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ORIGINAL RESEARCHES

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Reorganization and resilience of brain networks in focal epilepsy

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Abstract

Background: Epilepsy has been considered as a brain network disorder. Advanced computational tools have granted a non-invasive window to explore the brain networks in epilepsy. Studying the reorganization of brain networks can help in modelling the network topology changes related to focal epilepsy. The present study aimed to explore the reorganization and resilience of brain networks in patients with focal epilepsy.
Material and methods: The structural 3T T1-weighted MR images of 40 patients with focal epilepsy and 40 healthy subjects, were processed by using FreeSurfer. Cortical thickness values were used for the reconstruction of morphometric networks. The topological organization and resilience of brain networks were assessed by applying the graph theoretical analysis.
Results: The topological organization of the brain networks in patients was marked by a higher clustering coefficient, local efficiency and path length (all $p<0.05$) as compared to healthy individuals. The network hubs (i.e. brain regions responsible for network maintenance) were differently distributed in patients (left superior temporal and right paracentral) and healthy subjects (left anterior cingulate and right superior temporal). The brain networks in patients exhibited lower resilience ($p<0.05$) to targeted attacks (i.e. the removal of brain regions depending on their importance for network organization) and similar resilience ($p>0.05$) to random attacks (i.e. random brain area removal).
Conclusions: Brain networks in focal epilepsy were characterized by an increased segregability and a decreased integrability. Reduced resilience to targeted attacks in patients, as compared to healthy subjects, suggests an uneven importance of brain regions for network maintenance in the studied groups.
Key words: epilepsy, brain networks, reorganization, resilience, hubs.

Cite this article
Ciolac D. Reorganization and resilience of brain networks in focal epilepsy. *Mold Med J.* 2020;63(5):5-8. doi: 10.5281/zenodo.4018890.

Introduction

Epilepsy is one of the most common neurological disorders, characterized by susceptibility to generate recurrent seizures. It is widely accepted that focal seizures originate from a brain area and spread along the interconnected tracts to remote regions. However, extensive relevant studies have led to a paradigm shift from the "epileptogenic focus" to the "epileptogenic network". The pathways of interictal discharge propagation involving the thalamo-cortical networks in focal epilepsy have been previously shown [1]. Brain network modelling by using the graph theory is an emerging tool to explore the disease- and brain state-related reorganization processes that mirror the pathological alterations within the epileptogenic networks [2]. At the same time, the mechanisms underlying the vulnerability of networks to recurrent seizures remain poorly understood. A recent work has shown that patients with awake seizures display lower network vulnerability to repeated seizures than patients with sleep seizures [3].

This study aimed to identify the reorganization patterns of brain cortical networks in patients with focal epilepsy. It confirms the hypothesis that epilepsy patients show alterations in cortical networks that implies a higher vulnerability (lower resilience) to recurrent paroxysmal events. Therefore, a reconstruction of cortical networks was carried out, based on cortical thickness measurements from brain magnetic resonance imaging (MRI) and compared to the network topological parameters between the epilepsy patients and healthy subjects. Finally, a random and targeted attack analyses wese performed to assess the resilience of the networks.

Material and methods

Study participants. Forty patients with focal epilepsy (30 ± 6 years; 17 males) were included within the study. Seizure and epilepsy type of the patients were established according to the International League Against Epilepsy criteria [4, 5]. The control group included 40 healthy age- and

gender-matched subjects (28 ± 5 years, 14 males) without any history of neurological disorders. The study protocol was approved by the Ethics Research Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova (notification No 81 of 19.06.2018). All participants were provided with the written informed consent prior to being enrolled in the study.

MRI acquisition. Both patients with epilepsy and healthy subjects underwent a 3T MRI scanning (SIEMENS Skyra, Siemens Healthcare) with a 32-channel head coil according to an approved Epilepsy protocol [1, 3]. This protocol includes 3D T1-weighted (repetition time [TR] = 2000 ms, echo time [TE] = 9 ms, matrix size [MS] = 256 × 256, field of view [FoV] = 256 × 256 mm^2, slice thickness [ST] = 4 mm; T2-weighted (TR = 3800 ms, TE = 117 ms, MS = 256 × 256, FoV = 256 × 256 mm^2, ST = 4 mm) and fluid attenuated inversion recovery (TR = 5000 ms, TE = 388 ms, MS = 256 × 256, FoV = 256 × 256 mm^2, ST = 4 mm).

Image processing and cortical thickness reconstruction. The FreeSurfer software (version 5.3.0, http://surfer.nmr.mgh.harvard.edu/) was used to reconstruct the cortical surface from T1-weighted images. The FreeSurfer pipeline runs in a fully automated fashion, followed by visual inspection at various processing steps for quality control. Briefly, the surface-based processing stream consists of skull stripping, transformation into Talairach space, optimization of boundaries between gray matter and white matter and between gray matter and cerebrospinal fluid, segmentation of subcortical white matter and deep gray matter structures, and tessellation [6]. Cortical thickness at each vertex was calculated (in mm) as the average of the shortest distance between the gray matter-white matter surface and gray matter-cerebrospinal fluid surface. Afterwards, cerebral cortex was parcellated into anatomical labels according to the Desikan-Killiany atlas for regional cortical thickness measurements [7].

Cortical network reconstruction. The cortical thickness from each cortical region of interest (according to Desikan-Killiany atlas) was extracted and served for the construction of cortical connectivity matrices. For both groups, connectivity matrices (size 68 × 68 regions) were obtained by computing the Pearson's correlation coefficient between the anatomical regions. The Graph Analysis Toolbox was used to threshold the matrices into multiple densities, ranging from 0.38 to 0.48, and compute the network measures [8].

Network measures. Topological organization of cortical networks was assessed by computing the following parameters: clustering coefficient, path length, local efficiency and global efficiency [9]. Clustering coefficient represents the measure of network's local organization, which indicates the number of connections between the neighboring nodes. Path length is the minimal number of edges that must be passed to reach the given region (node). Local efficiency reflects the efficiency of neural communication within the network at local level. Global efficiency is the average inverse distance matrix of all brain networks and reflects the global network efficiency. The resilience of cortical networks was evaluated via random and targeted attack analysis.

Statistical analysis. All statistical analyses were performed in MATLAB R2012b (Mathworks, Natick, Mass). The normal distribution of the analyzed variables was assessed by using Shapiro-Wilk test. Assessment of between-group differences in parametric and non-parametric variables was based on t-test, Mann-Whitney U or Pearson's χ^2 tests, where appropriate. A p value of < 0.05 was considered statistically significant.

Results

Patients and healthy controls were comparable in terms of age ($t = 2.1$, $p = 0.23$) and gender ($\chi^2 = 0.04$, $p = 0.82$). Thirty-one patients had temporal and nine patients extra-temporal epilepsy.

The topological organization of brain networks in patients with epilepsy exhibited a higher clustering coefficient, local efficiency and path length (all $p < 0.05$) but lower global efficiency ($t = 2.8$, $p = 0.008$; fig. 1) as compared to the healthy subjects. The network hubs (i .e. brain regions, responsible for the functional maintenance of the whole network) had a different distribution in patients (left superior temporal, right paracentral cortex) and healthy subjects (left rostral anterior cingulate, right superior temporal, right supramarginal cortex).

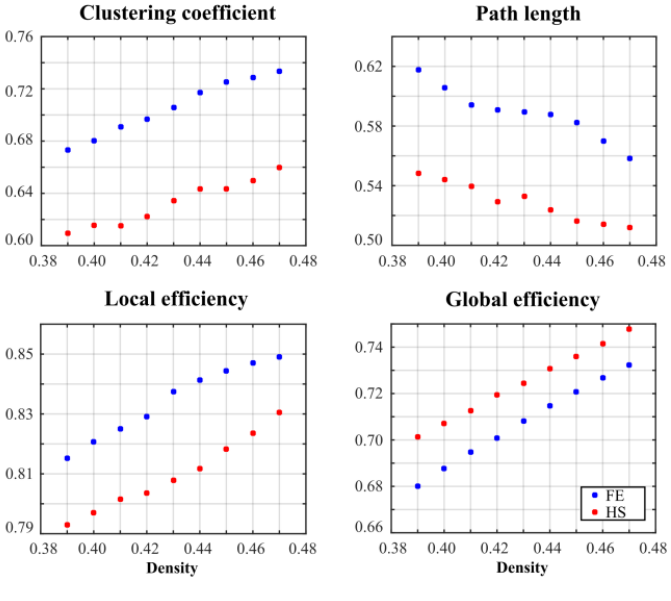

Fig. 1. Network topological parameters.

Mean values across densities of clustering coefficient, path length, local efficiency and global efficiency in patients with focal epilepsy (FE) as compared to healthy subjects (HS)

The brain networks in epilepsy patients were characterized by lower resilience (i.e. higher vulnerability) ($p < 0.05$) to targeted attacks (i.e. removal of brain regions depending on their importance for network organization) and similar resilience ($p > 0.05$) to random attacks (i.e. random removal of brain regions; fig. 2).

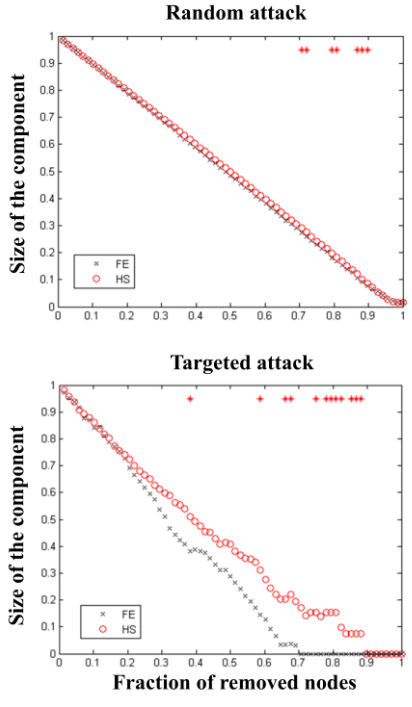

Fig. 2. Network attack analysis.

Patients with focal epilepsy (FE) were comparable to healthy subjects (HS) in terms of random attack but showed reduced resilience to targeted attack

Discussion

The current study analyzed the reorganization of cortical networks based on MRI-derived cortical morphometric measures in patients with focal epilepsy in order to identify the possible network mechanims of network vulnerability. The research results point towards a network reorganization pattern that is characterized by increased segregation (higher clustering coefficient and local efficiency) and decreased integration (higher path length and lower global efficiency). Additionally, patients with focal epilepsy had a different distribution of network hubs. The remodelling of brain networks in focal epilepsy might be due to an increased vulnerability to recurrent seizures as evidenced from the targeted network analysis.

The cortical networks in epilepsy patients displayed higher clustering coefficient, path length and local efficiency. Clustering coefficient is a parameter of network segregation that quantifies the number of connections between the neighbouring nodes [9]. The increased clustering coefficient in patients with focal epilepsy has been previously reported [2, 10]. In conditions of increased clustering coefficient, the network nodes are more likely to be connected to each other in order to maintain the local information processing. Thus, the increased clustering coefficient might be considered as a compensatory increase in the number of local connections as a response to the reduction of long-range connections [11]. The path length is a measure of network's integration and denotes the minimal number of connections that must be traversed to travel from one node to another [9]. An increased path length implies that the networks are less integrated [2]. As shown by Bernhardt et al. [10] the increased clustering coefficient and path length were associated with seizure recurrence after epilepsy surgery. Local efficiency is the average of the inverse distance in the network that describes the efficiency of information processing within a network [9]. Thus, it can be hypothesized that the identified increased local efficiency might also be a compensatory response to long-range disconnections that is directed to maintain local functionality of the network.

Different hubs were identified in patients with focal epilepsy (left superior temporal, right paracentral cortex) compared to healthy subjects. This suggests that along with local network reorganization, hub redistribution also occurs. In patients with temporal lobe epilepsy (TLE), 2 hubs were located in paralimbic and 3 hubs in primary cortical areas (left TLE) and 1 hub was identified in paralimbic and 5 in association areas (right TLE) [10]. The predominant distribution of the hubs in temporal association cortices might stem from the altered connectivity between temporolimbic and extratemporal networks [10].

To investigate the resilience properties of networks in epilepsy, the network attack analysis was performed. This implies virtual random or targeted removing of one node from the network and measuring the network alteration thereafter [8]. Patients with focal epilepsy displayed lower resilience to targeted attacks as compared to healthy subjects, thus suggesting an unequal importance of brain regions for network maintenance in the studied groups. Similar results were reported by Bernhardt et al. [10]. However, another study showed that epilepsy patients had a higher network resilience to random attack and targeted attack than the control group [12]. This might be explained by the study methodological differences – inclusion of children into the analysis and use of different image processing algorithms. It can be assumed that the increased vulnerability of brain networks in epilepsy patients might be the precondition for the recurrent generation of seizures. The altered distribution of the hub together with the increased path length and clustering coefficient may compromise the efficiency of global information transfer [13, 10].

Evaluation of the network measures was found to be useful to predict the clinical outcomes of the epilepsy patients [11]. In patients with TLE, both the decreased clustering coefficient and the increased path length were associated with lower cognitive performance [14]. These results suggest that local and global reduced information processing partially underlie the mechanisms of cognitive decline in TLE [11]. Consequently, these network measures may be used as biomarkers to predict the cognitive status in patients with epilepsy.

Several limitations were encountered within the present study. First, due to the group network analysis, individual values of the network topology were not available, thus, we couldn't relate the seizure frequency to the alterations of network topology. Second, the patients didn't undergo the

neuropsychological tests; hence, their cognitive performance couldn't be correlated with the network reorganization. Thirdly, the network parameters were derived only from structural MRI without analysing the functional data from electroencephalography. Fourthly, patients presented various structural causes of their focal seizures that could in a specific manner impact the reorganization of cortical networks. Patients' antiepileptic drugs could influence the network parameters, as well.

Conclusions

Patients with focal epilepsy show more segregated and less integrated network architecture. The increased vulnerability (reduced resilience) of brain networks in focal epilepsy may stem from the reorganized network topology and serve as mechanism facilitating seizure recurrence. Characterization of network topology reorganization patterns might be an important biomarker to assess individual epilepsy courses and treatment responses.

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Author's contribution
DC designed the study, collected, processed, and interpreted the data and drafted the manuscript.

Ethics approval and consent to participate
The research was approved by the Ethics Research Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 81 of 19.06.2018).

Conflict of Interests
Nothing to disclose.

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Functional features in interstitial lung diseases

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Abstract

Background: Interstitial lung diseases (ILD) are a group of disorders that are generally thought to commonly share a restrictive ventilatory defect and reduced diffusing capacity for carbon monoxide (DLCO). The aim was to find distinctive features of the pulmonary function tests (PFT) results in different types of ILD.
Material and methods: We conducted a retrospective study of 40 consecutive patients with ILD admitted to the Institute of Pthisiopneumology, Chisinau, the Republic of Moldova, during January 2019 – February 2020. The cohort included 10 cases of sarcoidosis patients, 8 cases of idiopathic pulmonary fibrosis (IPF) patients, 7 patients with nonspecific idiopathic interstitial pneumonia, 9 cases with hypersensitivity pneumonitis (HP) and 6 histiocytosis cases. All patients have been evaluated by pulmonary function tests (PFT), 6 minutes walk test, Medical Research Council scale for dyspnea, etc.
Results: Overall, we found normal mean spirometry parameters, a slightly increased mean residual volume (127.5±42.1), a mildly decreased mean total lung capacity (88.8±22.3) and moderately reduced DLCO (52.6±21.5). We found a dominant restrictive pattern in 75% of patients, and obstruction only in 7.5% when we used spirometry parameters. When we applied the bodyplethismographic values, we have found that an *air-trapping* pattern was identified in 32.5% cases of patients. This pattern has been identified in 1/3 of HP patients and in 10% of sarcoidosis patients.
Conclusions: PFT can help identifying individual features of different types of ILD being able to show even obstructive changes in a group of diseases thought to be strictly restrictive.
Key words: interstitial lung diseases, pulmonary function tests, obstruction, restriction.

Cite this article
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Introduction

Interstitial lung diseases (ILD) are defined as a variety of heterogeneous and diffuse parenchymal lung disorders associated with significant morbidity and mortality, sharing similar clinical, radiographical, physiological, or pathological manifestations [1]. These diseases are generally thought to share a common pattern of physiologic abnormality, characterized by a restrictive ventilatory defect and reduced diffusing capacity for carbon monoxide (DLCO) [2, 3].

Conflicting reports have been published regarding small airway function using more sophisticated testing [1, 4-6]. Unfortunately, these abnormalities are not specific for any particular ILD and the magnitude of the changes varies widely from patient to patient. DLCO typically is reduced in ILD to a greater extent than the lung volume at which it is measured. This statement is particularly true with IPF more than any other ILDs [7].

Hypersensitivity pneumonitis (HP) and sarcoidosis are two entities which have as a morphological marker the epithelioid granuloma. This seems to have a certain repercussion over the pulmonary function tests. It is not clear whether it is the granulomatous interstitial inflammation that imposes an obstructive defect in these diseases. Pulmonary function tests in HP typically demonstrate as any other ILD a restrictive defect, but some studies suggest obstructive, or mixed abnormality [8].

Pulmonary function tests (PFT) in sarcoidosis commonly reveal a restrictive pattern as well, with a reduction in the DLCO, although some studies suggested that airflow limitation may be the most common abnormality in newly diagnosed patients [4, 5, 9].

Adult Langerhans' cell histiocytosis (Hx) is a smoke related ILD and has different stages, which start with nodular lesions, which in the end transform into cysts [10]. Considering this, the functional abnormalities can vary in correspondence with the stage and also with the morphological changes. Similar to HP and sarcoidosis, in Hx the inflammation and the fibrotic process has a particular predilection for the peribronchiolar region [11], this is why we would expect that restrictive lesions would combine with air-trapping or air-flow obstruction.

Considering all the above mentioned, the exact physiology of pulmonary ventilation in different types of intersti-

tial lung diseases is unclear, this is why the aim of the study was to find distinctive features of the pulmonary function tests results in different types of ILD.

Material and methods

We have performed a retrospective study based on the data collected from 40 consecutive medical records of patients with ILD admitted to the Phthisiopneumology Ward of the *Chiril Draganiuc* Institute of Phthisiopneumology, Chisinau, the Republic of Moldova, during January 2019 – February 2020.

Patients who fulfilled the following criteria were considered eligible for inclusion in the study: age older than 18; diagnosis of a specific type of ILD established after a multidisciplinary ILD specialists discussion based on suitable clinical, imaging and/or morphology criteria; ILDs from the following list of entities: Idiopathic pulmonary fibrosis (IPF), Nonspecific Interstitial Pneumonia (NSIP), Sarcoidosis, Hypersensitivity pneumonitis (HP), and Adult Langherhans' cell Histiocytosis (Hx). Exclusion criteria were the following: patients with ILD and a high suspicion of a concomitant infectious disease (patients with positive sputum cultures and C reactive protein >20mg/dl); patients with ILD and a concomitant malignancy, patients with an ILD secondary to a collagen disease.

We have collected the following data from the medical records: major pulmonary symptoms, comorbidities, oxygen saturation (Sa02), Medical Research Council (MRC) scale for dyspnea, 6 minute walk test (6MWT), and pulmonary function test results (forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), the ratio FEV1/FVC, residual volume (RV), total lung capacity (TLC), and DLCO).

The statistical analysis was performed using the soft IBM SPSS statistics version 25. We have expressed the results in median and quartiles (for non-continuous variables, such as the MRC score) and the continuous variables (FEV1, FVC, FEV1/FVC, TLC, RV, DLCO) were expressed as mean and standard deviation. For multiple group analysis we used ANOVA test in case of normally distributed variables, and for group discrimination the Tuckey post hock analysis was used. For non-parametric variables we applied the Kruskal Wallis test and the post hock analysis. For calculation of correlation between non-parametric variables we used Spearman rank correlation. The level for statistical significance was $p<0.05$.

Results

After processing the data we have obtained 8 cases of IPF patients, 10 sarcoidosis patients, 7 NSIP patients, 9 HP patients and 6 Hx patients. The mean age in our cohort was 58.95±14.1 years, having the oldest patients (mean 69.7±8.3 years) in the IPF subgroup, and the youngest (mean 38.3±15.6 years) in the Hx group (tab. 1), $p<0.001$. In the general cohort 80% of the patients were older than 50 years.

Table 1

Age distribution through subgroups in the study cohort

Groups	Age Mean±SD
General cohort	58.95±14.11
IPF	69.75±8.26
NSIP	64.86±7.11
Sarcoidosis	61.40±8.81
HP	55.79±12.2
Hx*	38.33±15.58

* – Hx patients were significantly younger than the rest of the subgroups, $p<0.001$

IPF – idiopatic pulmonary fibrosis, **NSIP** – nonspecific indiopatic pneumonia, **HP** – hypersensitivity pneumonitis, **Hx** – Adult Langerhans cell histiocytosis.

In the gender distribution, women slightly prevailed over men (55% vs 45%). Although the gender was distributed statistically homogenous within subgroups ($p = 0.059$), fig. 1 shows a predominance of women in the Sarcoidosis subgroup (7 (70%)) and in HP subgroup (8 (89%)) while in IPF and Hx there was a male predominance.

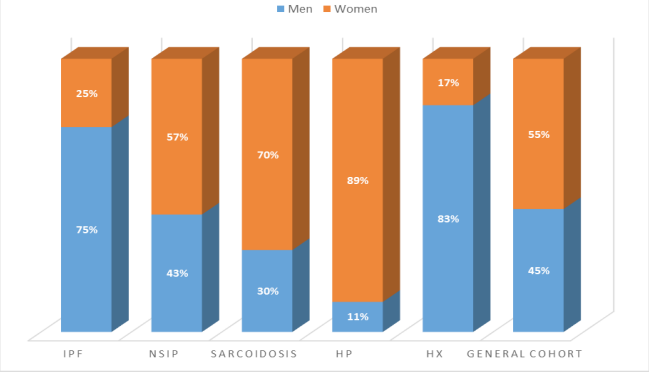

Fig. 1. Gender distribution through groups

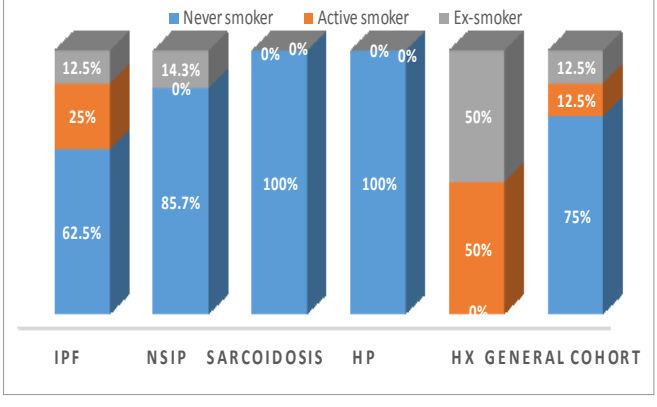

Fig. 2. Smoking status in patients in the general cohort and within subgroups

Since the study analyzes the pulmonary function test, presenting the smoking habits in our cohort is of great interest. As it can be seen from fig. 2, the great majority of our patients were non-smokers (75%). When compared be-

tween groups, there was a statistically significant difference of the prevalence of smokers and ex-smokers from the Hx subgroup (p<0.001) with the rest of them. Other prominent fact is that both sarcoidosis patients and HP patients are 100% never smokers.

Among clinical manifestations of ILD, dry cough is an important symptom, found in 31 (77.5%) cases. Dyspnea is another frequent symptom among patients with ILD. We have checked the impact of dyspnea in different types of ILD using the MRC scale. The median of MRC dyspnea score in the cohort was 3 [2,3]. When compared by subgroups, we found that patients with Hx and patients with sarcoidosis had the same level of dyspnea (p>0.05). Fig. 4 shows that the rank of dyspnea in patients with IPF, HP and NSIP is statistically simmilar and is the most severe. In this way, IPF patients presented significantly more dyspnea when compared to sarcoidosis (p=0.03), or Hx patients (p=0.007). Similarly, HP patients complained of significantly more severe dyspnea when compared to Hx patients (p=0.031) (fig. 3 and 4).

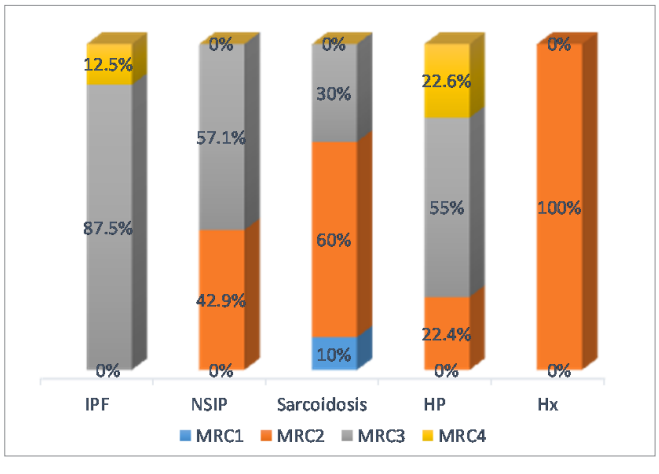

Fig. 3. Dyspnea scores distribution according to MRC scale among groups

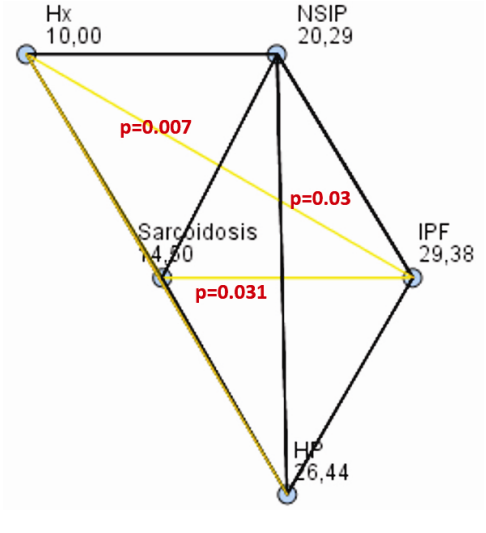

Fig. 4. Pairwise comparison of MRC scale of dyspnea according to groups of ILD

Weight loss of different degree can be also a symptom that may accompany ILD especially in episodes of exacerbation. So, 25 (62.5%) patients didn't experience any modifications in their body weight. Five (12.5%) patients have lost less than 5 kg, 7 (17.5%) patients have lost 5-10 kg, and more than 10kg loss of body weight within a short period of time was documented in 3 cases (7.5%). Both cough and weight loss variables were homogenously distributed among groups (p>0.05).

Analyzing the spectrum of comorbidities (fig. 5), we found that arterial hypertension was the most frequent comorbidity, being registered in almost half of the study cohort (19 (47.5%) patients), diabetes mellitus type II (DM type II) was documented in 10 (25%) cases and ischemic heart disease in 5(12.5%). One of the most severe complications of ILDs that portends a poor prognosis is pulmonary hypertension (PHT), which was found in 7 (17.5%) patients with no predilection for any of the subgroups (p>0.05). Still, PHT was found more frequent in HP patients (33.3%), while in sarcoidosis subgroup we didn't isolate this complication in any of patients. In fig. 5 we can see that arterial hypertension is a frequent comorbidity in the IPF subgroup of patients, probably related to the fact that these are also the oldest patients in our study group. The same age factor (younger age this time) can be attributed to the relative lack of comorbidities in Hx patients. There has been identified a statistically significant difference between the amount of comorbidities in IPF patients vs Hx patients (p = 0.014).

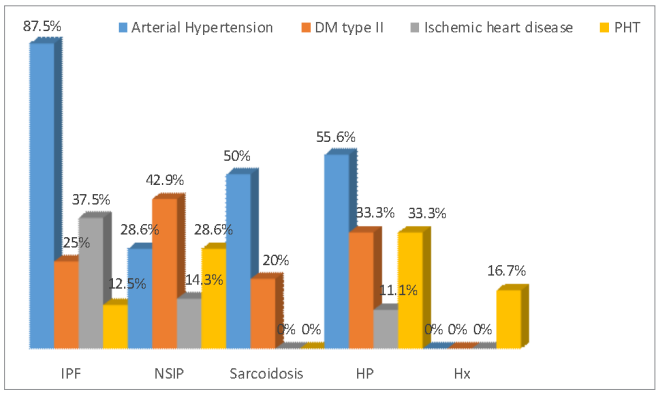

Fig. 5. Comorbidities prevalence within subgroups

In terms of pulmonary function tests we found normal FEV1 (80.8±21.7) and FVC mean values (78.4±21.5), a slightly increased mean RV (127.5±42.1), a mildly decreased mean TLC (88.8±22.3) and a moderately decreased DLCO (52.6±21.5).

According to the ERS/ATS series task force [12], we defined FEV1/FVC below 70% – **as obstructive pattern**, when above 80% – **as restriction**, and between 70% and 80% – **as normal range.** So, based on FEV1/FVC only, we found a predominant restrictive pattern present in 75% of patients from our cohort (fig. 6) with no significant differences among groups (p>0.05).

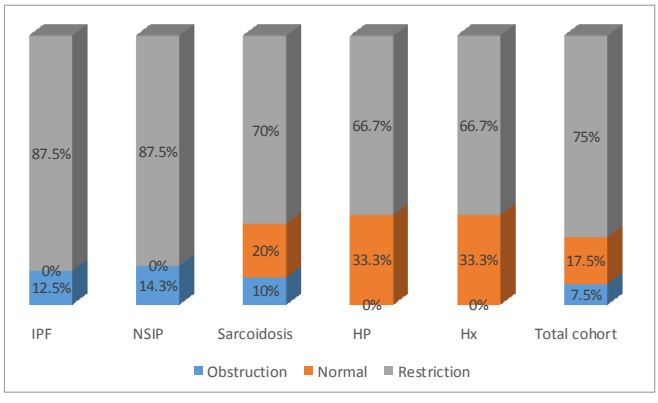

Fig. 6. Distribution of functional patterns based on FEV1/FVC

Similarly, we have analyzed the body-plethysmograph's parameters. We have found the presence of an *air-trapping* pattern, defined as elevated RV [12] (>140% of the predicted) in 13 (32.5%) cases (fig. 7). Hx patients have shown a clear cut statistical difference (p<0.001) in terms of *air-trapping* pattern presence, when compared especially with IPF patients (p<0.001) and with NSIP patients (p<0.002), in which this functional abnormality was absent. The other two diseases have demonstrated a different degree of *air-trapping* pattern, with 10% in the sarcoidosis subgroup and up to 1/3 in HP patients.

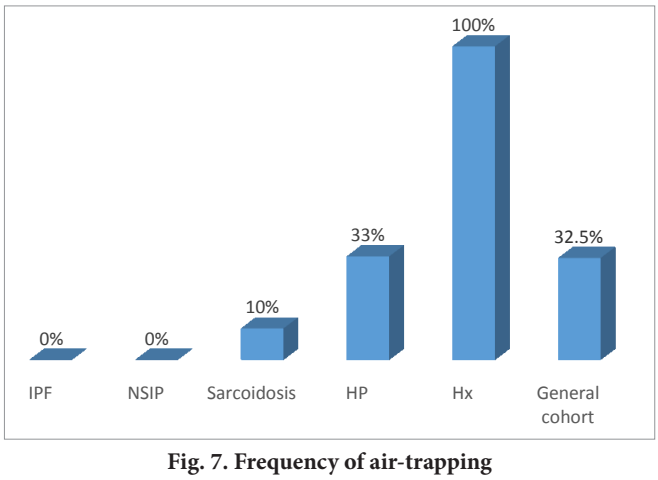

Fig. 7. Frequency of air-trapping

DLCO is one of the most important functional parameters for ILDs. Even though in the general cohort the mean value of DLCO has demonstrated only a mild decrease, we wanted to find out the variation of this parameter within the entities included in the subgroups.

First, we have scaled the severity of DLCO as follows: normal DLCO – values that range between 80 and 140% of the predicted, mild decrease – 80-60%, moderate decrease – 60-40% and severe decrease – less than 40%. In fig. 8 it is shown that we found 2 (5%) patients with normal DLCO in the general cohort, mainly sarcoidosis patients. Within the sarcoidosis subgroup, normal values of DLCO accounted for 20%, while the rest 8 (80%) were patients with mild decrease of DLCO. The highest rate of severely decreased DLCO was registered in the HP subgroup, phenomenon found in 7(78%) patients. We found a statistically significant difference in the distribution of severity of DLCO (p<0.001) between subgroups. The degree of DLCO impairment was significantly different in sarcoidosis patients when compared with IPF subgroup (p=0.042) and HP patients (p<0.001), while IPF patients and NSIP patients had almost identical proportion of categories of DLCO decrease.

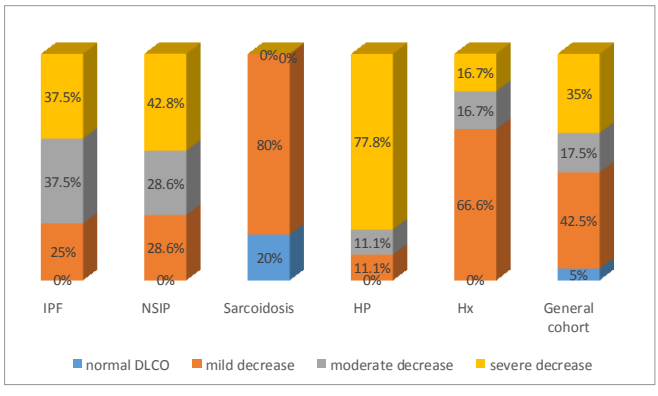

Fig. 8. Distribution of DLCO according to severity

After including the static pulmonary function test, we wanted also to analyze the features of these patients during exercise. So, 6MWT was the easiest test to assess. What we found was a mean of the predicted distance in the general cohort of 67.88±26.7%. So, we have set the cut-off value of 60% of the predicted distance and divided into two categories: under 60% and above 60%. In this way, we have found an acceptable exercise tolerance in 29 (72.5%) cases, while in almost 1/3 the physical tolerance was poor. The biggest proportion of patients with poor exercise tolerance was in the HP subgroup, while the NSIP subgroup had the best results, with statistically significant difference (p=0.041) (fig. 9).

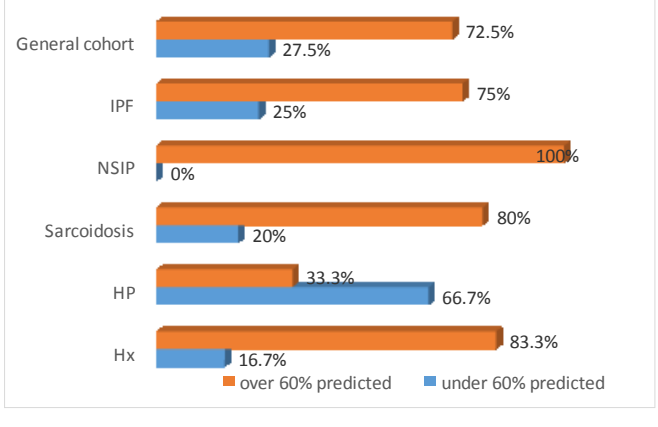

Fig. 9. 6MWT results in the general cohort and within subgroups

Having analyzed all these results, we wanted to find out if any of the clinical features of the ILD patients could reflect the functional abnormalities. Spearman rank correlation analysis (tab. 2) showed that dyspnea score correlates weakly with restriction and inversely with the 6MWT re-

sults, and also a moderate and inverse correlation with DLCO variations. Smoking status as it would be expected correlated weakly with the presence of air trapping changes. Moreover, the correlational analysis established a linear association between PHT and both DLCO and 6MWT.

Table 2

Correlation of clinical parameters with functional abnormalities

	Air-trapping	Restriction	DLCO	6MWT
Dyspnea score	-0.27	0.4*	- 0.57**	-0.43**
Smoking status	0.33*	-0.22	0.03	0.09
Cough	-0.16	0.11	-0.09	0.10
PHT	-0.04	-0.15	-0.43**	-0.45**

– correlation is significant at 0.05 level, ** – correlation is significant at 0.01 level,

PHT – pulmonary hypertension, DLCO – diffusing lung capacity for carbon monoxide, 6MWT – six minute walk test.

Discussion

Interstitial lung diseases commonly share a pattern of physiologic abnormality characterized by a restrictive ventilatory defect and reduced diffusing capacity. Various mechanisms can contribute to these changes, including loss of lung volume, reduced alveolar size, and increased surface tension because of surfactant abnormalities [13]. As a consequence, static lung volumes typically are reduced in ILDs. Our study found that IPF and NSIP in terms of ventilatory changes are very similar, showing a pure restrictive defect. The other 3 entities (sarcoidosis, HP and Hx) besides restriction, showed various degrees and incidence of obstruction. Considering that these 3 diseases share a peribronchiolar predilection of lesions, we supposed that pathological changes in the lung parenchyma and the interstitial space may be reflected in the lung function.

Supporting data also show that airflow limitation can be found in sarcoidosis [4, 9] and in some smoke related interstitial lung diseases, such as histiocytosis [10]. Residual volume is often elevated (likely related to small airways involvement) in sarcoidosis, and hypersensitivity pneumonitis, for example, but is normal or reduced in IPF [14]. In terms of gas exchange, *Boros et al.* stated that DLCO is reduced disproportionately in IPF compared with sarcoidosis, even at comparable lung volumes [15]. We found similar results, having a statistically significant difference between sarcoidosis and both IPF and HP subgroups, while IPF patients and NSIP patients had almost identical proportion of categories of DLCO decrease.

Several authors found that in hypersensitivity pneumonitis the most common finding seems to be reduced DLCO [16, 17]. In our HP patients we found gas exchange impairment in all the cases, with about 3/4 having a severely decreased DLCO. Some publications state that airflow reduction is a common feature for HP, and that it may reflect bronchiolitis, which is a prominent histopathologic feature of acute HP, while emphysema is a common feature among chronic HP patients [2, 18]. Having found the functional *air-trapping* pattern in 1/3 of our HP patients, our results confirm this statement. Moreover, the degree of DLCO reduction could also be explained in our group by the highest prevalence of PHT, an important comorbidity which portends a poor prognosis.

Pulmonary function tests in sarcoidosis typically reveal a restrictive pattern as well, with a reduction in the DLCO, although some studies suggested that airflow limitation may be the most common abnormality in newly diagnosed patients attributed either to narrowing of the bronchial wall because of granulomatous lesions or fibrotic scarring [4, 5, 9], compression by enlarged lymph nodes, airway distortion caused by pulmonary fibrosis, small airway disease, or bronchial hyperreactivity [4, 19, 20].

As we have previously stated in the cases of other entities, in Hx, as well, pulmonary function abnormalities are variable and depend on both the pathological lesions and disease duration [11]. Several studies suggest that, similar to HP and IPF, DLCO reduction in Hx patients is the most common functional abnormality, and can be found in 70–90% of cases [21, 22]. Even though we have registered decreased DLCO in all Hx cases, the degree of severity is significantly different from HP and IPF, having 2/3 of patients with only mildly reduced gas exchange. In fact, in our cohort a common finding was *air-trapping* as well, found in all patients. This could be explained by the fact that Hx is a smoking related disease, and in our subgroup 100% of patients were exposed to smoke, even though studies suggest that the degree of airway obstruction is predominantly related to the bronchiolar location of Hx lesions and not to the amount of total cigarette consumption [21]. In Hx patients, restrictive defect seems to be quite a rare finding [22].

Besides the functional changes, symptoms are a valuable piece in the diagnostic puzzle. We found that IPF, HP and NSIP patients express more symptoms with more severe degree of breathlessness when compared to Hx and sarcoidosis individuals. Our results also showed that the degree of dyspnea is reflected by the gas exchange impairment.

Although we had enough patients to make comparative statistical analysis of the proposed data, our study limitation was the small amount of patients within the subgroups. Due to this fact, we were not able to take into consideration the imaging features, and to identify dominant patterns within the subgroups that would have reflected or possibly predicted the functional disturbances.

Conclusions

Restriction is the dominant functional abnormality of most ILDs, but it coexists in various extents with *air-trapping*, found especially in patients with Hx, HP and sarcoidosis, this finding is related probably to the bronchiolocentric anatomical lesions in these entities. DLCO is almost universally decreased in ILDs, with the lowest levels registered in HP and in IPF patients, while mildly decreased and even normal values can be found in sarcoidosis and in Hx. IPF,

HP and NSIP patients are more dyspneic, while Hx subjects are less symptomatic. Also, IPF patients show more comorbidities, while PHT as a complication is more frequently found in HP. The 6MWT has shown the best results in the NSIP patients and the worst in HP subgroup. Clinical parameters like dyspnea and PHT are directly associated with low DLCO and less walked distance at the 6MWT.

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Authors' contribution
DC acquired, interpreted the data and drafted the first manuscript; DR acquired the data; AD acquired the data; VB designed the trial and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The research was approved by the Ethic Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 18 of November 21, 2017).

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

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S100 protein in molecular subtypes of breast cancer

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Abstract

Background: Cancer research is mainly focused on the tumor cells themselves, the tumor microenvironment being largely neglected. Antigen presenting cells are a heterogeneous population that infiltrates the tumor and can be identified due to the expression of the S100 protein. The aim of this study was to analyze the S100 protein expression (intratumoral vs peritumoral region) in different molecular subtypes, as well as its interrelations with various parameters (such as hormonal receptors expression and HER2 status, patients' age, tumor's grade).
Material and methods: 66 cases of breast carcinomas were examined in terms of their molecular profile (the expression of ER, PR, HER2) and the expression of S100 in the intra- (S100it) and peritumoral areas (S100pt). The data were analyzed using the SPSS program, the values being considered statistically significant in the case of $p < 0.05$.
Results: Maximum numerical values of S100it and S100pt were achieved in case of HER2+ and triple-negative carcinomas, respectively. In the case of luminal A subtype, an inverse correlation was established between S100it and age ($p=0.019$). In the HER2+ subtype, S100it correlated with HER2+ protein expression ($p=0.005$). In the triple negative subtype, the tumor grade influenced S100it ($p=0.022$), and S100it correlated positively with S100pt ($p=0.041$).
Conclusions: The dynamics of S100 positive intratumoral cells is strongly influenced by the HER2 status and age.
Key words: breast carcinoma, S100, HER2, peritumoral stroma, molecular subtypes, dendritic cells.

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Introduction

Cancer research is mainly focused on the tumor cells themselves, the tumor microenvironment being largely neglected. Latest studies suggest that tumors consist not only of neoplastic cells but also of a significantly altered surrounding stroma. Moreover, tumor microenvironment is considered to be a key player for tumor development and progression, as well as a measurable parameter of response to treatment. It is probably a combination of changes in both the epithelial and stromal elements which lead to tumor formation and progression [1].

The breast cancer microenvironment includes multiple cell types, such as fibroblasts, leukocytes, adipocytes, myoepithelial and endothelial cells. It also includes extracellular matrix (ECM), soluble factors (cytokines, hormones, growth factors and enzymes) and physical properties (pH and oxygen content). The interplay between epithelial and stromal cells is essential for the normal development and differentiation of the mammary gland. Physiological stroma maintains epithelial polarity and inhibits uncontrolled cell growth and neoplastic transformation [1, 2]. For example, myoepithelial cells form a natural border which is a semi-continuous protective sheet separating the human breast epithelium and the surrounding stroma. They suppress stromal invasion of tumor cells not only physically, but also by the secretion of various antiangiogenic and anti-invasive factors. Mast cells produce several proangiogenic (VEGFs – vascular endothelial growth factors) and lymphangiogenic factors. In addition, it was shown that VEGFs are chemotactic for mast cells, indicating that mast cells are a target, in addition to be a source for VEGF. Human mast cells produce different matrix metalloproteinases (e.g., MMP-9) and proteases (tryptase and chymase), which regulate the digestion of ECM favoring the migration of cancer cells [3]. Our previous study suggests that intratumoral mast cells increase especially in aggressive tumor types and serve as a worse prognostic factor [4].

Dendritic cells (DCs) are a heterogeneous population of leukocytes and play a crucial role in the initiation of an antitumor response because they are the most potent antigen-presenting cells to T lymphocytes, thus directing them to attack neoplastic cells [1, 5]. DCs are derived from hematopoietic bone marrow progenitor cells. These progenitor cells initially transform into immature dendritic cells, which are characterized by high endocytic activity and low T-cell activation potential [6]. Upon encounter with tumor antigens, immature DCs are induced to mature by inflammatory cytokines and prostaglandins released into the microenvironment. These mature DCs migrate in lymphoid organs where they interact with CD8+ and CD4+ T lymphocytes. They

also are able to stimulate and to generate memory T lymphocytes [5]. However, tumor-associated stroma shows an abundance of immature DCs with altered capacity to stimulate antitumor immunity. Moreover, immature DCs produce proangiogenic factors and increase endothelial cell migration, thus actively promoting tumor growth [1]. Studies revealed that in cancer patients, DCs present abnormalities that make T-cell activation against tumors difficult. On the other hand, the tumor microenvironment releases immunesuppressive factors that make antigen presentation difficult, with a negative impact on the immune response [5]. Despite the significant obstacles that T lymphocytes face in solid tumors, accumulating evidence indicates that natural/ induced/ and/ or engineered immune responses to cancer can dramatically change clinical outcomes [2]. As dendritic cells are considered the strongest stimulators of T-cell responses and play a crucial role in the initiation of primary immune response, different studies have exploited the potential effectiveness of DC-based vaccines in breast cancer [5].

DCs can be identified by immunohistochemistry due to their expression of S100 proteins, a class of protein with emerging roles in human cancers. The first member of the S100 family was documented in the nervous system by Moore et al. in 1965 and the name refers its nature of a soluble protein in saturated ammonium sulfate**.** It is a multigenic family of Ca^{2+} binding proteins comprising at least 20 members. These proteins exhibit a high degree of structural similarity, but are not functionally interchangeable. It is well documented that S100 proteins have a broad range of intracellular and extracellular functions, and are implicated in multiple biological functions, including cell division, motility, secretion, protein synthesis, and membrane permeability [7-10]. The aim of this study was to analyze the S100 protein expression (intratumoral vs peritumoral region) in different molecular subtypes of breast cancer, as well as its interrelations with various parameters, such as hormonal receptors expression and HER2 status, patients' age, tumor's grade.

Material and methods

66 cases of breast carcinomas were collected at Arad Clinical Hospital, Romania between 2013-2016. Mean age of patients was 64.9 years (range 37–83). All patients did not undergo chemo- or radiotherapy before surgery. Clinical data were obtained from the medical records of each patient. The current research is a part of a larger study of stromal changes in molecular subtypes of breast cancer that was approved by the Ethics Committee of Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, Moldova (no 33/ 37/ 12.02.2018).

Histological method. Specimens were obtained after surgery, fixed in 10% formalin and paraffin embedded (Paraplast High Melt, Leica Biosystems). Paraffin blocks were later used for creation of tissue microarrays by means of TMA Grand Master (3DHISTECH Ltd., Budapest, Hungary). Sections from these blocks were cut by using a Leica RM2245 microtome (Leica Biosystems, Newcastle UponTyne, UK) and mounted on glass slides (Surgipath X-tra Adhesive, Leica Biosystems, Newcastle UponTyne, UK).

Staining was accomplished by Leica Autostainer XL (Leica Biosystems, Newcastle UponTyne, UK). Mayer's hematoxylin (Merck, Germany) and aqueous eosin (Merck, Germany) were used. Slides were mounted automatically (Leica CV5030, Leica Biosystems, Newcastle UponTyne, UK). Tumor histology was reviewed by 3 independent pathologists and suitable sections were selected for immunohistochemical stains.

Immunohistochemistry. Immunohistochemical staining was performed automatically by Leica Bond-Max (Leica Biosystems, Newcastle UponTyne, UK). For staining, antigen retrieval was carried out using the Bond Epitope Retrieval Solution 1 (pH 6) and 2 (pH 9) (Leica Biosystems, Newcastle UponTyne, UK). Primary antibody (ER, PR, HER2, S100) was followed by 3% hydrogen peroxide in order to quench endogenous peroxidase activity. DAB (3, 3'-diaminobenzidine) was applied as a chromogen substrate for 10 minutes. Mayer's hematoxylin was the additional dye used for counterstaining (5 minutes). Then sections were placed in absolute alcohol for 5 minutes, dried and clarified in benzene for 5 minutes. Lastly, slides were mounted automatically (Leica CV5030, Leica Biosystems, Newcastle UponTyne, UK) using an ENTELLAN–like mounting medium (Leica CV Mount, Leica Biosystems, Newcastle UponTyne, UK).

Methods of quantification. Hormone receptors (ER – estrogen receptor and PR – progesterone receptor) were evaluated according to Allred score. This score accounts for the percentage of cells that test positive for hormone receptors, along with the intensity of staining [11]. HER2 protein was appreciated according to the recommendations of American Society of Clinical Oncology [12].

S100 requires cytoplasmic and nuclear staining for positive diagnosis. Positive staining is normal in case of neurons, Schwann cells, melanocytes, glial cells, myoepithelial cells, adipocytes, Langerhans cells, tissue dendritic cells and interdigitating dendritic cells, chondrocytes and notochordal cells [8].

Quantification of brown stained DCs was done by means of Axio Imager A2 microscope (Carl Zeiss, Germany). Sections were initially analyzed at a ×100 magnification in order to determine the most intensely stained regions. Then we analyzed intratumoral and peritumoral stroma, 2 microscopic fields for each one, at a ×400 magnification and counted DCs. The final value was the arithmetic mean of the values for the two fields. Expression was graded by two independent observers who were blinded to the patient's information.

Data analysis. We used a MS Excel 2010 database to store the data that were statistically analyzed using the SPSS statistical software package (SPSS Statistics 23.0; IBM, Chicago, IL, USA). We used Pearson's correlation coefficient (r) and in all analyses, p values <0.05 were considered significant.

Results

Most of tumors (46 cases out of 66/ 69.7%) were moderately differentiated (G2). 19 cases (28.8%) were poorly differentiated (G3) and only 1 case (1.5%) was well differentiated. We established the following molecular subtypes: luminal A (15 cases/ 22.7%), luminal B/ HER2+ (30 cases/ 45.5%), luminal B/HER2 – (2 cases/ 3%), HER2+ (8 cases/ 12.1%) and triple-negative (11 cases/ 16.7%). Histologically, we identified 60 cases of ductal invasive, 1 case of ductal *in situ,* 3 cases of lobular infiltrative and 2 cases of lobular *in situ* carcinomas.

We identified brown stained S100 positive cells in all the slides. In normal breast tissue adjacent to the tumor S100 protein expression was detected in a variety of structures: myoepithelial cells, adipocytes, nerves. These were used for internal positive control. Peritumoral DCs were usually accompanied by lymphocytes and had an irregular shape with a lot of cytoplasmic processes. They had a strong staining. Intratumoral DCs were less stained and had a foamy cytoplasm.

Intratumoral DCs were most numerous in case of HER2+ molecular subtype (maximum numerical value – 80.6). Peritumoral DCs were most numerous in the triple-negative subtype (maximum numerical number – 66.0).

For luminal A subtype, statistical analysis revealed a negative correlation between S100it and age (p=0.019, r=-0.594). In case of HER2+ subtype, S100it negatively correlated with the expression of HER2 protein (p=0.005, r=-871). In triple-negative carcinomas, S100it inversely correlated with tumor's grade (p=0.022, r=-0.678) and positively correlated with S100pt (p=0.041, r=0.621).

In G2 tumors, S100it negatively correlated with age (p=0.041, r=-0.302), while in G3 tumors S100it positively correlated with the molecular subtype (p=0.048, r=0.459).

Discussion

Breast cancer is the most common type of cancer among women. Despite the huge improvement in its outcome approximately 20–30% of patients still relapse, even many years after diagnosis [5]. Moreover, breast cancer remains one of the most enigmatic and poorly predictable cancers in its evolution due to the elevated biological heterogeneity along with varied responses to therapies across patients [6]. Thus, new biomarkers useful in clinical setting and for breast cancer management are coming up to explore [7].

Despite the promising potential of the S100 family as a biomarker panel, there are few studies that analyzed the interplay between the expression of S100 protein and different clinical parameters.

Masuda et al. showed that that expression of S100A2 (a member of S100 family) mRNA in colorectal cancer is significantly higher in cancerous tissue than in neighboring non-neoplastic tissue. The overexpression of S100A2 in colorectal cancer cells was associated with significantly worse overall survival and could be a biomarker of poor prognosis in stage II and III colorectal cancer recurrence. Their results suggest also the potential of the S100A2 protein as a target for molecular-targeted drugs for colorectal cancer [13]. This is supported by the idea that immunotherapy is an emerging and increasingly promising approach to treat cancer [2].

In lung adenocarcinoma, the expression of S100 proteins was higher in neoplastic cells than in bronchiolar epithelial cells. According to Tetsukan et al., S100A11 levels were significantly higher in adenocarcinomas with KRAS (Kirsten rat sarcoma viral oncogene homolog) gene mutations and strong proliferating activity. Their results suggested that the upregulation of S100A11 was involved in tumor progression and correlated with shorter disease-free survival [14].

As of breast cancer, Cancemi et al. demonstrated that patients which developed distant metastases showed a general tendency of higher S100 protein expression, compared to the disease-free group. They also found significantly higher S100 expression levels in ER negative tumors, in higher grade tumors and in basal-like and HER2 tumors, while lower S100 expression levels were found in Luminal A and Luminal B tumors [7].

Pedersen et al. found that high levels of S100A4 significantly correlated with histological grade and loss of estrogen receptor, but not to the time interval between surgery and development of distant metastasis or to patient's survival. They also demonstrated a significant correlation between the S100A4 immunoreactivity and the high histological grade. S100A4 staining was not correlated to the patients' age at the time of presentation, PR, lymph node involvement or tumor diameter [15]. Our study showed an inverse correlation between S100it and patients' age. However, the cited studies analyzed different S100 family members, while we payed attention to localization of S100 positive cells, thereby intratumoral and peritumoral areas.

Conclusions

S100 positive cells are more numerous in hormone-negative tumors (HER2+ and triple-negative molecular subtypes). The dynamics of S100 positive intratumoral cells is strongly influenced by the HER2 status and age.

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EC acquired, interpreted the data and drafted the first manuscript. The author revised manuscript critically and approved the final version.

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Ethics approval
This study was approved by the Ethics Committee of Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, Moldova (No 33/ 37/ 12.02.2018).

Conflict of interests
No competing interests were disclosed.

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Assessment of bronchiectasis in adult HIV/AIDS patients

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Abstract

Background: Immunodeficiencies should be suspected in cases of primary identified bronchiectasis in adults. Moldova is among the countries with a continuous increase in the number of HIV-infected adults. Impaired immune system and chronic inflammation contribute to the progression of bronchiectasis in HIV patients. The aim of the study was to present the clinical, imaging, bacteriological peculiarities and outcomes in adult patients with bronchiectasis and HIV/AIDS infection.

Material and methods: This case series involved 11 patients with HIV/AIDS and bronchiectasis, selected from a prospective study conducted on 490 patients diagnosed with non-cystic fibrosis bronchiectasis in a tertiary care hospital, between 2015–2019. Clinical, microbiological and radiological data, associated comorbidities and severity scores were analysed. Statistical analysis was performed using the SPSS 23 program.

Results: The mean age was 39 years (range 25-65 years), with a male predominance (54%). A CD4 count <200 *cells/mm*^{3} was identified in 6 cases. The mReiff score (6.8±4.6) showed a significant correlation with Bhalla score (9.72±4.5), r=0.66 (p<0.05). BSI score (11.7±3) reflects better the severity of the disease, showing a significant correlation with the Bhalla imaging score (r=0.62, p<0.05). Assessing the impact of comorbidities (BACI index 5.4±4.3 and Charlson index 6.9±1.3), the BACI index better reflected the severity of the disease in this group of patients, demonstrating a strong correlation with BSI (r =0.62, p<0.05). Only 3 patients (27%) were over 1-year follow-up.

Conclusions: Bronchiectasis is one of the common pulmonary manifestations of HIV/AIDS infection, being responsible for a number of chronic respiratory symptoms and the risk of premature death.

Key words: bronchiectasis, HIV/AIDS, mReiff score, Bhalla score, Charlson index, BACI index.

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Introduction

Recently, the Republic of Moldova is placed among the countries, which show a continuous increase in the number of adults infected with human immunodeficiency virus (HIV). Rates of newly-diagnosed HIV infections vary widely across countries of Europe, Moldova being ranked the 4th after Russia, Ukraine and Belarus (in 2018 – 22.3 per 100 000 population; 905 new cases) according to WHO data [1, 2]. There are alarming data regarding the delayed diagnosis (at the stage of acquired immunodeficiency syndrome), involving a large number of patients with CD4 T lymphocyte levels below 200/*mm*^{3}. In 2018 out of 696 primary identified cases, 85% (592 patients) were tested for CD4 level, of whom 223 were at the last HIV infection stage with CD4 below 200/*mm*^{3} [2]. Lung infections are among the main manifestations for which patients seek medical advice, it is also a common cause of mortality in AIDS (acquired immunodeficiency syndrome) cases.

Both primary and secondary immunodeficiencies should be suspected in all cases of identified primary bronchiectasis, especially in adult patients under 40 years of age [3, 4]. Untreated HIV infection is characterized by a progressive decrease level of helper T lymphocytes (CD4). Immune system damage accompanied by chronic inflammation contributes to the progression of bronchiectasis in HIV patients [5, 6], although in adult patients evidence is provided in favour of multifactorial involvement in both etiology and progression of bronchial wall lesions [5, 7]. More publications come up with arguments that show damage not only to the immune response (from helper T lymphocyte deficiency, impaired local response of macrophages and monocytes), but also the direct effect on bronchial walls due to intercurrent infections (pneumonia or tuberculosis) and the association of chronic obstructive pulmonary disease in adult patients with bronchiectasis and HIV infection [5, 7, 8].

The study aims to present the clinical, imaging, bacteriological peculiarities and outcomes in adult patients with bronchiectasis and HIV/AIDS infection.

Material and methods

A series of cases diagnosed with HIV/AIDS infection and bronchiectasis selected from the prospective study that included a group of 490 adult patients with non-cystic fibrosis (NCF) bronchiectasis evaluated at *Chiril Draganiuc* Hospital during 2015-2019. Each patient signed the informed consent. Demographics, clinical characteristics,

biological, imaging, and microbiological data (bacteriological examinations of sputum and bronchoalveolar lavage) were obtained and summarized in tables 1-4. The disease outcomes were analysed up to 1 year after being included in the study. HIV infection was confirmed by the positive WESTERN BLOT test, performed after obtaining two HIV-positive ELISA tests. All patients underwent the thoracic high-resolution computed tomography (HRCT) scan that assessed the morphology of bronchial dilatations (cylindrical, varicose and cystic), their distribution and the associated imaging lesions (cavities, calcification of lymph nodes, parenchymal calcifications, interstitial or consolidation syndromes). The Reiff (modified Reiff) [9, 10] and Bhalla [11] imaging scores were calculated. Bronchiectasis severity was evaluated using BSI (Bronchiectasis Severity Index) and FACED scores [12, 13]. Imaging signs of pulmonary hypertension on thoracic HRCT were also evaluated. Validated measuring instruments were used to assess comorbidities and their impact on the evolution of the disease, namely the Charlson Comorbidity Index [14] and the BACI index (Bronchiectasis Aetiology and CO-Morbidity Index) [15].

Table 1

Demographic characteristics and comorbidities of HIV/AIDS patients with bronchiectasis

	Age /Gender / Environment U/R	BMI, kg/m^2	Smoker/SI	Migrant worker	Comorbidities
Pt. 1	26/M/R	17.3	Yes/10 p/y	Yes, Russia	Oropharyngeal candidiasis Wasting syndrome
Pt. 2	42/F/R	17.3	No	husband was a migrant worker in Russia	Mastoidectomy Kidney stones Anaemia Keratitis Pulmonary cryptococcosis Wasting syndrome
Pt. 3	35/F/R	19.8	No	No	Pneumocystis pneumonia Multiple lung abscesses Anaemia
Pt. 4	32/F/U	11.4	Yes/15p/y	Yes, Russia	Anaemia Stomatitis Cutaneous mycosis Wasting syndrome
Pt. 5	22/M/R	20.1	No	No	Anaemia Pneumocystis pneumonia
Pt. 6	27/M/R	16	No	No	Anaemia Pneumocystis pneumonia Wasting syndrome Esophageal candidiasis
Pt. 7	65/F/R	22.6	Yes/20p/y	No	Pulmonary cryptococcosis Anaemia
Pt. 8	28/M/R	14.5	Yes/8p/y	Yes, Russia	Pulmonary cryptococcosis Anaemia Pneumocystis pneumonia Wasting syndrome Kaposi's sarcoma
Pt. 9	33/M/R	20	Yes/8p/y	Yes, Greece	Oropharyngeal candidiasis Pneumocystis pneumonia Anaemia
Pt.10	46/M/U	16.3	Yes/15 p/y	Yes, Russia	Anaemia Oropharyngeal candidiasis Pulmonary aspergillosis Pulmonary tuberculosis Wasting syndrome
Pt.11	35/F/R	16.3	No	Yes, Russia	Anaemia Oropharyngeal candidiasis Wasting syndrome Pneumocystis pneumonia

Note: U – urban, R – rural; SI – smoking index; p/y – pack/years.

Results

The prevalence of patients with bronchiectasis and HIV/AIDS infection in the group of patients with non-cystic fibrosis bronchiectasis (490 patients) was 2% (11 patients). The mean age was 39.2±13.5 (26-65) years, 63% – under 35 years old, 6 male patients (54%). No patient was a drug user, whereas 82% came from rural areas and 73% (8 patients) were married. Migrant worker status was identified in 64% of cases (tab. 1), migration to Russia being the most frequently reported (6 patients). All patients were hospitalized at least once during the last year in the departments of district medical facilities due to respiratory infections of unspecified etiology and diarrheic syndrome of unspecified etiology. Only two patients knew their HIV-infected status (though didn't use antiretroviral treatment) at the time of being included in our study, 82% have been primarily diagnosed with HIV/AIDS. All patients were at the last stage of HIV infection (clinical category C) with multiple comorbidities (tab. 2) and severe weight deficit, the mean BMI (body mass index) being of 17.4±3.4 kg/m^2. Only 3 patients (27%) had a BMI above 18.5 kg/m^2 (tab. 1). Out of 7 patients in whom the level of CD4 lymphocytes was evaluated (mean 82±125/ mm^3, range 4-350), 6 patients had a level below 200/mm^3 (3 cases had a critical level ≤ 5/mm^3 and also had more severe lung lesions). Cough with purulent sputum was identified in all cases (7 patients reported amounts of more than 50 *ml* of sputum/24 *hours*) and 4 patients had several episodes of hemoptysis. Most cases showed significant co-morbid conditions. Anaemia was found in 10 patients (91%), the most severe cases (haemoglobin level below 70 *g/l*) were associated with more extensive lung changes and chronic diarrhea.

The assessment of the impact of comorbidities on the severity of bronchiectasis revealed the BACI index 5.4±4.3 and the Charlson index 6.9±1.3. The BACI index better reflects the severity of the disease in patients with bronchiectasis and HIV/AIDS infection demonstrating a strong correlation with BSI (r=0.62, p <0.05).

The analysis of the thoracic HRCT scans showed an extremely polymorphic nature of the imaging lesions (tab. 3) at the time of inclusion in the study. The predominance of tubular (cylindrical) bronchiectasis was recorded (fig. 1-3). Six patients presenting exclusively cylindrical type of bronchiectasis, localized in all lobes revealed a 6-pont mReiff score and only 4 patients (36%) presented a score below 6 points. The severity of bronchiectasis extension was highlighted by the mReiff imaging score (6.8±4.6 points), which, although simplified, showed a strong correlation with the Bhalla score (9.72±4.5; r=0.66; p <0.05).

Bronchiolitis imaging features were present in 5 patients (fig. 1, 3). Only one patient presented lung parenchyma calcifications, and another one showed calcifications in the bronchial walls (fig. 3). Imaging signs of pulmonary hypertension (HTP) with pulmonary artery trunk diameter over 27 *mm* and pulmonary artery diameter ratio to ascending aortic diameter above 0.9 were present in 3 patients (fig. 4). Analysing the BSI (11.7±3) and FACED (2.9±0.9) severity scores, BSI better reflects the severity of the disease in this etiological group of NCF bronchiectasis, and a good correlation with the Bhalla imaging score was demonstrated (r=0.62, p <0.05).

Imaging signs suggestive of pneumonia caused by *Pneumocystis jiroveci* were identified in 7 patients (fig. 1), whereas the bacteriological confirmation was obtained only

Table 2

Individual characteristics and outcomes of HIV-infected patients with bronchiectasis

	HIV infection detected in the current hospitalization	CD4 cells/mm³	Duration of hospitalization/ antiretroviral treatment initiated during hospitalization	*Deceased (D)/Survived (S)
Pt. 1	Yes	N/D	7 days/No	S
Pt. 2	Yes	N/D	14 days /No	S
Pt. 3	No	5	36 days /No	D
Pt. 4	Yes	5	12 days /No	D
Pt. 5	Yes	122	18 days /No	D
Pt. 6	Yes	N/D	7 days /No	D
Pt. 7	Yes	N/D	29 days /No	D
Pt. 8	No	4	38 days /Yes	D
Pt. 9	Yes	39	21 days /Yes	S
Pt.10	No, he has known about HIV status for 3 years	350	32 days /No	D
Pt.11	Yes	50	8 days /No	D

Note: N/D – No data

* – Deceased (D)/Survived (S) one year after being included in the study.

in one case (tab. 3, 4). It is still challenging to identify the pathogens responsible for pulmonary infections in severely immunocompromised patients, requiring the exclusion of opportunistic infections, fungal infections, mycobacteria, and viruses in addition to Gram-positive and Gram-negative pathogenic bacteria.

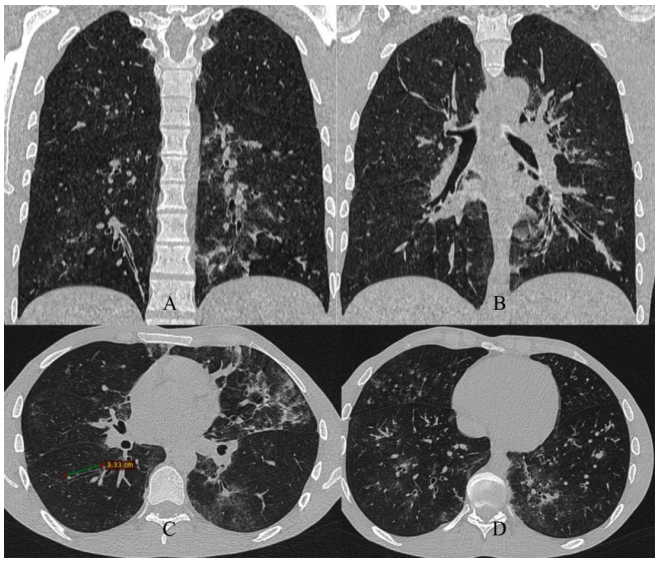

Fig. 1. Chest HRCT images of patient 1

Thoracic high-resolution CT in coronal (A, B) and axial (C, D) reconstructions of a 26-year-old man primarily diagnosed with HIV/AIDS shows bilateral cylindrical bronchiectasis and bronchial wall thickening that is more prominent in the lower lobes. A number of areas demonstrating the "signet ring" and the "tram tracks" signs, or lack of tapering when viewed in longitudinal cross-section (some of them are amputated due to endobronchial secretions). The presence of "ground glass" opacities was recorded that might be the imaging expression of an opportunistic infection (*P. jiroveci*) that was not actually confirmed in this case.

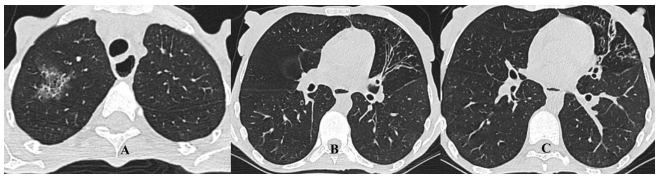

Fig. 2. Chest HRCT images of patient 4

Axial lung window HRCT sections (A, B, C) of a 32-year-old woman showing more severe bronchial dilatations (varicose and cystic) in the lingual segments (C). Tubular bronchiectasis was identified in the right upper lobe (A) as well as in the immediately subpleural areas of the left lung. The hypotrophy of the muscle and of the subcutaneous adipose tissue at the level of the thorax could be seen, the patient being with the lowest BMI 11 kg/m^2 (33 *kg* at a height of 175 *cm*) among all the patients included in the study. There was an evidence of dilation of the oesophagus (A).

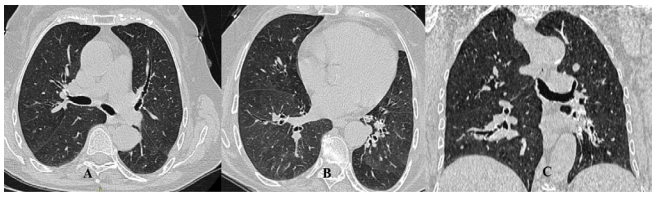

Fig. 3. Chest HRCT images of patient 7

HRCT images of the chest in the oldest patient aged 65 from this series, diagnosed primarily with HIV/AIDS infection, showing bilateral tubular bronchiectasis. There are focal areas of decreased attenuation with a mosaic aspect of the lung fields (B, C), findings consistent with constrictive obliterative bronchiolitis. Bronchial walls and aortic arch calcifications were recorded (A, C).

Table 3

Distribution of bronchiectasis and associated imaging lesions

	Bronchiectasis distribution /morphological type	m Reiff score	Bhalla score	Imaging signs of bronchiolitis	Consolidation syndrome	Interstitial syndrome	PA:Ao ratio > 0.9
Pt. 1	RUL, RML, RLL, LUL, lingula, LLL/ tubular	6	7	Yes	No	Yes	No
Pt. 2	RUL, RML, RLL, LUL, lingula, LLL/ tubular	6	7	No	No	No	No
Pt. 3	RUL, RML, RLL, LUL, lingula, LLL/ tubular, cystic	18	14	No	No	No	Yes
Pt. 4	RUL, RML, RLL, LUL, lingula, LLL/ tubular, cystic	8	16	No	Yes	Yes	No
Pt. 5	RUL, RML, RLL, LUL, lingula, LLL/ tubular	6	6	Yes	No	Yes	No
Pt. 6	RLL, LUL, lingula, LLL/ tubular, cystic	6	10	No	Yes	Yes	No
Pt. 7	RLL, lingula, LLL/ tubular	3	10	Yes	No	Yes	No
Pt. 8	RUL, RLL, LLL/ tubular, cystic	5	13	Yes	No	No	No
Pt. 9	RUL, LUL/ tubular	2	4	No	Yes	Yes	No
Pt.10	RUL, RML, RLL, lingula, LLL/ tubular, cystic	12	16	Yes	No	No	Yes
Pt.11	RUL, RML, LUL/ tubular	3	4	No	No	Yes	Yes

Note: RUL – right upper lobe, RML – right middle lobe, RLL – right lower lobe, LUL – left upper lobe, LLL – left lower lobe, PHT – pulmonary hypertension, CT – computer tomography, PA:Ao ratio – ratio of pulmonary artery diameter to ascending aortic diameter.

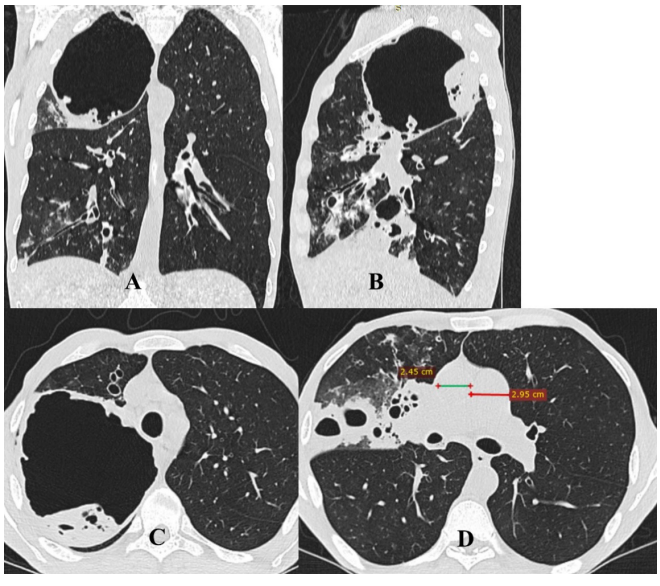

Fig. 4. Chest HRCT images of patient 10

Chest HRCT images of a 46-year-old patient, being HIV- infected for 3 years (without antiretroviral therapy) showed an extensive lung damage, the upper right lobe being replaced by a cavity (A-C), with an intracavitary content, (suggestive of *fungus ball*). Multiple tubular and cystic bronchiectasis, some with hydroaeric level, more prominent on the right. Dilatation of the pulmonary artery (2.95 *cm*) was noted in relation to the ascending aorta (D). Ratio of pulmonary artery diameter to ascending aortic diameter was 1.2 (> 0.9 suggestive of pulmonary hypertension).

Bacteriological confirmation of *M. tuberculosis* infection was confirmed in none of the suspected patients; however, in 4 cases presenting cavitary lesions and/or consolidations, the diagnosis of pulmonary tuberculosis was established based on clinical and radiological criteria. Two patients could not tolerate the anti-tuberculosis medication due to its side effects. Fungal infections viz. *Cryptococcus neoformans* was identified in 3 cases and *Candida spp*- in 7 cases (tab. 4).

Only 3 patients (27%) survived one year after being included in the study and continue the antiretroviral treatment (ART) and 2 patients died during hospitalization (patient 7 and patient 10).

Discussion

The prevalence of patients with immunodeficiencies in the aetiological structure of NCF bronchiectasis cases varies from 6% to 14% in adults, being higher among children 20–34% [16]. Recurrent respiratory tract infections, both viral and bacterial, along with HIV infection cause a decrease in innate immunity (due to a progressive loss of CD4 cells), which yields a persistent inflammatory state in the lower respiratory tract. Furthermore, in these patients, HIV infection may cause an inflammatory obliterative bronchiolitis, which would facilitate and contribute to the remodelling of the airways and the development of bronchiectasis [17].

Bronchiectasis is a progressive airway disease, anatomically defined by abnormal and progressive dilation of the bronchi, clinically manifested by persistent cough, sputum production, and recurrent respiratory tract infections, that are considered one of the most important aetiological factors in the development of bronchiectasis in HIV infected patients [17]. Microbial toxins and persistent inflammation compromise mucociliary clearance, which leads to increased susceptibility for microbial colonization, and

Table 4

Microbiology data and diagnosticated opportunistic infections

	Sputum cultures results	Bacteriological confirmation of *M. tuberculosis*	Clinically and radiologically diagnosed TB case	Clinically and radiologically diagnosed Pneumocystis pneumonia
Pt. 1	*Candida albicans*	No	No	No
Pt. 2	*Cryptococcus neoformans* *Candida albicans*	No	No	No
Pt. 3	*S. aureus* *E. coli* *Candida albicans*	No	Yes, anti-TB treatment not tolerated	Yes
Pt. 4	*Streptococcus gr D*	No	Yes, anti-TB treatment refused	No
Pt. 5	*Streptococcus gr D*	No	No	Yes
Pt. 6	*Kl. pneumoniae* *Candida krusei*	No	No	Yes
Pt. 7	*Cryptococcus neoformans*	No	No	Yes
Pt. 8	*Cryptococcus neoformans* *Citrobacter freundii*	No	Yes	Yes
Pt. 9	*Pseudomonas aeruginosa* *E. coli* *Candida albicans*	No	No	Yes
Pt.10	*Moraxella*	No	Yes	No
Pt.11	*Candida albicans* *Pneumocystis jiroveci*	No	No	Yes

create a self-perpetuating cycle [18]. Malnutrition, aspiration pneumonitis due to gastroesophageal reflux disease, esophageal candidiasis are among the multiple factors that have been incriminated to complement the appearance of bronchiectasis in this group of patients [18-20].

Patients with HIV infection became a common reality in daily clinical practice of a general physician and of a pneumologist as well. As the life expectancy of HIV-infected patients increased due to prevention and treatment of opportunistic infections, an early recognition of bronchiectasis and its associated features may have an important role for disease outcome. The key element for the diagnosis of bronchiectasis is the imaging technique. Thoracic HRCT scan, replacing the more invasive bronchography, is considered more sensitive to identify and assess the severity of bronchiectasis.

Plenty of scientific papers have been published, describing pulmonary complications in HIV-infected patients, most of them including advanced immunosuppression cases, have focused on the evaluation and treatment of infections with opportunistic germs. First studies presenting bronchiectasis in HIV-infected patients included a small series of cases, Holmes and co-authors published the first 5 cases in 1992 [5, 21, 22]. Several authors have concluded that acute bronchitis is the most common pulmonary manifestation in HIV-infected patients and retrospective studies have shown that in AIDS patients bronchiectasis has a higher incidence [19, 23]. Similar to the results from the case series analysed by McGuinness (12 patients) [22] in our study, the airway lesions, as well as those identified in the lung parenchyma, were found to be much more extensive than would have been expected according the history of lung infections reported by patients. Verghese and co-authors concluded that recurrent bacterial infections (especially *S. aureus, H. influenza, B. cepacia*, and *S. pneumoniae*) contribute to the development of bronchiectasis in HIV-infected individuals much more frequently than previously considered [21].

In 1997 King and co-authors, analysing chest CT images of a group of 50 HIV-infected patients (without history of AIDS or lung infections until inclusion in the study) and another group of 11 HIV-negative control subjects, demonstrated the presence of bronchiectasis in 36% of HIV infected patients and none in HIV-negative subjects. The authors hypothesised that lung and airway lesions in HIV-infected patients were present before the onset and development of lung infectious complications. Bronchial dilatation was found to be caused by bronchiolitis obliterans, elevated level of neutrophils in the airways and lymphocytic interstitial pneumonia [23].

Despite significant progress in understanding HIV infection, including prevention strategies, chemoprophylaxis, and antiretroviral therapy, the incidence of HIV-related diseases remains high in Moldova [1, 2]. Although the patients included in our study had a history of lung infections, the diagnosis, evaluation of the etiology and severity of bronchiectasis were neglected, most patients being identified with severe lung damage at the stage of establishing HIV status. Among the pulmonary complications, HIV/AIDS patients showed a higher occurrence for infections (especially pulmonary tuberculosis, pneumocystis pneumonia and various other opportunistic infections) thus, the development of bronchiectasis might be accelerated in this patient population.

Patients often underestimate their symptoms and refer to a doctor at the stage of advanced disease with limited management opportunities. Unfortunately, even after establishing the diagnosis of HIV/AIDS infection in the patients of our study, in some cases it was not possible to initiate ART, due to various difficulties (wasting syndrome, dyspeptic syndrome, religious and cultural considerations, poverty, inadequate referral system and poor adherence to treatment) which may have contributed to a high mortality rate, thus leading to 73% of deaths in this case series. Countries with limited resources face more difficulties in managing chronic diseases and bronchiectasis and HIV/AIDS are no exception. It would be advisable to test for HIV infection as part of the diagnostic process not only the patients with HIV risk factors, but also the group of primary identified patients with bronchiectasis, especially in countries with high incidence of HIV infection, such as the Republic of Moldova.

Conclusions

Bronchiectasis is one of the common pulmonary manifestations of HIV/AIDS infection, being responsible for a number of chronic respiratory symptoms and a risk factor for premature death.

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Authors' contribution
OM drafted the first manuscript and interpreted the data, DR acquired the data, DT acquired the data, VB 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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Neuromodulatory approach in paroxysmal neurological disorders

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Abstract

Background: Nowadays, neuro-modulation offers different devices and techniques in the treatment of neurological patients suffering from paroxysmal disorders, such as epilepsy and migraine. Among non-pharmacologic therapies, rTMS shows good results.
Material and methods: A longitudinal, double-blinded, rTMS-intervention study was conducted on 42 subjects with episodic migraine (with and without aura, 2-14 attacks per month). After a baseline follow-up for 1 month, subjects had 6 sessions of rTMS during 2 weeks and received multifocal rTMS or sham stimulation, with further 3-month assessment via questionnaires on headache frequency .
Results: After stimulation, the real rTMS group showed a reduction in the number of attacks – 7.5 ± 3.7 at baseline to 3.8 ± 2.7 attacks at 3 months' period ($p<0.05$) with an effect lasting at least three months. The number of attacks was also reduced in the placebo group (7.3 ± 3.6 to 4.4 ± 2.9) ($p>0.05$). There was a significant reduction in the intensity of attacks over 4-week therapy in the treatment group (6.7 ± 1.5 at baseline; 5.3 ± 2.5 at 4 weeks ($p<0.05$). The conducted questionnaires revealed a positive impact on quality of life and functional outcomes. There were no serious adverse events reported.
Conclusions: Our study showed evidence that the experimental rTMS protocol significantly reduced the frequency and intensity of migraine attacks compared to placebo treatment with no serious adverse events.
Key words: transcranial magnetic stimulation, multifocal, migraine.

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Introduction

Nowadays, neuromodulation offers different devices and techniques in the treatment of neurological patients suffering from paroxysmal disorders, such as epilepsy and migraine. rTMS has shown good results among other non-pharmacologic therapies. Transcranial magnetic stimulation (TMS) was introduced for the first time in 1985, as a method of noninvasive stimulation of the human cortex [1, 2], offering the possibility of studying the connection between the anatomical and functional elements of the human cortex [3]. Currently, rTMS is considered a useful tool in the management and treatment of several disorders originating in the cerebral cortex [4]. The small intensity currents induced by the magnetic field have an impact on various mechanisms at cellular level being able to change the expression of neurotransmitters, thus resulting in modulation of pathophysiological pathway of migraine.

The primary mechanisms causing migraine attacks still remain largely unrecognized due to the complex and dynamic organization of processes in the brain neuronal networks. Cortical excitability has been suggested to be dysfunctional in patients with migraine [5]. The ability to modulate cortical activity and induce persistent, plastic effects renders repetitive transcranial magnetic stimulation (rTMS) as a potential therapeutic approach.

Several studies demonstrate that TMS can reduce the frequency and severity of migraine attacks [6, 7]. Possible mechanisms involve induction effects on blood-flow, peripheral nerve sensing, cortical excitability and the release of cytokines or inflammatory neuropeptides [8-10].

The purpose of our study was to evaluate the efficacy and tolerability of multifocal rTMS for migraine prevention. The study hypothesis states that multifocal rTMS reduces the frequency and intensity of migraine attacks in comparison to a baseline period, and that this effect exceeds a possible placebo effect. Furthermore, it hypothesized that this stimulation protocol can induce improvements in quality of life scores: Headache impact test 6 (HIT-6), Migraine disability index score (MDIS), and Headache disability index (HDI).

Material and methods

A longitudinal, double-blinded, rTMS-intervention study was conducted on subjects with episodic migraine (both with and without aura, 2-14 attacks per month). The research project was approved by the Research Ethics Committee of Nicolae Testemitanu State University of Medicine and Pharmacy (No 90 of June 19, 2018). After a 4-week baseline period, the subjects underwent 6 intervention sessions within 2 weeks to receive either multifocal experimental rTMS or a placebo-treatment (randomized trial

was performed by a researcher blinded to every aspect of the study except randomization codes). The blinding of subjects was performed by means of a specific round biconcave active/placebo coil, which depending on the randomization code could act as an active coil (applying the experimental protocol) or sham (that was vibrating and making sounds imitating the real rTMS stimulation). A total number of forty-two subjects were eligible to participate in the study. The overall group baseline description is presented in table 1 and age-related group distribution in figure 1.

The test findings were evaluated via the IBM SPSS Statistics v. 23, Microsoft Office Excel program; the Student-test was applied to process the statistical mean values, repeated measures ANOVAs were performed separately for both groups. To determine the statistical significance, the P value should have been less than 0.05 [11].

Table 1

Group baseline characteristics

Variables	Total (n=42)	Real (n=22)	Sham (n=20)
Female, n (%)		19 (86.3%)	20 (100%)
Age in years (M ± SD)		38.4 ± 10.2	41 ± 12.6
Range		20 – 58	22 - 62
Headache frequency per month (M ± SD)		7.5 ± 3.7	7.3 ± 3.6
Range		2 – 14	3 – 14
Pain intensity (M ± SD)		6.7 ± 1.5	6.2 ± 1.2
*HIT-6 (M ± SD)		63.4 ± 6.3	64.2 ± 4.4
• HDI (M ± SD)		64.2 ± 17.4	55.4 ± 22
†MIDAS (M ± SD)		36.5 ± 22.9	35.9 ± 23.9

* – Headache Impact Test, • – Headache Disability Index, † – Migraine Disability Assessment Score.

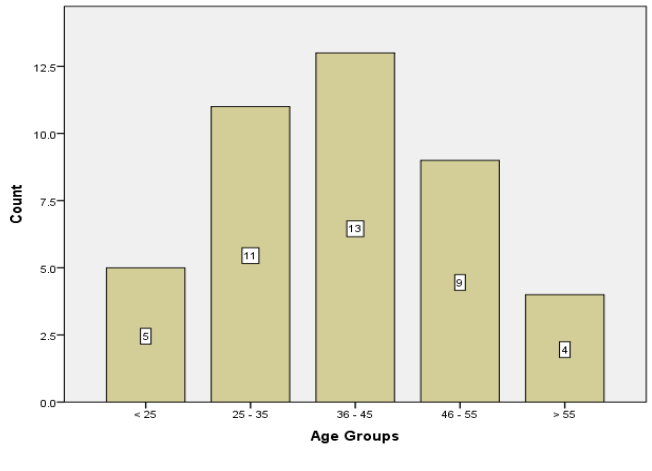

Fig. 1. Age group distribution. Subjects aged 36-45 years old were registered as the dominant age group, data similar to those presented in other studies

Study design

After signing the informed consent, subjects were asked to fill out a headache diary for 4 weeks and complete the HDI, HIT-6, and MDIS questionnaire prior to the first stimulation session. Frequency and severity of migraine attacks assessed within the 8 weeks, following the intervention serve as primary outcome variables. Quality of life questionnaires were conducted on follow-up dates (fig. 2).

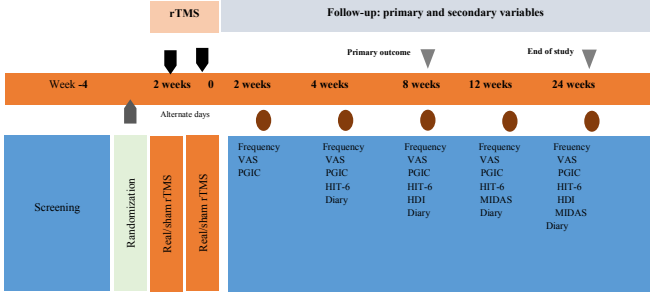

Fig. 2. Study design

Stimulation protocol

The stimulation protocol consisted of 2 steps, a swipe-stimulation and a spot burst stimulation. High frequency rTMS comprised 140 pulses/train in trains at 60% of motor threshold, followed by 5 pulses/train in trains at 85% of motor threshold, applied over cortex within a predefined multifocal delivery scheme consisting of 11 points marked on individual caps according to the 10-20 EEG system during the first session (fig. 3).

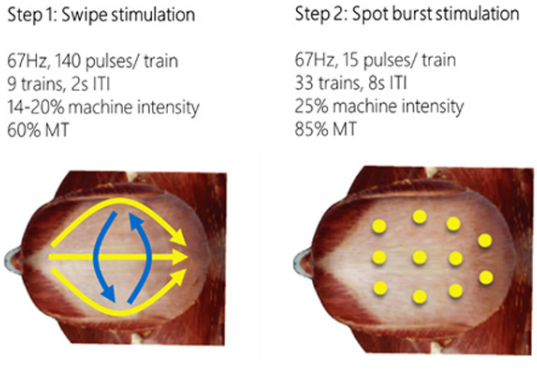

Fig. 3. Graphic representation of the experimental stimulation protocol (Neurophysiology Laboratory, Department of Neurology, Emergency Medicine Institute)

Safety

Stimulation procedures had been performed respecting the IFCN committee safety protocols and recommendations [12].

Results

42 eligible subjects were included in the data analysis. After stimulation, the real rTMS group showed a reduction in the number of attacks – 7.5 ± 3.7 at baseline to 3.8 ± 2.7 attacks at 3 months' period ($p<0.05$). The effect lasted at least three months.

The number of attacks was also reduced in the placebo group (7.3 ± 3.6 to 4.4 ± 2.9) ($p>0.05$). There was a significant reduction in the intensity of attacks at 4 weeks after the treatment in the treatment group (6.7 ± 1.5 at baseline; 5.3 ± 2.5 at 4 weeks ($p<0.05$). The primary outcome results are presented in fig. 5. The assessment of secondary outcomes

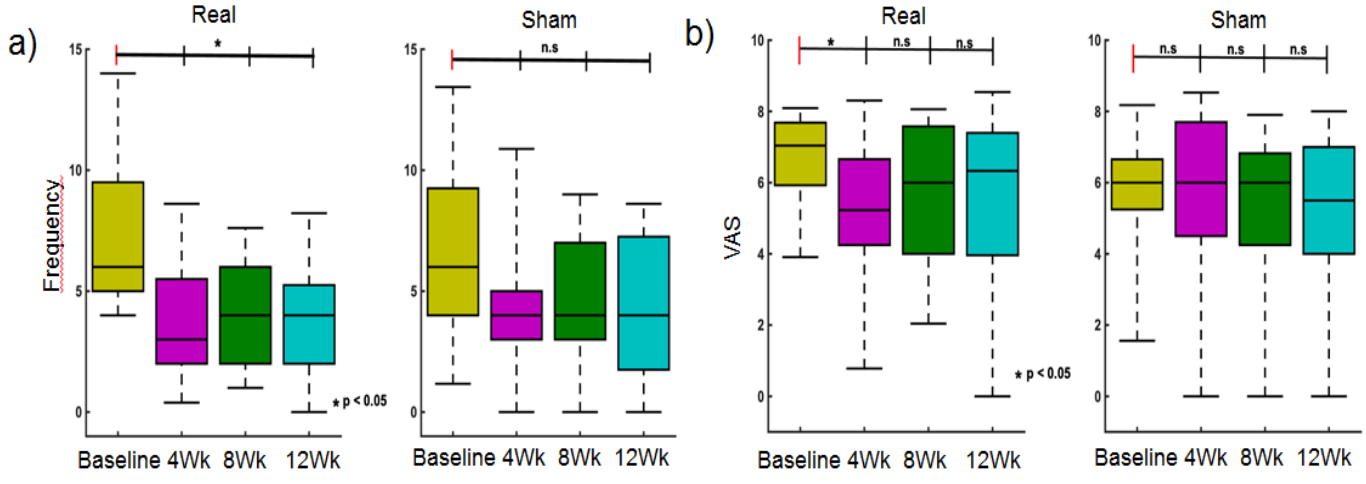

Fig. 4. Primary outcomes. Mean changes in headache frequency (a) and intensity (b) at baseline and follow-up. The frequency of migraine attacks was significantly reduced in the treatment group for 3-month following stimulation. The severity of the attacks was markedly reduced over 4 weeks after stimulation (p<0.05) in the treatment group, whereas the sham group showed a slight reduction

in real rTMS group had shown an overall reduction in all variables: HIT-6 scores – 63.4 ± 6.3 at baseline to 54.1 ± 8.3 at 12 weeks, compared to sham group – 64.2 ± 4.4 at baseline to 56.7 ± 8.9 at 12 weeks follow-up; HDI real rTMS 64.2 ± 17.4 at baseline to 48.5 ± 24.5 at 8 weeks vs 55.4 ± 22.1 at baseline to 40.7 ± 24.1 at 8 weeks; the same effect was observed in MIDAS scores – real rTMS group 36.5 ± 22.9 at baseline to 20.9 ± 23.2 at 12 weeks vs 35.9 ± 25.9 at baseline to 19.4 ± 19.2 at 12 weeks in sham rTMS group. The conducted questionnaires revealed a positive impact on quality of life and functional outcome in both groups, more prominent in the real rTMS group but with no statistical inter-group difference (p>0.05). There were no serious adverse events reported.

Discussion

This present study hypothesized that the observed positive effect in the reduction of headache frequency and intensity of the real (experimental) rTMS protocol compared to placebo could be explained by the changes in the cortical excitability and function obtained by direct cortical magnetic stimulation [8] as well as by the modulatory effect on peripheral nerve sensing activity (ophthalmic branch of the trigeminal nerve and greater occipital nerve (C2)) [9]. The changes in the assessment questionnaires of quality of life (HIT-6, HDI, MIDAS) could be partially explained by the improvement in primary outcomes (headache frequency and intensity) [13] as well as by the modulation of cortical areas engaged in mood and affective behavior [14-17]. One of the limitations of the study is the relatively small number of analyzed subjects, as well as the fact that assessment by such scales as HIT-6, HDI and MIDAS, though a standard in migraine research, carries a subjective recall bias in both groups [18]. In addition, based on the novelty of the rTMS as a treatment option, another possible bias could be considered high subject treatment expectations [19]. Further research is needed in order to confirm the experimental rTMS protocol usefulness and non-inferiority to already existing therapeutic TMS protocols [20].

Conclusions

Our study showed compelling evidence that the experimental rTMS paradigm reduces the number and severity of migraine attacks compared to placebo treatment. Multifocal rTMS should be considered a novel and effective prevention treatment approach for paroxysmal disorders, such as episodic migraine in adults. An important fact is that the experimental protocol was well tolerated and showed no serious adverse events.

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PL carried out the study, elaborated the manuscript. SG was the principal investigator and supervised with due diligence the course of the study. Both authors revised and approved the final version of the manuscript.

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The research protocol No 90 (of June 19, 2018) was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy.

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The development of the automated information system of pharmaceutical staff management

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Abstract

Background: The automated evidence of any systemic personal data represents an important tool to provide good functionality of that system. It absolutely refers to the pharmaceutical system as well, which is a part of the health system. Purpose of the study: To elaborate and argue the need to implement the automated information system of pharmaceutical staff management (AIS PSM) within the health system of the Republic of Moldova.
Material and methods: Statistical data on the pharmaceutical system; systemic approach by applying statistical analysis, and system programming methods.
Results: The automated system contains and ensures the processing of the following categories of personal data: first name, last name; date of birth; gender; occupation; graduation diploma; graduated institution; the employee workplace /the pharmaceutical company address; continuous education training; professional association membership fee; special references. The confidentiality of the personal data and the possibility of extending the categories of data is ensured, as well as the possibility of integration the developed system in the national health systems and statistical systems. Recommendations regarding the need of implementation of the AIS PSM in pharmaceutical units were worked out.
Conclusions: In the Republic of Moldova, the automated pharmaceutical staff management information system was developed and proposed, though its implementation was largely discussed. There were also arguments on the recommendations to ensure the functionality of the system by elaborating sequential diagrams.
Key words: automated information system, pharmaceutical staff.

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Introduction

Currently the Information Technology register is well known for its most dynamic evolution, which is applied more and more frequently in the data processing processes, in the decisional, management, computerizing processes etc.

Practically, there is no field in which at least one information system is used, as a support in ensuring the functionality and / or control of different parts of the activity in those fields [1].

Medicine compared to other science fields is the one of the domains, which implements modern information technologies more cautiously and also with a delay. This is determined by the major responsibility in the use of medical and a pharmaceutical information technology, which is related to human health and life.

Unlike some of medical staff, pharmacists have a great experience in using computer systems, both regarding the issuance of medicines and collaboration with insurance companies, as well as for keeping stock records and performing orders to pharmaceutical warehouses. Due to the diverse use of information technologies in the pharmaceutical activity, pharmacists can be considered leaders in the implementation of IT in their professional activity.

The role of the pharmacist in society is constantly growing; pharmacy itself is a constantly changing profession. Over the last 100 years, the profession of pharmacy has evolved from a dispensing model focused on the formulation and delivery of a drug product to a patient, care model focused on individualizing drug therapy and delivering direct patient care [2].

Nowadays, there is a shortage of pharmaceutical staff in the Republic of Moldova, which significantly diminishes the quality of pharmaceutical services provided.

At the same time, the lack of a register of pharmacists does not allow the registration of pharmaceutical staff.

In the current operating conditions of the pharmaceutical system, the operative and statistical recording of the pharmaceutical staff is of great importance.

The purpose of this study was to develop and reason the need to implement the automated information system of pharmaceutical staff management (AIS PSM) within the health system of the Republic of Moldova.

Material and methods

The statistical data in the field of the pharmaceutical system as well as the "pharmaceutical framework" subsystem served as study materials. The methods applied included the systems approach, study of factors and processes, statistical analysis, decomposition and construction of systems, elaboration of information system design and components and programming the automated information system of pharmaceutical staff management.

Results and discussions

Some fields of activity in the Republic of Moldova have developed and operate IT systems for personnel records. Thus, the personnel record in the public authority, which contains general data, was elaborated as a methodological support in the process of organizing and carrying out the personnel record activities in the public authorities [3].

The information system for registering health personnel was developed and integrated in collaboration with the International Organization for Migration. The database of this system was created for the correct management of resources by local authorities [4]. The system was created following a similar model of a Finnish software, and its purpose was to monitor the activity and migration of medical staff [5].

The "Human Resources Management Information System" ensures the collection, administration, processing and interpretation of data by issuing lists, text reports, statistical and comparative data, as well as improving communication within the organization by better organizing the flow of information between departments of Human Resources and other subdivisions. The system ensures good data accessibility and significantly reduces the time required for administrative activities on personnel management [6].

The experience gained in other areas, as well as the following general principles were considered during the process of developing the AIS PSM:

- The principle of *legality* of the system implies the operation of the system in accordance with the legislation in force;
- The principle of *respect for human rights* provides system operating in strict accordance with national normative documents and within the limits of the stipulations of international treaties and conventions on human rights, to which the Republic of Moldova is a party;
- The principle of the *first person* / *of the unique center* implies the existence of a highly qualified leader who is adequately empowered to adopt decisions and coordinate system creating and operating works;
- The principle of *data authenticity* implies the introduction of data in the system only on the basis of entries in qualified documents as sources of information;
- The principle of *data integrity*, completeness and veracity, according to which:

1) Data *integrity* means that data keeps its content and its uniform interpretation under the influence of random factors. Data is considered to maintain its integrity if it has not been distorted or destroyed (not deleted);

2) Data completeness means the volume of information collected, registered and authorized in accordance with the normative acts;

3) Data *veracity* means its degree of correspondence to the computer memory or to documents which render the real situation of the reflected objects from a certain system domain.

- The principle of *state identification of the objects of registration*, according to which each subject of registration is given a unique identification number [7, 8].

The processes of creation, implementation and operation of AIS PSM must not contradict the normative acts on the pharmaceutical activity in force at the time of elaboration [9, 10].

The developed system is provided with a user-server architecture, based on web technology. It is designed modularly and the development of the modules can be done simultaneously. Any user can connect to the application server and use the system according to their rights.

The authentication module guarantees safe access of users to the system. To log in, users have a username and password, which they use to access the system. The authentication model guarantees the user exclusivity within the system:

- The system ensures that the authentication module is an operating one and provides messages / helps in case of incorrect entry of authentication data (incorrect username / password). The messages are explicit, short and coherent, in Romanian version, so that they do not confuse the users;
- The login interface contains information on the access conditions of the users in the system and a message which informs the users on non-compliance with access conditions that might be sanctioned according to the law;
- Once logged in, users have exclusively those rights, which they need to carry out their activity;
- The system has an access control mechanism, which allows users, by default, a minimum number of actions without the intervention of administrators, this being included only for the granting of special rights when necessary [9].

In the following approaches, the notion of "user" refers to a person with valid permissions to operate within the system, and the notion of "roles" defines some responsibilities. Thus, the role of "user" belongs to a responsible person within the pharmaceutical unit, and the role of "administrative" – to a responsible person within the institution responsible for the management and maintenance of the information system.

Users will have usage restrictions, so they will only be able to access certain fields to complete them, and administrative roles by performing actions on the management

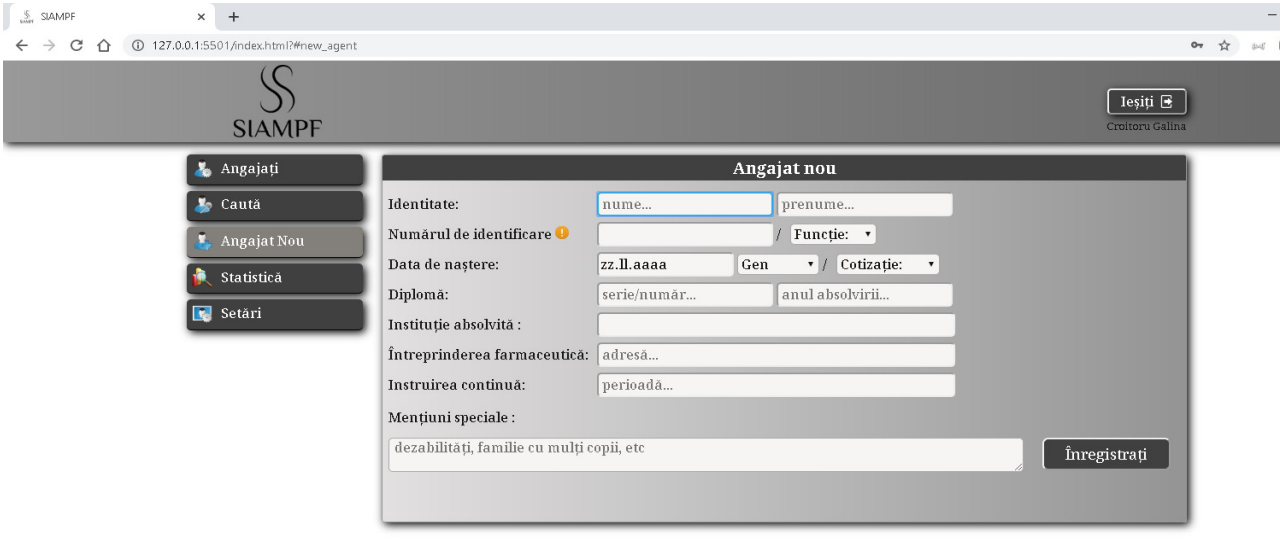

Fig. 1. Graphical interface of the pharmacy user when a new employee is being introduced

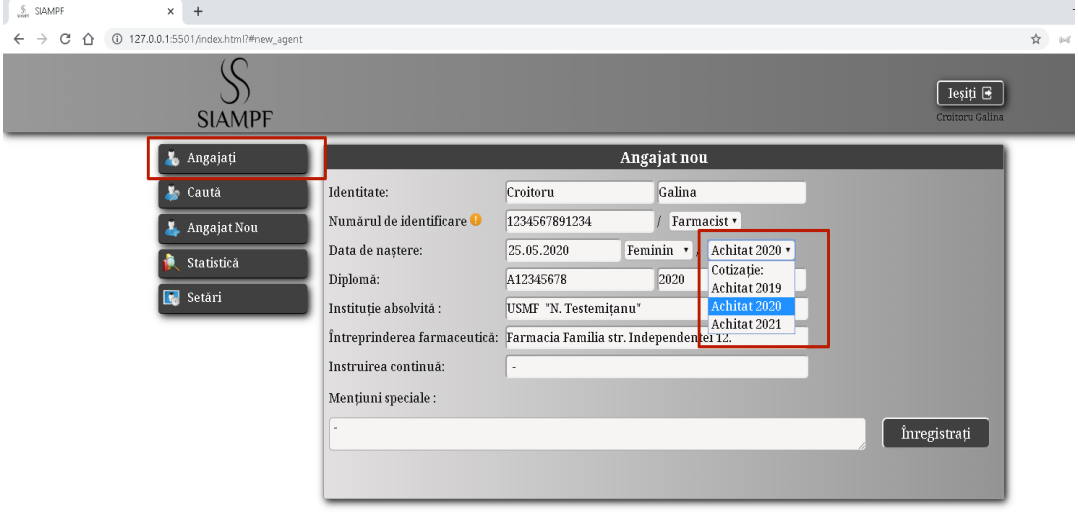

Fig. 2. Graphical user interface for the person responsible for the APhRM

of already registered information, including filling in new information, as well as searching and retrieving the necessary information.

The user of the pharmacy computer system has the function of entering the personal data regarding the new employee engagement within a definite unit. Fig. 1 shows the interface for the pharmacy user when enrolling a new employee. When accessing the "*New Employee*" button, the personal data entry appears.

The following categories of personal data are processed within the AIS PSM:

- Name, surname;
- Date of birth; Gender;
- Pharmacist / Assistant Pharmacist position;
- Diploma (series, number, year of graduation);
- Graduated institution;
- Pharmaceutical company / address;
- Continuous training (period);
- Membership fee of the Association of Pharmacists of the Republic of Moldova (APhRM);
- Special mentions (disabilities, family with many children, etc.).

If the employee is already introduced into the system, access the "Employees" button and enter the name and surname of the employee. It will enable to access the complete personal file, allowing the user to modify only the necessary data.

The graphical interface of the responsible person within the Association of Pharmacists of the Republic of Moldova is presented in fig. 2. It ensures the management of the data system access in order to modify the information referring to the payment of the annual fee.

In order for the information managed by the system to be truthful and current, the data is constantly renewed, for example: when changing the address / company where the person works; when paying the annual fee; when conduct-

ing continuous training; changes in personal data; when special references are required, etc. After each data change, the system is updated and presents a new version.

The system functionality cycle contains 4 stages (fig. 3):

I. *Data entry* represents the process of data collection, verification, coding and transmission;

II. Data *processing* involves various activities of classification, sorting, performing mathematical-logical calculations, selective archiving of data and processing results, in order to find and further process them;

III. *Information extraction* that is performed in three steps: 1 – retrieving the results from memory; 2 – decoding the results and presenting them in a comprehensible format; 3 – sending the information to the place requested by the user;

IV. *Feedback mechanism* is the information obtained after processing that may or may not meet the requirements; Therefore, an evaluation of the processing results may take place, according to which a series of changes will be made in the data entry or processing phase. It can be considered that this stage has the role of a feedback mechanism, which allows proper functioning of the system, giving it the characteristics of a cybernetic system [11].

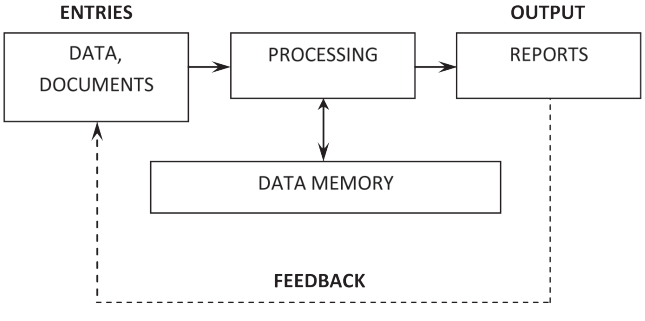

Fig. 3. Data processing flow in AIS PSM

The improvement of the existing information system must be considered and elaborated continuously. The requirements imposed at the national level must also be complied with the existing information systems at the level of economic agent, territorial region or field. Consequently, the modernization of the information system will also enhance the improvement of the information systems with which it interferes. The data exchange between different compartments, hierarchical levels or external partners should be provided through computer networks, in conditions of uniformity regarding the way of preparation and presentation of reports and in order to achieve data comparability.

Soon, due to additional data included within the system, it will be possible to generalize data related to other personnel procedures. The implementation of this database will allow a faster obtaining of the requested information and its truthful reporting.

AIS PSM will be able to be integrated within the health system of the Republic of Moldova as well as in the national statistical system.

The implementation of the AIS PSM, developed for recording the pharmaceutical staff, will provide benefit for the parties involved from the following aspects:

- *Medicines and Medical Devices Agency:*
  - Fast and guaranteed access to accurate data referring to pharmaceutical staff;
  - Access to statistical data;
  - Accessing and verifying in real time the data regarding the pharmaceutical staff working in the pharmaceutical enterprises / units;
  - Monitoring information on the pharmaceutical unit related to the licensing, assessing and accreditation process;
  - Accurate assessment of the continuous introduction of data regarding human resources from the pharmaceutical system.
- *Association of Pharmacists of the Republic of Moldova:*
  - Managing the information regarding the continuous development of professional activity of the pharmaceutical staff;
  - Analysis of staff stability in pharmaceutical units;
  - Checking the payment of the fee and data update;
  - Extraction of statistical reports;
- *Faculty of Pharmacy of Nicolae Testemitanu* State University of Medicine and Pharmacy:
  - Providing possibility of strategic planning of the pharmaceutical staff training within the health system;
  - Evidence of employment and evolution of graduates in employment;
- *Pharmaceutical units, enterprises and institutions:*
  - Introducing information on pharmaceutical staff by the duty-bound person;
  - Updating the information regarding the pharmaceutical staff;
  - Reporting the necessary information to the Agency for Medicines and Medical Devices.

Conclusions

The Automated Information System of "Pharmaceutical staff management" was developed for the first time in the Republic of Moldova and proposed for its implementation.

In order to facilitate the use of AIS PSM, the stages of data entry in the developed system and the way of their management by various users were developed and described.

Recommendations were made to ensure the functionality of the system by sequentially describing the stages of the cycle.

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Authors' contribution
VS, SA drafted the first manuscript; GC designed the compartments of the automated information system of pharmaceutical staff management; VS, SA developed and piloted the automated information system of pharmaceutical staff management and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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

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

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Early detection of urinary bladder tumors with narrow band imaging

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Abstract

Background: Early diagnosis at the incipient stages of bladder tumors is one of the current issues discussed in modern urology. Although the main method of diagnosis remains cystoscopy, new methods of visualization and detection of bladder tumors have been proposed over the last decade. The purpose of the study was to determine the impact of narrow band imaging (NBI) cystoscopy in the detection of non-muscular invasive bladder tumors in relation to white light (WL) cystoscopy.
Material and methods: 57 patients with bladder tumor pathology were diagnosed within the Urology Clinic of *Nicolae Testemitanu* State University of Medicine and Pharmacy during February 2016 – March 2018. All patients underwent white light cystoscopy, followed by narrow band imaging cystoscopy. The obtained data were comparatively analyzed.
Results: Out of the total number of 57 patients diagnosed with bladder tumors, 49 (86%) patients were diagnosed via WL cystoscopy and NBI cystoscopy performed after WL; tumor pathology was also detected in other 8 (14%) patients. The quantitative assessment of tumor lesions revealed 102 lesions, of which 75 (73.5%) were determined through WL and 27 (26.5%) tumor lesions were identified by using the NBI method.
Conclusions: Narrow band imaging cystoscopy determines more favorable results in the early diagnosis of non-muscular invasive bladder tumors compared to white light cystoscopy.
Key words: narrow band imaging cystoscopy.

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Introduction

Bladder cancer is the 11th most common malignancy worldwide and one of the most common cancers of the urinary tract. There are two types of bladder cancer: about 75% is non-muscle-invasive bladder cancer (NMIBC), the remaining 25% is muscle-invasive bladder cancer (MIBC) [1].

In the Republic of Moldova, bladder cancer ranks second after prostate cancer, according to the nosological structure of tumors of the urogenital system. Over 15 years, cancer morbidity has almost doubled. Since 2000, due to the implementation of new diagnostic methods, the number of patients detected at the early stages has increased, which allows the improvement of treatment results and the quality of life of patients [2].

The initial treatment of all bladder tumors includes an accurate transurethral resection of the tumor, which is commonly performed by white light (WL) cystoscopy. Full resection of all visible tumors with the histological examination is the standard medical care that must be performed. However, white light cystoscopy may fail to detect flat and small lesions [3]. A small, flat lesion that was, in fact, a tumor or carcinoma *in situ* (CIS) and that was missed at the time of primary diagnosis and surgery can result in recurrence [4]. These overlooked flat lesions have the chance to recur: about 61% in the 1st year and 78% within the 5th year, which may even progress into invasive bladder cancer: approximately 17% in the first year and 45% during the 5th year [5, 6]. Because of these great rates of recurrence and prevalence, bladder cancer is a large burden on the economy and medical insurance [7].

To optimize the therapeutic and diagnostic approach of NMIBC narrow band imaging (NBI) cystoscopy has been introduced into the medical practical use. NBI is an optical image enhancement technique that uses wavelengths in the blue – 415 nm and green – 540 nm zone of the electromagnetic spectrum. These specific wavelengths are strongly absorbed by hemoglobin and vascular structures, such as tumors and areas of carcinoma *in situ*, making them appear dark brown or green against a pink or white normal mucosal background, without the use of any dye [8]. NBI is useful in endoscopy detection of early stages of gastrointestinal cancer that is, therefore, expected to play a significant role in the diagnosis of NMIBC [9].

The aim of the study is to determine the impact of narrow band imaging cystoscopy in the detection of non-muscular invasive bladder tumors if compared to white light cystoscopy.

Material and methods

The study was conducted on 57 patients, diagnosed with bladder tumor pathology within the Department of Urology and Surgical Nephrology of *Nicolae Testemitanu* State University of Medicine and Pharmacy, in *Timofei Mosneaga* Republican Clinical Hospital during February 2016 – March 2018. A transversal descriptive study was performed. The patients were selected from all amount of bladder tumor patients treated in our department by cystoscopy with transurethral resection of the bladder tumors, according to the following criteria. The patients inclusion criteria were primary non-muscular invasive bladder cancer, patients aged over 18 and the Eastern Cooperative Oncology Group (ECOG) score 0-2. The exclusion criteria were identified as follows: other non-urothelial tumors, severe comorbidities, ECOG score ≥ 3, and pregnancy. White light cystoscopy was performed in all patients, followed by narrow band imaging cystoscopy; the obtained data were comparatively analyzed. Descriptive statistics was applied. The results of the study are presented as absolute and relative values.

Results

The demographic data of the patients with NMIBC are presented in table 1.

Of the 57 patients included in the study, based on gender distribution, 48 (84%) were men and 9 (16%) were women. The age ranged between 24-85 years old, the mean age was 65.4 years. The number of patients, included in the 18- 30-year-old age group was 4 (7%), confirming that bladder cancer is an older age-related disease. In 17 (30%) of the patients included in the research, age varied from 31 to 60 years. However, the most common study age group was over 60, which consisted of 36 patients (63%). Another important risk factor for the development of bladder cancer is tobacco smoking. 24 (42%) patients out of 57 are tobacco users.

Tumor analysis showed that the majority of the patient's single bladder tumor was detected in 36 (63%) cases. 2 tumors were discovered in 9 (16%) cases and multiple tumors – 3 cases and more – in 12 (21%) patients. According to bladder tumor volume, 49 (86%) patients included in the study had up to 3 cm size, and massive tumors of over 3 cm were recorded in 8 (14%) cases.

The results of the histopathological examination after the T stage showed that stage Tis was observed in 3 (5%) cases, stage Ta was detected in the majority of the patients that made up 35 (61.5%) cases and stage T1 was in 19 (33.5%) cases.

According to the WHO/ISUP 2004 classification: PUNLMP – papillary urothelial neoplasm of low malignant potential was detected in 3 (5%) cases, low-grade papillary urothelial carcinoma – in 25 (44%) patients and high-grade papillary urothelial carcinoma – in 29 (51%) cases of patients included in research.

Out of the total number of 57 patients, diagnosed with

Table 1

Patient and tumor demographics

Parameters	Categories	Patients (n=57)
Gender:	Men, n (%)	48 (84%)
	Women, n (%)	9 (16%)
Age, years	Mean age (CI 95%)	65.4 (26-83)
Age group:	18-30 years, n (%)	4 (7%)
	31- 60 years, n (%)	17 (30%)
	60 years and more, n (%)	36 (63%)
Tobacco/Smoking	Yes, n (%)	24 (42%)
Tumor size:	< 1 cm, n (%)	22 (38.5%)
	1-3 cm, n (%)	27 (47.5%)
	> 3 cm, n (%)	8 (14%)
Number of tumors:	Single tumors, n (%)	36 (63%)
	2 tumors, n (%)	9 (16%)
	> 2 tumors, n (%)	12 (21%)
T stage:	Tis (CIS), n (%)	3 (5%)
	Ta, n (%)	35 (61.5%)
	T1, n (%)	19 (33.5%)
Histopathology grade:	PUNLMP, n (%)	3 (5%)
	Low-grade, n (%)	25 (44%)
	High-grade, n (%)	29 (51%)

Note: CI — Confidence Interval, Tis (CIS) — Carcinoma *in situ*, "flat tumor", Ta — Noninvasive papillary tumor, T1 — Invades subepithelial connective tissue, PUNLMP — papillary urothelial neoplasm of low malignant potential, Low-grade — Low-grade papillary urothelial carcinoma, High-grade — High-grade papillary urothelial carcinoma.

Table 2

Detection of tumor lesions during white light cystoscopy and narrow band imaging

Characteristics	White light cystoscopy (WL)		Narrow band imaging cystoscopy (NBI)		Total	
	n	%	n	%	n	%
Tumor lesions	75	73.5%	***+27***	***+26.5%***	102	100%
Number of patients	49	86%	***+8***	***+14%***	57	100%

bladder tumors (tab. 2), this diagnosis was established by WL cystoscopy in 49 (86%) patients, and by NBI cystoscopy performed after WL; the tumor pathology was detected in 8 (14%) patients. The quantitative assessment of tumor lesions revealed 102 lesions, of which 75 (73.5%) were determined through WL and 27 (26.5%) tumor lesions were identified using the NBI method. Of CIS lesions, 1 lesion was detected during WL and other 2 with NBI.

Discussion

Despite being introduced within a urologic setting more than 10 years ago, NBI is still not being routinely utilized in the detection of NMIBC. Nevertheless, some researches have shown that NBI is more efficient in detecting NMIBC than WL. Bryan et al., who first introduced NBI in the urologic setting, found that 15 additional urothelial carcinomas were detected in 12 of 29 patients (41%) [10].

The proposal of a new cystoscopy visualization technique, should meet at least two requirements: first, it must improve the diagnostic precision in the detection of bladder cancer and second, the implementation of the technique in the transurethral treatment should be capable to decrease the risk of progression and/or recurrence. Different studies have demonstrated an improvement in the diagnostic rate of bladder tumors using NBI, but it remains unclear whether the increase of detection rate is due to the second accurate examination of the bladder only [11].

According to the data of the specialized literature, compared to WL, NBI can detect more tumors in 9%-56% additional patients [3, 10, 12-16]. In our study, NBI discovered additional pathologies in extra 14% of patients.

According to the acknowledged superiority of NBI in the detection of extra tumors, as well as in our research, NBI has revealed additional 26.5% of tumor lesions. However, the site of the resection with NBI control in the transurethral resection algorithm remains to be clearly determined [17].

However, it has been observed that visibility during NBI was restricted due to inflammation and bleeding, making it hard to reveal and resect tumor lesions. It may be the case because wavelengths during NBI are actively absorbed by free hemoglobin, which occurs in bleeding [14].

In the detection of additional tumors, NBI cystoscopy is superior to WL cystoscopy. NBI is not perfect for the primary resection of multiple and large tumors due to poor visualization. However, NBI can influence the finding of residual or missed tumors after initial resection under WL. It may be right to propose the adage "NBI to detect, WL to resect" for future use [18].

Conclusions

Narrow band imaging cystoscopy determines more favorable results in the early diagnosis of non-muscular-invasive bladder tumors compared to white light cystoscopy. Only adequate equipment is required to perform the NBI cystoscopy. The technique is easy to apply, just pushing on a single button, without using additional medical substances. In our research, NBI was able to detect additional pathologies in 8 (14%) patients and detected 27 (26.5%) additional tumor lesions.

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IV and AP acquired, interpreted the data, drafted the first manuscript, GS performed most of the analyzed interventions, VG designed the trial and revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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The research was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 4 of December 16, 2019). Written informed consent was obtained from all participants in the study.

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

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The morbidity rate of acute stroke among adult population in both Moldova and India

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Abstract

Background: According to the WHO, over 15 million people worldwide suffer from stroke annually, 5.5 million die and 5 million become permanently disabled. According to the latest WHO data published in 2017, Stroke Deaths in the Republic of Moldova made up 15.87% of total deaths.
Material and methods: A retrospective hospital-based study was conducted at the clinics of the Department of Emergency Medicine of *Nicolae Testemitanu* SUMPh and at the tertiary Care Hospital of Assam Medical College, the Republic of India, during January 01, 2019 – December 31, 2019. Two groups of patients were enrolled in the research, viz. lot 1 (80 patients) treated in the Institute of Emergency Medicine of the Republic of Moldova and lot 2 (80 patients) treated at the tertiary Hospital of Assam Medical College, India. The purpose of the study was to assess the major risk factors for developing ischemic stroke, as well as to evaluate the impediments in providing patients with modern treatment strategies among adult population.
Results: The study group included 44 (55.0%) males and 36 (45.0%) females. 66 patients (83.0%) suffered an ischemic stroke and 14 patients (17.0%) had hemorrhagic stroke. The risk factor providing access to modern treatment strategies in acute stroke cases is taken in consideration.
Conclusions: The high incidence of stroke suggests that primary prevention strategies used in the Republic of Moldova and the Republic of India are either not widely implemented or not sufficiently effective.
Key words: ischemic stroke, thrombolysis, incidence, risk factors.

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Introduction

According to the WHO, over15 million people worldwide suffer from stroke annually, 5.5 million die and 5 million become disabled. The ischemic strokes commonly account for about 80% of stroke cases while hemorrhagic strokes make up 20% but the actual occurrence of stroke types depend on the population [1]. Approximately 1.1 million inhabitants of Europe suffer a stroke each year due to the aging population, the absolute number of stroke is expected to increase by 2025 to 1.5 million people [2]. According to the latest WHO data published in 2017, Stroke Deaths in the Republic of Moldova (RM) reached 6638 cases or 15.87% of total number of deaths. The age-adjusted Death Rate is 121.53 per 100 000 of Moldovan population and ranks 39 in the world. 33% of strokes occur in working age population. Stroke mortality is three to four times higher in RM than in the EU [3-6].

The Republic of India has been experiencing significant demographic and epidemiological transition during the past two decades. These have resulted in an increase in life expectancy and consequently led to an increase in aging population. Reliable stroke-related morbidity and mortality rate assessment in India is very limited. The cumulative incidence of stroke ranged from 105 to 152/100 000 persons per year, whereas the estimated prevalence of stroke ranged from 44.29 to 559/100 000 persons within different parts of the country during the past decade [7, 8]. In India, 1.5 million people suffer from acute stroke every year and 1880 people die every day. The cerebrovascular diseases prevalence accounts for 400-625 per 100 000 persons, an incidence of 145 per 100 000 and a 1-month case-fatality of 41% [8].

Almost half of stroke-related mortality may be attributed to variable risk factors (i.e. hypertension, diabetes, dietary risks, impaired glucose intolerance, obesity, smoking, air pollution, alcohol use, hypercholesterolemia, and physical inactivity), which are mostly due to poor clinical management, limited access to health care, and late detection of underlying risk factors [9, 10]. This requires resource allocation to those variable risk factors that show the highest impact on stroke for each region. Moreover, social and economic policies to reduce inequalities in stroke care should become a health priority, particularly in less developed countries. These policies should focus on the treat-

ment of early predisposing factors and on early educational programs since childhood, which have long-lasting impacts on adulthood health [11]. Likewise, improving worldwide primary healthcare services may have an important impact on post-stroke outcomes. It is essential to improve stroke awareness among socio-economically deprived individuals and societies and provide equitable post-stroke medical care [11].

The high burden of strokes suggests that primary preventive strategies in the Republic of Moldova and the Republic of India are either not widely implemented or not sufficiently effective. Moreover, the behavioral risk factors and an effective screening for conditions that increase stroke risk, such as hypertension, atrial fibrillation, and diabetes mellitus should also be considered[12].

Most guidelines are based on high-income countries data, uncertainty remains regarding best management of stroke of unknown type in low-and middle-income countries. For example, in low-and middle-income countries, 34% of strokes (versus 9% in high-income countries) are of haemorrhagic subtype. Current guidelines for the management of acute stroke recommend a course of treatment based on the diagnosis of ischaemic stroke (versus haemorrhagic stroke) using CT scanners. In low-resource settings, CT scanners are either unavailable or unaffordable, forcing clinicians to make difficult clinical decisions, such as whether to anticoagulate patients or not, and to what level to control their blood pressure without means of distinguishing between ischaemic and haemorrhagic stroke. These patient management challenges, combined with inadequate rehabilitation services, lack of preventive measures, as well as poor understanding of the possible unique risk factors 'ass' ociated with stroke in low-and middle-income countries, may account for the disproportionately large stroke burden borne by these countries [13, 14].

Material and methods

A retrospective hospital-based study was conducted at the clinic of the Department of Emergency Medicine of *Nicolae Testemitanu* State University of Medicine and Pharmacy and a tertiary care Hospital of Assam Medical College, the Republic of India. All the medical records with stroke diagnosis were identified based on the ICD, R- X, from January 01, 2019 to December 31, 2019. Two groups of patients were enrolled in the research, lot 1 (80 patients) treated in Institute of Emergency Medicine (Chisinau, the Republic of Moldova) and lot 2 (80 patients) treated in the tertiary Hospital of Assam Medical College, Dibrugarh, India.

Inclusion Criteria:

1. Subjects aged older than 20 years;
2. The diagnosis of acute stroke (ischemic/hemorrhagic) based on clinical and imaging (computed tomography (CT) – head/cerebral magnetic resonance imaging (MRI)) assessment;
3. Patient's written consent.

Exclusion Criteria:

1. Patients with stroke-like conditions due to systemic diseases, such as infections and trauma;
2. All hemorrhagic stroke patients who have posttraumatic, drug-induced (e.g., anticoagulant-induced), and those with bleeding diathesis-related etiologies;
3. Patients for whom the whole investigation protocol was not possible;
4. Patients with malignant tumors and end-stage organ failure;
5. Pregnant women in II-III trimester.

The purpose of the study was to estimate the clinical and epidemiological profile of acute stroke, prevalence of risk factors and impediments for providing access s to modern treatment strategies among the adult population of the Republic of Moldova and the Republic of India.

Objectives of research

1. To study the clinical-epidemiological trends of strokes in the population and the accessibility to modern treatment strategies in the acute phase;
2. To study the prevalence of risk factors for stroke in the adult population of the Republic of Moldova;
3. To study the major risk factors for developing stroke among the adult population of the Republic of India;
4. To assess the impediments for providing medical access for patients with ischemic stroke to modern treatment strategies. The statistical data processing was performed by using SPSS 22.0 (SPSS inc)programs.

Two study groups were included in the research, lot 1(80 patients) treated at the Institute of Emergency Medicine, RM and Lot 2 (80 patients) treated in the tertiary Hospital of Assam Medical College, India. All the patients were clinically assessed by performing a detailed medical history and clinical examination. Various demographic variables were collected from the history, inclu-ding age, sex, history of transient ischemic attack/stroke, hypertension, diabetes mellitus, coronary artery disease, pre-stroke disability, smoking, and family history of stroke. Routine hematological and biochemical tests including Hb, total leukocyte count, erythrocyte sedimentation rate, blood sugar, and lipid profile were carried out. All patients underwent the electrocardiogram (ECG), echocardiography, CT, cerebral MRI, intracranial MR angiography, transthoracic echocardiography, and carotid Doppler study.

Results and discussion

The morbidity rate of acute stroke among adult population in the Republic of Moldova

Out of 80 patients 44 (54.4%) were males and 36 (45.6%) were females; the patients' mean age was 59.8±17.4 years and the mean age at stroke onset was 58.4 ±15.9 years. The age range of the study group was 20-88 years.

The study group included 44 (55.0%) males and 36 (45.0%) females. 66 patients (83.0%) had ischemic stroke and 14 patients (17.0%) had hemorrhagic stroke. The mean age was 56.4 ± 14.38 years in ischemic stroke group and 53.24 ± 12.45 years in hemorrhagic stroke group.

Exceedance of time window – 34.2%;

Low or improved NIHSS – 19.8%;

Uncontrolled blood pressure – 6.8%;

Stroke mimics – 5.6%;

History of stroke in the past 3 months – 4.2%;

History of gastrointestinal bleeding – 2.8%;

Uncertain onset – 2.6%;

Family refusal – 1.4%;

No alteplase availability – 2.0%.

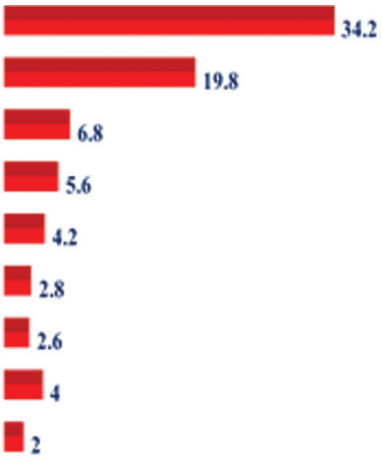

Fig. 1. Current status of intravenous thrombolysis for acute ischemic stroke patients who did not receive IV thrombolytic therapy in the Republic of Moldova

35 (53.0%) were males and 31 (47.0%) were females in ischemic stroke group, 9 (64.0%) were males and 5 (36.0%) were females in hemorrhagic group.

Stroke is predominantly a problem of aging population, the most affected categories being patients aged 61-70 years (23.0%), 71-80 years (33.0%) and patients over 80 years (17.0%), and patients over 70 years (50.0%). Our study of age distribution showed that 67.0% of stroke patients were in the 61-80-year age group, 17.0% patients were in 20-60-year age group, and 18.0% of patients were aged more than 80 years.

The analysis of prevalence of stroke-related risk factors and their distribution based on stroke subtypes among population of the Republic of Moldova showed that the incidence of different risk factors in ischemic stroke (IS) were as follows: 42.6% of cases are due to hypertension, 32.7% – smoking, 32.2% – alcohol intake, 24.8% – diabetes mellitus, 22.6% – coronary artery disease, 18.6% – dyslipidemia, 16.6% – dysrhythmia, 13.4% – previous stroke, 10% -inactivity, 8.8% – past transient ischemic stroke. The major risk factors for developing a hemorrhagic stroke (HS) included 57.0% of cases due to hypertension, 39.3% – smoking, 36 – alcohol intake, 26.8% – coronary artery disease, 26.3% – dyslipidemia, 21.2% – obesity, 26.3% – dysrhythmia, 20% – diabetes mellitus, and 19.8 % – inactivity. CT scan showed 83.0% (66) cases of ischemic stroke, while intracerebral hemorrhage was found in 17.0% of patients.

The present study revealed that the most common clinical presentation was motor weakness (90.0%) followed by headache (39.0%), speech involvement (35.0%), and impaired sensorium (33.0%). The ischemic stroke was characterized by motor weakness in 92.0%, speech involvement (38.0%), headache (33.0%), and impaired sensorium (20%). The hemorrhagic stroke incidence included patients with impaired sensorium in 93.0%, motor weakness in 79.0%, headache and vomiting in 64% of patients, that showed a statistically high significant value ($p<0.001$). In the present study, headache was present in 39.0% of the cases, headache was more common in ICH patients (64.0%) as compared to ischemic stroke patients (33.0%), showing significant statistical value ($p<0.05$). Vomiting was present in 20.0% of patients, including 11.0% of cases of ischemic stroke and 64.0% of cases with ICH. This result was highly significant ($p<0.001$). Seizures were present only in 4.0% of the total patients included in the study.

On clinical examination, right hemiparesis was found in 32 cases (40%), left hemiparesis in 36 cases (40%), facial nerve palsy in 35 cases (44%), aphasia in 23% and dysarthria in 28.0%, respectively [15]. In the RM, only 20.6% of ischemic stroke patients currently receive thrombolytic therapy (fig. 1).

The most common reasons for not receiving thrombolytic therapy were the exceedance of time window 34.2%, low or improved NIHSS 19.8%, uncontrolled blood pressure 6.8%, stroke mimics 5.6% and history of stroke in the past 3 months 4.2%, history of gastrointestinal bleeding 2.8%, uncertain onset 2.6%; family refusal 1.4%, and no alteplase availability 2.0% [16, 17].

Morbidity rate of acute stroke among adult population in the Republic of India

The age range of the study group was 24-88 years. The study group included 49 (61.6%) males and 31 (38.4%) females (M:F = 1.75:1). 25 patients (31.0%) had ischemic stroke and 55 patients (69.0%) had hemorrhagic stroke. The mean age was 53.02 ± 14.38 years in ischemic stroke group and 52.84 ± 12.45 years in hemorrhagic stroke group. In ischemic stroke patients, 15(60.0%) were males and 10 (40.0%) were females. In hemorrhagic group, 36 (66.1%) were males and 19 (33.9%) were females. 74 (92.9%) patients came from rural and semi urban-areas.

Socio-demographic profile was represented by 66.7% of patients with none and primary school, whereas 78% of patients were self-employed or unemployed and 78.9% of pa-

76.3% of acute stroke patients did not receive thrombolysis in present study.
Hemorrhage on computed tomography (CT) scan – 42.3%.
Exceedance of time window – 24.1%.
Low or improved NIHSS score – 18.7%.
Family refusal – 10.9%.
Symptom onset is unknown, or if patient awoke with stroke – 17.2%.
Persistent elevated blood pressure – 12.6%.
Intracranial neoplasm – 2.7%.
Infective endocarditis – 0.4%.
GI malignancy or GI bleeding within 21 days – 3.7%.
Severe head trauma, or intracranial/intraspinal surgery within 3 months – 2.7%.
No alteplase availability – 7.9%.

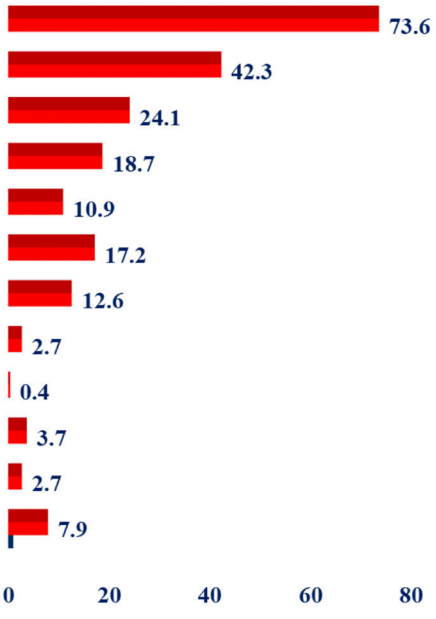

Fig. 2. Current status of intravenous thrombolysis for acute ischemic stroke patients who did not receive IV thrombolytic therapy in the Republic of India

tients from lower middle and poor classes. Age distribution of patients with ischemic stroke was the following: about half (55%) of stroke patients aged 30-60 years, 32.4% of patients aged 60-70 years, and 13% of patients aged over 70 years. Age distribution of patients with hemorrhagic stroke included about 56% of stroke patients aged 30-60 years, 29% of patients aged 60-70 years, and 15% of patients ages over 70 years.

Education status of patients with stroke: 69.8% of patients with ischemic stroke and 60.0% in the group of patients with hemorrhagic stroke were with none and primary school.

Risk factor assessment of ischemic stroke among ischemic stroke patients showed that 66.3% of patients were diabetic, 63.9% of patients – atrial fibrillation, 63.5% – ischemic heart angina, 61.3% of patients were hypertensive and 59.1% suffered from headaches. The risk factor assessment among ischemic stroke patients found that past history of stroke was present in 63.9% of cases, tabacco consumption in 36.4% and alcohol consumption in 66.0% of cases.

The risk factor assessment of hemorrhagic stroke among hemorrhagic stroke patients revealed that 39.9% had headache, 38.7% of patients were hypertensive, 35.5% of patients had atrial fibrillation, 35.5% of patients had ischemic heartangina, and 33.7% of patients were diabetic. The risk factor aassessment among hemorrhagic stroke patients, found that history of stroke was present in 35.5% of cases, tabacco consumption in 45.2% and alcohol consumption in 66.0% of cases.

Clinical signs and symptoms of ischemic stroke were featured by history of TIA (64.1%), disphagia (63.9%),speech problems (61.9%),ocular/visual impairment (63.8%), weakness of the face/limbs (56%)and impaired consciousness (50%). Clinical signs and symptoms of hemorrhagic stroke were characterized by impaired consciousness (49.3%), weakness of the face/limbs (43.6%), history of TIA (36.0%), speech difficulties (39.1%), ocular/visual impairment (36.2%), and disphagia (32.0%). CT scan showed 30.7% (24) of patients had ischemic stroke, while intracerebral hemorrhage and subarachnoid hemorrhage were found in 69.3% (55) and 4.0% (3) of cases, respectively. The most common reason for not receiving thrombolytic therapy in the Republic of India were the following: presence of hemorrhage on computed tomography (CT) scan (42.3%), exceedance of time window (24.1%), low or improved NIHSS score (18.7%), family refusal (10.9%), unknown symptom onset, or if patient awoke with stroke (17.2%), persistent elevated blood pressure (12.6%), intracranial neoplasm (2.7%), infectious endocarditis(0.4%) GI malignancy or GI bleeding within 21 days (3.7%), severe head trauma, or intracranial/intraspinal surgery within 3 months (2.7%), and no alteplase availabi-lity (7.0%).

76.3% of acute stroke patients did not receive thrombolysis in present study, (fig. 2).

The hospital-based retrospective study conducted in Kolkata, reported approximately equal numbers of hemorrhagic (399) and ischemic stroke (393) in 792 patients who underwent CT scan. Hypertension was registered in 77.3% of ICH cases. The unusual finding of this study was a remarkably high number of ICH among the admitted patients. The possible cause of very high hemorrhagic stroke (69.3%) in our population study may be a feature of lifestyle rather than genetics and possibly linked with economic transition of the general population [18-21].

The Indian Government launched National Program for Prevention & Control of Cancer, Diabetes, Cardiovascular

Diseases and Stroke to address high prevalence of non-communicable diseases. Risk factor control requires, multidisciplinary approach, which includes approaching social determinants of health, health-care financing, improving medical education, and health system strengthening [22, 23].

Conclusions

Stroke remains one of the leading causes of death and the largest cause of disability in the RM. According to the latest WHO data published in 2017, Stroke Deaths in RM reached 6638 cases or 15.87% of total death number. The age-adjusted Death Rate is 121.53 per 100000 of Moldovan population and ranks 39 in the world. However, no exact estimation of the incidence and clinical consequence of stroke in India is unavailable, the epidemiological survey covering 52577 people reported an estimated standardized prevalence of 545 per 100000, an annual incidence of 145 per 100000 and a 1-month case-fatality of 41%.

In India, the Stroke-related age onset is the highest in 40–49 year-old population, which is the most productive period of life. Hemorrhagic stroke showed the commonest occurrence in our study. Our population is younger and mostly come from a lower social and economic strata. The possible cause of very high hemorrhagic stroke (69.3%) in our population study might be due to the lifestyle rather than genetics and possibly linked with economic transition of the general population. In the Republic of Moldova, stroke is a predominant problem of aging population, the most affected being persons aged 61-70 years (23.0%), 71-80 years (33.0%) and patients over 80 years (17.0%), patients over 70 years (50.0%). The analysis of different risk factor incidence in ischemic stroke (IS) was as follows: 42.6% – hypertension, 32.7% – smoking, 32.2% – alcohol abuse, 24.8% – diabetes mellitus, 22.6% – coronary artery disease, 18.6% – dyslipidemia, 16.6 % – dysrhythmia, 13.4% – previous stroke, 10% – inactivity, 8.8% – transient ischemic stroke in the past. Risk factor aassessment of ischemic stroke among ischemic stroke patients in the RI showed: 66.3% of patients had diabetes mellitus, 63.9% – atrial fibrillation, 63.5% – ischemic heart disease, 61.3% – hypertension, history of stroke – in 63.9% of cases, tabacco consumption in 36.4% and alcohol consumption in 66.0% of cases.

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Authors' contribution
GC interpreted the data and revised the manuscript critically; VM designed the study; NN drafted the first manuscript; All the authors revised and approved the final version of the manuscript.

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The risk factors for developing primarily detected pulmonary tuberculosis requiring hospitalization

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Abstract

Background: The risk factors assessment and updating will enable to establish an efficient and targeted policy in the fight against tuberculosis, thus providing a much more efficient management of the limited resources available in the Republic of Moldova. The purpose of the research is to study the impact of risk factors in patients with pulmonary tuberculosis treated within inpatient and outpatient units, as well as the effectiveness of treatment.
Material and methods: A case-control analytical, cross-sectional, retrospective study was conducted on 243 patients with pulmonary tuberculosis, with negative and positive microbiological results, which were sensitive to treatment. The patients were divided into two groups: the study group (190), the inpatients and the control group (53) that were treated in the outpatient setting.
Results: The risk factors for developing TB that require hospital admission include the following: demographic factors: men (Odds Ratio) (OR) = 3.29, confidence interval (CI) 95% 1.75-6.17), and passive detection method (OR = 3.25, CI95% 1.72-6.11) epidemiological – contact (OR = 3.66, CI95% 1.63-8.21); socio-economic: unfavorable living conditions (OR = 7.4, CI95% 3.63-15.09), unemployment (OR = 4.77, CI95% 2.27-10.06), primary education (OR = 4, 59, CI95% 1.05-19.91), secondary education (OR = 5.02, CI95% 1.49-16.89), smoking (OR = 13.86, CI95% 1.86-103.4), alcohol and smoking abuse (OR = 3.47, CI95% 1.18-10.18); medical and biological data: two chronic pathologies (OR = 13.86, CI95% 1.86-103.41), liver pathologies (OR = 3.06, CI95% 1.04-9.01).
Conclusions: Inpatients exhibit more risk factors than outpatients, which leads to a more serious development of TB pathogenesis. The efficient sorting of patients according to hospitalization criteria has contributed to a highly successful treatment rate.
Key words: pulmonary tuberculosis, risk factors.

Cite this article
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Introduction

Tuberculosis is an infectious disease, which according to mortality rate is overcome only by HIV. According to WHO data, 1/3 of the global population is infected with *Mycobacterium tuberculosis*. The risk of developing tuberculosis during life have 10% of the population; however, immunocompromised people show a higher risk of disease. The prevalence of tuberculosis infection and the disease itself, as well as mortality from this disease remains a major global health problem [1].

According to the results stated in the WHO report on the fight against TB for 2018, 10 million people have contracted tuberculosis in the world (confidence interval) (CI) = 9.0-11.1 million); this indicator has remained relatively stable lately. In the Republic of Moldova there were 3500 cases of tuberculosis (the incidence of tuberculosis represents 84 cases per 100 000 population), 1400 of them being multidrug-resistant tuberculosis (MDR) (34 per 100 000). Therefore, the Republic of Moldova is one of the 30 countries in the world with a high burden of multidrug-resistant tuberculosis [1].

Assessment of risk factor for contraction of TB and of its unfavorable evolution, might justify the opportunity to change the groups structure that are at high risk for developing this disease, thus, a prompt identification of tuberculosis patients will reduce the number and need for their specific inpatient treatment.

Material and methods

In order to achieve the purpose and objectives of the study a documentary research was performed, which included a retrospective cross-sectional analytical study of case-control type to assess the risk factors involved in the development of primary pulmonary tuberculosis detected. The information was collected by extracting and analyzing the data from the observation sheets of patients admitted to the Municipal Clinical Hospital of Phthisiopneomology and from patient's medical records who received outpa-

tient treatment in the Medical-Territorial Associations of Chisinau.

243 new cases of pulmonary tuberculosis showing negative and positive sensitivity to treatment bacteriological results were included in the study during 01/01/2017-31/12/2017. All the study parameters were compared between two groups: the study group (190, 78.2%) included patients, treated in inpatient settings during the intensive stage, and the control group (53, 21.8%) included patients, treated at both stages within outpatient conditions.

Based on the study results, the database was established. The primary data collected were verified and computer-processed by using Excel of the 2007 Microsoft Office site and the Epi Info 7.2 program. The data were found as absolute, relative (rates, proportions and ratios) and mean values. The statistical data significance was determined by calculating the confidence interval for the significance of the results of 95% (CI95%). The significance of the relative values was assessed by determining the "p" value using the "t" – Student test for assessing the quantitative parameters and the "Chi-square" non-parametric test (criterion $\chi 2$) or the Fisher exact test for the qualitative ones. The probability ratio – Odds Ratio (OR) was calculated to assess the strength of the epidemiological association of risk factors and assigned risk (AR) via the Epi Info 7.2 program. The Excel program helped to calculate the means, ratios, standard deviation (SD), followed by graphical representation.

Results

In 2017, 310 of new cases were registered in Chisinau. Of these – 112 (36.1%) showed negative bacteriological results, – 130 (41.9%) were positive, sensitive to anti-tuberculosis treatment and 68 (22.00%) were positively resistant to treatment.

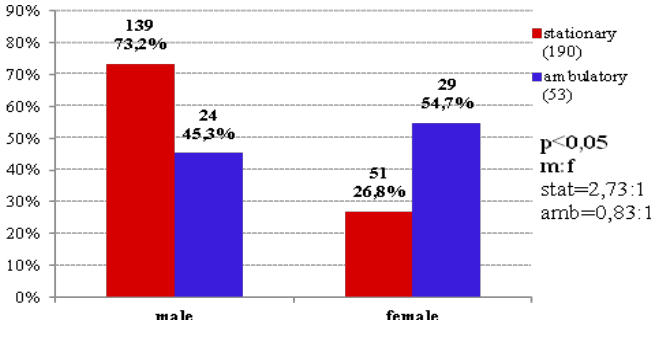

Fig. 1. Gender distribution

Almost 3/4 of the hospitalized patients were men (fig. 1), the men: women ratio being of 2.73: 1, while more than half were women from outpatient department (p <0.05).

In both groups, more than half of the patients were aged between 25-44 years (fig. 2) (p> 0.05). The average age (SD) of inpatients was 42.4 years (14.3), and of outpatients was 41.7 years (15.8).

The inpatients were mostly detected by a passive method (by referral), accounting for 2/3 of the patients, whereas only 1/3 of cases were identified by the active method (by prophylactics) (fig. 3). Both methods show an equal occurrence within outpatient settings, viz. half of the cases per each (p<0.05).

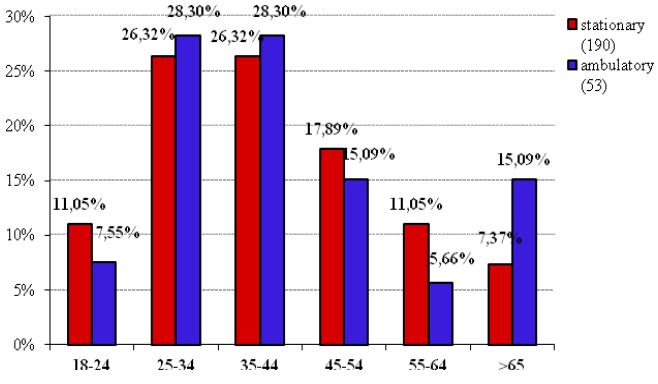

Fig. 2. Age distribution

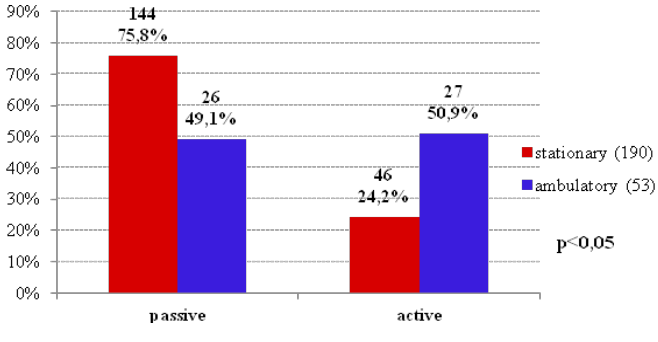

Fig. 3. Detection method

From an epidemiological point of view, the study assessed the possible contact of patients on being migrants from countries with a high-burden of tuberculosis. The present study also analyzed data of homeless people, given the fact that they have a much higher probability to contact tuberculosis, as well as they are more prone to a multitude of risk factors: epidemiological, socio-economic and medico-biological.

Table 1

Epidemiological factors

Epidemiological factors	Inpatients (190)		Outpatients (53)		p
	N	%	N	%	</>0,05
Contact	75	39,47	8	15,09	<0,05
Migrants	49	25,79	7	13,21	>0,05
Homeless	19	10,00	0	0	<0,05
Penitentiary	20	11	2	4	>0,05
No factor	92	48,42	38	71,70	<0,05

Based on the table data, 39.47% of the inpatients and only 15.09% of outpatients had contact with a tuberculosis patient (p <0.05). There were 19 homeless patients who

received treatment at both stages of inpatient treatment (p<0.05). 1/4 of the patients from the hospital had a migrant status, whereas 7 (13.21%) patients underwent outpatient treatment (p>0.05). 20 patients who received inpatient treatment returned from the penitentiary, which represents 10.5%, and only 2 (3.7%) patients were treated in outpatient conditions (p>0.05). A large number of patients showed no epidemiological risk factors. Thus, almost 1/2 of the inpatients and almost 3/4 of the outpatient did not register any epidemiological factor (p<0.05).

The socio-economic aspects of the patients were examined in terms of living conditions, marital status, occupation and level of education.

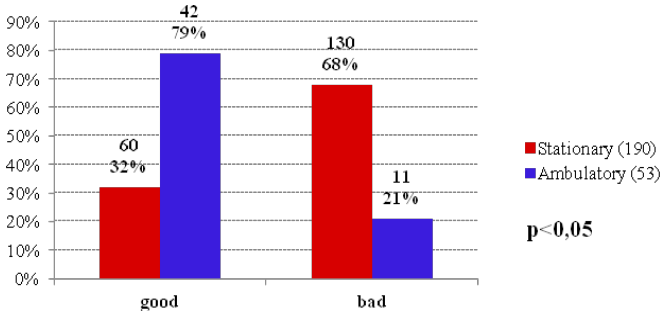

Fig. 4. Living conditions

2/3 of inpatients and only 1/5 of cases from outpatient department had unsatisfactory living conditions (fig. 4), (p <0.05).

Table 2

Occupation and level of education

Occupation / education	Inpatients (190)		Outpatients (53)		p
	N	%	N	%	</>0,05
Employed	58	30,53	34	64,15	<0,05
Unemployed	100	52,63	10	18,87	<0,05
Limited work capacity	9	4,74	0		>0,05
Retired	16	8,42	6	11,32	>0,05
Student	5	2,63	2	3,77	>0,05
Maternity leave	2	1,05	1	1,89	>0,05
Primary education	29	15,26	2	3,77	<0,05
Gymnasium studies	44	23,16	3	5,66	<0,05
Secondary education	86	45,26	33	62,26	<0,05
Higher education	21	11,05	14	26,42	<0,05
Incomplete higher education	10	5,26	1	1,89	>0,05

Only 30% of inpatients were employed and more than a half were unemployed. However, almost 2/3 of patients from outpatient department were employed and less than 1/5 unemployed (p<0.05). There were registered 9 people with limited work capacity, who received treatment under inpatient conditions (tab. 2).

Almost 40% of the inpatients had a low level of education, thus accounting for about 10%, compared to most of

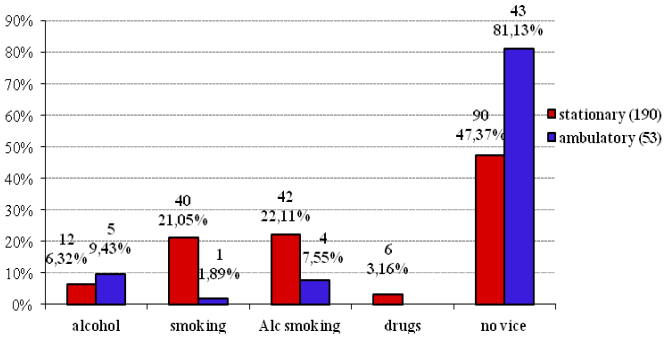

Fig. 5. Vice-aassociated risk factors

patients who had a profession or higher education (p<0.05) (tab. 2).

More than half of the inpatients had at least one associated bad habit, whereas only every fifth of individuals was from the outpatient department (p<0.05). Among the inpatients, 6.32% recorded an alcohol abuse, 22.11% – tobacco smoking and 21.05% of cases combined these two bad habits. The ratio of patients from outpatient department showed a more significantly reduced number of alcohol users and smokers compared to the inpatients. Drug users were registered among those admitted to hospital as compared to the outpatients. The distribution difference between these two groups shows statistically significant values (p<0.05) in cases of smoking-associated and both smoking and alcohol-associated risk factors (fig. 5).

The associations of chronic pathologies, such as HIV, diabetes mellitus, lung diseases, liver pathologies, gastrointestinal tract diseases, cardiovascular diseases, psychiatric disorders, etc. were studied from both medical and biological point of view. In addition, the study analyzed whether there is a difference when patients have one, two or three associated pathologies.

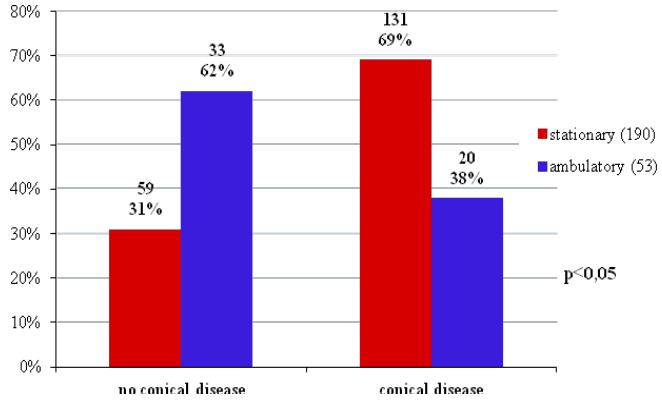

Fig. 6. Associated chronic pathologies

Almost 70% of inpatients had at least one associated chronic pathology, while less than 40% were from the outpatient settings (p<0.05) (fig. 6).

More than 1/3 of the inpatients and outpatients presented with an associated chronic pathology, but in the inpatients each fifth patient had associated 2 chronic pathologies

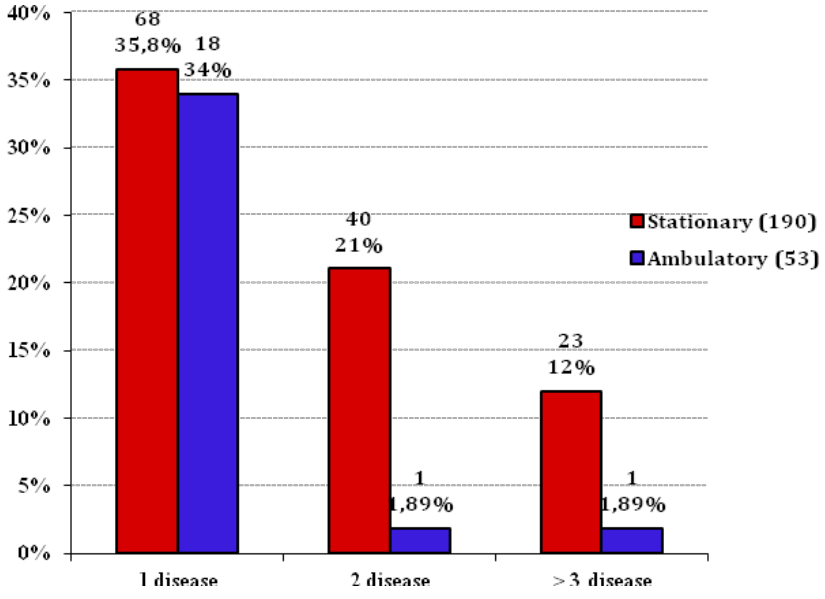

Fig. 7. Number of associated chronic pathologies

Table 3

Associated chronic pathologies

Associated chronic pathologies	Inpatiens (190)		Outpatients (53)		p
	N	%	N	%	</>0,05
Liver diseases	38	20	4	7,55	<0,05
Lung diseases	29	15,26	3	5,66	>0,05
Cardiovascular diseases	25	13,16	2	3,77	>0,05
HIV	23	12,11	0	0	<0,05
Diseases of the gastrointestinal tract	20	10,53	2	3,77	>0,05
Neurological diseases	21	11,05	1	1,89	>0,05
Diabetes mellitus	15	7,89	1	1,89	>0,05
Immunosuppressive therapy	9	4,74	4	7,55	>0,05
Anemia	10	5,2	0	0	>0,05
Lues	7	3,68	0	0	>0,05
Psychiatric diseases	6	3,16	0	0	>0,05
Vascular diseases	3	1,58	1	1,89	>0,05
Other diseases	31	16,32	2	3,77	<0,05

(p <0.05), and each ninth three or more (fig. 7).

The most common pathologies associated among hospitalized patients were liver (p <0.05), pulmonary and cardiovascular disorders. There were registered 23 patients with HIV, all of them received inpatient treatment (tab. 3).

The present study is a comparative assessment of the epidemiological associations of determinants in tuberculosis, performed in patients diagnosed with primary pulmonary tuberculosis detected in 2017, who received inpatient treatment, as well as in patients who received outpatient treatment. For this purpose, 46 risk factors were determined and analyzed, of which, 11 showed a statistically significant

Table 4

Ranking of risk factors

Parameter	OR, CI95	AR (%)	Ranking
Smoking	13,86 (1,85-103,41)	92,78	I
2 chronic diseases	13,86 (1,85-103,41)	92,78	I
Bad living conditions	7,4 (3,63-15,09)	86,50	II
Gymnasium studies	5,02 (1,49-16,89)	80	III
Unemployed	4,77 (2,27-10,06)	79,03	IV
Primary education	4,59 (1,05-19,91)	78,21	V
Contact	3,66 (1,63-8,21)	72,67	VI
Alcohol and smoking	3,47 (1,18-10,18)	71,18	VII
Men	3,29 (1,75-6,17)	69,60	VIII
Passive detection	3,25 (1,72-6,11)	69,23	IX
Liver diseases	3,06 (1,04-9,01)	67,3	X

difference between samples (p <0.05). Thus, it was possible to calculate the ratio of the probability – Odds Ratio (OR) and the assigned risk (AR). The present study assessed and analyzed the epidemiological relationship of the determinant factors for tuberculosis, requiring inpatient treatment, among patients who are mostly exposed to their action, followed by their ranking.

The highest risk is attributed to social, medical and biological factors (tab. 4). Thus, smokers and people with two associated chronic pathologies had a 13.86 (OR = 13.86; CI95 1.85-103.41) or higher probability to develop tuberculosis, requiring hospital admission. These are followed by patients who exhibited unfavorable living conditions (OR = 7.4; CI95 3.63-15.09), low level of education (OR = 5.02; CI95 1.49-16.89), were unemployed (OR = 4.77; CI95 2.27-10.06), contacted other persons (OR = 3.66; CI95 1.63-

8.21), as well as alcohol and tobacco consumers (OR = 3.47; CI95 1.18-10.18), men (OR = 3.29; CI95 1.75-6.17), subjects detected by passive method (OR = 3.25; CI95 1, 72-6.11), people suffering from chronic liver diseases (OR = 3.06; CI95 1.04-9.01).

Table 5

The treatment outcomes

The result of the treatment		Inpatients (190)		Outpatients(53)		p
		N	%	N	%	</>0,05
Successful treatment		157	82,63	50	94,34	<0,05
	Treated	90	47,37	12	22,64	<0,05
	Completed	67	35,26	38	71,7	<0,05
Lost surveillance		14	7,37	2	3,77	>0,05
Died		19	10	1	1,89	>0,05
	TB	11	5,79	0		>0,05
	Other causes	8	4,21	1	1,89	>0,05

The successful treatment rate (tab. 5) was high in both hospitalized patients (82.63%) and outpatients (94.34%). Every tenth inpatient died, while only one death was registered in the outpatient department (1.89%).

Discussion

The study analyzed the impact of risk factors on the development of tuberculosis in hospital and outpatient departments in 2017. It recorded a predominant incidence of males compared to females. The data obtained are similar to the results provided by the WHO [2]. Most patients were of working age, between 25-54 years.

Most patients were passively detected; this factor determined the progression of the disease in a more advanced evolution. Specialists at international and national level recommend the active detection of tuberculosis cases, in order to intervene promptly in its treatment and avoid the spread of infection [2, 3].

The socioeconomic, biomedical and epidemiological risk factors were assessed within this study. Thus, from a socio-economic point of view, the marital status, the patients' occupation, the level of schooling and the associated vices were studied as risk factors in the development of tuberculosis. Statistically significant results were obtained for unemployed patients, those with primary and secondary education, in smokers, but also in patients who excessively consume alcohol and tobacco. Studies conducted by researchers say that tuberculosis is a social pathology and largely affects the socially vulnerable population. The excessive smoking and alcohol consumption of 40 g per day are also reported as risk factors in contacting tuberculosis [4-9].

From a biomedical point of view, the associated pathologies of the patients were analyzed. The study tried to find a causal link between the area affected by a chronic disease and the development of tuberculosis. Patients living with chronic pathologies have a higher risk of developing tuberculosis that requires hospitalization, however contrary to expectations most patients suffer from liver, lung and cardiovascular pathologies. The pathologies, such as diabetes, renal failure, and immunosuppressive treatment showed statistically insignificant results. Although the study group (inpatients) had a statistically significant distribution (p <0.05) for HIV/AIDS it was impossible to determine the strength of the epidemiological association and the risk assigned due to the absence of patients living with HIV/AIDS in the control group (outpatients) [4, 7, 9, 10-13].

From an epidemiological point of view, the patients who were in contact with a tuberculosis patient, the phenomenon of migration to / from countries with a high tuberculosis load, homeless patients and those released from detention were studied. Epidemiological factors were more common in inpatients; however, statistically significant results were obtained for patients who had contacts with TB-infected persons [4, 7, 9, 12, 14].

Conclusions

1. Most inpatient cases included men of working age, detected by the passive method, 1/3 of which having a contact with a TB-infected person, 1/4 being migrants, and every tenth was homeless or released from the jail. The outpatient clinic revealed a predominant number of women and people detected by the active method.

2. The social factors were more highlighted in the patients treated in the inpatient departments, such as unfavorable living conditions, lack of employment, low level of education, and vicious habits.

3. Hospitalized patients had 2-3 comorbidities, often at a decompensation stage that required permanent and thorough medical monitoring, whereas most patients in outpatient conditions did not present or have only one associated disease.

4. According to the ranking of risk factors it was established that patients who are exposed to socio-economic factors (smoking, unsatisfactory living conditions, unemployment, low level of education) and medico-biological (association of two chronic pathologies), are more likely to develop tuberculosis that requires hospitalization.

5. A multitude of risk factors present in inpatients, compared to outpatients, leads to the development of TB processes with more serious evolution, and efficient sorting of patients according to hospitalization criteria has contributed to achieving a high success rate of treatment.

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Authors' contribution
VF acquired and interpreted the data and drafted the first manuscript, AU interpreted the data, revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The research was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy (protocol No 55 of June 18, 2015).

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

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Acute transverse myelitis in a HIV-positive patient with COVID-19

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Abstract

Background: Immunocompromised status keeps on being a challenge for a physician, especially in the context of the coronavirus disease – 19 (COVID-19) pandemic. The predominant clinical presentations are related to the respiratory system, but neurological manifestations are recognized increasingly. Cases of myelitis associated with the new coronavirus infection have already been published, but no cases of HIV-positive patients with myelitis and COVID-19 have been reported yet.
Material and methods: This study described a clinical case of a human-immunodeficiency virus (HIV) – positive patient, who developed an acute transverse myelitis with confirmed SARS-CoV-2 infection.
Results: Magnetic Resonance Imaging examination showed longitudinally extensive spinal cord abnormality, and laboratory tests confirmed SARS-CoV-2 infection. The patient responded to methylprednisolone pulse therapy, followed by oral corticosteroids and therapeutic plasma exchange.
Conclusions: Continuing pandemic and the expectation that a large part of the world population will be infected suggest that the number of patients with neurological manifestations could become large. Curious neurologic constellations can appear which complicate the diagnostic process and treatment in certain patients.
Key words: COVID-19, transverse myelitis, neuromyelitis optica spectrum disorder.

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Introduction

By September 1, 2020 the coronavirus disease – 19 (COVID-19) pandemic has resulted in more than 25 million confirmed cases and more than 850000 deaths [1]. The predominant clinical presentations are related to the respiratory system; however, neurological manifestations are recognized increasingly. These features are determined by the effects of a combination of direct viral infection, inflammation of the structures of the nervous system and vasculature, nonspecific complications of systemic disease, which can be para-infectious or post-infectious [2].

Transverse myelitis (TM) is a focal disorder of the spinal cord presenting in acute or subacute manner (hours/days) resulting in motor, sensory, and autonomic dysfunction of varying degrees of expression depending on the type, location and surface of the lesion longitudinally and transversely. The term longitudinally extensive transverse myelitis is used when the spinal cord lesion extends over three or more vertebral segments.

The possible causes of myelitis are as follows: infections (viruses, bacteria, fungi, and parasites), post-infectious or post-vaccination, paraneoplastic, demyelinating (multiple sclerosis (MS), neuromyelitis optica (NMO), acute disseminated encephalomyelitis, other inflammatory disorders (neurosarcoidosis, systemic lupus erythematosus, Sjögren syndrome, Behcet's disease), congestive edema due to dural arteriovenous fistula, and tumor [3, 4]. TM may be idiopathic in 15-30% of cases. Post-infectious and idiopathic myelitis incidence varies between 1.3 - 8 cases / 1 million, but can reach 24.6 cases / million in patients with MS [5]. Recent case reports of myelitis associated with the new coronavirus infection were published, but the mechanism of spinal cord injury remains unclear [6, 7].

Human-immunodeficiency virus (HIV) causes spinal cord injury both by direct HIV virus invasion, presenting in vacuolar myelitis in most cases, as well as manifestations of opportunistic virus-induced infections (Herpes simplex virus (HSV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Varicella Zoster virus, or bacteria (Mycobacterium

tuberculosis, Treponema pallidum), as well as due to vitamin B12 deficiency. Vacuolar myelopathy (VM) is symptomatic in 5-10% of acquired immune deficiency syndrome (AIDS) patients, shows a progressive evolution and is pathologically characterized by the presence of intralamellar white matter vacuoles in the posterior and lateral columns of the thoracic spinal cord [8].

SARS-CoV-2 causing the COVID-19 pandemic is proven to be neurotropic and may affect the central and peripheral nervous system. The mechanism of the new type of coronavirus neuroinvasion has not yet been fully identified [2, 9]. Short- and long-term health effects for patients who have been infected have not yet been assessed. The changes induced by the new type of coronavirus in the coagulation status resulted in an impressive number of fatal thromboembolic events, including the cerebral ones. Guillain-Barré syndrome reported in COVID-positive patients is explained as a possible parainfectious manifestation, whereas cases of necrotizing hemorrhagic meningoencephalitis, altered mental status, and respiratory distress syndrome are referred to both direct virus action on brain neurons, as well as cytokine storm syndrome [10, 11].

Material and methods

The present study reported a clinical case of a human-immunodeficiency virus (HIV) – positive patient who developed an acute TM with confirmed SARS-CoV-2 infection.

Case report

A 27-year-old male, with known HIV infection for the past 1 year, treated with anti-retroviral therapy, was hospitalized on June 7, 2020 due to paresthesia and numbness in legs and in the right arm, paralysis in lower extremities, and bladder and bowel dysfunction (retention). All these symptoms developed quickly, reaching the nadir in 15 hours.

Clinical evaluation revealed normal cranial nerves function, spastic tetraparesis, with 4/4.5 Medical Research Council (MRC) in upper and 0.5/2MRC in lower extremities, Th7 superficial and C7 deep sensory level disturbances. The patient was subfebrile 37.5C without other systemic abnormalities.

An extensive paraclinical workup was obtained. Routine laboratory data showed a slight general blood inflammatory abnormalities (WBC 13.7 x10^9, ESR 14 mm/h) but with negative CRP. His CD4 count at admission was 310 cells/µl, and viral load was less than 40 copies/ml. Cerebrospinal fluid (CSF) analysis was normal. Blood serology and CSF polymerase chain reaction (PCR) for HSV 1,2,6, CMV, EBV, Borrelia burgdorferi, Treponema pallidum, Toxoplasma gondii, Chlamydia trachomatis, Mycoplasma pneumoniae, Ureaplasma urealyticum were negative. Autoimmune markers tests (autoantibodies ANA, ANCA), tumor marker tests (CA 19.9, Ca 15.3, PSA, CEA, Alpha Fetoprotein) and hepatitis viral serology all were negative. Thyroid function tests were normal. The first swab for SARS-CoV-2 at admission was negative. CSF oligoclonal bands (OCB) and serum anti-aquaporin-4 antibody (AQP4-IgG), anti-MOG antibodies were negative. Vitamin B12, methylmalonic acid and angiotensin-converting enzyme levels were normal. Folic acid level test was 4.62 nmol/L (normal range 6.00-39.0). Brain MRI did not show any abnormalities. Spinal cord MRI revealed an extensive C4-Th5 lesion mainly in posterior columns and right lateral column without gadolinium enhancement (fig. 1).

The patient was treated with IV methylprednisolone 1g/day for 5 days, followed by oral corticosteroids and five procedures of plasma exchanges. His condition improved with a significant reduction in paresis. Lung CT performed on the 19th day of illness showed slight patchy ground-glass opacity basal on the left side. No other typical symptoms for CoV-2 infection were noted. A repeated swab for SARS-CoV-2 was positive, followed by negative COVID-19 PCR in the CSF. The 1-month follow-up spinal MRI did not reveal significant changes.

Two-month follow-up revealed significant improvement of muscle strength allowing the patient to walk. Sphincter disturbances became less severe. At the same time, the CD4 count dropped to less than 200 cells/µl.

Discussion

The presented case is unique for several reasons. All these circumstances – transverse myelitis, HIV infection and COVID-19 may be a random combination, as well as a range of three successive elements or an interrelationship that influenced the onset of clinical signs and imaging changes. Normal CSF examination does not meet the TM diagnostic criteria [12]. Negative data for OCB and AQP4-IgG, and normal brain imaging disclaim the most common demyelinating causes of TM-MS and NMO, but do not rule out a possible isolated clinical syndrome or NMO spectrum disorders.

Spinal cord injury presented in posterior and lateral columns suggested a subacute combined degeneration due to vitamin B12 deficiency. Neurosyphilis and neurosarcoidosis were excluded by laboratory and imaging studies.

Despite the patient's CD4 cells count that did not indicate a severe degree of immunosuppression, the risk of opportunistic infections as a cause of myelitis had to be considered. However, the absence of CSF pleocytosis and normal infectious disease tests did not confirm this hypothesis. HIV-associated spinal cord disease cannot be excluded, particularly vacuolar myelitis, which is slowly progressive and that predominantly occur at AIDS stage. The clinical examination usually demonstrates motor and sphincter disorders, sensitive ataxia, though without a sensory level, as well as normal or discreetly modified MRI that often develops a simultaneous cognitive impairment [8].

The patient's SARS-CoV-2 infection role is controversial. This could be an accidental co-infection during hospitalization, a fact supported by the negative test results at admission and the lack of typical blood changes. At the same time, a false-negative result cannot be denied. High-dose corticosteroids therapy and plasma exchange might influence

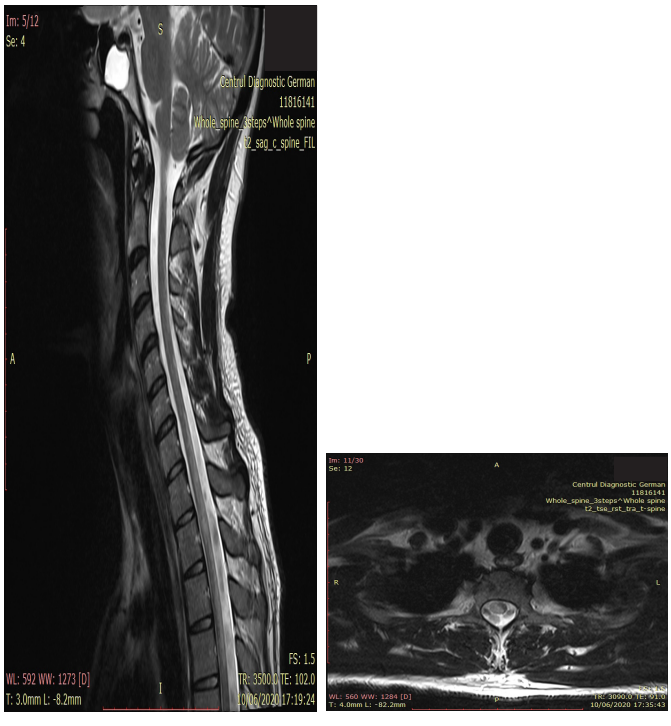

Fig. 1. Sagittal and axial MRI shows the spinal cord segmental myelopathy area at C4-T5 level, ~15 cm in length, with a predominant involvement of the posterior and right lateral columns, without contrast uptake

the relatively benign course of SARS-CoV-2 pneumonia. Myelitis may resemble a direct virus injury, or a parainfectious autoimmune process, as well due to molecular mimicry, the fact sustained by the positive response to corticosteroid therapy.

Conclusions

Continuing pandemic and the expectation that a large part of the world population will be infected, suggest that the number of patients with neurological manifestations could become greater. Curious neurologic constellations can appear which complicate the diagnostic process and treatment in certain patients.

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REVIEW ARTICLES

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Applicability of next generation genetic testing in epilepsy through whole exome sequencing

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Abstract

Background: Epilepsy affects around 1% of the general population. With already acknowledged strong genetic contributions, >50% of epilepsy cases still remain undiagnosed. This is primordially due to the multifactorial condition of epilepsy that makes it a challenge to select the optimal genetic test for each specific case. Recently, next-generation sequencing (NGS) led to massive gene discovery, including epilepsy that also imposed serious financial burdens on healthcare systems. This study review highlights the progress in the field of epilepsy genetics and argues on how the genetic architecture of common epilepsies is progressively being unraveled. Since the 1995 finding of *CHRNA4* mutation, more than 500 genes were estimated to play a significant role in epilepsy. To date, the majority of diagnostic genetic testing is conducted in the pediatric population, while the utility of such testing is less well understood in adults with epilepsy. A broad range in the diagnostic rate of NGS, especially of the Whole Exome Sequencing (WES), in epilepsy has been described. However, NGS introduces new challenges, yet to be resolved.
Conclusions: Epilepsy's genetic background is nowadays undeniable; however, the complexity of this condition makes it difficult to be solved. WES has increasingly been used to uncover the role of the coding genetic material in the human genome and is nowadays considered one of the most cost-effective genetic tests for epilepsy, being a prerequisite for personalized treatment approaches and for reducing the epilepsy patient's "diagnostic odyssey".
Key words: epilepsy genetics, next-generation sequencing, whole exome sequencing.

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Introduction

Epilepsy affects approximately 0.6% to 0.8% of the general population and it is a disorder with strong genetic contributions [1]. Globally, the idiopathic epilepsy, a term introduced in 1985, within the International League Against Epilepsy (ILAE)'s proposal for classification of epilepsies and epileptic syndrome [2], means epilepsy of genetic origin or without a definite structural, metabolic, infective, or immune cause / or when diagnostic assessment did not reveal a causative factor) – ranked the 5th among neurological disorders after stroke, migraine, dementia, and meningitis and even the 2nd in some particular areas (southern sub-Saharan Africa) [3].

The incidence of epilepsy is nearly 70 per 100000 children younger than 2 years and genetic epilepsies account for more than 0.4% of the general population, constituting 30% of all epilepsies [4]. A study on a larger group of severe epilepsy cases starting before the 18month-age found an incidence of one in 2000 births [5-7].

Globally, in 2016, there were 45.9 million patients with all-active epilepsy (both idiopathic and secondary epilepsy globally). Of these patients, 24 million had active idiopathic epilepsy (prevalence 326.7 per 100000 population) [8].

Idiopathic epilepsy accounted for 0.23% of deaths and 0.56% of disability-adjusted life-years (DALYs) from all causes. Global age-standardized mortality rates of idiopathic epilepsy were 1.74 per 100 000 population (1.40 per 100000 population for women and 2.09 per 100000 population for men) [8]. A decrease in death and DALYs rates in patients with epilepsy between 1990 and 2016 was recorded, however the changes varied across geographical areas and based on the available data within countries. Furthermore, changes were linked to the socio-demographic development status, which should prompt more action in economically deprived areas. The success of reducing the burden of idiopathic epilepsy relies mostly on access to treatment and diagnostic techniques [3, 8].

Several diseases and injuries are involved in the origin of

epileptic seizures, showing a variable distribution worldwide [9]. Meanwhile, 4% to 78% of selected patients with initially unknown epilepsy etiology have genetic variants of probable or definitive etiologic significance [10]. The estimated proportion of individuals who carry a pathogenic variant that contributes substantially or causes epilepsy is approximately 17% of patients for epileptic encephalopathies, 5% of patients with genetic generalized epilepsies, and 2% for non-lesional focal epilepsies [11]. However, more than 50% of patients with developmental and epileptic encephalopathies (DEEs) cannot be genetically diagnosed despite state-of-the-art genetic testing techniques [9, 12].

In 2019, more than 140 epilepsy-associated genes or loci have been listed within the Online Mendelian Inheritance in Man database [13].

Familial analysis in epilepsy

Human genetics research has established that a genetic basis contributes to the susceptibility to epilepsy in most cases. However, the multifactorial condition of epilepsy that subsumes a variety of epilepsy types, seizures, levels of severity, and comorbidity has made it a core challenge to disentangle the genetic architecture for different types of epilepsy and to determine the specific genetic risks for each individual with epilepsy [14].

Early epilepsy gene discoveries used the strategy of ascertaining very large families, typically with 10 or more affected individuals, where the family history supported the presence of simple inheritance, and success utilizing parametric linkage analysis was likely [15]. This approach led to the recognition of a number of familial epilepsies and some of their genetic determinants.

The epilepsy diathesis hypothesis suggested that a familial predisposition for epilepsy exists due to the inheritance of susceptibility variants. In support of this was the discovery that rare inherited copy number variants can increase risk for different epilepsy syndromes [16].

Since the historical finding of a CHRNA4 mutation causing autosomal dominant sleep-related hypermotor epilepsy (formerly known as autosomal dominant nocturnal frontal lobe epilepsy) in 1995 [17], discoveries of epilepsy genes have advanced greatly and accelerated further with the advent of next generation sequencing [10, 18].

Most genes identified to date come from monogenic families of focal epilepsies, and attempts to identify risk genes associated with genetic generalized epilepsies (GGE) have been largely unsuccessful [19]. Besides that, to date, reports from largescale Whole Exome Sequencing (WES) projects in epilepsy have focused mainly on cohorts with severe epilepsies of infancy and childhood, particularly the epileptic encephalopathies [20, 21]. These studies have reported diagnostic, monogenic causes in almost 27% of cases, identifiable via exome sequencing [22-24].

Fakhro et al. recently confirmed the benefit of working with families whose large sizes facilitate the assessment of multiple siblings [25]. The effect of adding siblings to the analysis of recessive variants was even more drastic than for *de novo* variation. Between 12 to 42% of recessive variants discovered in an index case were shared by a single sibling, and only 1.3 to 11% were shared by two siblings. For families where there were 3 affected siblings, for example, GD001, the only variant remaining after filtration was the disease-causing variant. Conversely, in settings where siblings do not share the phenotype, the additional siblings can help sort benign family-specific polymorphisms from *bona fide* disease variants [25]. At the same time, index cases may appear to have as many as 10 *de novo* protein-altering variants when compared only with their parents, requiring significant time and resource investment for experimental validation. Therefore, introduction of a single sibling will reduce that number by more than half, while introduction of two siblings reduced the mean number of high quality protein-altering *de novo* variants to 0.5 per individual, consistent with previous reports [26].

Relatives of people with epilepsy have shown an increased incidence of epilepsy, even in families without Mendelian (monogenic) patterns of inheritance [27]. Moreover, studies on twins and families have shown that specific features of epilepsy are themselves heritable traits, including specific epilepsy syndromes [28], seizure types and symptoms [29], and EEG patterns [30]. Furthermore, the risk of epilepsy appears to be higher in the relatives of probands with generalized epilepsy than in the relatives of probands with focal epilepsy [27].

A lot of other, still incompletely studied family features may have genetic determinants that are distinct from the genetic determinants of epilepsy per se, just as in a recent study that proved the age at seizure onset to be an independent familial trait, with possible genetic determinants distinct from the determinants of particular epilepsy syndromes [13].

Several novel genes and disorders associated with DEE have been identified in the last few years [31-33]. Many of the genes causing epilepsy encode components of neuronal ion channels leading to neuronal hyperexcitability or depletion of inhibitory mechanisms [34, 35]. However, recently, several new genes coding for proteins other than ion channels have been identified, such as chromatin remodelers, intracellular signaling molecules, metabolic enzymes, transcription factors, and mitochondrial complex genes [6, 36].

Genetic testing in epilepsy

Clinical features often drive the choice of a particular genetic test or testing strategy, but in many patients, their presentation is not suggestive of a specific gene, or set of genes. WES and epilepsy panels (EP) are nowadays considered the most cost-effective genetic tests for epilepsy [37].

Gene panels provide a higher sequencing depth and lower cost when compared to the exome or genome sequencing, but restrict the diagnosis to specific genes in the panel, commercially available EPs typically targeting from 70 to 465 genes [38].

Importantly, some large panels are now based on WES, with restricted analysis of only the "panel" genes, so the benefit of higher depth of coverage is lost, but this opens

up the possibility of future reanalysis to include the whole exome [12].

Considering the fact that copy-number variants (CNVs) contribute significantly to variation in the human genome and estimating that they cause 1.2% difference for every reference genome [39], previous recommendations used the stepwise chromosomal microarrays method (CMA) ± EP ± WES testing strategy in epilepsy. CNVs can be detected by several genomic methods including conventional karyotype (deletions/duplications >5 Mb), CMA (~100 kb–5 Mb) and/or other methods, such as quantitative PCR and multiplex ligation-dependent probe amplification that target to detect smaller variations (<1 kb) [12].

Although less expensive, CMA has a lower diagnostic yield in epilepsy, and its use as the first-tier test is thus not anymore supported from a cost-effectiveness perspective [37]. However, in specific scenarios like epilepsy plus intellectual disability, epilepsy plus autism spectrum disorder, epilepsy with dysmorphic features – CMA is still considered be the most cost-effective and clinically useful test [37]. Studies using CMA have shown that pathogenic CNVs account for 5–10% of childhood epilepsies including DEE [40, 41]. Besides that, the most common types of genetic causes of DEE are sequence changes, responsible for 30–40% of cases, and chromosomal deletions or duplications, responsible for 5-10% of cases [10, 42]. Thus, an individualized evaluation of cost-effectiveness based on prior diagnostic yields for each of the targeted populations and costs for each test should be considered that is expected to optimize the diagnostic yield and use of resource. It is worth mentioning that the diagnostic yield of copy number variants (CNVs) is better understood in paediatric epilepsy compared to adult patients with epilepsy [43, 44].

More recently, *de novo* mutagenesis has emerged as the major genetic mechanism in epileptic encephalopathies and rapid progress in identifying them has been facilitated by WES [45, 46].

An increasingly appreciated and clinically important subtlety for the *de novo* paradigm is the role of mosaicism – post-zygotic mutations not present in every cell in the body. This kind of somatic mosaicism might contribute to the phenotypic heterogeneity seen with many epilepsy genes [13]. This new genetic mechanism has been recently identified as playing a larger role in focal epilepsies than it was previously thought. The repeated expansions in intronic regions – identified as the cause of a familial epilepsy syndrome associated with myoclonus [47] and tremor [48] suggest the role of these type of variants in epilepsies, an important aspect that is not easily detected by current sequencing technologies, the vast non-coding portion of the genome (including intronic and intergenic regions) that are currently explored in neurodevelopmental disorders and the analysis of the regulatory regions (e.g., promoters and enhancers) in patients with autism and developmental delay [49]. Another aspect is represented by the genes, the mutations in which they evoke a range of different phenotypes, yet to be described, starting with complex, neonatal onset diseases at the severe end and a childhood onset at the milder end of the spectrum, including or excluding epilepsy from the picture, depending on the type of the mutation [50].

Another issue to be discussed is the use of Next Generation Sequencing (NGS) methods to identify disease-causing variants in poorly characterized populations that presents several challenges. For example, it was recently discovered that up to 15% of "variants" detected in >1000 Arabian people when aligned to reference genome GRCh37/hg19 had a minor allele frequency (MAF) >50% in the same cohort and therefore should to be considered reference alleles for this population [51].

Despite all the previously mentioned challenges, the need to identify causative genes for genetic disorders is an urgent issue, given that Mendelian diseases on aggregate affect ~8% of live births and are the leading cause of morbidity and mortality in children worldwide [52]. This also poses serious financial burdens on healthcare systems – in the cases where healthcare intervention is available, the total cost of care over an individual's lifetime may exceed $5 000 000 [53].

Whole Exome Sequencing in epilepsy

Over the last decade, NGS has significantly advanced the field of human genetics and genomics [54], leading to an explosion of gene discovery across many human disorders. The number of disease-associated genes has grown to 4132, and over 50 genes have been newly associated with epilepsy in the last three years alone [55].

It was previously established that WES, in combination with array-comparative genomic hybridization (aCGH), provides a diagnostic rate of 27% in unrelated adult epilepsy patients, 42% in unrelated paediatric patients, and 31% in a combined adult and paediatric cohort of unrelated patients with medically refractory epilepsy and co-morbid intellectual disability, that indicates that WES has similar utility in both adult and paediatric cohorts and is appropriate for diagnostic testing in both epilepsy patient groups [56]. To date, the majority of diagnostic genetic testing is conducted in the paediatric population, while the utility of such testing is less well understood in adults with epilepsy.

Another recent meta-analysis comprising more than 20000 children proved the diagnostic and clinical utility of whole exome/genome sequencing to be greater than chromosomal microarray alone, and that it should be considered as the first-line genomic test for children with suspected genetic diseases [57]. WES alone, judging on the previous studies, in mixed-age populations with multiple seizure types, has a diagnostic yield of 33–38% [10, 24, 58].

WES is not yet a match for CMA for CNV detection, as it can provide data about only the protein coding or exonic regions, but it is an increasingly powerful diagnostic tool, since a growing number of algorithms are being developed to aid the detection of CNVs by NGS and it is now possible to detect both single nucleotide variations (SNVs) and CNVs using an exome – or genome-wide approach with a single test [59].

A broad range in the diagnostic rate of WES in epilepsy has been described, the result of the variable definition of

each cohort depending on factors, such as type of epilepsy, phenotypic features, disease severity or prior genetic screening. In focal epilepsy, genetic diagnostic rate varies between 12.5% of cases [60] to 43% of cases with epileptic encephalopathy (EE) and in 33% of epilepsy cohort overall [10].

In 2011, the International League Against Epilepsy (ILAE) launched the Consortium on Complex Epilepsies, to facilitate meta-analysis in epilepsy genomics. In 2014, the first such meta-analysis was reported comprising 8696 cases and 26157 controls. This led to the identification of 2q24.3, 4p15.1, and 2p16.1 as epilepsy loci [61].

A recent analysis of exome sequencing in unrelated individuals with a family history of epilepsy shows an increased burden of ultra-rare variants among the currently known epilepsy genes [62]. However, the relevance of variants in these genes to common epilepsies, where inheritance is complex, remains uncertain, and molecular genetics advances have been modest [63].

In 2016, Afawi Z. et al. published their results on 211 families ascertained over an 11-year period in Israel, and pathogenic variants were identified in 49/211 families (23%). The majority were found in established epilepsy genes (e.g., SCN1A, KCNQ2, CSTB), however in 11 families, this cohort contributed to the initial discovery (e.g., KCNT1, PCDH19, TBC1D24) [63].

In 2017, the Epi4K Consortium, assembled and analyzed a cohort of 303 families. These findings suggested that specific patterns of syndromic familial aggregation occur, including newly recognized forms of familial focal epilepsy; although syndrome-specificity usually occurs in multiplex families, the one-third of families with features of both focal and generalized epilepsy is suggestive of shared genetic determinants; and that patterns of features observed across families including pedigree structure, sex, and age of onset may hold clues for future gene identification [64].

Recently, International League Against Epilepsy Consortium on Complex Epilepsies, performed a Genome-wide mega-analysis, and identified new 16 epilepsy loci. Importantly, 11 of these loci are associated with the genetic generalized epilepsies; the group of epilepsies where despite having the highest heritability there were made the least genetic progress to date [65].

The largest exome study of epilepsies to date showed that deleterious ultra-rare variants (URVs) – variation absent in a large population-based exome database – is enriched across the severity spectrum for epilepsy syndromes, when individuals with these syndromes are compared to ancestrally matched controls. Specifically, they observed a significant excess of deleterious URVs in constrained genes, established epilepsy-associated genes, and GABAA receptor subunit genes, a larger group of genes delineating the GABAergic pathway, and also in all cation-channel-encoding genes. The evidence that URVs contribute partially to genetic generalized epilepsies and non-acquired focal epilepsies is clear, but what remains unclear is the extent to which the excess rate of URVs observed in individuals with epilepsy that is a consequence of a small subset of affected individuals carrying highly penetrant mutations or a result of URVs that confer risk, yet instead of rising to the level of Mendelian acting mutations, simply contribute to an overall polygenic risk for these syndromes [14].

Single gene causes of the more common forms of epilepsy appear to be relatively rare [64]. These common forms are likely multifactorial, with a significant and complex genetic architecture [66]. Solving the genetic architecture of common complex diseases remains a major challenge in the genetics field, since these findings might highlight that genes commonly involved in epilepsy span a wider range of epilepsy phenotypes than previously assumed [67].

Despite recent molecular advances in epilepsy, genetic investigation is often overlooked in adult practice. Diagnostic yields of different genetic testing methods have not yet been established for adult epilepsy patients. Further studies including larger population samples could be aimed to assess more prevalent genes related to epilepsy in adulthood, and whether these are similar to or different from those previously reported in paediatric cohorts [68]. Less is known about the diagnostic yield of WES in adult epilepsy populations, and it is unknown if adult patients with epileptic encephalopathy who survive into adulthood have a different genetic etiology compared to a paediatric patient cohort [56].

The reanalysis can increase the diagnostic yield in larger cohorts. Re-analysis and diagnosis are particularly important in epilepsy due to the rapid rate of gene discovery and potential for treatment implications [10]. For example, recently, a study identified intragenic, multi-exon deletions in TANGO2 by reanalysis of ES data [69, 70].

The Epilepsy Genetics Initiative (EGI) was formed in 2014 to create a centrally managed database of clinically generated exome sequence data. EGI performs systematic research-based reanalysis to identify new molecular diagnoses that were not possible at the time of initial sequencing and to aid in novel gene discovery. They recently showed a diagnostic rate of 5.8% in previously negative cases – a considerable increase in diagnostic yield demonstrating the value of periodic reinterrogation of whole exome data [8].

Whole Genome Sequencing (WGS) is increasingly being used to uncover the role of non-coding genetic material in the human genome [71, 72].

Several studies have proposed a genetic testing strategy to achieve the highest clinical utility, cost-effectiveness, and diagnostic yield for individuals with epilepsy [24, 37, 73], but specific testing algorithms are likely to change over time as new tests are introduced and the costs of existing tests decrease. New assays may be required to detect lesser-known but important molecular mechanisms [12].

Risk prediction in epilepsy

For most common epilepsies not caused by a single gene mutation, the relative risk to first-degree family members is 6–8 times greater for generalized epilepsy and 2–3 times greater for focal epilepsy, relative to a baseline cumulative incidence around 1% by age 20 years [27].

If a dominant monogenic cause is identified by genetic

testing, or strongly suspected from the family history, then a recurrence risk approaching 50% is expected (slightly reduced by incomplete penetrance, which is approximately 60–80% for most dominant Mendelian epilepsies) [74]. For children with *de novo* mutations, the recurrence risk in siblings should theoretically be zero. However, parental mosaicism elevates that risk and might be more common than previously suspected [7].

Among relatives of all probands (patient zero with epilepsy), cumulative incidence of epilepsy up to the age of 40 is 4.7%, and the risk shows a 3.3-fold increase compared with population incidence. The risk is largely higher in relatives of probands with idiopathic generalized epilepsies and epilepsies associated with intellectual or motor disability presumably present since birth ('prenatal/developmental cause'). Among relatives of probands with epilepsy without an identified cause (including epilepsies classified as 'idiopathic' or 'unknown cause'), the risk was significantly higher for epilepsy of prenatal/developmental cause. In relatives of probands with generalized epilepsy, standardized incidence ratios were 8.3 for generalized epilepsy and 2.5 for focal epilepsy. In relatives of probands with focal epilepsy, standardized incidence ratios were 1.0 for generalized epilepsy and 2.6 for focal epilepsy [27].

Gender analysis showed that epilepsy incidence was greater in offspring of female probands than in offspring of male probands, and this "maternal effect" was restricted to offspring of probands with focal epilepsy [75].

The results suggest that risks for epilepsies of unknown and prenatal/developmental cause may be influenced by shared genetic mechanisms. They also suggest that some of the genetic influences on generalized and focal epilepsies are distinct. However, a similar increase in risk for focal epilepsy among relatives of probands with either generalized (2.5-fold) or focal epilepsy (2.6-fold) may reflect some coexisting shared genetic influences [27].

In addition to single-gene Mendelian inheritance, there is an ample evidence for gene variants conferring risk of disease due to variable alterations in cellular function, sometimes modulated by other genes or epigenetic and environmental cues [76]. Consequently, many variants occur among population with minor degrees of potential influence on disease. Separately, they might not be enough to cause the disease in most circumstances. They would rather probably affect health by altering the risk of sporadic disease, in combination with other factors. Additional research is needed to realize the potential of linking strategies for genetic risk assessment to disease prevention and therapy.

Limitations, such as referral and reporting biases, small sample size, ambiguous disease definitions in probands and relatives, lack of controls, and failure to control adequately for age in the relatives should be considered when interpreting historical genetic studies in epilepsy.

Precision medicine in epilepsy

There are ample data to support the use of next-generation sequencing in reducing the patient's time to diagnosis, often referred to as the "diagnostic odyssey". Precision health encompasses the use of patient-specific data to tailor patient-specific care [77].

We are now entering the era of genomics-driven personalized medicine, whereby novel treatments can be designed which are not solely symptomatic, but address the underlying cause of the epilepsy in the individual person and offer opportunities for truly disease modifying effects [78].

An increasing body of evidence indicates that identifying the pathogenic variant in individual patients with genetic epilepsies is relevant not only for diagnosis and prognosis, but also for treatment selection [79, 80]. This finding is not surprising, because responses to specific treatments can vary depending on the disease's underlying mechanisms which, in turn, may differ even across individuals sharing the same phenotype [6].

Precision approaches have also helped progress in the diagnosis and treatment of epilepsy syndromes. For example, in genetic epilepsy syndromes due to single-gene Mendelian mutations (about 1% of paediatric epilepsies), the efficacy of specific anti-epileptic drugs can be directly related to the underlying mutation, as is the case in Dravet syndrome, for which treating patients with sodium channel blockers is contraindicated [81]. Also, a more recent report of Kim et al. [32] described the discovery, development, and administration of an antisense oligonucleotide (ASO) therapy specifically designed for a single patient with CLN7 neuronal ceroid lipofuscinosis (a form of Batten's disease), a fatal genetic neurodegenerative disorder. The most remarkable is the fact that some neurological diseases, previously of unknown etiology, are nowadays proved as being treatable, without too much effort, as in case of vitamin B6 utility in neuropathies characterized by reduced PLP levels [82].

Reaching a genetic diagnosis in epilepsy may modify treatment, although this occurs in a minority of cases. The most frequent benefits of a genetic diagnosis of epilepsy are difficult to quantify, though this might include the answer to what is causing the disease, the ability to search for other symptoms associated with the gene variant, additional prognostic information, a sense of belonging to a specific support group for the families, informed reproductive choices, and possibly enrollment in clinical trials that are genotype specific [41].

Common problems in refractory epilepsy include the challenges of trial-and-error drug selection that can result in undesirable polytherapy, seizure-related injury, side effects, cost and even the development of some structural changes under the influence of the medication in some patients [60, 83-85].

The advanced knowledge of the molecular mechanisms leading to the development of epilepsy and its comorbidities might facilitate the patients' management by applying truly personalized therapies. Rather than relying on empirical observations relating genotypes to response to specific drugs, further prevailing paradigms will involve characterization of the functional consequences of the pathogenic gene variant and thus searching for available treatments that could correct the specific dysfunction responsible for the manifestations of the disease in each individual patient [86].

If no available treatment is identified, then new treatments may be designed and developed to address the pathogenic defect or the resulting functional abnormalities [6, 78, 87]. The alternative to drug repurposing consists in developing totally novel treatments, which can be designed once the mechanisms of the disease have been sufficiently characterized. The development of effective therapies for genetic CNS disorders is facilitated by advances in gene therapies, sense and antisense oligonucleotides, and other innovative therapeutics [6, 88, 89]. Applied research in this area also benefits from improved understanding of structure-activity relationships, and from access to 3D structural information on thousands of protein molecules through the Protein Data Bank [90].

The availability of animal models, which reproduce the targeted genetic defect is especially highly valuable to streamline preclinical development [91, 92].

Finally yet importantly, the application of pharmacogenetics to treatment and diagnosis extends beyond epilepsy and is a clinical area that is still under development. Over time, the use of patient's genetic data to predict drug efficacy and minimize side-effects will probably expand as research into these areas progresses. With unprecedented amounts of human data being generated from patients and healthy individuals, coupled with major developments in technology and large-scale data analysis, advances in genomics and precision health are creating new opportunities for evidence-based and patient-centered care. The next decade provides major shifts in the translation of these technologies into the clinical setting that will certainly benefit patients with neurological diseases.

Conclusions

Epilepsy's genetic background is nowadays undeniable; more than 140 genes or loci being already associated with this worldwide spread disease. However, the complexity of this health burden makes it a challenge to rapidly determine the cause and to pursue the best treatment management.

It is already proved that relatives of people with epilepsy have an increased risk to develop epilepsy, even in families without Mendelian inheritance.

Whole Exome Sequencing (WES) and epilepsy panels (EP) are nowadays considered the most cost-effective genetic tests for epilepsy, though the familial genetic analysis is an approach that could furthermore reduce the epilepsy patient's "diagnostic odyssey", by increasing the chances of identifying the truly disease-causing variant after filtration.

Despite recent molecular advances in epilepsy, genetic investigation is often overlooked in adult practice and much more details should be considered when interpreting historical genetic studies in epilepsy.

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DC and VC conceptualized the project and designed the research; DC drafted the first manuscript; VC and SG revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Precancerous gastric lesions: pathophysiology and symptomatology

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Abstract

Background: Independent risk factors for chronic atrophic gastritis, gastric intestinal metaplasia and gastric cancer are: *Helicobacter pylori* infection (especially virulent CagA strains), genetic factors (advanced age, reflecting the duration of *Helicobacter pylori* infection, male gender, family history of gastric cancer in first-degree relatives), gastric ulcer, biliary reflux, acute or chronic gastric inflammation, smoking, alcohol consumption, prolonged use of proton pump inhibitors or non-steroidal anti-inflammatory drugs, diet low in fruits, vegetables and vitamin C, excessive salt intake and consumption of canned foods with salt). *Helicobacter pylori* infection and inflammatory cells induce the expression of inducible nitric oxide synthase in the gastroduodenal mucosa, which causes various clinical lesions (duodenal ulcer, gastric ulcer and chronic gastritis without ulcer). Another important condition associated with *Helicobacter pylori* infection is gastric cancer. Overproduction of nitric oxide, through inducible nitric oxide synthase and oxidative stress, is a genotoxic and mutagenic metabolism which plays a crucial role in the process of gastric carcinogenesis.
Conclusions: Chronic atrophic gastritis is considered a multifaceted condition because it can manifest itself through a variable spectrum of nonspecific gastric and extra-gastric symptoms, with an overlap of the clinical features of the two entities of chronic atrophic gastritis – autoimmune and associated with *Helicobacter pylori* infection. Thus, in contrast to the classic perception of a silent condition, patients with chronic atrophic gastritis report a wide range of gastrointestinal symptoms, ranging from dyspeptic symptoms to those of gastroesophageal reflux.
Key words: gastric intestinal metaplasia, epithelial dysplasia, gastric cancer, *Helicobacter pylori*.

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Introduction

In many cases, the development of intestinal gastric adenocarcinoma is the final stage of the inflammation-atrophy-metaplasia-dysplasia-carcinoma sequence, also called Correa's multi-stage cascade of gastric oncogenesis, a model confirmed by a considerable number of longitudinal clinical-pathological and epidemiological studies [1-5]. According to this well-defined cascade of premalignant conditions or lesions, gastric cancer (GC) develops as a result of a gradual progression. The first real step in the precancerous cascade is from the normal gastric mucosa to a chronic active inflammation associated with *Helicobacter pylori* (HP) infection, which may persist (chronic non-atrophic gastritis without loss of glands) or may progress to chronic atrophic gastritis (CAG) – mild, moderate and severe. The next steps are: gastric intestinal metaplasia (IM), initially "complete" and then "incomplete", and dysplasia of the gastric mucosal epithelium (DGME), initially low-grade and then high-grade. GC is the last step in this multi-stage cascade, triggered by long-term inflammatory conditions (especially HP infection) [3-7]. According to estimates, Correa Waterfall is involved in about 50% of cases of GC, especially intestinal type [8].

Material and methods

The aim of the paper is to develop a narrative synthesis of contemporary studies on the pathophysiology and clinical picture of precancerous gastric lesions and their role in the development of GC. The publications were selected from the PubMed, Hinari and SpringerLink databases by keywords: chronic atrophic gastritis, gastric intestinal metaplasia, gastric mucosal epithelial dysplasia, gastric cancer, *Helicobacter pylori*. After processing the information from the databases, we selected all publications in English starting from January 1990. After a preliminary analysis of the titles, the final bibliography included original articles, editorials, articles of narrative synthesis, systematic and meta-analysis that contained information on the pathophysiology and clinical picture of gastric precancerous lesions. Additionally, the bibliography of the selected articles was studied in order to find other relevant articles on this topic. According to the search criteria, 563 complete articles were found. The final bibliography contains 46 relevant sources, which were considered representative for the materials published on the topic of this synthesis article.

***Helicobacter pylori* infection.** HP is located in the mucous layer along the surface of the gastric epithelium and in

the luminal portion of the gastric fovea, being rarely present in the deeper glands. The infection is usually contracted in childhood and progresses throughout life in the absence of proper treatment. The host responds to the presence of the bacterium by activating B and T lymphocytes, followed by infiltration of the *lamina propria* and gastric epithelium with polymorphonuclear and mononuclear inflammatory cells, which phagocytose the bacterium. The release of toxic bacterial and inflammatory products causes damage to gastric epithelial cells, which progress to atrophy. Some glandular units develop an intestinal-type epithelium. MIG subsequently occurs in several areas of the atrophied gastric mucosa. Other glands are replaced by fibrous tissue from the expansion of the *lamina propria.* The loss of the glands of the gastric body produces functional changes with the loss of the ability to secrete acid, pepsin and intrinsic factor and the increase of gastric pH. There is a good, but not complete, correlation between the severity of gastric mucosal atrophy (GMA) and depression of gastric function [9,10].

Most HP strains can be classified into 3 major types: type 1 – highly infectious, have the gene encoding CagA antigen and VacA antigen, type 2 – transitional, expressing CagA antigen independent of VacA antigen or vice versa and type 3 – with low resistance, which does not express any antigen [11].

Gastritis associated with HP infection progresses on two topographic models that have different clinico-pathological consequences. The first model, most common in Western countries, is antral CAG, characterized by inflammation located predominantly in the gastric antrum. Peptic ulcer usually overlaps with this type of lesion [9, 10]. The second model is the multifocal CAG. The special virulence of HP CagA-positive strains, with a predominant role in CAG and the evolution towards GC, is widely accepted. GMA involves the body, fundus and antral regions, with the progressive evolution of CAG and the gradual replacement of the gastric glands with intestinal-type epithelium (IM). This pattern is more common in developing countries and in Asia [9, 10].

Multiple studies and meta-analyses have highlighted the very strong association between HP infection and CAG. CAG develops late in the course of chronic non-atrophic gastritis associated with HP infection, even in HP-negative patients. On average, about 50% of people infected with HP will develop CAG of some degree or type during their lifetime. In addition, HP is also the leading cause of GC [12].

HP is the most important and significant risk factor in establishing CAG and IM, often associated with GCA, but there are other clinical, environmental, and genetic conditions that are important risk factors (RF) for the progression of IM to GC. CAG is not a normal aging process, but it is the result of HP infection, and IM is caused by both the aging process and HP infection. A low risk of IM among HP-negative women may partially explain the lower prevalence of GC in women compared to men [13, 14, 15, 16]. It is well documented that long-term exposure to HP infection is a RF for the development, worsening and progression of precancerous lesions (CAG and IM). HP CagA-positive strains are associated with increased prevalence and severity of CAG and IM [14-18].

It was found that bacterial factors are important RF for CAG, and environmental and host factors are more important for IM [14-18]. These include age, male gender, gastric ulcer, biliary reflux, severe acute and chronic gastric inflammation, smoking, alcohol consumption, diet low in fruits, vegetables and vitamin C diet, and a high salt intake [14, 19]. Therefore, independent RFs for CAG, IM and GC are considered: HP infection (especially CagA virulent strains), severe CAG, autoimmune CAG, certain rheumatic diseases (Sjögren's syndrome), genetic factors (advanced age, which reflects the duration of infection with HP, male gender, family history of GC in first-degree relatives), gastric ulcer, biliary reflux, severe acute and chronic gastric inflammation, smoking, alcohol consumption, prolonged use of proton pump inhibitors or non-steroidal anti-inflammatory drugs, alimentation (diet low in fruits, vegetables and vitamin C, excessive salt intake and consumption of canned foods with salt) [11, 18].

A key RF of chronic inflammation is the release of large amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are associated with DNA damage and increased rates of mutations. Previous studies have shown that ROS and RNS, secreted by inflammatory and epithelial cells, can cause oxidative and nitrative damage to DNA, including the production of nitric oxide (NO) – a known mutagenic substance derived from inducible nitric oxide synthase (iNOS). Deterioration of cellular components results in increased mutations, disorders of the functions of important enzymes and proteins in premalignant tissues, thus contributing to the process of multistage and multifactorial carcinogenesis [13, 20].

NO is an important intracellular and intercellular signaling molecule involved in the regulation of various physiological and pathophysiological mechanisms in the cardiovascular, nervous and immune systems. According to experimental data, NO is normally involved in the physiological regulation of gastric microcirculation and its integrity. NO is also a SRA that acts as a cytotoxic agent in pathological processes, especially in inflammatory disorders, not only for the microorganism, but also for cells and tissues. When the generation of ROS and RNS exceeds the antioxidant capacity of the cell, they play an important role in cell damage and carcinogenesis of HP-infected gastric mucosa. Simultaneously with oxidative damage (ROS and RNS) of tissue and DNA with modification of target proteins, NO overproduction during chronic inflammation, including HP infection, increases histamine secretion and the formation of carcinogenic compounds with increased risk of carcinogenesis (initiation and progression of carcinogenesis to GC). Thus, long-term inflammation of the gastric mucosa generates significant amounts of NO, which contributes not only to the deterioration of basic nucleotides in DNA and proteins, but, by hypermethylation of promoter sequences, leads to epigenetic changes in gene expression and suppression of gene activity [1, 20-24].

Recent advances in basic research on HP-associated carcinogenesis have explained that iNOS-derived NO plays a crucial role in the process of gastric carcinogenesis. iNOS expression has been reported to be absent in the normal gastric mucosa, increases significantly in HP-negative patients with chronic gastritis, and increases significantly in HP-positive patients with chronic gastritis. This expression is closely related to the infiltration of inflammatory cells in the gastric mucosa, modulates inflammation and epithelial changes. In HP-positive patients, high levels of RNS in the gastric mucosa contribute to neoplastic transformation. A number of activities may contribute to the tumor-modifying effects of NO, including damage to DNA and DNA-repairing proteins, endogenous mutagenesis, increased angiogenesis and increased blood flow, inhibition of apoptosis with genetically modified cell survival, enzyme activation cyclooxygenase-2 and suppression of the immune system [14, 20].

Increased expression of iNOS has been observed in inflamed human gastric mucosa (in inflammatory cells – olymorphonuclear, plasma, lymphocytes and macrophages), as well as in some gastrointestinal, gynecological and central nervous system tumors. It is known that inflammatory cell infiltrate is generated in the gastric epithelium and *lamina propria* during the development of chronic gastritis, including chronic gastritis associated with HP. The results of the studies support the hypothesis that HP infection, especially HP CagA-positive strains and certain VacA-positive alleles, induces iNOS expression and activity and causes NO overproduction in the gastro-duodenal mucosa in order to regulate the inflammatory process. NO is a factor in the oxygen-dependent system for antiviral and antibacterial protection. Thus, NO has an essential role in inflammatory processes, but the excessive accumulation of this metabolite in tissues causes toxic effect on cells, severe destructive changes and dysregenerative disorders. As a result, significant destructive changes of the gastric mucosa, erosions and regenerative disorders, including IM, occur. However, the latest studies have shown that eradication of HP causes a significant reduction in iNOS expression [20-25]. In the gastrointestinal tract, excess NO aggravates mucosal lesions. Prolonged oxidative and nitrosative stress in severe CAG contributes to the development of IM and DGME and subsequently to intestinal GC. In atrophic gastric mucosa, associated with HP infection, a significant increase in iNOS expression and NO-modified proteins has been found, and hypergastrinemia is a feature of iNOS-producing gastritis, which has an increased risk for carcinogenesis [20-25].

Evaluation of the relationship between oxidative stress and early onset of GC, especially poorly differentiated intramucosal adenocarcinoma in young people, revealed a significant reduction in iNOS expression in cancer cells compared to non-cancer cells, which may play an important role in CAG-associated carcinogenesis induced by HP [25].

In addition, research has detected an increased expression of iNOS in the gastric mucosa adjacent to HP-infected sites and in the non-cancerous gastric mucosa adjacent to intestinal cancer tissue. After successful eradication of HP, iNOS expression decreases. Persistence of IM, a precancerous lesion, is probably a source of continuous induction of iNOS even after eradication of HP infection [21, 22].

Therefore, HP infection induces iNOS expression in the gastroduodenal mucosa, an important element in lesions associated with HP infection. The expression of iNOS, stimulated by HP and inflammatory cells, contributes to the mechanisms by which HP causes various clinical lesions (duodenal ulcer, gastric ulcer and chronic gastritis without ulcer). Another important condition associated with HP infection is GC. NO is genotoxic and mutagenic, suggesting that NO overproduction via iNOS and ROS, derived from polymorphoneutrophils in HP-infected gastric mucosa, is involved in carcinogenesis.

Chronic atrophic gastritis. CAG is a prevalent condition, the final consequence of an inflammatory process that eventually leads to the loss of the corresponding mucous glands with reduced gastric secretory function. This histological change is the result of an autoimmune-mediated reaction directed to parietal cells or may be associated with HP infection [26, 27].

In the last two decades there has been a wide shift in the paradigm of understanding GC and its premalignant states from histological models to increasingly accurate molecular descriptions. Despite recent advances in the molecular and cellular understanding of the events involved in GC, little is known about how premalignant gastric lesions contribute to carcinogenesis [13]. Intestinal type GC carcinogenesis is an example of a malignant disease with a well-described cascade of precancerous lesions (Correa cascade). GMA and IM pose a high risk for the development of GC, a risk that increases with the severity of precancerous lesions, as it is the background in which DGME and intestinal gastric adenocarcinoma may develop, although the molecular mechanisms responsible for this progression are not yet well understood. For this reason, CAG and IM are considered the main histological precursors, which exponentially increase the risk of intestinal GC. However, only a minor proportion of lesions (except DGME) progress to cancer [3, 6, 13, 17, 28, 29]. The extent and topographic distribution of GMA are parallel to the risk of developing GC, which theoretically allows the application of either non-invasive (serological) or invasive (endoscopic / histological) methods to quantify GMA in order to assess the risk of CG [30 , 31].

Gastric intestinal metaplasia. Numerous studies, systematic reviews and meta-analyses have evaluated the association between IM and GC risk. Patients with incomplete IM, compared to complete IM, have a higher risk of DGME and GC [17, 19, 29, 32-34]. Several studies have shown that IM is associated with a higher risk of cancer in the gastric body than in the gastric antrum alone, which suggests that IM progresses concomitantly with GMA and predicts the risk of GC [19, 33]. Therefore, patients with IM, especially incomplete IM and in the gastric body, have a higher risk of GC [33].

Dysplasia of the epithelium of the gastric mucosa. IM is a precursor to low-grade DGME, which can culminate in high-grade DGME and gastric carcinoma. DGME is the penultimate stage in the succession of gastric carcinogenesis. This lesion is a combination of three basic morphological abnormalities: epithelial atypia without evidence of tissue invasion (variation in size, shape, and orientation of epithelial cells), loss of native epithelial engagement, and disorganized glandular architecture. Thus, DGME is considered a direct precancerous lesion [2, 3, 6, 28, 32, 34].

Gastric cancer. The high prevalence of GC in HP-positive subjects probably occurs because HP infection contributes to the progression of CAG to IM and DGME with a significant increase in the risk of GC [7, 12, 35]. For the development of GC, especially intestinal type, the end result of chronic inflammation caused by bacterial colonization, is more important than HP infection itself [35]. The risk of developing CG depends on the degree of GMA at the time of eradication: it is 0.31-0.62% per year for successful eradication of HP in cases of severe CAG (cases of unintentional eradication in the treatment of other infections and cases of unreported eradication) and 0.53-0.87% annually for cases with severe CAG and spontaneous regression of HP due to CAG progression. The prevalence of GC in HP-negative people is extremely low – 0.66% [12]. Among people with IM, the cumulative rate of progression to DGME was 15% at 3 and 5 years, and the cumulative rates of 3, 5 and 10 years incidence of GC were 0.4%, 1.1% and 1.6–2.0%, respectively [32, 35]. In general, among people with IM the annual rate of progression in GC exceeds 0.5-1% [35].

Among 98000 patients with premalignant gastric lesions in the Netherlands, the average risk of GC was 2-3% over 10 years. This risk varied concomitantly with the baseline stage of premalignant lesions: 0.8%, 1.8%, 3.9% and 32.7% for patients with CAG, IM, mild-to-moderate DGME and severe DGME, respectively [36].

All the above data suggest that there are other factors than GMA and IM, which have a role in gastric carcinogenesis. In addition to infection with HP, CAG, and IM, there are several RFs for GC – sex, age, blood type, HP infection, family history of GC, smoking, alcohol consumption, and eating habits [2, 28].

Chronic atrophic gastritis symptoms. Gastritis is an inflammation of the gastric mucosa, most often accompanied by structural changes. The term gastritis most often refers to dyspeptic symptoms, defined as disorders of the upper gastrointestinal tract. Dyspepsia is the most common gastrointestinal problem in general practice with prevalence rates between 5.3% and 20.2% [37], and 20-40% of the population report these symptoms at least once in a lifetime [38]. About 80-90% of people with HP remain asymptomatic throughout their lives. Chronic HP infection contributes to the development of CAG, which has also traditionally been considered asymptomatic or with nonspecific symptoms and diagnosed incidentally, especially autoimmune CAG [27, 39, 40, 41]. However, due to the marked decrease in gastric functional activity, dyspeptic syndrome (anorexia, belching, nausea, postprandial fullness and early satiety), bacterial overcrowding syndrome (noise and flatulence in the abdomen, belching, unstable stool, with frequent diarrhea, weight loss and anemia may occur), anemic syndrome, pain syndrome, dystrophic syndrome. Gastrointestinal manifestations are associated with non-acid reflux and are not specific [27, 40].

Typical symptoms of reflux (heartburn and / or acid regurgitation), epigastric pain syndrome (epigastric pain and / or epigastric heartburn) and postprandial distress syndrome (postprandial fullness and / or early satiety) were present at 10.5% of patients with non-atrophic gastritis or mild CAG, in 19.8% of patients with predominantly antral CAG and in 16.2% of patients with CAG predominantly in the gastric body. Symptoms of epigastric pain syndrome and postprandial distress syndrome were significantly more common in male patients with predominantly CAG in the gastric body and in female patients with predominantly antral CAG. Thus, the extent and severity of CAG affect the generation of specific dyspeptic symptoms and this influence was different depending on gender. The reason why there is a gender difference in the results cannot be clearly explained [42].

At least one typical symptom of gastroesophageal reflux was reported in 24.1% of patients with CAG in the gastric body, including 9.2% reported epigastric heartburn and 18.5% – regurgitation. These data showed that gastroesophageal reflux disease is present in about ¼ of these patients, which suggests that hypochlorhydria does not exclude, in itself, the occurrence of esophageal symptoms [43]. However, there is no correlation or overlap between symptoms and endoscopic or morphopathological data [44].

CAG is considered a multifaceted condition because it can manifest through a variable spectrum of gastric and extra-gastric symptoms. The clinical spectrum of CAG is not clearly defined and is often nonspecific, with an overlap of clinical features between the two CAG entities. Studies have been conducted mainly on autoimmune CAG, while data on the clinical presentation of CAG associated with HP infection are limited [27, 45]. Thus, in contrast to the classic perception of a silent condition, patients with CAG report a wide range of gastrointestinal symptoms, ranging from dyspeptic symptoms to symptoms of gastroesophageal reflux [46].

Conclusions

1. Independent risk factors for chronic atrophic gastritis, gastric intestinal metaplasia and gastric cancer are: *Helicobacter pylori* infection (especially CagA virulent strains), genetic factors (advanced age, reflecting the duration of *Helicobacter pylori* infection, male history, gastric cancer in first-degree relatives), gastric ulcer, biliary reflux, acute or chronic gastric inflammation, smoking, alcohol consumption, prolonged use of proton pump inhibitors or non-steroidal anti-inflammatory drugs, diet low in fruits,

vegetables and vitamin C, excessive salt intake and consumption of canned foods with salt).

2. *Helicobacter pylori* infection and inflammatory cells induce the expression of inducible nitric oxide synthase in the gastroduodenal mucosa, which causes various clinical lesions (duodenal ulcer, gastric ulcer and chronic gastritis without ulcer). Another important condition associated with *Helicobacter pylori* infection is gastric cancer. Nitric oxide overproduction, through inducible nitric oxide synthase and oxidative stress, is a genotoxic and mutagenic metabolism with direct involvement in carcinogenesis.

3. Chronic atrophic gastritis is considered a multifaceted condition, because it can manifest itself through a variable spectrum of nonspecific gastric and extra-gastric symptoms, with an overlap of the clinical features of the two entities of chronic atrophic gastritis – autoimmune and associated with infection with *Helicobacter pylori*. Thus, in contrast to the classic perception of a silent condition, patients with chronic atrophic gastritis report a wide range of gastrointestinal symptoms, ranging from dyspeptic symptoms to symptoms of gastroesophageal reflux.

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AB designed the trial and drafted the first manuscript; NB and VI interpreted the data and revised the manuscript critically.
The authors revised and approved the final version of the manuscript.

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