers to the negative experience during intrauterine life and the child's fantasy of non-existence related to his prenatal experience [24].

Clinical case study

Patient S. was first consulted at the National Center for Epileptology, when he was 14 years old. He was sent to the consultation after being hospitalized six times for epileptic seizures. Treatment with anticonvulsant drugs was ineffective at that period. S. continued to have seizures with a frequency of about twice a week. According to his parents, S.'s epilepsy could result from neurosurgical intervention. It was difficult to establish any contacts with S. He ignored the people around him.

The attitude of his parents was special: they expressed their concern about epilepsy, but, on the other hand, com-

pletely denied the pathological behavior of S. The anamnesis showed that S. was a child whose birth was eagerly awaited. The mother's pregnancy went well. Parents reported a decrease in his development after the onset of the first seizure, which occurred at the age of 11 months. S. had psychotherapeutic sessions once a week, over a year. These sessions took place in the presence of his mother. For a few weeks, S.'s behavior did not change. The sessions were always the same: the teenager mostly kept silence. It was very difficult to get any feedback from him: he was completely indifferent. The interviews focused on the seizures he had. The surgery was rejected and only drug solutions were recommended, though they were ineffective.

Taking into account little progress, the father was recommended to attend the consultations. It was found out during a consultation with the father that S.'s sister died at the age of four. The father claimed that they were shocked by the death of their first child. The patient's father considered that the soul of his first child lived in his sick son, and epilepsy took for him the meaning of repeating the death experiences that S. was suffering from some kind of death conspiracy. His wife, S.'s mother, shared these ideas. Later, sessions allowed discussing the differentiation between S... and his deceased sister and about the need to leave it in the past. Subsequently, S.'s behavior changed a lot. His parents began to perceive him as a definite separate personality, although epileptic seizures continued regularly.

In this observation, a trauma, that of the experience of S.'s first crisis, awakened another, associated with the daughter's death. The effect of the crisis on the parents, through this traumatic repetition, had disastrous consequences for the mental structure of the child, who did not exist for his parents in their own perception. Awareness of the trauma and its repetitions had the effect of "thawing" the representations and allowed S. to exist as a person.

Discussion

We often underestimate the impact of trauma of announcing a disability on the parental process, and therefore, on the child's psychological structuring. In fact, it has been established that the disclosure of disability interrupts the fragile process of the parenthood. The imaginary reverie that existed around the child interrupts the foreign representation of anything that had been imagined until then. It is a brutal separation: the child becomes another personality, a stranger. This is a real psychological trauma. Parents, caught between guilt and an attempt to remedy, must build a story contrary to this emptiness: for them it is about the meaning of this event, in an attempt to overcome the trauma [25-27].

At present the task of professionals to offer parents their capacity for empathy and emotional sharing to overcome trauma. Parents are usually diagnosed with depression at the time when the effects of trauma which, in Ferenczi's words, "acts as an anesthetic in the face of shock, unexpected, unprepared and overwhelming..." [28]. This traumatic shock leads to the inability to work out the psychic connection and the inability to experience this emotional catastrophe. Dialogue with parents is difficult: "they know everything, but they feel nothing", according to Gianna Tissier (Sarfaty et al., 2000). Hence the need to create the safe environment for the child, in which parents and professionals share the emotions aroused by the child. From this experience the trauma can be recognized, spoken about and overcome [29].

Epileptic seizures (when generalized) cause parents to experience their child's death. This reinforces the trauma and leads to overprotective attitudes towards the child. These are the classic prohibitions that apply to all patients with epilepsy. Managing the autonomy of the child with a certain level of risk-taking is often difficult for professionals who have often had to experience accidents, sometimes fatal, related to crises. And yet risk-taking is a condition of empowerment.

As described in Freud's article, mourning and melancholy find their complement in Totem and Taboo, where it is indicated that the fundamental ambivalence of love relationships suggests that the work of mourning will never be simple. Mourning is indelible: new investments will always have a deep connection with those that came before them, which are never completely abandoned. The success or failure of suffering is a matter of degree [30].

However, the difficulties and anxieties of parents in the face of disability are not the only factors that influence mental construction. The other equally important factor is the effect of seizures on the child's construction. The child builds the world around him through a continuous connecting activity in which he matches his perceptions with the memory traces of previous perceptions in order to build the coherent representative system. The conditions of this constructive work are both a functional perceptual system, an ability to analyze the various sensory channels and to synthesize the received signals. Therefore, continuity must be internal and external. We know the role of the environment in the construction of the child, and all the works on the lack of maternal care showed the need for this continuity. It is ensured especially by the mother or the person who takes her place and by the connections between the different people who take care of the child [31].

Parents of children with epilepsy have a personal difficulty in performing mourning work. This difficulty manifests itself in different ways: the case is particularly serious if parents suffered mourning before the birth of the child. The child could have felt it, therefore, mainly through the reactions of his parents, he will feel the effects. When parents do not react adequately enough to the loss of one of their objects of love, the child feels it and may be disturbed by it. On the other hand, it is possible to think that the deficiencies that the parents of this child will manifest later, when faced with a regression, will be the testimony of a psychological difficulty that could have already intervened in the patient's epileptic predisposition [32].

The child's representative system must function well enough to integrate the various events experienced. Howe-

ver, epilepsy produces a very significant discontinuity. The crisis creates such mental chaos that it takes several hours and sometimes several days for the child to return to the normal functioning. This disorganizing effect is greater when the child is smaller and the seizures are more frequent. For example, epileptic diseases that occur at a very early age, such as West syndrome, have a mental prognosis that is worse the earlier the disease begins. Hence the importance of taking action on crises as early as possible [33, 34].

Usually, several brain disorders that include epilepsy in a large number of cases generate ubiquitous developmental disorders. This is the case with West syndrome, as we have mentioned, but also with Lennox-Gastaut syndrome. It can also be hypothesized that autism, when associated with Bourneville's tuberotic sclerosis, is directly related to early epilepsy which is often observed in this disease [34, 35].

All these questions should be identified and considered by the doctor. Care should allow this patient to integrate the representation of the disease, at the same time he must integrate all the changes that puberty makes to appear in his body. For example, to give the child the opportunity to express the subjective sensations that accompanies seizures. This internalization and intrapsychic delimitation of the body cannot be solved by an intellectualization of the phenomenon, but rather by a true intrapsychic work [36-38]. The work of the psychotherapist is complex in the case of epilepsy, since the patient feels the disease, to a large extent in the eyes of others (parents, friends, society), and this stigma is the possible cause of regression to an infantile self-image [39].

This situation requires the doctor to question his own projections and to remember that the epilepsy he treats cannot be considered in general. Supporting a patient with epilepsy and his family allows the reorganization of expectations and life plans, sometimes even values. This professional, collective or individual approach focuses on the personality and meaning of epilepsy for patients [40-42].

Conclusions

Patients with epilepsy are particularly vulnerable. Supporting a patient with epilepsy requires consideration of all the data presented in order to be able to respond effectively to his request. What is his request? In order for the doctor to be able to react to his illness competently, to answer the concrete questions that patient asks, whom he considers a person whose place he affirms in society. It is well understood that the physician, in this framework thus defined, cannot escape his emotional involvement in this human relationship. This requires a lot of discussion, advice, sometimes negotiations, intelligible medical explanations and real health education. It is essential for the doctor the collaboration with other medical specialists, social workers or psychologists; he is the interface between the patient and a specialized team. This is the priority function of the medical activities.

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The author has no conflicts of interests to declare.

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Presurgical and postsurgical neuropsychological assessment in epilepsy

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Abstract

Background: Epilepsy surgery represents a valuable treatment for people with drug-resistant epilepsy, which often leads to a substantial improvement in the cognitive-behavioral domains and to a better quality of life, especially in children. A neuropsychological assessment is considered mandatory and should form an integral component of the presurgical evaluation and assessment of postoperative outcome for all epilepsy surgery patients. In this context, the presurgical neuropsychological assessment in combination, as well as other relevant neurological investigations are important for assessing the risk of potential postsurgical cognitive deficits, to determine the dominant hemisphere responsible for language function and to predict the risk of memory decline and of visual and motor deficits. A postsurgical neuropsychological assessment is necessary in assessing the outcomes because cognitive decline is one of the most significant sequelae of epilepsy surgery.
Conclusions: The neuropsychological assessment remains an obligatory and valuable part of the presurgical and postsurgical assessment. This article provides a comprehensive overview of the role of neuropsychological assessment in the pre- and postsurgical evaluation of epilepsy surgery patients. The neuropsychological profile may have a predictive role for the identification of the cognitive risk, prognosis, and treatment. New researches about neuropsychological assessment may provide many relevant answers about the outcome of the epilepsy surgery as well as to influence the quality of life.
Key words: epilepsy, presurgical, postsurgical psychological assessment.

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Introduction

According to International League Against Epilepsy (ILAE) definition, "epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition" [1]. Cognitive impairments, as well as mood and behavioral issues, represent common comorbidities of epilepsy [2, 3]. Despite the availability of antiseizure drugs for symptomatic treatment of epileptic seizures, about one-third of patients have drug-resistant epilepsy [4, 5]. Epilepsy surgery represents an elective treatment and cognitive decline is the most frequent comorbidity associated with this procedure [6-10].

The main aim of the surgery procedure is to improve the person's health-related quality of life and to obtain the seizure freedom [11]. Moreover, early epilepsy surgery improves the quality of life, cognitive and developmental outcomes and allows the person to regain a normal life [12]. Furthermore, a successful epilepsy surgery focuses to preserve or even to improve the patient's functional capabilities, emotional state, and behavior, including social cognitive function [13]. However, 30% to 50% of surgery patients have a risk of additional postoperative memory impairment [14]. The major determinant of surgical cognitive outcome is the "functionality" of brain areas affected by epilepsy which needs to be resected and the second determinant is the "functionality" of brain areas and functions that are not affected by epilepsy or surgery, also called patient's mental reserve capacity.

In the context of epilepsy surgery, a neuropsychological assessment is considered mandatory and should form an integral component of the presurgical evaluation and assessment of postoperative outcome for all epilepsy surgery patients [6, 15, 16]. In addition, the determination of language lateralization is very important in planning surgical resections and predicting cognitive outcomes [6, 17, 18].

According to the special report of the ILAE Neuropsychology Task Force, Diagnostic Methods Commission: 2017-2021, Neuropsychological assessment in epilepsy surgery [6], a neuropsychological assessment plays a vital role during the two main phases of the neurosurgical management: the first phase is the preoperative assessment, which implies the diagnosis of the impact of a lesion on cognitive functions and the second phase includes the postoperative or post-traumatic one, which will evaluate the cognitive result of the injury or the surgical treatment.

A neuropsychological assessment is a comprehensive and exhaustive assessment of skills and abilities linked to

brain function, which provide an overview of a person's functioning, drawing on the person's history, the clinician's observations, and test scores in various cognitive domains [7, 19, 20]. The evaluation quantifies such domains as memory, IQ, language, attention, executive functioning, visuospatial skills, cognitive abilities, emotional functioning, and behavior [14, 20, 21]. The aim of a neuropsychological evaluation is to assess and identify attentively and comprehensively the behavioral strengths and weaknesses beyond the multiple cognitive areas [22, 23].

The neuropsychological assessment contributes to a number of important decisions in medical aspects, such as identification of candidates for surgery, potential risk, benefits and efficacy of treatments and rehabilitation, as well as, identification of epilepsy-related cognitive impairments and their etiologic attribution to lesions [24, 25]. The assessment also determines whether developmental problems are present, establishes a diagnosis, guides treatment and educational planning, measures progress and demonstrates eligibility for special education services [21]. According to K.B. Casaletto and R.K. Heaton [26], the primordial purposes of neuropsychological assessment remain constant, viz: (1) detect cognitive dysfunction and guide differential diagnosis, (2) characterize changes in cognitive strengths and weaknesses over time, and (3) guide recommendations regarding everyday life and treatment planning.

Presurgical neuropsychological assessment

The primary role of neuropsychological assessment is to assess all cognitive, emotional and behavioral domains and to use the results from the presurgical assessment to establish a baseline assessment against which cognitive change can be measured after the surgery [6, 24]. The presurgical assessment also provides the teamwork with seizure description, lateralization, and localization, as well as with evidence-based predictions of cognitive results associated with the proposed surgery, including risk of amnesia, psychologic and psychiatric issues [27-29]. The assessment should include formal measures of psychosocial function and health-related quality of life, and it is important to include the parental/caregiver or teacher evaluations of behavior, mainly in children [6].

After the assessment, it is vital to provide feedback and preoperative counseling including investigations of patient and family expectations of surgical treatment [6]. Communication of the results of the neuropsychological assessment to the patient is an integral part of the presurgical evaluation. This will help the surgical candidate and their family understand the etiology of any cognitive or behavioral difficulties identified. The results of neuropsychological assessment contribute to the prediction of the postsurgical deficit risk. The most considerable predictors of neuropsychological outcomes include the performance of presurgical tests, which reflect the functional integrity of the resected tissues and cognitive reserve capacities [7].

A preoperative neuropsychological assessment should include standardized tests of cognitive function and behavioral, emotional, and psychosocial functions [6, 21]. In general, a neuropsychological assessment will typically include assessment of intellectual functioning (IQ), verbal and visual memory, language, attention, executive function, visual-spatial and visual-perceptual skills, visual-motor and fine motor coordination as well as emotional and behavioral functioning [8, 20, 21, 24, 25, 30].

Postsurgical neuropsychological assessment

A postsurgical neuropsychological assessment is necessary in evaluating the outcome because cognitive decline is one of the most significant sequelae of the epilepsy surgery. A postsurgical neuropsychological assessment should be an integral part of the epilepsy surgery [6]. The same principles that inform the comprehensive nature of the preoperative neuropsychological assessment should guide the assessment of postoperative outcome. The postoperative assessment should address all aspects of cognitive and behavioral function, as assessed prior to the surgery [6, 7]. In addition, a detailed picture of postoperative changes in seizure control should form an important part of the postoperative neuropsychological assessment since the relationship between postoperative seizure control and cognitive change is a complex one [31]. There is some evidence that cognition and memory improves with seizure control following successful epilepsy surgery, at least in some patients, while other studies have found no association or report greater cognitive declines in those with ongoing seizures following surgery [10, 31-35]. After surgery, children demonstrate faster rehabilitation from surgically caused impairments than adults; there is evidence of a greater plasticity and compensational capacity in childhood [36, 37].

Additionally, the psychiatric comorbidities can occur independently or arise from the same organic substrate as the seizures and cognitive impairment [14]. Risk factors for psychiatric illness include a previous patient or family history, a structural brain abnormality, seizure frequency, medication effects, cognitive impairment, personality traits, and social and family functioning [22, 25, 38, 39]. In some cases, psychotherapeutic support is welcome to help surgical candidates maximize their postoperative potential [6, 32].

Clinical case study

Neuropsychological assessment is recognized as a core investigation in presurgical planning for children with epilepsy. The present case study evaluated an 11-year-old child, left-handed. The study was carried out at the Institute of Emergency Medicine and National Center for Epileptology during 2019-2020. The patient was referred by his epileptologist for a neuropsychological assessment because of his epileptic seizures and the diagnosis of a tumor in the temporal-parietal left hemisphere. As a result, the patient underwent an epilepsy/tumor neurosurgery. To assess the risk of cognitive decline and emotional and behavioral risk, the patient's cognitive function was evaluated before and 6 months after the neurosurgery. The neuropsychological assessment in this case, comprised 6 presurgical and post-

Table 1

Neuropsychological assessment. Presurgical and postsurgical results organized by domain of function

Presurgical assessment		Postsurgical assessment	
Domain of function	Scores	Domain of function	Scores
Intellectual functioning		Intellectual functioning	
Matrices Progressive Raven	IQ=118	Matrices Progressive Raven	IQ=121
Memory function		Memory function	
Rey auditory verbal memory test (RAVLT)	8/15; 8/15; 12/15; 11/15; 12/15 List B – 8/15 List A – 11/15 after 30 min 15 words from 15	Rey auditory verbal memory test (RAVLT)	9/15; 11/15; 11/15; 13/15; 15/15 list B 5/15 List A – 13/15 after 30 min 12 words from 15
Visual memory. Rey Complex Figure Test		Visual memory. Rey Complex Figure Test	
Copy - 90 percentiles Immediate recall - 90 percentiles Time – 3 min 18 sec		Copy - 80 percentiles Immediate recall - 80 percentiles Time – 4 min 8 sec	
Language. Verbal Fluency		Language. Verbal Fluency	
Phonemic: COWAT test – F-5; A-12; S – 9 Semantic: Animals – 15		Phonemic: COWAT test – F-5; A-11; S -8 Semantic: Animals - 13	
Visual spatial skills		Visual spatial skills	
Clock drawing test	Copied from the 3-rd trial. Minutes – incorrect	Clock drawing test	Drawing from the 1st trial. Minutes – incorrect
Cube copy test	Copied from the 3-rd trial	Cube copy test	Copied from the 2-nd trial.

surgical standardized tests. To assess the cognitive function, we used the following psychological instruments: Clinical interview; The Raven's Progressive Matrices Test [40]; Rey auditory verbal memory test (RAVLT) [41, 42]; Rey Complex Figure for visual memory test [43]; COWAT test for verbal fluency [44], the Cube coping and the Clock drawing test [45, 46].

Results

The neuropsychological test results are presented in table1 2 and 3.

Table 2

Visual spatial skills, constructive disabilities

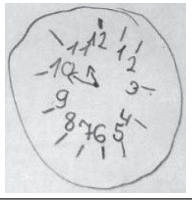
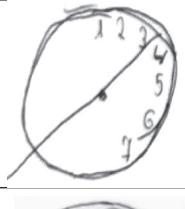
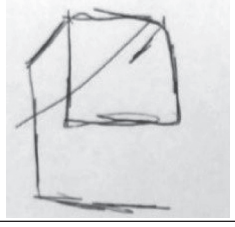
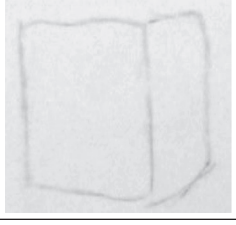
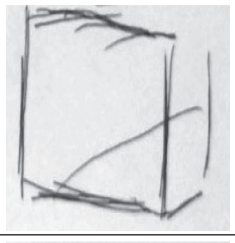
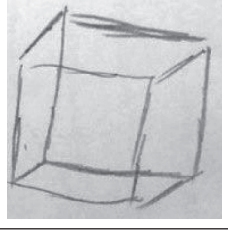
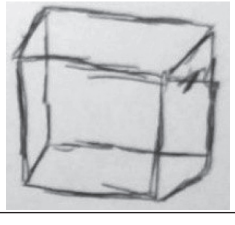
Image 1. Presurgical Clock Drawing Test		Image 2. Postsurgical Clock Drawing Test	
1st trial		1st trial	
2nd trial			
3rd trial			

Table 3

Visual spatial skills, constructive disabilities

Image 1. Presurgical Cube Coping Test		Image 2. Postsurgical Cube Coping Test	
1st trial		1st trial	
2nd trial		2nd trial	
3rd trial			

Discussion

According to data shown in tab.1, a significant postsurgical improvement in the intellectual performance can be observed. Before surgery the IQ of the patient was 118 which corresponds to high average level, subsequently after the surgery the patient reached superior higher level with IQ=121.

According to the results of the Rey auditory verbal learning test (RAVLT), it can be concluded that the patient proved improvement in the first five recalling trials after the surgery; however, a decrease was noticed at the interference trial and after 30 min. It can be presumed that the concentration and the short verbal memory improved, however the long-term verbal memory slightly decreased.

On the Rey complex figure test, the presurgical results were better than the postsurgical outcomes. Before surgery, the patient obtained 90 percentiles at reproducing and immediate recall test and after the surgery the score was 80 percentiles at the same test trials. Another important observation regarding this test is the time for performing the Rey figure. Prior to the surgery, the patient copied the Rey figure in 3 min. 18 sec. and after the surgery; he copied and recalled the same figure in 4 min. 8 sec. It can be hypothesized that the processing and reaction speed became slower after the surgery. Additionally, this test revealed a high level of anxiety before surgery, this was emphasized by the manner of drawing, whereas the lines of the figure were repeatedly aggressively accentuated. Later, the patient's mother confirmed that the patient did not know about his health condition and his future surgery. After the surgery, the patient manifested irritability, which disappeared over a few months.

Nevertheless, the patient faced difficulties at the language test (COWAT); his performance on confrontation naming, phonemic and semantic fluency was impaired equally before and after the surgery. However, it is difficult to assume if his verbal fluency was affected by epileptic seizures, tumor or both.

While performing the Clock test, the patient was confused, thus, before surgery (tab. 2, image 1) he had 3 attempts to draw the clock and he accomplished it from the third trial with mild error at ticking the minutes. Postsurgically (tab. 2, image 2), he drew the clock from the first trial and repeated the same mistake prior the surgery, and he reproduced more exactly the third presurgical trial. Almost the same difficulties were encountered when coping the cube test. Before surgery (tab. 3, image 1) the patient reproduced the cube from the third trial and after the surgery (tab. 3, Image 2) from the second trial.

Thus, the neuropsychological assessment before the surgery, revealed impairment in visual spatial skills, namely in the clock drawing and cube coping test. His performance was a borderline, with soft decline on measures of psychomotor processing speed, confrontation naming and semantic fluency. Otherwise, the verbal, visual memory and intelligence level were in the normal limits. Postsurgically, we noted an improvement in intelligence level and visual spatial skills, however a slight decline but in normal limits were registered in verbal and visual memory.

In conclusion, we can affirm that epilepsy surgery provided seizure freedom, improvement of intellectual level, positive changes in behavioral and emotional domains and a better quality of life of the patient and his family.

This result confirms the findings of previous study conducted by C. Cunningham et al. [47], who described an 8-year-old boy with a history of intractable epilepsy who underwent a left frontal temporal-parietal resection. He passed a neuropsychological testing prior to and following surgery. Presurgical results indicated that his IQ was within the low-average range, whereas visual-perceptual abilities, the motor tasks and attention domains indicated some difficulties. Postsurgical neuropsychological evaluation revealed a positive outcome. IQ remained in the low average range and there was a mild improvement in visual-perceptual/visual-constructional areas.

Regarding intelligence, E. Wyllie et al. [48] studied seizure outcome in 136 pediatric patients who underwent surgery for intractable epilepsy and showed that IQ level tended to be higher in adolescents (85) than in children (76), whereas the full scale IQ tended to be highest for patients who underwent temporal resection. Another study [49] concluded that intelligence level remained stable two years after epilepsy surgery in 94 children and adolescents. A seizure-free outcome was the most important factor for the prognosis of cognitive development, regardless of the intellectual level of the child before surgery. On the other hand, U. Gleissner et al. [50] revealed in their study that the postoperative cognitive result was not dependent on seizure outcome, while IQ alone is not a good predictor of postoperative outcome in pediatric patients with epilepsy.

A long-term follow-up study of 42 children, who underwent temporal lobe surgery after an average postoperative period of 9 years, reported that the surgery performed in childhood results in excellent long-term seizure control and favorable cognitive outcome along with positive effects on brain development [51]. A study from Khajavi et al. [52], which included 34 pediatric patients with medically refractory epilepsy and primary brain tumors who underwent the neurosurgery, reported that completeness of tumor resection is the most important factor in determining seizure outcome.

A retrospective study comparing preoperative evaluation and postoperative outcomes demonstrated that the resection surgery is an effective and safe intervention in early stages and in strictly selected pediatric patients with refractory epilepsy [53].

A. Miserocchi et al. [54] evaluated the relevance of the presurgical workup and the postoperative outcome in 68 children who underwent temporal lobe epilepsy surgery (TLE), and concluded postoperatively that the percentage of patients with a pathological score invariably decreased compared with the results from the preoperative evaluation in all cognitive domains. Results of the neuropsychological evaluation indicated a long-term improvement in cognitive performance in all the explored domains after TLE surgery.

Conclusions

This article shows the importance of the neuropsychological assessment in epilepsy surgery as an obligatory and valuable part of the presurgical and postsurgical assessment. The article provides a comprehensive overview of the role of neuropsychological assessment in the presurgical and postsurgical evaluation of epilepsy patients undergoing epilepsy surgery. The neuropsychological profile may have a predictive role for the identification of the cognitive risks, outcomes, and treatment. The neuropsychologist, along with other specialists from the multidisciplinary team, plays an important role in both the presurgical process and postsurgical rehabilitation and is a support to the patient and his family.

Therefore, new researches about neuropsychological assessment may provide many relevant answers regarding the outcome of the epilepsy surgery as well as to influence the quality of life of the patient and his family.

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Clostridium difficile infection in the intensive care unit

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Abstract

Background: *Clostridium difficile* (CD) infection is widespread throughout the world, showing an increased incidence over the recent years and may cause severe forms of disease. This infection most commonly affects patients whom were administered antibiotics. An increased resistance to commonly used antibiotics is associated with *Clostridium difficile infection* (CDI). CD has a generally recognized infectious potential on a clinical ground. CDI is unpleasant and may sometimes cause serious bowel disorders that are usually treated with another course of antibiotics. The evolution of CD infection depends on the individual characteristics of the patient along with risk factors, associated diseases as well as the particularities of the recommended treatment. However, even under the conditions of a correct and complete treatment the risk of the disease relapse is estimated to occur depending on risk factors. Many clinical instruments that are designated for the purposes to treat non-infectious diseases can be useful in estimating the severity of an infection. This review is important for understanding the abusive and irrational prescription of various groups of antibiotics, often unjustified, including the ones used in the treatment of an infection with SARS-CoV-2.

Conclusions: These infections mostly occur in people aged 65 and older that receive medical care, including antibiotics administration, people with a long-term hospital stay, people with a weakened immune system or with a previous CD infection. The following measures, in order to reduce the risk of CDI in patients, should be considered: hand hygiene, avoidance of unnecessary administration of antibiotics – the antibiotic treatment is recommended only if it is prescribed by an experienced specialist, avoidance of unnecessary administration of drugs that reduce gastric acidity, because it favors the invasion of the gastrointestinal tract with CD.

Key words: *clostridium difficile*, risk factors, treatment options.

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Introduction

Clostridium difficile (CD) is a gram positive, anaerobic and spore-forming bacillus [1]. CD causes a toxin-mediated colitis by expressing the toxin A and toxin B [2, 3] that are encoded by the tcdA and tcdB genes. These toxins lead to intercellular gap cytoskeleton destruction that results in cell death via apoptosis and necrosis [3] and in turn to pseudomembranous colitis [4, 5]. Sometimes a binary *Clostridium difficile* toxin (CDT) is expressed in hypervirulent CD species [2, 5]. The A+B+CDT-genotype was identified in 87.13% of cases in a brief study made in Shanghai (China) [6]. This microorganism may be transmitted within a community, causing a community-acquired *Clostridium difficile* infection (CDI), or within the hospitals, leading to a healthcare-associated CDI [7-10]. A study conducted in Spain revealed that 16% of the CDI were acquired before Intensive Care Unit (ICU) admission or before hospital admission and are commonly more severe [11]. The incidence of CDI is decreasing over the last 10 years in Spain [11]. A study showed that less than 1% of patients developed healthcare-associated CDI, but a high risk of recurrence and complications was associated with a long ICU stay [12].

On the other hand, the incidence and severity of *Clostridium difficile*-associated diarrhea (CDAD) have increased in the United States of America (USA) [13]. Up to 50% of newborns have a CDI in the first 10 days of life, and up to 70% in the first 30 days of life [9]. Other sources inform that up to 35% of infants had CDI within the ICU [10]. It is reported that CD causes 12-12.1% [14, 15] or 21-22.4%, according to another source, [16] of all healthcare-associated infections. The incidence of CDAD in hospitalized patients ranges between 3-29% [17, 18]. The prevalence for CDI in India was 5.6-15.4% [19], in Kuwait – 6.42% [20].

There is a seasonal incidence for CDI, being higher in March-June then in October-December in the Northern Hemisphere and Asia [21]. Approximately 1/3 of the patients who develop antibiotic-associated diarrhea will become infected with CD [22]. Almost one-third of hospital-associated CD infections referred to asymptomatic adult

carriers [9] in which the mortality incidence ranges between 3.4-15.1% [23].

Bacterial antibiotic resistance is a growing global concern [24], this fact being also observed for the CD occurence [25]. ICU patients have a higher risk to develop CDI [26]. Antibiotic-associated diarrhea (AAD) is present in 25-30% of the patients who take antibiotics, which is defined as 3 or more loose stools over 24 h [27].

The purpose of this study is to identify the most important risk factors and treatment options that are currently available for a CDI. The PubMed database was used in order to identify the relevant scientific articles. The following keywords were used: "*Clostridium difficile* in the intensive care unit", March 7, 2020. The study was carried out by analyzing the 100 scientific articles that were identified using the PubMed search engine, after excluding the scientific articles that were published before 2015 and were irrelevant (42 articles), finally 58 scientific articles remained. 13 sources were identified in a non-systematic manner after using the keywords "*Clostridium difficile* diagnostic". The study was performed on the prevalence, incidence and regional specificity of CD management including the risk factors, treatment options and the prevention methods within the ICU.

Results and discussion

Risk factors

The present study identified the following risk factors that were mentioned in one or more sources that were associated with the ICU:

1. Proton pump inhibitors [1, 3, 27-33]. One of the literature sources proves it wrong [14].
2. The use of some antibiotics is proven to be the leading cause of CDI [1, 14, 33-37] as well as administration of antimicrobial drugs [38] or long-term and multiple use of antimicrobials [13, 39, 40]. Clindamycin, cephalosporins, penicillins and fluoroquinolones are the antibiotics that commonly increase the risk of a CDI [3].
3. Advanced age (>65 years) [1, 3, 27, 39] and a long hospital stay [1, 3, 27, 39, 41].
4. Healthy adults, peripartum women and young children that are admitted to hospitals are the most incident carriers of CD [42].
5. Chemotherapy [3, 27] and H_2 receptor blockers [3].
6. Several comorbidities or comorbidity-related conditions, such as an increased serum creatinine level (an indicator of renal dysfunction) at the time of ICU admission [13, 14], admission to a neurological ICU [14], immunocompromising conditions, inflammatory bowel disease (IBD) [13], diabetes mellitus [18, 27], malignant neoplasms [13, 38], cirrhosis, hypoalbuminemia [36], fever, metabolic disorders [40].
7. Surgical intervention is a known risk factor for CDI, at the same time a known treatment option for patients with severe CDI [44], preoperative acute renal failure and postoperative acute hyperglycemia are regarded as isolated risk factors [45].
8. Chronic obstructive pulmonary disease (COPD) was positively associated with CDI in trauma/surgery patients compared with medical patients [41].
9. Positive toxin test, because it may not always indicate a CDI [43].

In addition, were identified unexpected neutral factors:

1. Trauma doesn't increase the CDI incidence [46, 47].
2. Metronidazole was not associated with a high CDI risk [48].

Prevention, diagnosis and treatment methods

The prevention methods involve hand hygiene and reduction of enviormental contamination [33, 49, 50]. Most sources attest that Chlorhexidine has no effect on the incidence of CDI [51], [52], while another source states that it is unclear [39] and only one source has reported a decreasing incidence, with 7% [53]. The probiotics usage is not a widely used preventive measure [33].

The diagnostic methods include the cell cytotoxicity test (CCT), which is the measurement of toxins in the feces or the cytotoxigenic culture (CC) study [54]. RT-PCR is a useful method in order to identify the CD toxins in the stool, though a very expensive one compared to other methods [5]. Nucleic acid amplification test is used in order to identify the CD strains [33, 54] with Glutamate dehydrogenase tests [54].

A strategy for identifying patients with a high risk for CDI is proven to be efficient for reducing the mortality rate in the ICU [55]. Therefore, the physiologic scores used for the assessment of the patients in the ICU may be quite useful – Acute physiology and chronic health evaluation II (APACHE II) [56-60], APACHE III [61], APACHE IV [34], Zar's score [23, 62, 63] and Charlson comorbidity index (CCI) [38, 64-66].

The treatment methods include basic antimicrobial programs and antimicrobial management, the usage of antibiotics (e.g. vancomycin) [67]. The first-line treatment is represented by metronidazole administration, while the second-line treatment is represented by administration of vancomycin or fidaxomycin [3, 33]. Mild or moderate CDI should be treated with oral vancomycin [3] or metronidazole [3, 40, 48, 68], other studies reported no effectiveness for metronidazole [33]. Severe cases were treated with intravenous/ileostoma-administered vancomycin [68]. Vancomycin is administered in 48.3% of cases, whereas metronidazole is administered in 34.5% of cases according to one study [69]. Other sources report that vancomycin usage should be revised [67]. Recent new treatment options involve fecal microbiota transplant [3] which was reported as an effective treatment option [70, 71]. Bezlotoxumab – antibody specific for *Clostridium difficile* is proven to be effective in the treatment of this infection [33].

Conclusions

1. Clostridium difficile remains an actual controversial issue according to the articles reviewed within this study. The present study concluded that the most reported risk factors were the following:

- Antibiotic usage (12 sources);
- Proton pump inhibitors (9 sources);
- Long duration of hospitalization (5 sources);
- Advanced age (4 sources).

2. No adequate or sufficiently proven preventive measures were found, the only exception accounts for the reduction of in-hospital circulation of different doctors and visitors.
3. The following useful tools, for the diagnostic act, were identified – APACHE scales, CCI and the Zar's score. The most efficient, though the most expensive, clinical test is RT-PCR.
4. The most efficient and wide-spread treatment options were as follows:
   - Oral vancomycin and metronidazole given in mild cases, as well as administered through the intravenous/ileostoma route in severe cases;
   - Fecal microbiota transplant.

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