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REVIEW

Public Health in the Framework of the International Security. A Constructive Approach

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The article highlights the fact that public health is an element of the security dimension that must be included on the priority agenda of specialists in the fields of international relations and security studies. There are arguments in favor of this theory. The costs of materializing threats to human security in general and public health in particular are particularly high, with serious long-term consequences. Global trends and prospects for the implications that can be generated are likely to change the world's security landscape, and increasing global connectivity increases the degree of uncertainty about public health implications. Non-traditional issues arising from technological change can induce risks, whose management may go beyond institutional capacities. On the other hand, the new types of wars, increasingly interconnected with various forms of risk materialization, make this mission more difficult. The final conclusion is that these risks need to be assessed to ensure national, regional or even global security, and international cooperation for prevention and counseling.

Keywords: human security, public health, international security, globalization

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Introduction

The current dynamics of the international environment entail certain dilemmas for international relations, imposing new approaches in the realm of security, from the perspective of the *human security* paradigm. This allows for the emergence of new actors in the concept framework, such as the health status, a field of the utmost importance for human beings, and the threats it might be subject to. As a matter of fact, human security, a concept which has been consecrated in the 1994 Report on Human Development of the United Nations Development Program [1], is identified based on its six components: health security, food security, environmental security, personal security, economic security and political security.

Despite the traditionalist view in security studies, which aims to limit the issue to the military and political sectors, the importance of public health and of the challenges it represents in the framework of international security has been well proven on the occasion of the Special Session on the HIV Epidemic organized by the United Nations Security Council in 2000, for the first time in the agenda of the foremost international body. [2]

The impact of globalization on public health is manifest in the series of uncertainties regarding its consequences on the health of the population. Generally speaking, globalization entails a blurring of state borders in the face of such new threats as terrorism, cybercrime, trans-border organized crime, human trafficking, drug crime or trafficking of strategic materiel. It is even more obvious that, in the case of threats to public health, the etiologic agents responsible for the spread of disease would not stop at any state's frontier. Therefore, public health should be approached as a component of international security, taking into account the fact that globalization has caused the threats to evolve, entering a transnational dimension.

Human security

Promoting the paradigm of human security can be justified discursively on the basis of the idea that all human lives are equally valuable, as we all belong to the human species. This can be contrasted with the national security paradigm, which is based on the principle of privileged security of nationals. [3] Human security is concerned with the security of individuals and communities, rather than with that of nation states, and builds upon both human rights and human development; it is the supremacy of human rights which differentiates it from the traditional, state-centric approach. [4] A substantial body of literature on human security uses the notion of threat in order to describe a long (and growing) list of challenges to human security. So as to allow the classification of these issues – from pandemics to human-induced environmental disasters, nuclear weapons and small-arms proliferation – all these threats are included in the list, with no prioritization and with no estimate on their respective probabilities to occur; furthermore, an estimate of the costs associated with such distinct sectors is required. The rate of AIDS mortality or that of HIV infection illustrates the direct human costs of such diseases, with no indication of the consequences or the costs of public policies or preventive strategies, especially in such instances where the social and economic costs are significant in the long term. [5]

Human life is subject to various conflicts. The rate of civilian casualties was 10% during the First World War, 50% in the Second World War, and 80-85% in more re-

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cent wars. Many of these victims were children, women, sick or elderly.

Such „new wars" are increasingly interwoven with other global risks – the spread of diseases, an increased vulnerability to natural disasters, poverty and homelessness. A significant percentage of casualties in times of war is indirectly caused by the lack of access to sanitation and by the prevalence of disease, hunger and the destruction of residences. [4] (Table I)

Although some threats are associated with very large costs in human lives, such as the potential use of a nuclear weapon by a terrorist organization against a major population center, the actual probability of such an occurrence may be quite low, at least relative to the human insecurity situations which impact certain people on a day-to-day basis. A series of human security indicators made available by the United Nations in 2002 [6] show that the main threat sources are structural:

– Every day, more than 30,000 children throughout the world succumb to preventable diseases, for a total of over 11 million each year;
– 5% of the world's richest people earn 114 times more than the income of the world's poorest 5%. One percent of the richest have as much as 57% of the poorest;
– 2.8 billion people live on less than US$ 2/day, and of those, 1.2 billion must survive on less than US$ 1/day; Between 1997 and 1999, approximately 815 million people suffered from malnutrition;
– In the 1990s, the absolute number of people in Sub-Saharan Africa living in extreme poverty increased from 242 million to 300 million;
– Towards the end of year 2000, nearly 22 million people (and currently, according to the United Nations Development Programme, 24.8 million) died of AIDS, 13 million children were orphaned of their mothers or both parents, more than 40 million people were infected with HIV, with 90% of them living in the developing world, and 75% in Sub-Saharan Africa;
– 100 million baby girls would have been born alive but for the practice of selective abortions (due to gender preferences), or died because of infanticide or neglect;
– Each year, there are 300 million cases of malaria infection, 90% of them in Sub-Saharan Africa;
– More than 500,000 women die each year during pregnancy, while giving birth or in the immediate term. [7]

Institutionally-determined mortality is separate from the casualties of military conflicts. The high rate of mortality in children younger than 5 years of age is a consequence of conscious policies: it is a consequence of socially-constructed bio-poverty and is the product of those national and international public policies which prevent the population's access to such prerequisites of life as water, sanitation and otherwise cheap vaccines. [8]

An analysis by the Office of the Director of National Intelligence points to certain global tendencies and their key implications until 2035, capable of drastically changing the picture on a global scale, such as the following:

Climate change, environmental and health issues which require extraordinary attention. Extreme weather phenomena, inadequate water and soil management and food insecurity will impact societies.

Sea level rises, ocean acidification, icecap melting and pollution will change life patterns. Climate change induced tensions will increase. Advances in global population mobility and precarious healthcare infrastructure will make it increasingly difficult to manage infectious diseases.

The silent and chronic threats of air pollution, water deficits and climate change will become more visible, leading to more frequent conflicts, as the study and prevention of these issues remain partial and individual endeavors, instead of a global effort.

Demographic changes will impact employment, social welfare and social stability. The population of developed countries is aging, whereas in many of the poorest countries, the number of males is on the rise and migration increases: people follow their hopes of finding a better life, or escape the horrors of conflicts.

World population will continue to grow, to turn increasingly older and more urban, even if the rate of increase will slow down. The effects on individual countries will vary substantially however, because the world's major economies will grow older, while the developing countries will remain comparatively younger. From the current 7.3 billion, the world population is expected to reach 8.8 billion before 2035. The population of Africa – with a fertility rate double that of the rest of the world – and that in certain parts of Asia will increase. This might lead to economic advances or to disasters, depending on how much those respective governments and societies invest in education, infrastructure, infrastructure and other key sectors.

The number of displaced or mobile persons will stay high or may even further increase, as environmental issues become more stringent.

Table I. Statistical data on the number of deaths resulting from structural causes, respectively as a result of acts of direct violence

	Military and civilian casualties of violent conflict – deaths due to direct violence	Deaths due to smallpox	Deaths due to malaria	Deaths due to cholera	Deaths due to parasite-borne diseases and to respiratory infections
2002	21,405	611,000	1,272,000	1,798,000	14,866,870
2003	47,351	530,000	1,000,000	1,788,500	-
2004	41,586	454,000	1,000,000	1,820,007	-
2005	31,013	345,000	1,000,000	-	14,018,871

(Source: World health report 2004)

Changing weather, the increasing pressure on natural and environmental resources, and the deepening interdependence of human and animal health reflect complex systemic risks, capable of overrunning current management approaches.

Extreme weather may lead to crop failures, wildfires, energy depletion, infrastructure and supply chain breakdown, migrations and epidemics.

In the long term, global climatic stress will change the known habitation patterns, but also the types of diseases currently threatening humans. Such factors include sea level rises, ocean acidification, melting icecaps, degraded air quality, cloud capacity changes, and sustained modifications of global temperature and rainfall patterns.

Nearly all Earth systems suffer natural or anthropogenic crises which overcome the national and international environmental protection efforts. Institutions will have to fight harder and harder to manage the complex interdependencies between water, food, energy, land, health, infrastructure and workforce.

Before 2035, it is estimated that air pollution will become the main environmentally-related cause of death on a global scale, due to non-implementation of recent air quality measures. More than 80% of urban residents are already exposed to air pollution levels which surpass safety limits, according to the World Health Organization. [9]

Public Health – between challenges and moral/ethical responsibilities

Public health – a multidisciplinary concept, situated at the crossroads of life sciences and social science – is aiming towards prevention of disease, lifespan extension and health promotion, via an organized, conjugated effort of all society. It utilizes means inherent to the field of medicine, but it also borrows from elsewhere: sociology, psychology, statistics, communication science, anthropology, economics, marketing, political science. In a globalized world, the increasing connectivity and changing environment will bear a significant toll on the geographic distribution of both pathogens and hosts, which will, in its own turn, impact the emergence, transmission and spread of many infectious diseases, affecting both the human and the animal population. The health of both populations will be increasingly interconnected. The deficiencies of national and global healthcare systems will make it increasingly difficult to identify and manage the hotspots of infectious diseases, increasing the risk for epidemics to potentially spread beyond their original areas. Nevertheless, non-transmissible diseases, such as heart disease, cerebral vascular incidents, diabetes and mental disorders, will greatly surpass infectious diseases in the coming decades, due to certain demographic and cultural factors, such as population aging, poor nutrition and hygiene, urbanization and increasing inequalities. [10] In the Report on Human Security, Amartya Sen conceptualizes human security by referring to the exposure to disease or pandemics uncertainties, or to persons vulnerable to sudden poverty. [4]

Recently, threats considered hypothetical have become historical fact. The bioterrorism phenomenon represents a real, present threat to the future of humanity, due to its consequences.

The threat of biological attacks is considered a public health issue, as the damage it poses is considerable even in such a scenario where the number of infected persons is small. Military force is lacking effectiveness in countering this threat, therefore it is becoming crucial that defensive measures be deployed within the healthcare system.

Scientists have issued warnings that the current measures against biological attacks are insufficient and, probably, ineffective. To support such views, we only need to consider the rapid spread of some viral infections, confirmed by the statistics of the World Health Organization. An attack using biological weapons might have catastrophic consequences for the future of humanity. [11] Considering that biological weapons ("the poor man's nuke") do not require sophisticated technologies or significant quantities of offensive material, we are facing a somber picture of the risks generated by the exposure to such an attack.

The risk of chemical, biological or nuclear terrorism is on the rise, in a world where there is increasing interethnic and religious violence and human rights abuse. International treaties governing such weaponry are lacking in control measures. Therefore, the fight against terrorism imposes the involvement of non-governmental organizations, as well. [12]

The proliferation of advanced technologies, especially biotechnologies, will further lower the threshold for new actors to obtain weapons of mass destruction. Biotechnologies such as genome sequencing will revolutionize medicine and other fields as well, yet the moral aspects involved will become ever more acute. The recent discoveries in gene reproduction and manipulation such as Clustered Regularly Interspaced Short Palindromic Repeats open huge new possibilities in biotechnology. [9]

Technology will continue to strengthen the position of individuals, small groups, corporations and state entities, and to accelerate the rate of change, introducing new complex challenges, discontinuities and tensions. The development and deployment of advanced technologies, especially Artificial Intelligence, innovative materials and manufacturing capabilities, robotics and automation, will modify the current paradigms governing the pharmaceutical and medical systems. They will also pose fundamental questions about what is the meaning of being human. Such evolutions will increase the divide between various society values, hindering a progress of international regulation of such sectors. The existential risks associated with some of these applications, especially synthetic biology, genome manipulation and Artificial Intelligence, are already real. [9]

A few years ago, the Clustered Regularly Interspaced Short Palindromic Repeats technology was revealed to be

applicable in connection to a set of enzymes which accelerate or catalyze the chemical reactions involved in modifying specific Deoxyribonucleic Acid sequences. Such a capacity revolutionizes biology, and it accelerates the rate in which applications of biotechnology are developed for responding to medical, healthcare, industrial, environmental or agricultural problems, but at the same time, it raises significant ethical and security issues.

Biotechnologies have reached a turning point where progress in gene testing and editing, catalyzed by new manipulation technologies, turn science fiction into reality. The time and costs required to sequence the human genome have been greatly reduced. Such possibilities open the door to new approaches in human adaptation, treatment of diseases, lifespan extension or food production. [9]

It is highly likely that extant institutions will face non-traditional issues, such as genome reproduction, AI and human enhancement, because technological advances will have significantly surpassed the capacity of the states, agencies and international bodies to regulate and standardize in these matters. And, as if all this would not suffice, world epidemics, sanitation disasters, food crises and economy crashes, Genetically Modified Organisms, junk food, dangerous drugs, pollution of all types, nothing will prevent the 21st century Homo erect to be the most subject to malnutrition and the most in danger of poisoning, of all human beings since the dawn of time. [13, 14]

Conclusions

In the context of globalization and alarming evolutions of risks generated by climate change, transmission and spread of infectious diseases, proliferation of chemical, biological or nuclear weapons, and uncertainties regarding emergent technologies, there is a clear need to assess these risks in the benefit of national, regional and global security.

The manifestation of such threats in a globalized world imposes international cooperation and common programs for their prevention and countering, by initiating action independently of the territory.

The dynamics of the international security environment, together with the application and the acceptance of the constructivist theory/perspective on security, imposes adaptive approaches of the concept.

Social rules, norms, principles, institutions and organizations capable of clarifying and providing resolution to the "dilemmas of security deeply engrained in human condition, multiplying and in increasing complexity, due to the emergence of a world society in the evolution of an armed species which for the first time has both the knowledge and the means required for self-annihilation".

Conflict of interest

None to declare

Author's contribution

Sergiu Viorel Borsa (Conceptualization; Formal analysis; Writing – original draft; Writing – review & editing)

References

1. Human Development Report. Published for the United Nations Development Programme. Oxford University Press, NY Oxford, 1994, 22-38.
2. United Nations Security Council Resolution 1325(2000), http://www.un.org/womenwatch/osagi/cdrom/documents/Background_Paper_Africa.pdf
3. Brown C. Borders and identity in International Political Theory, in Mathias A, Jacobson D, Lapid Y (eds): Identities, Orders, Borders. Rethimking International Relations Theory, Univ Minnesota Press, Mineapolis, London, 2001,128-129.
4. Kaldor M. Securitatea umană, CA Publishing House, Cluj-Napoca, 2010, 214-218.
5. Hampson FO. Human Security, in Williams P (eds): Security Studies. An Introduction, Routledge, NY, 2008, 238-240.
6. Tim D, Wheeler JN. We the Peoples`: Contending Discourses of Security in Human Rights Theory and Practice in David C, Hynek N (eds): Critical perspectives on Human Security. Rethinking Emancipation and Power in International Relations, Routledge, London and NY, 2011, 20-22.
7. Leucea I. Constructivism și securitate umană. European Institute Iași, 2013, 122-123.
8. David R. Global Governance and Biopolitics. Regulating Human Security. Zed Books, London and NY, 2010, 9-14.
9. https://www.dni.gov/index.php/global-trends/trends-transforming-the-global-landscape.
10. Curta AI. Politici de sănătate în noile State Membre ale Uniunii Europene. Cazul României. Cluj University Press, Cluj-Napoca, 2008, 25-29.
11. Curis C. Bioterorismul – bomba nucleară a secolului XXI. Intelligence. 2010; https://intelligence.sri.ro/bioterorismul-bomba-nucleara-secolului-xxi/
12. Ciobanu OM. Bioterorismul – inamicul invizibil. Intelligence. 2009; https://intelligence.sri.ro/bioterorismul-inamicul-invizibil/
13. Severac C. Complotul mondial împotriva sănătății. Lucman Publishing House, București, 2010, 9-12.
14. Kolodziej AE. Securitatea și relațiile internaționale, Polirom, București, 2007, 391-392.

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REVIEW

New Insights in Treatment Options in Pediatric Urinary Tract Infection

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Urinary tract infection (UTI) represents one of the most frequent infections with bacterial etiology during childhood. In infants and toddlers with fever without source UTI' investigation should be carried out, since signs and symptoms are nonspecific. However, obtaining uncontaminated urine samples from these patients can be challenging and time consuming; all current collection methods (clean-catch, plastic collection bag, catheterization, etc) have disadvantages. Criteria for UTI definition are represented by the presence of significant number of a single uropathogen, this number being different depending on the collection method: at least 1000 colony-forming unit (CFU/ml) for catheter samples and at least 100.000 CFU/ml from midstream clean-catch samples or 50.000 CFU/ml and significant pyuria in a symptomatic or febrile child. Accurate diagnosis of UTI is essential to avoid any antibiotic overuse and expensive investigations. UTI caused by resistant bacterial strains has an increasing prevalence in children. In pediatric population, extended spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) represent the etiology of around 15% of UTIs. Because of limited therapeutic options the reintroduction of some old antimicrobial agents is necessary, therefore Nitrofurantoin and Fosfomycin, can represent alternatives for oral treatment and prophylaxis of UTIs in children or in case of resistance suspicion to other drug classes. It is important to recognize patients at risk, such as children with recurrent UTIs, kidney abnormalities, like vesicoureteral reflux and previous antibiotherapy, in order to recommend adequate empiric treatment, especially against resistant bacteria.

Keywords: child, fever, urinary tract infection, treatment

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Introduction

Urinary tract infection (UTI) is considered as the second infections with bacteria in children. The diagnosis of UTI in infants and children can be difficult, as clinical picture varies with the patient's age, lack of characteristic symptoms in young infants, different collecting methods of urine sample, and interpreting the result of urinalysis and urine culture. In infants and toddlers with fever without source UTI' investigation should be carried out, since signs and symptoms are nonspecific. Careful anamnesis and complete physical examination are key-elements for the suspicion and diagnosis of UTI.

A detailed anamnesis to identify risk factors or an underlying renal pathology is essential (poor urine flow, dysfunctional voiding, enlarged bladder, previous UTI, recurrent fever without source, renal abnormality diagnosed antenatally, family history of renal disease, constipation, spine abnormalities, poor growth) (1).

Recently, many guidelines have tried to settle several debates in diagnosis of pediatric UTI. UTI is defined as a positive urine culture and urinalysis, plus clinical symptoms (1, 2). Significant pyuria is defined as ≥10 leucocytes/mm^3 on an uncentrifuged urine sample or ≥5 leucocytes/ high-power field on a centrifuged urine sample or presence of leukocyte esterase on urine dipstick (3). A positive nitrite test result is another argument for UTI, but a negative one has little value in ruling out UTI as it is not an accurate marker for infant' UTI, who urinate frequently and not all uropathogens reduce nitrate to nitrite (3).

Clinical picture

Neonates and young infants with febrile UTI are more likely to have bacteriemia or sepsis than older children and should be carefully evaluated and managed. UTI in newborn and infant can manifest as different "picture" and has non-specific symptoms (Table I).

Laboratory

To confirm a UTI, a proper collected urine sample for culture is mandatory, this being a challenge in non-toilet trained children. However, obtaining uncontaminated urine samples from these patients can be difficult and time consuming; all current collection methods (clean-catch, plastic collection bag, bladder catheterization and suprapubic aspiration) have disadvantages (Table II). We have to take into consideration that not all collection methods of the urine are equals! The final bacterial concentration is directly related to urine output, collection method, storage and shipment of the urine sample to laboratory (1, 3).

For many years the same cut-off value of 100.000 CFU/mL of a single uropathogen has been the criteria for UTI diagnosis in schoolchildren and adults. It was based on the

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Table I. Clinical features in pediatric UTI based on patient' age

Neonates, infants< 2 months	Infants and children aged 2 months to 2 years	Toddler, preschool children (2-6 years)	School-aged children, teenagers (>6 years)
fever, prolonged jaundice, failure to thrive, poor feeding, vomiting, diarrhea, irritability, lethargy sepsis, hematuria, cloudy or malodorous urine	systemic symptoms: poor feeding, high temperature, nausea and vomiting, abdominal pain or discomfort, irritability, malaise, strong-smelling urine, hematuria, ±crying on passing urine	gastrointestinal signs, such as vomiting and diarrhea, abdominal pain; fever; urinary symptoms: dysuria, frequency, urgency, new onset urinary incontinence, enuresis, abdominal or flank pain, suprapubic discomfort, strong-smelling urine, hematuria, cloudy urine	fever, vomiting, abdominal pain, flank/back pain with or without fever, urinary symptoms (dysuria, urgency, frequent voiding), enuresis, incontinence; suprapubic pain, strong-smelling urine, hematuria, cloudy urine

Table II. Disadvantages of urine specimen collection method

Collection method	Disadvantages
Suprapubic aspiration	invasive method, painful
Bladder catheterization	the risk of introduction of nosocomial pathogens
Clean voided midstream urine sample	first morning sample
Clean-catch urine	time consuming (average waiting times of 30-70 minutes), contamination rates of 5-27%
Urine collection bags	high false-positive and contamination rates (85%)

study of Kass in the 1950s that comprised adult asymptomatic and symptomatic women (4).

According to recent guidelines in infants and young children any bacterial growth in urine sample obtained by suprapubic aspiration confirms UTI (5, 6). There is a difference between European and American guidelines regarding bacterial number cut-off for UTI diagnosis. European guidelines consider that growth of 10.000 CFU/mL or even 1000 CFU/mL are sufficient for UTI diagnosis from a catheterized urine sample (1, 5), whereas United States and Canadian guidelines require the presence of at least 50.000 CFU/mL (3, 6). It is recommended to remove the first few milliliters of urine drained by bladder catheterization in order to reduce the contamination rate (3).

Use of the lower criteria of 10.000 UFC/ml is important in pediatric population, as an infant or small child with urgency or frequent voiding is not able to hold urine long enough to allow bacterial multiplication to reach 50.000 or 100.000 CFU/mL, leading to fewer diagnosed cases, and possible morbidities (4).

A new study suggest that reducing the bacteriologic criteria to diagnose a UTI in infants, toddlers and preschool children with fever or UTI symptoms plus leucocyturia from previous cut-off >100.000 CFU/mL or >50.000 CFU/mL to newer cut-off >10.000 CFU/mL in an appropriately obtained urine sample would have no consequence on diagnostic specificity and sensitivity in accordance with the new European guidelines, but allow to recognize UTIs in this age population group (4).

A recent study concluded that dipstick examinations of clean-catch urine sample through standardized stimulation technique are a useful screening test in febrile infants younger than 3 months old for diagnosing UTIs and it may represent a good alternative to invasive method like bladder catheterization or suprapubic aspiration (7).

According to American Academy of Pediatrics (AAP) guidelines (2011) and revised in 2017, criteria for a true and definitive diagnosis of UTI are the presence of both quantitative urine culture and positive results on urinalysis (presence of white blood cells). A febrile UTI produces both fever or clinical symptoms as well as leucocyturia or pyuria (8).

Asymptomatic bacteriuria (ASB) is defined by significant bacteria count in urine specimen (>100.000 UFC/ml or positive urine culture) in children with absence of any symptoms of UTI. ASB will have no pyuria, despite the positive urine culture. Pyuria is a hallmark of UTI (8). It occurs more frequently in preschool and school age girls. Incidence declines with increasing age. Because it does not cause kidney damage, screening and treatment for ASB should be discouraged (8).

In children with febrile UTIs complete cell blood counts and blood smear, acute phase reactants (the erythrocyte sedimentation rate, C-reactive protein, procalcitonin), blood culture when necessary, kidney function studies (creatinine, blood urea nitrogen) and serum electrolytes are recommended.

Imaging studies

Urinary tract ultrasound is recommended in all children with febrile UTI or recurrent lower or nonfebrile UTIs. Voiding cystourethrography (VCUG), to detect or rule out vesicoureteral reflux presence, is recommended in case of: recurrent UTIs, abnormal ultrasound, atypical UTI. Sometimes, in selected cases, other imaging studies may be necessary (1, 3, 6).

Treatment

After urine sample has been obtained and, when necessary, blood samples collected, antibiotic therapy should be started as soon as possible. According to recent studies, early treatment is reported to reduce bacteraemia, improve clinical outcome and reduce the incidence of renal scar formation, while any delay in therapy initiation awaiting a new urine specimen for culture, as recommended, may have severe consequences (9-11). Empiric antibiotic treatment should be started for suspected UTI in a sick child, and if necessary, changed later according to sensitivity results for the isolated uropathogen. Guidelines recommend that empiric antibiotic treatment for suspected UTI should be

based on local susceptibilities derived from available epidemiologic local information (1). Waiting for urine culture results, all patients will receive empirical antibiotics for at least 48-72 hours which can be changed later based on *antibiotic sensitivity***.** Avoiding the use of antibiotics with known resistance and wide spectrum drugs, we may assist to a partial sensitivity recovery of uropathogens. A recent study confirmed that resistance pattern of the bacterial strains isolated from urine samples collected from routinely submitted outpatient and inpatient urine samples is different (12).

According to current guidelines, antibiotics should not be used in infants and children to treat asymptomatic bacteriuria, otherwise we may select resistant bacteria.

In infants and children older ≥3 months with ***lower or nonfebrile UTI*** a short oral antibiotic course (3-5 days) is recommended (Trimethoprim/Sulfamethoxazole, Nitrofurantoin, 1st or 2nd generation Cephalosporin, Amoxicillin/clavulanic acid; and sometimes, based on local sensibility, even: Ampicillin, Amoxicillin), but not shorter than 1- 2 days. An old drug, Fosfomycin on oral route, widely used in adults, in pediatrics having just few published data, is recommended as a single 2-g dose for cystitis in children and adolescents. High urinary concentrations are observed in approximately 4 hours after drug intake that persist for several days. The dose may be repeated every 2-3 days for 3-7 times (13, 14).

Parenteral antibiotic therapy is recommended in case of: complicated UTI (kidney abnormalities, decreased kidney function, and sepsis), gastric intolerance, febrile UTI, urinary sepsis, noncompliance to oral treatment and in infants younger than 3 months old with UTI suspicion.

In ***febrile UTI*** antibiotic treatment is recommended for 10 to 14 days, at beginning with an intravenous antibiotic for 2 to 4 days then changed to oral antibiotics in case of good clinical evolution (if fever disappears, general state is improving, etc).

In infants and children with first UTI episode antibiotic prophylaxis should not be routinely recommended, but this may be considered in those with recurrent UTIs or if kidney abnormalities are present.

According to recent guidelines, infants and children who receive aminoglycosides (Amikacin, Tobramycin, Gentamicin), one single dose/day is recommended, this frequency being safer and equally effective as twice daily (1, 11). Also, Amikacin in a single intramuscularly dose/day could be a therapeutic option in case of suspected multidrug resistant (MDR) uropathogen as the UTI ethiological agent until the culture result is revealed. To prevent the occurrence of aminoglycosides side-effects (nephro- and ototoxicity), Amikacin should be given as a total single daily dose of 15 mg/kg with a treatment length of 7 days (15).

Based on above mentioned data, the antibiotic choice in acute UTI treatment in outpatient children is illustrated in Table III.

Discussion

UTI can be the first sign in 30% of children with urinary tract abnormalities. During the first 6–12 months after an initial UTI episode nearly 30% of infants and children, with urinary tract abnormality, namely vesicoureteral reflux, suffer recurrent UTIs (5).

UTI caused by resistant bacterial strains has an increasing prevalence in children. ESBL-producing gram-negative bacteria etiology in children UTIs represents an important therapeutic challenge. Another issue is represented by the growing percentage of UTIs caused by MDR pathogens for which there are limited therapeutic options.

Results of a recent meta-analysis highlights that in the pediatric population 14% UTIs (1 out of 7) are caused by ESBL-producing Enterobacteriaceae (ESBL-PE). Risk factors for UTIs caused by ESBL-PE are: history of vesicouretral reflux, previous UTI as well as recent antibiotic therapy during the previous month or even in the last 6 months. These pathogens are associated with higher length of hospital stay, more than 1.5 times compared to other uropathogens, higher costs (more likely to be managed in inpatient settings) as well as the exposure of the patient to the hospital acquired infection risk (16).

The global spread of ESBL-PE among pediatric UTIs is different, the ESBL-PE UTIs rate being about 40% in Asia compared to Europe where this rate is close to 20% and 5% in North America (16). This difference is assigned to local variability in antibiotic use as well as the enormous consumption of broad-spectrum cephalosporins as first line treatment, leading to the appearance of MDR bacteria.

ESBL-PE etiology leads to limited therapeutic options resulting in an important obstacle in the clinical treatment of UTIs (6). ESBL-PE are resistant to majority of beta-lactam antimicrobials, the therapeutic options being more restricted by the associated co-resistance to other antimicrobials. Beta-lactam-beta-lactamase inhibitor combinations, Aminoglycosides, Carbapenems and Tigecycline, represent alternative treatment options for ESBL-PE UTIs. The big disadvantage of all these drugs is that they have to be administered parenterally, so patients need to be treated as inpatients (16). Febrile children who fail to respond to antibiotherapy within 2-3 days are prone to kidney scar

Table III. Antibiotic options in case of lower versus febrile UTIs

Diagnosis	First line	Alternative
Lower UTI/acute cystitis	Amoxicillin/clavulanic acid Trimethoprim/sulfamethoxazole Nitrofurantoin Fosfomycin trometamol	1st or 2nd generation of Cephalosporin (Cefuroxim, Cephalexin) Nitrofurantoin
Febrile UTI	Amoxicillin/clavulanic acid	2nd or 3rd generation Cephalosporin (Cefuroxim, Ceftibuten, Cefpodoxim, etc)

formation, especially if they have a history of vesicoureteral reflux or recurrent UTIs. These findings are particularly important in pediatric population. To prevent long-term sequelae such as kidney scarring, high blood pressure and chronic kidney disease a precocious diagnosis and appropriate treatment of ESBL-PE and MDR uropathogen as UTIs ethiological agent is essential (16).

In the study conducted by Duffy et al. a time-related association between previous Trimethoprim-Sulfamethoxazole treatment and *Escherichia coli* resistant strains from urine specimens in children was found, with stronger associations for more recent Trimethoprim prescriptions (in the previous year) (17).

A very recent prospective study that included almost 4000 UTI samples (32% pediatric patients) demonstrated that Fosfomycin is considerable active against both Gram-positive and Gram-negative bacteria including resistant organisms like ESBL-PE (>90%) and Methicillin-resistant *Staphylococcus aureus* (MRSA) (88%) except for *Acinetobacter* spp. Fosfomycin, an old drug, may be an oral option in the era of MDR bacteria and should be kept in mind in pediatric UTIs treatment (2).

Given the lack of new antibiotics, there is a justifiable motivation to test older drugs that maintain some activity against MDR bacteria. Of these, Fosfomycin seems to be a promising choice because of its wide susceptibility against both Gram-positive and Gram-negative bacteria. Published microbiological data support this option, as susceptibility rate is very high in *Klebsiella pneumoniae* and, especially, in *E. coli* (18).

Data on antibiotic resistance patterns of Gram-negative organisms in Romania are incomplete. A recent study performed in central Romania, that included 107 small infants with UTI, concluded that there is a terrifying high antibiotic resistance rate of *E. coli* and *Klebsiella* spp. for Aminopenicillins, Ceftriaxone, Cefuroxime, Gentamicin and Ciprofloxacin, respectively. Almost 81% of the *E. coli* and 43% of the *Klebsiella* spp. isolates were ESBL-producers. The resistance rates of this 2 major uropathogens to Piperacillin/Tazobactam, Meropenem, Nalidixic acid, Chloramphenicol and Colistin were low (19). The resistance rate in this research was higher than that reported by European Centre for Disease Prevention and Control (20). Based on the latest European Union reports; Romania has a high resistance rate to antibiotics for *E. coli* and for *Klebsiella pneumonie* (table IV) while the highest resistance rate from Europe were reported for *Pseudomonas* spp. to Piperacillin±Tazobactam, Fluoroquinolones, Ceftazidime, Aminoglycosides, Carbapenems, or combined resistance to these drugs (20).

The results of a recent study that comprised 31.000 urine isolates showed that uropathogens resistance to many antibiotics was higher in the inpatient vs. outpatient (table V), (16). A recent meta-analysis stated that Nitrofurantoin represents the most appropriate therapeutic option as first line treatment for lower or nonfebrile UTI. The same study underlined that some drugs commonly used in primary care, including Ampicillin or Amoxicillin and Trimethoprim, may have no effectiveness as first-line treatment (20). Also we should not be tempted to prescribe broad-spectrum second line antibiotics, such as Amoxicillin/Clavulanic acid, Cephalosporins and Fluoroquinolones (21).

In contradiction with previous data, another study demonstrated that most UTIs in preschool children retained susceptibility to Nitrofurantoin, Amoxicillin/Clavulanic acid and Cephalexin (86-98%) and Trimethroprim (74%) (22).

In a recent study, Polat and Kara proved the usefulness of once-daily intramuscular Amikacin in children with lower UTIs caused by ESBL-producing *E. coli* strains susceptible to this drug, without any oral therapy option, treated as outpatients (15).

However, when choosing an antibiotic for first line empirical treatment of UTI the main criteria should be the local prevalence of resistance to antibiotics smaller than 20% (21).

Conclusion

The UTI diagnosis in children should be based on clinical presentation, physical examination, urinalysis, cut-off of urine culture, methods of urine collection, inflammatory markers, and sometimes imaging studies. Prompt and appropriate diagnosis and treatment of a febrile UTI is important (in particular, in infants younger than 3 months). The significant incidence of MDR uropathogen strains in pediatric population should be taken into consideration in the attempt to suggest empiric treatment protocols. Nitrofurantoin and Fosfomycin, considered as old drugs, can represent alternatives for oral treatment and/or prophylaxis of UTIs in children or in case of resistance suspicion to

Table IV. Uropathogen resistance to antibiotics in Romania according to European Centre for Disease Prevention and Control report (20)

Uropathogen	Drug	Resistance rate
E. coli	Aminopenicillins	72,3%
	Fluoroquinolones	30,6%
	3rd generation Cephalosporins	23,4%
	Aminoglycosides	15%
	Carbapenems	1%
	MDR	11%
Klebsiella pneumoniae	3rd generation of Cephalosporins	68%
	Aminoglycosides	62%
	Carbapenemes	31%
	MDR	55%

Table V. Uropathogen resistance to antibiotics in the inpatient vs. outpatient (16).

Uropathogen	Drug	Inpatient	Outpatient
E. coli	Trimethoprim/ Sulfamethoxazole	30%	24%
	Cephalothin	22%	16%
Klebsiella spp.	Cephalothin	14%	7%
Enterobacter spp.	Ceftriaxone	24%	12%
	Ceftazidime	33%	15%
Enterococcus spp.	Ampicillin	13%	3%
	Ciprofloxacin	12%	5%

other drug classes. This is probably due to the very scarce use of these drugs in the last years.

Assessment of the risk factors for MDR uropathogens is mandatory as it may help to choose appropriate empirical antibiotic therapy. If antibiotic exposure has been occurred in the preceding 3-6 months different antibiotic classes should be recommended for childhood UTI' treatment.

Authors' contribution

Carmen Duicu (Conceptualization; Investigation; Supervision; Writing –original draft; Writing – review & editing)
Iulia Armean (Data curation; Funding acquisition; Writing – original draft)
Cornel Aldea (Supervision; Validation; Writing – review & editing)

Conflict of interest

The authors confirm that this article content has no conflict of interest.

References

1. NICE guideline. Urinary tract infection in under 16s: diagnosis and management (CG54). Updated 2017. https://www.nice.org.uk/guidance/cg54
2. Patwardhan V, Singh S. Fosfomycin for the treatment of drug-resistant urinary tract infections: potential of an old drug not explored fully. Int Urol Nephrol. 2017 Sep;49:1637-43.
3. AAP SUBCOMMITTEE ON URINARY TRACT INFECTION. Reaffirmation of AAP Clinical Practice Guideline: The Diagnosis and Management of the Initial Urinary Tract Infection in Febrile Infants and Young Children 2–24 Months of Age. Pediatrics. 2016 Dec;138(6): pii: e20163026.
4. Primak W, Bukowski T, Shuterland R, et al. What Urinary Colony Count Indicates a Urinary Tract Infection in Children? J Pediatr. 2017 Dec;191:259-61.
5. Stein R, Dogan HS, Hoebeke P, et al. Urinary tract infections in children: EAU/ESPU guidelines. Eur Urol. 2015 Mar;67(3):546-58.
6. Ammenti A, Cataldi L, Chimenz R, et al. Febrile urinary tract infections in young children: recommendations for the diagnosis, treatment and follow-up. Acta Paediatr. 2012 May;101(5):451-7.
7. Herreros ML, Tagarro A, García-Pose A, et al.. Performing a urine dipstick test with a clean-catch urine sample is an accurate screening method for urinary tract infections in young infants. Acta Paediatr. 2018 Jan;107(1):145-50.
8. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management, Roberts KB. Urinary tract infection: Clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics 2011 Sep;128(3):595-610.
9. Coulthard MG, Lambert HJ, Vernon SJ, et al. Does prompt treatment of a urinary tract infection in preschool children prevent renal scarring: mixed retrospective and prospective audits. Arch Dis Child. 2014 Apr;99(4):342-7.
10. Karavanaki KA, Soldatou A, Koufadaki AM, et al. Delayed treatment of the first febrile urinary tract infection in early childhood increased the risk of renal scarring. Acta Paediatr. 2017 Jan;106(1):149-54.
11. Stein R, Dogan HS, Hoebeke P, et al. Urinary tract infections in children: EAU/ESPU guidelines. Eur Urol. 2015 Mar;67(3):546-58.
12. Saperston KN, Shapiro DJ, Hersh AL, et al. A comparison of inpatient versus outpatient resistance patterns of pediatric urinary tract infection. J Urol. 2014 May;191(5 Suppl):1608-13.
13. Calzi A, Grignolo S, Caviglia I, et al. Resistance to oral antibiotics in 4569 Gramnegative rods isolated from urinary tract infection in children. Eur J Pediatr. 2016 Sep;175(9):1219-25.
14. Hsu AJ, Tamma PD. Treatment of multidrug-resistant Gram-negative infections in children. Clin Infect Dis. 2014 May;58(10):1439-48.
15. Polat M, Kara SS. Once-daily intramuscular amikacin for outpatient treatment of lower urinary tract infections caused by extended-spectrum β-lactamase-producing Escherichia coli in children. Infect Drug Resist. 2017 Nov;10:393-399.
16. Flokas ME, Detsis M, Alevizakos M, et al. Prevalence of ESBL-producing Enterobacteriaceae in paediatric urinary tract infections: A systematic review and meta-analysis. J Infect. 2016 Dec;73(6):547-57.
17. Duffy MA, Hernandez-Santiago V, Orange G, et al. Trimethoprim prescription and subsequent resistance in childhood urinary infection: multilevel modelling analysis. Br J Gen Pract. 2013 Apr;63(609):e238-e243.
18. Mazzariol A, Bazaj A, Cornaglia G. Multidrug-resistant Gram-negative bacteria causing urinary tract infections: a review. J Chemother. 2017 Dec;29(sup1):2-9.
19. Falup-Pecurariu O, Leibovitz E, Bucur M, et al. High resistance rates to 2nd and 3rd generation cephalosporins, ciprofloxacin and gentamicin of the uropathogens isolated in young infants hospitalized with first urinary tract infection. Biomed Res. 2017;28(20):8774-8779
20. EARSS. Antimicrobial resistance surveillance in Europe 2016. https://ecdc.europa.eu/sites/portal/files/documents/AMR-surveillance-Europe-2016.pdf
21. Bryce A, Hay AD, Lane IF, et al. Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis. BMJ. 2016 Mar;352:i939.
22. Butler CC, O'Brien K, Wootton M, et al. Empiric antibiotic treatment for urinary tract infection in preschool children: susceptibilities of urine sample isolates. Fam Pract. 2016 Apr;33(2):127-32.

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RESEARCH ARTICLE

Interferon Beta-1b for the Treatment of Multiple Sclerosis – More than 10 Years of Experience

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Objective: Interferon beta-1b (IFNβ-1b) was the first disease-modifying agent (DMT) used for the treatment of multiple sclerosis (MS). We aimed to evaluate the first patients with MS that started treatment in our clinic. **Methods**: An observational, retrospective study was performed on 78 patients that had continuous treatment with IFNβ-1b for more than 10 years. The collection of the demographical data and periodical clinical evaluation was performed on all patients. The disability was quantified using the Expanded Disability Status Scale (EDSS), creating two groups of patients, G1: EDSS < 4.0 and G2: EDSS ≥ 4.0. The hallmarks of the disability evolution were gathered by direct patient interview, such as the symptoms at onset and relapse frequency. **Results**: After more than 17 years of disease evolution, more than half (65.38%) of the patients present a mild disability score. The majority (54.90%) started treatment in the first three years after the onset, while the patients in G2 started treatment after more than 3 years from the onset. The initiation of IFNβ-1b lead to a significant reduction of the relapse rates. A reduced number of patients (<25%) transitioned from RRMS to SPMS. **Discussion**: Continuous evaluation of MS patients allows us to assess the possibility of prolonged treatment with IFNβ-1b and to differentiate the responders from non-responders. The clear reduction in relapse rates and disability progression, notably in patients that started treatment early ensure us into continuing administering this medication. Compared to historical cohorts, our lot had a slower disability evolution and a significant proportion hadn't reach an important disability score.

Keywords: multiple sclerosis, interferonβ-1b, disability, evolution, disease-modifying therapy

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Introduction

Multiple sclerosis (MS), a chronic, immune-mediated disease of the central nervous system (CNS), which predominantly affects the young adults, is one of the most frequent causes of neurological disability in the modern world. Relapsing-remitting MS (RRMS) is the most common form of evolution, which accounts for most of the cases and usually converts into the secondary-progressive MS (SPMS) in 10 to 15 years. [1, 2] Accumulation of disability in MS is secondary to both inflammatory and neurodegenerative processes. From a clinical perspective, active inflammation will trigger a relapse and recovery may be complete or partial, resulting in neurological sequelae. Neurodegenerative processes will lead to progressive disability and mark the onset of the SPMS [3]. The available treatments for MS exert their actions mainly in the inflammatory phase by modulating the immune response, thus being known as disease-modifying therapies (DMT).

The interferons were discovered by accident in 1957 by Alick Isaacs and Jean Lindenmann, two British researchers who were actively following the viral response to the Influenza viruses in animal models (chicken embryos) [4]. While the interferon gamma was the first agent used for the treatment of MS, it proved to have a contrasting effect by incrementing the relapse rates and overall augmenting the disease evolution [5], being quickly replaced by the interferon beta. One of the most studied DMTs - the interferon beta was the first immunomodulatory agent used for the treatment of MS, with a well-known safety profile and a good impact upon the general clinical evolution of the patients. The interferon beta-1b (IFNβ-1b) was approved for the treatment of RRMS in USA in 1993, and in EU in 1995 [6], and shortly after, in 1999, it was approved for use in SPMS [7] after extensive studies that focused upon disease's evolution determined that it also reduces the disability progression and [8] controls the relapse rates in patients with an SPMS [9, 10].

Despite the increasing number of DMTs appearing on the market, the IFNβ-1b remains one of the most prescribed agents for MS' treatment. Whether this drug can significantly delay the disability accumulation when being used for long periods of time is still under dispute, but due to its worldwide availability, the increasing number of long-term treated cohorts of patients starts to answer one question at the time. In order to bring our contribution to the MS world, we examined the clinical impact of IFNβ-1b in MS patients treated continuously for more than 10 years.

Methods

Lot selection

For this study, patients treated with IFNβ-1b subcutaneously once every two days were selected. In our clinic we treat more than 400 patients with MS, from which 140 are treated with IFNβ-1b.

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An observational, retrospective study was performed on 78 patients that had continuous, uninterrupted treatment with IFNβ-1b for more than a decade. Data collection and patient evaluation took place between 2016-2017. The patients have been followed ever since the treatment was instituted in the Neurology 1 Clinic of the Emergency Clinical County Hospital of Targu Mures, Romania. All the patients that are included in the MS treatment programme were evaluated every six months or when needed in case of relapses or other symptoms, as well as brain and spinal cord magnetic resonance imagining (MRI) together with complete neurological follow-ups, as per diagnosis protocol based on the revised McDonalds criteria (2010). [11]

The inclusion criteria were: a) patients diagnosed with MS based on McDonalds criteria revised in 2010, b) were treated continuously for more than 10 years with IFNβ-1b, c) had no prior immunomodulatory treatment and d) consented to regular follow-ups and to be a part of the study. The exclusion criteria were applied for the remaining 62 patients, as following: a) patients interrupted at one point, either voluntarily (pregnancy) or because of adverse effects the treatment with IFNβ-1b, b) had prior immunomodulatory treatment with other DMT, c) they did not consent to be a part of the study, d) had less than 10 years of continuous treatment with IFNβ-1b.

Lot evaluation

The patient's demographical and clinical data, including date of the clinical onset, symptoms at onset, relapses and disability were recorded at the first visit and historical information about the course of the disease was retrospectively collected and analysed. The neurological disability was quantified using the *Expanded Disability Status Scale* (EDSS) [12], actively following the disability progression and moment of conversion from a RR to a SP. The functional systems (FS) affected at onset were noted as optic (O-FS): onset with optic neuritis, pyramidal (P-FS): onset with pure motor deficit, brainstem (B-FS): onset with diplopia or alternate syndromes, cerebellar (C-FS): onset with coordination impairment and instability, sensory (S-FS): onset with either paraesthesias or loss of sensibility and polysymptomatic onset(X-FS).

The total number of relapses was calculated as annual relapse ratio (ARR), by using the following formula: ARR = number of relapses/number of treatment years. ARR was defined as the total number of relapses, ARR_0 as relapses before the treatment respectively, ARR_1, relapses on treatment. A relapse was considered as the sudden onset of new neurological symptoms with a concomitant worsening of the clinical picture, that occurred in the absence of fever or any other kind of active infection. The events lasted more than 24 hours and alleviated spontaneously or with the use of corticosteroids [13].

The disability score was noted according to EDSS, and to simplify the patient selection two groups were defined: G1 – patients with an EDSS < 4.0 and G2 – patients with an EDSS ≥ 4.0 at the study inclusion. The patients' evolutions were followed accordingly, thus the progression was subsequently assessed by consulting the old records, mainly the transition from a lesser EDSS to a score of 4.0 or 6.0. The milestones 4.0 and 6.0 were chose because of the impact they have upon the disability progression. Thus, an irreversible EDSS of 4.0 marks the first clinical signs of ambulatory restriction, limiting the walking perimeter within 500 meters (without any physical aid), and the EDSS of 6.0 is defined by the necessity of unilateral assistance for ambulation. By evaluating the patient files, we were able to indicate, when applicable, the moment of progression, keeping in mind that the ambulatory capacity is the main determinant for the EDSS. The EDSS at the start of the treatment is EDSS_0, respectively at the study inclusion EDSS_1.

Statistical Analysis

The data was centralized using the Excel Platform incorporated in Microsoft Office 2016 and the statistical analysis was performed using Graph Pad Prism 6. We used mean and standard deviation (SD) when assessing gaussian population, and median and range defined by 25%-75% percentile when analysing non-gaussian populations. The normality tests used were Shapiro-Wilk and Kolmogorov-Smirnov, and the correlations were performed using Spearman or Pearson test, after assessing the normality distribution. The statistical significance was defined when $p<0.05$.

Results

A total of 78 MS patients that had continuous treatment with IFNβ-1b for more than 10 years were evaluated. Patient demographic and general clinical data are presented in *Table I*. At the moment of study inclusion, 51 (65.38%) of the patients had an EDSS lower than 4.0, while 27 (34.61%) had a disability score higher or equal to 4.0. The female gender is dominant in both groups, 68.62% in G1 and 70.37% in G2. There was no statistically significant difference ($p=0.259$) when comparing the age at onset of the disease for both groups, (*Fig.1)* but statistical significant data was found when comparing the age at the beginning of the treatment between the two groups ($p<0.0001$), with a median for G1 of 35 years, respectively 42 years for G2 (*Fig 1*). Most of the patients in G1 (54.90%) started treatment between 1 and 3 years from the onset of the symptoms, while in G2, most of them started treatment after 3 years (70.22%).

From the cohort of patients, 63 (80.75%) started treatment with an EDSS between 0 and 3.5 and 48 (76.16%) of them still have an EDSS < 4.0 at the moment of study inclusion, signifying that more than three quarters of the patients with a mild/moderate degree of disability still have a good clinical course. 15 (23.80%) patients converted to SPMS.

The patients had been treated with IFNβ-1b approximately 13 years for both groups. The mean duration of

Table I. Demographic and clinical data of the patients

	All patients - G n= 78	EDSS < 4.0 - G1 n= 51 (65.38%)	EDSS ≥ 4.0 - G2 n= 27 (34.61%)	EDSS ≤ 2.0 n=29 (38.46%)
Female	54 (69.23%)	35 (68.62%)	19 (70.37%)	23 (79.31%)
Male	24 (30.76%)	16 (31.37%)	8 (29.62%)	6 (20.68%)
Mean age at the MS' onset (years)				
< 20 years old	4 (5.12%)	3 (5.88%)	1 (3.70%)	1 (3.44%)
20-30 years old	35 (44.87%)	21 (41.17%)	14 (51.85%)	13 (44.82%)
31-40 years old	30 (38.46%)	26 (50.98%)	4 (14.81%)	12 (41.37%)
> 41 years old	9 (11.53%)	1 (1.96%)	8 (29.62%)	3 (10.34%)
Mean age at the beginning of treatment (± SD*) (years)	36.73 ± 8.53	29.82 ± 7.19	42.04 ± 7.52	29.72 ± 7.26
Mean duration of the disease (years) (± SD)	18.90 ± 7.10	16.90 ± 5.43	22.67 ± 8.36	16.10 ± 5.010
Mean treatment duration (years) (± SD)	13.08 ± 2.46	12.80 ± 2.38	13.59 ± 2.59	12.34 ± 2.05
Start treatment				
Same year	9 (11.53%)	7 (13.72%)	2 (7.40%)	2 (6.89%)
1-3 years	34 (43.58%)	28 (54.90%)	6 (22.22%)	18 (62.06%)
> 3 years	35 (44.87%)	16 (31.37%)	19 (70.22%)	9 (31.03%)
FS at onset				
Optic (O-FS)	16 (20.51%)	13 (25.49%)	3 (11.11%)	9 (31.03%)
Pyramidal (P-FS)	19 (24.35%)	12 (23.52%)	7 (25.92%)	5 (17.24%)
Cerebellar (C-FS)	6 (7.69%)	2 (3.92%)	4 (14.81%)	1 (3.44%)
Sensory (S-FS)	11 (14.10%)	8 (15.68%)	3 (11.11%)	5 (17.24%)
Brainstem (B-FS)	20 (25.64%)	13 (25.49%)	7 (25.92%)	6 (20.68%)
Polysymptomatic (X-FS)	6 (7.69%)	3 (5.88%)	3 (11.11%)	3 (10.34%)
Median EDSS_0 (25%-75% percentile)	2.0 (1.0 – 3.0)	1.5 (1.0 – 2.5)	3.5 (2.5 – 4.5)	1.5 (1.0 – 2.0)
Median EDSS_1 (25% - 75% percentile)	3.5 (2.0 – 5.5)	2.0 (1.5 – 3.5)	6.0 (5.0 – 6.5)	1.5 (1.0 – 2.0)

*SD: standard deviation

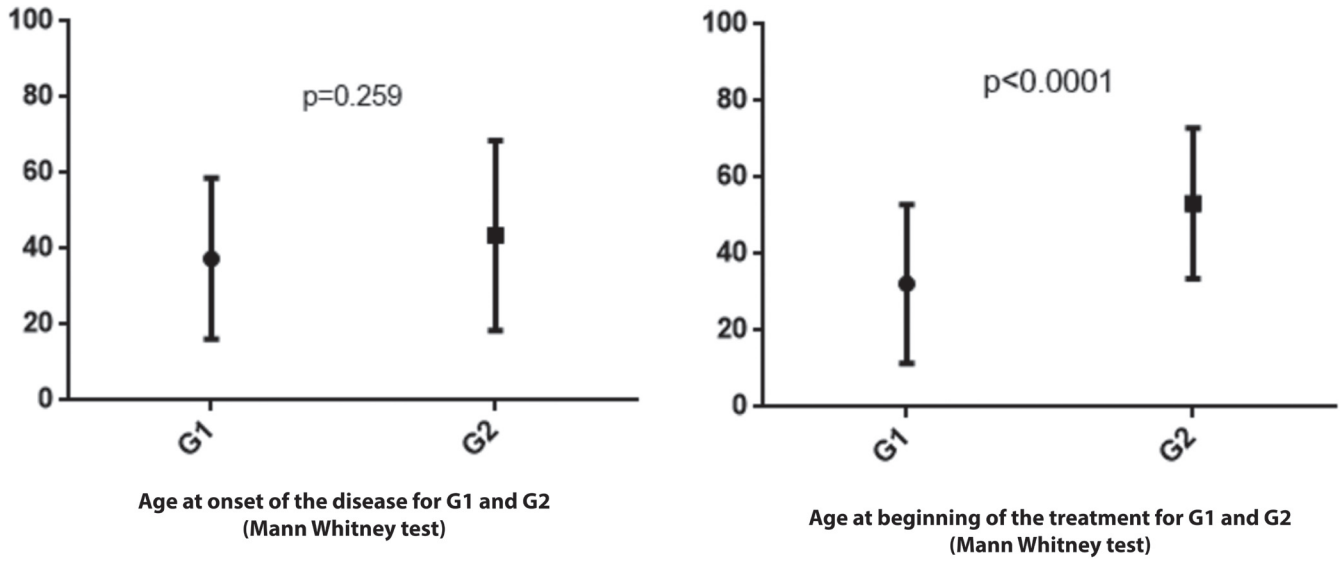

Fig. 1. Age values analysis for G1 and G2, based on the onset and moment of treatment initiation

the disease was 16.90 years for G1 and 22.67 years for G2. Most of our patients in G1 presented at onset with B-FS and O-FS (25.49%) symptoms, while in G2, the predominance was between B-FS and P-FS (25.92%). The EDSS_0 ranged from 1.5 in G1 to 3.5 in G2. At the study inclusion, the EDSS_1 for G1 was 2.0, while for G2 was 6.0.

By comparing the ARR before and after the treatment was instituted, statistical significance was found for all the groups: G, G1, and G2, but with a stronger p-value for G1 ($p<0.0001$), signifying a reduced relapse ratio after treatment with IFNβ-1b was started (Fig 2).

The overview of the clinical evolution of the patients is presented in *Table II*. This included the 15 patients that converted to an SPMS during the treatment. We did not account for patients that already had a higher disability level when they started treatment, because of the difficulty in correctly assessing the moment of conversion historically. The median time from the MS' onset to an EDSS of 4.0 was 12 years and for an EDSS of 6.0 was 16 years. The mean treatment duration was 15 ± 2.63 years. This can be interpreted as following in our study: a patient will reach

Table II: Conversion of the RRMS patients into SPMS

n=15	EDSS 4.0	EDSS 6.0
MS' onset (years) Median (25% - 75% percentile)	12 (8 – 17)	16 (9 – 22)
Start of treatment (years) Median (25% - 75% percentile)	5 (1 – 7)	6 (1.5 – 10)

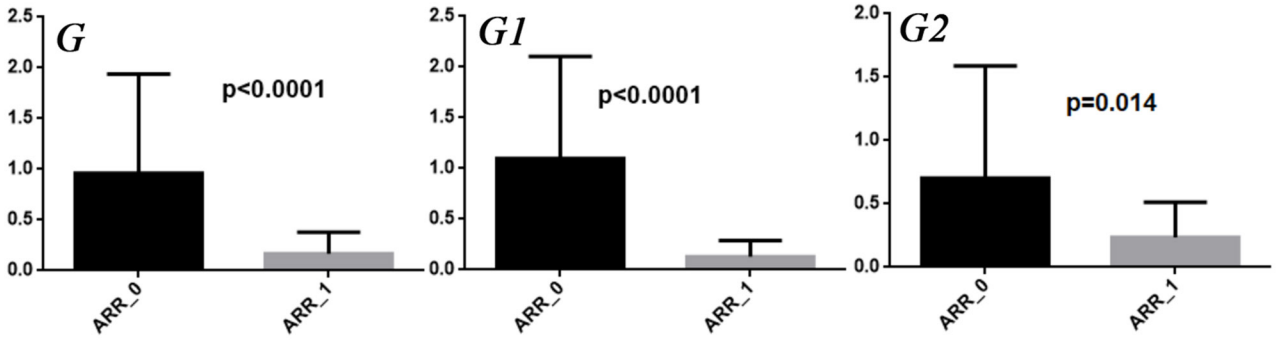

Fig. 2. Analysis of the relapse rates using Wilcoxon matched paired signed ranked test.

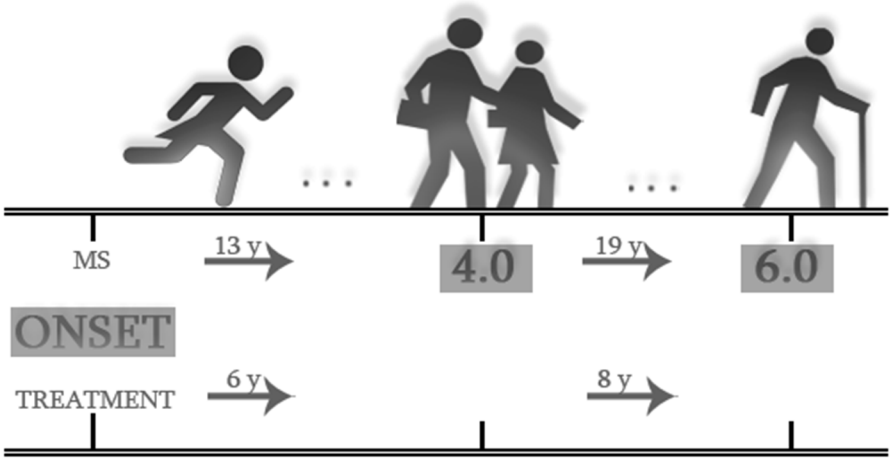

Fig. 3. Representation of the evolution of disability

an irreversible EDSS of 6.0 in 4 years after the start of the progressive phase. The median time from the start of the treatment to an EDSS of 4.0 was 5 years and to an EDSS of 6.0 was 6 years. The evolution towards an SP phase is represented in Fig.3.

The patients were evaluated every 6 months for the entire duration of the treatment. The retrospective information allowed us also to explore the subgroup with the lesser degree of disability and to assess the clinical data (*Table I*). 29 (38.46%) patients present with an EDSS < 2.0 at the moment of study inclusion. Their mean age at MS' onset, the mean age at the beginning of the treatment, mean duration of the disease and mean treatment duration were similar between the G1 and G2. Most of the patients started IFNβ-1b in the first three years from the onset (62.06%) and the dominant FS present at onset was O-FS (31.03%), followed by B-FS (20.68%). The median EDSS_0 was equal to EDSS_1, with a low disability score of 1.5.

Discussion

The efficiency of IFNβ-1b has been the subject of numerous individual and collective studies, mainly due to its availability and ease of monitoring. Ever since its approval in 1993, numerous extensions followed, which demonstrated that continuous administration has a persistent effect upon relapse rate reduction [14], thus diminishing the disability accumulation. In our present study, we focused on clinically evaluating those patients that started the treatment as soon as it became available in our country, knowing that IFNβ-1b was first DMT approved in Romania in our center in 2000 for the RRMS and, shortly after, for the SPMS.

The pathophysiological mechanisms of MS, though not being fully understood yet, are characterized by two entwining processes: demyelination and neurodegeneration, defining MS as a two-stage disease [15]. Demyelination is the result of an over-active auto-immunity and is characteristic for the active form of the disease, RRMS, where inflammation is the dominating pathophysiological process and usually manifests as clinical relapses. The available DMTs will exert their action modulating the inflammation and have a modest effect on the second stage, the neurodegeneration, marked by axonal loss. But the evolution of disability in the first phase seems to be independent than the progression that manifests in the second phase of the disease [16].

IFNβ-1b mechanism of action is partially known, but it's main effects are targeted against lymphocyte T proliferation and a reduction of endogenous IFN-gamma. It also blocks the class II major histocompatibility complex (MHC) and reduces the subsequent expression of the antigen presentation, blocking the activity of the adhesion molecules, reducing thus the inflammation in the CNS [17]. At a umoral level, the agents act upon reduction of the pro-inflammatory cytokines and augment the expression of anti-inflammatory agents [18].

The demographic data of our patients were similar to the results reported by other studies. The obvious dominance

was for female patients [19]. There is a genetic susceptibility for female patients to have a higher risk of developing auto-immune conditions [20], some linked to MHC genes on chromosome 6 [21] and some explained by the various HLA complexes [22]. We found no significant differences between the age of the patients at the MS onset. What was expected was the strong statistical significance when we evaluated the age of patients at the start of the treatment for both groups, knowing that the onset in younger patients tends to be correlated with a better evolution [23, 24].

The importance of early treatment was easily stratified in the present study. More than half of the patients from the G1 started treatment in the first 3 years, while for the G2, most of the patients started treatment over 3 years after onset. Considering the times, it was not uncommon, due to the absence of DMTs in our country when most of our patients presented their first symptoms, but it is clear that early treatment equals better evolution, from the perspective of relapse reduction and disability control [10, 25]

Probably one of the most important effects that IFNβ-1b exerted was the reduction in the relapse rates, noticed for both groups but with a stronger statistical significance for the G1. Subsequent relapses will favor disability burden and thus, disability progression, therefore, by controlling relapse rates one can delay the conversion to an SP phase. Several studies hold unto the idea that relapses will increment disability progression [26] while others affirm that the data cannot be validated in order to reproduce this argument [27]. But it is certain that being relapse free is wished for in clinical practice.

IFNβ-1b proved its efficiency in significantly delaying the conversion from clinically isolated syndrome (CIS) to clinically defined MS [28]. IFNβ-1b effects are also beneficial upon patients with an SP form [17] because even though the effects are mainly anti-inflammatory, by adequately modulating the immune response the agents act upon the residual inflammation that is also present in the second phase of the MS, dominated by neurodegeneration. One study reported a reduction in ARR in SP patients up to a 43% [29].

The conversion rate from RRMS to SPSM was low, less than 25% from the patients with RRMS progressed to SPMS. Our patients reached a moderate disability, an EDSS of 4.0 in a median of 12 years and 6.0 in 16 years from the MS' onset. Studies upon the evolution of the disease mention a rate of conversion as high as 54% [3] with a median time of evolution of 19 years. These patients had a mean time of treatment of 13 years, so we would have anticipated a higher conversion rate. Another study comparing disabilities showed that MS patients will normally reach an EDSS of 6.0 after approximately 17 years of evolution [30], and in our case, this happened at 16 years after the first symptom. The natural history of MS states that, in about 11 years from the onset of the disease, the progression is unavoidable [31]. The mean age of patients that converted to SP in early historical studies was around 40 years [32], but in our study, the patients progressed and reached an irreversible EDSS close to 48 years of age, finding consistent to other international studies on cohorts of long term treated patients [33].

Depending on the onset of the disease, for the G1 the clinical picture was dominated by optic and brainstem symptoms, while the G2 patients the predominance was pyramidal and brainstem symptoms. It has been reported that the onset with ocular [34] or sensory symptoms [35] is usually associated with a lesser degree of disability. A polysymptomatic onset is usually an indicator of a poor prognosis [36].

Seeing how in some of the cases we are faced with a slow or steady evolution, we hypothesized about *benign MS*. Even though the diagnosis of a benign MS can be made only after a long evolution of the disease and many authors still disapprove of the concept, it's important to evaluate this specific lot of patients in order to at least determine the favourable predictors. *Razzolini L et al* implied that to maintain a benign MS status, the EDSS should be lower than 2.0 and the diseases' evolution should exceed 10-12 years. Also, brain volume as measured in T1 is an important biomarker that can aid in the diagnosis of a truly benign MS [37]. The selected patients from our cohort with a mild disability level had no significantly different demographic data compared to the whole G1/G2 groups, most of them presented at onset O-FS and B-FS symptoms and they started treatment early in the first 3 years from the onset. The only clinical difference was their EDSS level, at study inclusion their mean EDSS was 1.5, signifying only signs of disability, with no clinical impact. While we cannot say that we have obtained new prognostic factors to aid in the definition of benign MS, the follow-up of the abovementioned patients including imaging studies will aid us into completing the missing diagnosis pieces.

Being one of the most used MS treatment, the safety profile of IFNβ is well known. One study that followed patients treated with IFNβ-1b for over 16 years [38] showed that adverse effects (AE) tend to diminish with time. The most frequent AE are local inflammatory reactions such as pain or tenderness around the area of injections and flu-like syndrome, both of which are easily manageable with intermittent anti-inflammatory medications [39]. Comparing the IFNβs, IFNβ-1b with subcutaneous administration is better tolerated than IFNβ-1a with subcutaneous/intramuscular administration. [40, 41].

The limitations of our study are represented by the classical method of determining the neurological status of our patients, the EDSS. This scale mainly implies the physical disability and mobility and doesn't quantify the whole clinical picture of an MS patient, such as fatigue, depression, anxiety etc, given the fact that disability might not always be perceived by the patient as the inability to walk. The clinical data of our patients from the first moment they entered the clinic is accurate, as trained MS specialists were performing the clinical evaluations, but the be-

fore clinical status of the patients, most importantly the ones that joined us already in an SP phase is being left to speculation, since we cannot exactly pinpoint the moment of conversion. An important characteristic of the present study is the uninterrupted IFNβ-1b treatment in a rather homogenous population which displays a heterogeneous response.

Conclusions

Long-term treatment with IFNβ-1b for MS patients remains a safe and optimal option. It's well-known safety profile and ease of monitoring, manageable AE and good tolerability makes it one of the most widely prescribed agents for the treatment of MS.

By reducing the relapse rates and disability progression, IFNβ-1b will delay the evolution of the disease, focusing its effects mainly in the inflammatory phase. It is important to start IFNβ-1b treatment as early as possible, ideally when the diagnosis of MS has been established, this being universally recommended for any other kind of DMT. Extension of the indication for the treatment of not only RRMS but SPMS makes IFNβ-1b an excellent first-line treatment.

However, one must always remember that clinical monitoring it's essential and that if our patients show signs of progression or bad tolerance, either a switch or an escalation of therapy is mandatory in order to ensure the best quality of medical care.

Authors'contributrion

Laura Iulia Barcutean, MD (Conceptualization; Investigation; Methodology; Writing – original draft; Data analysis)
Smaranda Maier, MD, PhD (Reviewing)
Zoltan Bajko, MD, PhD (Statistical analysis of the data)
Anca Motataianu, MD, PhD (Writing – review & editing)
Andreea Romaniuc, MD (Investigation)
Sebastian Razvan Andone, MD (Investigation; Writing – review & editing)
Rodica Ioana Balasa, MD, PhD, Professor (Conceptualization; Investigation; Writing –review & editing)

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Conflict of interest

The authors have no conflict of interests.

References

1. Scalfari A, Neuhaus A, Degenhardt A et al - The natural history of multiple sclerosis: a geographically based study 10: relapses and long term disability. Brain. 2010;133:1914-29.
2. Ciccarelli O, Thompson A - Multiple sclerosis in 2015: Managing the complexity of multiple sclerosis. Nat Rev Neurol. 2016;12:70-2.
3. Tremlett H, Yinshan Z, Devonshire V - Natural history of secondary-progressive multiple sclerosis. Mult Scler. 2008;14:314-24.
4. Isaacs A, Lindenmann J - Virus Interference: I. The Interferon. Proc R Soc Lond B Biol Sci. 1957;147:258-67.
5. Panitch HS, Hirsch RL, Schindler J et Johnson KP - Treatment of multiple sclerosis with gamma interferon: exacerbations associated with the activation of the immune system. Neurology. 1987;37:1097-102.
6. Kappos L, Polman CH, Freedman MS, et al - Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. Neurology. 2006;67:1242-9.
7. European Study Group on Interferon Beta-1b in Secondary Progressive MS - Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. Lancet. 1998;1491-7.
8. Filippini G, Munari L, Incorvaia B, et al - Interferons in relapsing remitting multiple sclerosis: a systematic review. Lancet. 2003;361:545–52.
9. Kappos L, Polman C, Pozzilli C, et al - Final analysis of the European multicenter trial on IFNbeta-1b in secondary-progressive MS. Neurology. 2001;57:1969-75.
10. Kappos L, Freedman MS, Polman CH, et al - Long-term effect of early treatment with interferon beta-1b after a first clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENFIT trial. Lancet Neurol. 2009;8:987-997.
11. Polman CH, Reingold SC, Banwell B et al - Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann Neurol. 2011;69:292-302.
12. Kurtzke JF - Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-52.
13. Confavreux C, Vukusic S, Thibault M et al. – Relapses and progression of disability in multiple sclerosis. The New England Journal of Medicine. 2000; 343:1430-8.
14. IFNB Multiple sclerosis study group and the University of British Columbia. MS/MRI Analysis group - Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. Neurology. 1995; 45:1277-85.
15. Steinman L - Multiple sclerosis: a two stage disease. Nat Immunol. 2001;2:762-4.
16. Leray E, Yaouaq J, Le Page E et al - Evidence for a two-stage disability progression in multiple sclerosis. Brain. 2010.1900-13.
17. Paolicelli D, Direnzo V, Trojano M - Review of interferon beta 1b in the treatment of early and relapsing multiple sclerosis, Biologics. 2009;3:369-76.
18. Barcutean LI, Romaniuc A, Maier S et al - Clinical and serological biomarkers of treatment's response in patients treated continuosly with interferon β-1b for more than a decade. CNS Neurol Disord Drug Targets. 2018. DOI: 10.2174/1871527317666180917095256. E-pub ahead of print.
19. Harbo JH, Gold R, Tintore M - Sex and gender issues in multiple sclerosis. Ther Adv Neurol Disord. 2013; 6:237-48.
20. Dunn SE, Gunde E, Lee H - Sex -based differences in multiple sclerosis (MS): Part II: Rising incidence of multiple sclerosis women and the vulnerability of men to progression of this disease. Current Topics in behavioural neurosciences. 2015;26:57-86.
21. Bell J, Lathrop G - Multiple loci for multiple sclerosis. Nat Genet. 13;1996:377-8.
22. Oksenberg J, Barcellos L, Cree L et al - Mapping multiple sclerosis susceptibility to the HLA-DR locus in African americans. Am J Hum Genet. 2004;74:160-7.
23. Tremlett H, Paty D, Devonshire V - Disability progression in multiple sclerosis is slower than previously reported. Neurology 2006. 24; 66:172-7.
24. Weinshenker BG, Rice GP, Nosworthy JH - The natural history of multiple sclerosis: a geographically based study. 3. Multivariate analysis of predictive factors and models of outcome. Brain. 1991; 114:1045-56.
25. Palace J, Duddy M, Lawton M et al – Assessing the long-term effectiveness of interferon-beta and glatiramer acetate in multiple sclerosis: final 10-year results from the UK multiple sclerosis risk-sharing scheme. J Neurol Neurosurg Psychiatry. 2018; DOI: 10.1136/jnnp-2018-318360.
26. Lublin FD, Baier M, Cutter G - Effect on relapses on developmental of residual deficit in multiple sclerosis. Neurology. 2003; 9:1528-1532.
27. Young PJ, Leaderer C, Eder K et al - Relapses and subsequent worsening of disability in relapsing-remitting multiple sclerosis. Neurology 2006; 12: 804-8.
28. Kappos L, Freedman MS, Polman CH, et al - Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: a 3-year follow-up analysis of the BENEFIT study. Lancet. 2007;370;389-97.

29. Panitch H, Miller A, Paty D, Weinshenker B - Interferon beta-1b in secondary progressive MS: results from a 3-year controlled study. Neurology. 2004;63:1788-1795.
30. Cree BA, Gourraud PA, Oksenberg JR, et al - Long term evolution of multiple sclerosis disability in the treatment era. Ann Neurol. 2016;80:499-510.
31. Vukusic S, Confavreux C - Prognostic factors for progression of disability in the secondary progressive phase of multiple sclerosis. J Neurol Sci. 2003;206:135-7.
32. Confavreux C, Vuksic S - Age at disability milestones in multiple sclerosis. Brain. 2006;129:595-605.
33. Trojano M, Pellegrini F, Fuiani A et al - New natural history of Interferon-β-treated relapsing multiple sclerosis. Ann Neurol. 2007;61:300-6.
34. Bergamaschi R - Prognostic factors in multiple sclerosis. Int Rev Neurobiol. 2007;79:423-47.
35. Degenhardt A, Ramagopalan SV, Scalfari A, Ebers GC - Clinical prognostic factors in multiple sclerosis: a natural history review. Nat Rev Neurol. 2009; 5:672-82.
36. Visscher B, Liu KS, Clark VA, Detels R, Malmgren RM, Dudley JP - Onset symptoms as predictors of mortality and disability in multiple sclerosis. Acta Neurol Scand. 1984;70:321-28.
37. Razzolini L, Portaccio E, Stromillo ML et al - The dilemma of benign multiple sclerosis: Can we predict the risk of losing the “benign status”? A 12-year follow-up study. Mult Scler Relat Disord. 2018; DOI: 10.1016/j.msaed.2018.08.011 Epub ahead of print.
38. Reader AT, Ebers GC, Traboulsee A, et al - Cross-sectional study assessing long-term safety of interferon-beta-1b for relapsing-remitting MS. Neurology. 2010;74:1877-85.
39. Zettl UK, Hecker M, Aktas O, Wagner T, Rommer PS – Interferon β-1a and β-1b for patients with multiple sclerosis: updates to current knowledge. Expert Rev Clin Immunol. 2018;14:137-53.
40. Baum K, O’Leary C, Coret Ferrer F, et al - Comparison of injection site pain and injection site reactions in relapsing-remitting multiple sclerosis patients treated with interferon beta-1a or 1b. Mult Scler. 2007;13:1153-60.
41. Harris C, Billisberger K, Tillotson L et al - Injection-site pain in patients with multiple sclerosis: interferon beta-1b versus interferon beta-1a. Int J MS Care. 2005/2006;7:132-6.

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RESEARCH ARTICLE

Congestive Heart Failure and Upper Digestive Endoscopic Lesions

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Objective: To evaluate the impact of congestive heart failure and the most important clinical and pathological factors on severe upper digestive mucosal lesions. **Methods**: The study included 749 patients referred for upper digestive endoscopy, divided into two groups: 140 subjects with congestive heart failure (study group) and 609 subjects without heart failure (control group). **Results**: Severe endoscopic lesions quantified according to Lanza score (OR = 3.84, 95% IC: 2.62-5.62), active/inactive gastritis (OR = 2.07, 95% CI: 1.36-3.14), intestinal metaplasia and/or gastric atrophy (OR = 2.42, 95% CI: 1.67-3.52) were significant more frequent among patients with heart failure. Anemia (OR = 3.65, 95% IC: 2.48-5.37) and all investigated comorbidities, as well as alcohol consumption (OR = 1.60, 95% IC: 1.10-2.34) and smoking (OR = 1.76, 95% IC: 1.17-2.64) were more frequent in the study-group. Dividing the patients with cardiac insufficiency according to the severity of their endoscopic lesions, the male gender (OR = 2.76, 95% IC: 1.35–5.61) and daily low-dose aspirin consumption were found to be more frequent among patients with severe endoscopic lesions (OR = 7.71, 95% IC: 3.62–16.40), while anticoagulant therapy and alcohol consumption were borderline associated with mucosal lesions (p=0.08). **Conclusions**: Male patients and aspirin consumers with heart failure, but not those with *H. pylori* infection seem to be more prone to develop upper digestive endoscopic lesions, while alcohol consumption or anticoagulant therapy could be other modifiable factors associated with severe endoscopic lesions in a congestive gastro-duodenal mucosa.

Keywords: congestive heart failure, endoscopic gastro-duodenal lesions, anemia

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Introduction

Heart failure is a common and potentially fatal condition, being one of the most frequent causes of hospitalization today, with a poor prognosis despite the improvements in diagnosis and medical treatment [1,2]. In 2016 it affected over 60 million people worldwide and despite improvements in modern device-, and pharmacotherapy, it continues to have a high mortality [3].

Congestive heart failure (CHF) can be described as a multi-organ disorder caused by the incapacity of the heart to keep adequate cardiac output to satisfy the body's metabolic needs [4]. It has been established that the cardiovascular system is not the only one affected by heart failure. In CHF, the increased systemic venous congestion is transmitted to the inferior vena cava, which leads to congestion in its draining territories, such as the gastrointestinal tract (GIT) mucosa [5]. Over the last decade, several studies investigated the gastrointestinal changes associated with CHF [5,6]. Structural changes have been previously described in the gastric mucosa, such as mosaic pattern in the stomach, mucosal thickening, antral vascular ectasia, and areas of telangiectasias [6].

Mechanisms of gastrointestinal-related symptoms remain poorly understood despite their common presence and increased morbidity and mortality correlated with their coexistence. The specific involvement of the gastrointestinal system in CHF results in a bidirectional relationship. The systemic volume overload characteristic of CHF is generally associated with concomitant gastrointestinal edema, which can result bacterial translocation into the systemic circulation. Consequent activation of monocytes and excessive release of cytokines lead to systemic inflammation, increased symptoms, and therefore, progression of the disease [4].

Anemia is a very common and well-known comorbidity in patients with CHF and its prevalence increases with the severity of the disease. The true frequency of anemia in CHF patients varies widely, but it has been reported to range between 30% - 50%, depending on the severity of CHF and the population studied [6, 7, 8,9]. Anemia in CHF patients is a multifactorial and multidimensional problem. However, there has been an increasing appreciation for the significance of anemia in the pathophysiology, treatment, and prognosis of CHF [9].

The aim of the present study is to evaluate the influence of CHF and associated clinical and pathological factors on the severity of upper digestive endoscopic lesions.

Methods

The study included 749 patients divided as follows: 140 patients with congestive heart failure (CHF group) and 609

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subjects without congestive heart failure (control group). All patients were hospitalized in Medical Clinic Nr. 3 in Tîrgu Mureș Emergency County Hospital, Romania, and underwent an upper digestive endoscopy. The reasons for endoscopy were specific digestive symptoms, anemia, or screening before initiating an antithrombotic therapy or a major cardiovascular surgery.

Written informed consent was obtained from all subjects before being included in the study. The research was approved by the Ethical Committee of the University of Medicine and Pharmacy of Tîrgu Mureș, Romania. Demographical and clinical data were collected from each patient after structured interviews and clinical examinations.

The diagnosis of CHF was derived from a careful history and based on present and past medical records of the patients. Digestive symptoms questioned were epigastric pain, heartburn, regurgitation, nausea/vomiting. Alcohol consumption was considered at the use of at least 10 units (10 mL) of pure alcohol weekly, while smoking at more than 5 cigarettes/day. To conduct an investigation into drug exposure, medical records of the patients and a structured interview was performed. Patients taking low-dose aspirin (LDA, 75 -100 mg/day), regular daily doses of non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs-ibuprofen, ketoprofen, dexketoprofen, diclofenac) and proton pump inhibitors (omeprazole, pantoprazole, esomeprazole) for more than 2 weeks were considered as exposed to drug. Cut-off values of hemoglobin level for anemia (hemoglobin level of < 12g/dl (7.5mmol/l) in women and <13g/dl (<8.1 mmol/l) in men,) were used according to the WHO definitions [7].

Each endoscopy was carried out by an endoscopist who was not informed about the symptoms and drug exposure. The mucosal lesions were described as erythema, petechiae (hemorrhagic area without mucosal defect), erosions (mucosal defect smaller than 5 mm), or ulcers in both gastric and duodenal mucosa. We used the modified Lanza score (MLS), as followed: Lanza score 0 for no mucosal lesions, Lanza score 1 for one erosion or petechiae, Lanza score 2 for 2–10 erosions or petechiae, Lanza score 3 for more than 10 erosions or petechiae and Lanza score 4 when an ulcer was present [10]. To investigate factors associated with endoscopic lesions we stratified patients in CHF group according to their lesions in severe endoscopic lesions group (Lanza score 2,3,4 - 77 subjects) and controls (Lanza score 0,1- 63 subjects). The Los Angeles Classification System for the endoscopic assessment of reflux esophagitis was used to define esophagitis, but patients in the present study were assigned as having or not any mucosal lesions. During endoscopy, four biopsy specimens were taken, two from the antrum and two from the corpus (from the greater and the lesser curvature). The specimens were routinely processed and examined by a pathologist blinded to symptoms and drug exposure. Mucosal changes in the gastric biopsies were described and classified using the Updated Sydney System. *H. pylori* infection was considered present if the germ was identified on histologic examination in at least one biopsy sample.

All collected data was recorded in a specially designed database.

Statistical Analysis

Qualitative nominal variables were summarized using absolute frequencies (number of cases) and relative frequencies (%). Chi-square and Fisher's exact tests were performed to analyze the associations between possible predictors for congestive heart failure. Value of p lower than 0.05 was considered statistically significant. The odds ratio (OR) and 95% confidence intervals were calculated to quantify the magnitude of the association. GraphPad Prism 6 was used for the statistical analysis.

Results

Bivariate Analysis

Distribution of demographic and clinical characteristics of the patients in two groups are showed in Table I. Male patients were more frequent in the CHF group. Anemia and severe endoscopic lesions were with statistically significant higher frequency in the CHF group (OR = 3.65, 95% IC: 2.48-5.37), (OR = 3.84, 95% IC: 2.62-5.62). LDA and NSAIDs consumption was statistically lower among CHF patients compared with the control group (OR = 0.41, 95% IC: 0.74-0.71).

Gastritis (active/inactive) (OR = 2.07, 95% CI: 1.36-3.14), intestinal metaplasia and/or gastric atrophy in biopsy samples (OR = 2.42, 95% CI: 1.67-3.52) were significantly associated with congestive heart failure, but not with active H. pylori infection (Table I).

The history of ulcer, as well as concomitant diseases (respiratory, liver, renal, or cerebrovascular disease) were significantly more frequent in patients with congestive heart failure (OR = 23.96, 95% CI: 14.75–38.94),. Consumption of gastrotoxic drugs (LDA and NSAIDs) was statistically significant less frequent among patients with CHF, while gastroprotective drugs (PPI) were more frequently taken. Epigastric pain and heartburn were found to be less frequent among patients with heart failure. Alcohol consumption (more than 2 units/day) and smoking (over 5 cigarettes/day) also showed significant association with congestive heart failure.

Dividing patients from CHF group according to the severity of endoscopic lesions (Lanza score) we observed that from all considered predictors, the male gender was positively associated with the severity of endoscopic lesions (OR = 2.76, 95% IC: 1.35–5.61), while alcohol consumption had a tendency toward statistical significance (Table II). LDA consumption was found to be more frequent among patients with CHF and severe endoscopic lesions (OR = 7.71, 95% IC: 3.62–16.40), mean while anticoagulants tended to have a tendency toward significance. Anemia was more frequent in patients with CHF and severe

Table I. The distribution of demographical, clinical, endoscopic and pathological variables in studied groups

Variables	Congestive heart failure group N= 140 (18.70%)		Control Group N=609 (81.30%)		p* value	OR	95% CI
	N	%	N	%			
Male gender	89	63.57	263	43.18	< 0.0001	2.29	1.57-3.35
Anemia	68	48.57	125	20.52	< 0.0001	3.65	2.48-5.37
Drug consumption							
Anticoagulants	65	46.42	30	4.92	< 0.0001	16.73	10.20-27.44
NSAIDs	25	17.85	512	84.07	< 0.0001	0.04	0.02-0.06
PPIs	83	59.28	285	46.79	0.0086	1.65	1.14-2.40
LDAa	17	12.14	151	24.79	0.0010	0.41	0.24-0.71
Endoscopic findings							
Severe endoscopic lesions	77	55	147	24.13	< 0.0001	3.84	2.62- 5.62
Esophagitis	30	21.42	141	23.15	0.73	0.90	0.57- 1.41
Biliary reflux	48	34.28	218	35.79	0.76	0.93	0.63-1.37
Histologic findings							
Reactive gastropathy	34	24.28	154	25.28	0.91	0.94	0.61-1.45
Active/inactive gastritis	106	75.71	366	60.09	0.0005	2.07	1.36-3.14
GA/IMb	78	55.71	208	34.15	< 0.0001	2.42	1.67-3.52
H. pylori infection	47	33.57	220	36.12	0.62	0.89	0.60-1.31
Comorbidities							
Ulcer history	81	57.85	33	5.41	< 0.0001	23.96	14.75-38.94
Cerebrovascular disease	13	8.66	15	2.46	0.001	3.75	1.74-8.08
Renal disease	64	45.71	41	6.73	< 0.0001	11.67	7.36-18.47
Liver disease	80	57.14	213	34.97	< 0.0001	2.47	1.70-3.60
Respiratory disease	69	49.28	77	12.64	< 0.0001	6.71	4.46-10.10
Osteoarticular disease	61	43.57	155	25.45	< 0.0001	2.26	1.54-3.30
Symptoms							
Epigastric pain	61	43.57	367	60.26	0.0004	0.50	0.35-0.73
Heartburn	14	10	193	31.69	< 0.0001	0.23	0.13-0.42
Regurgitation	7	5	42	6.89	0.56	0.71	0.31-1.61
Nausea/vomiting	34	24.28	132	21.67	0.49	1.15	0.75-1.78
Social behaviours							
Alcohol consumptionc	58	41.42	186	30.54	0.0162	1.60	1.10-2.34
Smokingd	45	32.14	129	21.18	0.0076	1.76	1.17-2.64

* Obtained from Chi-square or Fisher's exact tests; [a] Low-dose aspirin; [b] Glandular atrophy/ Intestinal metaplasia; [c] Over 5 cigarettes/day; [d] More than 2 units/day, 1 unit = 10mL pure alcohol.
OR: odds ratio; CI: 95% confidence interval; NSAIDs: nonsteroidal anti-inflammatory drugs; PPI: proton pump inhibitors.

endoscopic lesions, in comparison with no-lesions group, but without statistical significance, while *H. pylori* infection was not associated with the severity of endoscopic lesions.

Discussion

Cardiovascular diseases (CV), including CHF, are the leading cause of morbidity and mortality worldwide. Physiologically, the gastrointestinal tract is one of the most intensely perfused organ, and mucosal gastric congestion is expected in CHF, due to the increase systemic venous pressure, which is transferred to the portal circulation through the hepatic venous bed [6,11]. Our results sustain that CHF by itself seems to be a factor influencing the severe gastro-duodenal endoscopic lesions, but not esophageal ones. Reactive gastropathy changes involve the congestion of superficial mucosal capillaries alongside with prominent mucin depletion, foveolar hyperplasia, and fibro-muscular replacement of the lamina propria. These changes appear after various type of aggressors, like alcohol consumption, biliary reflux and gastrotoxic drug consumption, having an impact on the balance of the gastric epithelium developing a constellation of mucosal changes [12]. In our study, the frequency of reactive gastropathy histologic changes were comparable in patients with and without CHF and were non-significant less frequent in patients with CHF and severe endoscopic lesions. Our results support the possible role of other aggressive factors (*H. pylori* infection, aging mucosa) on endoscopic lesions occurrence, not only the congestion in upper digestive tract.

Infection with *H. pylori* may be directly or indirectly involved in the pathogenesis of cardiovascular diseases. Altered iron metabolism is one of the leading mechanism of H. pylori, which can contribute to cardiovascular diseases [13]. In our study, *H. pylori* was not more frequent in patients diagnosed with congestive heart failure (p=0.62). In a geographical area with a high prevalence of *H. pylori* infection, the histologic changes of gastric mucosa in elderly patients are usually related to early acquisition of infection. In our research, the inflammatory and premalignant histological gastric changes were more common in patients with heart failure (OR = 2.42, 95% IC: 1.67–3.52) as they were older age than patients in the control group (69.75 ± 0.76 years old for cases vs. 54.10 ± 0.55 years old for controls), but these findings seemed to not influence the frequency of endoscopic lesions.

Table II: The distribution of studied variables in patients with CHF divided according to their endoscopic lesions

Variables	Severe endoscopic lesions group N=77 (55%)		No lesions group N=63 (45%)		p* value	OR	95% CI
	N	%	N	%			
Male gender	57	74.02	32	50.79	0.0051	2.76	1.35-5.61
Age >70	43	55.84	36	57.14	1.00	0.94	0.48-1.85
Anemia	40	51.94	28	44.44	0.39	1.35	0.69-2.63
Drug consumption							
Anticoagulants	41	53.24	24	38.09	0.08	1.85	0.93-3.64
NSAIDs	17	22.07	8	12.69	0.18	1.94	0.77-4.87
PPIs	48	62.33	35	55.55	0.48	1.32	0.67-2.60
LDAa	57	74.02	17	26.98	< 0.0001	7.71	3.62-16.40
Histologic findings							
Active/inactive gastritis	62	80.51	44	69.84	0.16	1.78	0.81-3.89
GA/IMb	45	58.44	33	52.38	0.49	1.27	0.65-2.50
Reactive gastropathy	15	19.48	19	30.15	0.16	0.56	0.25-1.22
H. pylori infection	29	37.66	18	28.57	0.28	1.51	0.73-3.08
Comorbidities							
Ulcer history	48	62.33	33	52.38	0.30	1.50	0.76-2.95
Cerebrovascular disease	10	12.98	3	90.47	0.14	2.98	0.78-11.36
Renal disease	35	45.45	29	46.03	1.00	0.97	0.50-1.90
Liver disease	48	62.33	32	50.79	0.17	1.60	0.81-3.15
Respiratory disease	42	54.54	27	42.85	0.17	1.60	0.81-3.13
Osteoarticular disease	34	44.15	27	42.85	1.00	1.05	0.53-2.06
Symptoms							
Epigastric pain	29	37.66	32	50.79	0.12	0.58	0.29-1.15
Heartburn	7	9.09	7	11.11	0.78	0.80	0.26-2.41
Social behaviours							
Alcohol consumptionc	37	48.05	21	33.33	0.08	1.85	0.92-3.68
Smokingd	29	37.66	16	25.39	0.14	1.77	0.85-3.68

* Obtained from Chi-square or Fisher's exact tests; [a] Low-dose aspirin; [b] Glandular atrophy/ Intestinal metaplasia; [c] Over 5 cigarettes/day; [d] More than 2 units/day, 1 unit = 10mL pure alcohol; OR: odds ratio; CI: 95% confidence interval; NSAIDs: nonsteroidal anti-inflammatory drugs; PPI: proton pump inhibitors.

In our present study, anemia appeared to be more frequent in patients with CHF, but not in those with endoscopic lesions. The approach of the underlying mechanism of anemia in patients with CHF is very difficult, in most cases, more than one etiology is involved. Such factors are bone marrow dysfunction, renal dysfunction, abnormal steroid metabolism, hemodilution, resistance to erythropoietin, the use of drugs for the treatment of CHF, chronic inflammation, hematinic deficiencies, decrease of food intake, reduction of intestinal absorption, and blood losses by the GIT [6, 7, 8, 9]. Among anemic CHF patients the most frequent form of hematinic deficiency, besides folate and vitamin B12 deficiency, is represented by iron deficiency [14, 15, 16]. An other important factor which may induce anemia is chronic gastrointestinal blood loss. Many patients with CHF use antithrombotic treatments, antiplatelet and/or anticoagulants. These drugs promote the blood loss by the entire GIT from various mucosal lesions [17, 18, 19, 20]. Furthermore, gastrointestinal conditions that do not usually induce bleeding, frequently associate iron deficiency anemia due to impairment of iron metabolism [21]. Our results support the important role of other combined mechanism except for bleeding from upper digestive endoscopic lesions in anemic patients with CHF that required a more complex approach.

The consumption of gastro-toxic drugs (NSAIDs, LDA), was less frequent in patients with CHF, while anticoagulants were more frequent, as the international therapeutic guideline recommends their use in treatment or secondary prevention of the underlying conditions. Among all questioned variable, the aggressive effect of antiplatelet therapy (LDA), in a vulnerable congestive gastric mucosa was supported by our present and past results, while the role of anticoagulants should be further investigated in larger studies [22]. It has been demonstrated that daily LDA consumption reduces the risk of cardiovascular diseases, however, it also associates adverse effects, mostly in the gastrointestinal tract. These complications can range from mild upper events (dyspepsia, petechiae, or erosions) to severe events (peptic ulcer disease and bleeding) [23,24]. Based on present observations, antiplatelet therapy should be cautioned in patients diagnosed with CHF, and gastroprotective therapy should be offered in high risk patients.

The presence of epigastric pain and heartburn were negatively associated with CHF, probably due to the selection of the cases: controls usually referred for symptoms, while cases for bleeding risk assessment. Different results were obtained in a study of 57 patients with congestive heart failure complaining from GI symptoms [5,11].

Our findings suggest that male gender was more frequently associated with CHF and severe lesions on endoscopy. In our previous research we obtained similar results regarding the severity of endoscopic lesions in patients consuming LDA [17]. Male gender presents cardiovascular diseases more commonly than females, due to hormonal differences [25]. On the other hand, they are more

frequently affected by duodenal ulcer in relationship with *H. pylori* infection [26]. This association should be further questioned in larger studies adjusted for the most important confounding factors (smoking, alcohol consumption) [27,28].

Patients suffering from CHF usually have at least one comorbidity and the severity of the heart failure leads to increasing numbers of comorbidities. The high number of comorbidities in our study was associated with the elderly population suffering from heart failure. Renal disease and anemia were the most common comorbidities found in a study conducted by van Deursen [29]. Similar results were found in our study, but the comorbidities did not appear to influence the severity of endoscopic lesions.

In this study, alcohol use was borderline correlated with endoscopic lesions, probably due to its additive aggressive effect on the gastrointestinal mucosa. Similar findings were described in a research that investigated bleeding in aspirin consumers [30]. Alcohol is considered to contribute to the development of cardiovascular diseases. Regular light alcohol drinking (< three drinks per day) may confer protective effects on heart failure associated with coronary heart disease. The protective effect disappears in heavy drinking (> three drinks per day) with an increases risks to develop cardiomyopathy, supraventricular arrhythmias, and systemic hypertension [31,32].

To the best of our knowledge this is the first study investigating histological and endoscopic upper digestive findings in a Romanian population with CHF. It's limitations are represented by the lack of regressions and adjustments based on confounding factors that will be approached in further studies. The present research questioning the impact of various demographical, clinical and histologic parameters on endoscopic lesions in patients with CHF may offer important clues for preventive strategy development in Romanian population, characterized by a high frequency of *H. pylori* infection and its consequences (ulcer, premalignant lesions, cancer).

Conclusions

Based on our findings, we can conclude that male patients and low-dose aspirin consumers with CHF, but not those with *H. pylori* infection seem to be more prone to develop upper digestive endoscopic lesions, while anticoagulants and alcohol consumption could be associated with severe endoscopic lesions in a congestive gastro-duodenal mucosa.

Authors' contribution

Adriana-Stela Cosma (Conceptualization; Data curation; Formal analysis; Writing –original draft)
Claudia Bănescu (Conceptualization; Data curation; Methodology; Supervision; Validation; Writing – review & editing)
Simona Mocan (Investigation; Validation)
Beáta Balla (Investigation; Writing – review & editing)
Anca Negovan (Conceptualization; Data curation; Investigation; Methodology; Supervision; Writing – review & editing)

Conflict of interest

None to declare.

References

1. Scott MC, Winters ME - Congestive Heart Failure. Emerg Med Clin North Am. 2015;33(3):553-62.
2. Ezekowitz JA, McAlister FA, Armstrong PW - Anemia is common in heart failure and is associated with poor outcomes: insights from a cohort of 12 065 patients with new-onset heart failure. Circulation. 2003;107(2):223-5.
3. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators - Disease and Injury Incidence and Prevalence Collaborators, Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016 GBD 2016. Lancet. 2017;390(10100):1211-1259.
4. Sundaram V, Fang JC - Gastrointestinal and Liver Issues in Heart Failure. Circulation. 2016;133(17):1696-703.
5. Zain EAS, Mohammad AG, Lobna AW, Elham AH, Khaled MA - Upper Gastrointestinal Mucosal Changes in Patients with Congestive Heart Failure. Med. J. Cairo Univ. 2013;81(1):1009-1014.
6. Romeiro FG, Okoshi K, Zornoff LA, Okoshi MP - Gastrointestinal changes associated to heart failure. Arq Bras Cardiol. 2012;98(3):273-7.
7. van Veldhuisen DJ, Anker SD, Ponikowski P, Macdougall IC - Anemia and iron deficiency in heart failure: mechanisms and therapeutic approaches. Nat Rev Cardiol. 2011;8(9):485-93.
8. Krack A, Sharma R, Figulla HR, Anker SD - The importance of the gastrointestinal system in the pathogenesis of heart failure. Eur Heart J. 2005;26(22):2368-74.
9. Shah R, Agarwal AK - Anemia associated with chronic heart failure: current concepts. Clin Interv Aging. 2013;8:111-22.
10. Lanza FL - Endoscopic Studies of Gastric and Duodenal Injury after the Use of Ibuprofen, Aspirin, and Other Nonsteroidal Anti-Inflammatory Agents. Am J Med. 1984;77(1A):19-24.
11. Raja K, Kochhar R, Sethy PK, Dutta U, Bali HK, Varma JS - An endoscopic study of upper-GI mucosal changes in patients with congestive heart failure. Gastrointest Endosc. 2004;60(6):887-93.
12. Sonnenberg A, Genta RM - Changes in the Gastric Mucosa with Aging. Clin Gastroenterol Hepatol. 2015;13(13):2276-81.
13. Jamkhande PG, Gattani SG, Farhat SA - Helicobacter pylori and cardiovascular complications: a mechanism based review on role of Helicobacter pylori in cardiovascular diseases. Integr Med Res. 2016;5(4):244–249.
14. Anand IS, Gupta P - Anemia and Iron Deficiency in Heart Failure: Current Concepts and Emerging Therapies. Circulation. 2018;138:80–98.
15. Arora NP, Ghali JK - Anemia and Iron Deficiency in Heart Failure. Heart Failure Clin. 2014;10(2):281-94.
16. Klip IT, Comin-Colet J, Voors AA, et al - Iron deficiency in chronic heart failure: an international pooled analysis. Am Heart J. 2013;165(4):575-582.e3.
17. Negovan A, Iancu M, Moldovan V, et al - The contribution of clinical and pathological predisposing factors to severe gastro-duodenal lesions in patients with long-term low-dose aspirin and proton pump inhibitor therapy. Eur J Intern Med. 2017;44:62-66.
18. Stein J, Connor S, Virgin G, Ong DE, Pereyra L - Anemia and iron deficiency in gastrointestinal and liver conditions. World J Gastroenterol. 2016;22(35):7908-25.
19. Negovan A, Iancu M, Moldovan V, Mocan S, Banescu C - The Interaction between GSTT1, GSTM1, and GSTP1 Ile105Val Gene Polymorphisms and Environmental Risk Factors in Premalignant Gastric Lesions Risk. Biomed Res Int. 2017;2017:7365080.
20. Negovan A, Iancu M, Moldovan V, et al - Influence of MDR1 C3435T, CYP2C19*2 and CYP2C19*3 gene polymorphisms and clinical characteristics on the severity of gastric lesions: a case-control study. J Gastrointestin Liver Dis. 2016;25(2):258-60.
21. Annibale B, Capurso G, Chistolini A, et al - Gastrointestinal causes of refractory iron deficiency anemia in patients without gastrointestinal symptoms. Am J Med. 2001;111(6):439-45.
22. Negovan A, Iancu M, Moldovan V,et al - Clinical Risk Factors for

Gastroduodenal Ulcer in Romanian Low-Dose Aspirin Consumers. Gastroenterol Res Pract. 2016;2016:7230626.

23. Valkhoff VE, Sturkenboom MC, Kuipers EJ - Risk factors for gastrointestinal bleeding associated with low-dose aspirin. Best Pract Res Clin Gastroenterol. 2012;26(2):125-40.
24. García-Rayado G, Sostres C, Lanas A - Aspirin and Omeprazole for Secondary Prevention of Cardiovascular Disease in Patients at Risk for Aspirin-associated Gastric Ulcers. Expert Rev Clin Pharmacol. 2017;10(8):875-888.
25. Jousilahti P, Vartiainen E, Tuomilehto J, Puska P - Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. Circulation. 1999;99(9):1165-72.
26. Shiotani A, Sakakibara T, Yamanaka Y, et al - Upper gastrointestinal ulcer in Japanese patients taking low-dose aspirin. Journal of Gastroenterology. 2009;44(2):126–31.
27. Wen L - Upper Gastrointestinal Complications and Cardiovascular/ Gastrointestinal Risk Calculator in Patients with Myocardial Infarction Treated with Aspirin. Chin Med J(Engl). 2017;130(16):1909-1913.
28. Nema H, Kato M, Katsurada T, et al - Endoscopic survey of low-dose-aspirin-induced gastroduodenal mucosal injuries in patients with ischemic heart disease. J Gastroenterol Hepatol. 2008;23 Suppl 2:S234-6.
29. van Deursen VM, Urso R, Laroche C, et al - Co-morbidities in patients with heart failure: an analysis of the European Heart Failure Pilot Survey. Eur J Heart Fail. 2014;16(1):103-11.
30. Serrano P, Lanas A, Arroyo MT, Ferreira IJ - Risk of upper gastrointesninal bleeding in patients taking low-dose aspirin for the prevention of cardiovascular diseases. Aliment Pharmacol Ther. 2002;16(11):1945-53.
31. Bardach AE, Caporale JE, Rubinstein AL, Danaei G - Impact of level and patterns of alcohol drinking on coronary heart disease and stroke burden in Argentina. PloS One. 2017;12(3):e017704.
32. Klatsky AL - Alcohol and cardiovascular diseases. Expert Rev Cardiovasc Ther. 2009;7(5):499-506.

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RESEARCH ARTICLE

The Influence of GPX1 Pro198Leu, CAT C262T and MnSOD Ala16Val Gene Polymorphisms on Susceptibility for Non-Hodgkin Lymphoma and Overall Survival Rate at Five Years from Diagnosis

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Objective: The aim of the current study was to investigate possible associations between catalase C262T (*CAT* C262T), glutathione peroxidase 1 Pro198Leu (*GPX1* Pro198Leu), manganese superoxide dismutase Ala16Val (*MnSOD* Ala16Val) gene polymorphisms and non-Hodgkin Lymphoma risk (NHL) in a Romanian population and the five-year overall survival rate of the NHL patients. **Methods**: We included in this case-control study 406 individuals, divided into two groups: the control group (n=315) and the patients group (n=91). The DNA was extracted from peripheral blood and amplified using specific techniques. **Results**: The variant homozygous genotype of *GPX1* Pro198Leu represents a risk factor for NHL development and no associations regarding the risk for NHL were found for *MnSOD* Ala16Val and *CAT* C262T gene polymorphisms. Two of the studied polymorphisms were associated with the overall survival rate thus: negative association regarding *MnSOD* Ala16Val, associated with higher overall survival rate and a positive one regarding *CAT* C262T, associated with lower overall survival rate. **Conclusions**: According to our results, the mentioned polymorphisms may be considered as susceptible markers of the five-year overall survival rate for NHL patients. Future studies with a larger number of patients are needed to confirm our results.

Keywords: NHL, GPX1, CAT, MnSOD polymorphism

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Introduction

Non-Hodgkin Lymphoma (NHL) is a heterogeneous type of blood cancer characterized, like all malignancies, by an abnormal cellular proliferation [1]. NHL represents the most common type of lymphomas (90%) and may be classified into two groups, mature B-cell neoplasms and mature T-cell and natural killer (NK)-cell neoplasms [1,2]. One study conducted by Perry et al. demonstrated that the frequency of NHL subtypes is significantly different in some geographical regions. Also, the median age of patients with low-grade and high-grade of NHL in North America is significantly higher than in the Far East [3]. These findings suggest that differences between some etiological aspects and host risk factors are likely responsible, but for a better understanding of these differences, more epidemiological studies are needed [3,4].

In Romania or Eastern and Central Europe, only a few studies are being involved in analyzing new risk factors for NHL and even fewer studies are addressing the role and the risk of different genetic variants [5,6]. The distribution of NHL subtypes in South-eastern Europe reported by Dotlic et al. in 2015 was: 91.1% of B-cell lymphomas and 8.9% of NK-cell lymphomas [7].

Chronic inflammation is a major risk factor which was reported to be connected to NHL, although the mechanism underlying this connection is still ambiguous [8]. An aspect that could sustain this matter is the fact that neoplasia is known to be in some cases induced by oxidative stress caused by inflammation**,** which can generate reactive oxygen species (ROS) that lead to oxidative DNA damage [9]. In addition, ROS propagate pro-inflammatory cytokines, including interleukin-1 which stimulates B cells to produce antibodies that trigger a signal for B-cell activation [10].

Furthermore, a number of studies highlighted that several diseases such as arthritis, systemic lupus erythematosus, and Sjögren's syndrome, characterized all by a chronic B-cell activated phenotype, have been associated with an increased risk of developing NHL [11,12,13].

ROS can also be inactivated by anti-oxidant enzymes such as catalase (*CAT*), glutathione peroxidase (*GPX*) and manganese superoxide dismutase (*MnSOD*) which protect the cells from the negative effects of oxidative stress [10,14].

According to SNPedia [15] and NCBI [16], there is a consistent number of single nucleotide polymorphisms (SNPs) of the human catalase gene. In the last few years, there has been a significant number of case-control and meta-analysis studies [17,18] focusing on the *CAT* C262T polymorphism (substitution of Cytosine with Thymine in the 262 position of the promotor) involved in the progression of neoplasia, but more studies regarding NHL are still needed [10,19].

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GPX1 is an additional antioxidant enzyme involved in the cell protection mechanisms against the ROS effects, reducing lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water. A specific polymorphism caused by the substitution of Proline (Pro) with Leucine (Leu) at position 198 (*GPX1* Pro198Leu, rs1050450), has previously been reported to be associated with decreased enzyme activity and increased risk for developing cancer [20].

Manganese superoxide dismutase (*MnSOD*), another antioxidant enzyme, protects the cells against oxidative stress. The superoxide radicals are eliminated after their conversion to water and oxygen [18]. *MnSOD* Ala16Val is one of the few polymorphisms previously investigated in NHL patients [10, 21] and correlated with enzyme activity and cancer risk [18, 20, 22].

To the best of our knowledge, none of the published studies have evaluated *CAT*, *GPX1* and *MnSOD* gene polymorphisms in patients with NHL in Central or Eastern Europe.

The aim of the current study was to investigate possible associations between *CAT* C262T, *GPX1* Pro198Leu, *MnSOD* Ala16Val gene polymorphisms and NHL risk in a Romanian population and their influence on the overall survival rate of NHL diagnosed patients.

Method

Patients and Controls

We conducted a population based case-control study, which consisted of 406 individuals from the same geographical area with a similar ethnical background (central region of Romania), divided into two groups: the control and the patients group. The patients group consisted of 91 adults with a confirmed diagnosis of NHL, admitted to the Hematology Clinics, of Emergency Clinical County Hospital of Târgu Mureş, Romania, between 2010 and 2016. Our control group included 315 healthy participants with no hematological malignancies and no history of cancer or other chronic diseases.

The approval of the present study was obtained from the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureş and was conducted according to the principles of the Declaration of Helsinki.

Clinical and histological diagnosis of NHL has been made according to the WHO standards [2], with the following distribution of NHL subtypes: 35 patients with Diffuse large B-cell lymphoma (DLBCL), 16 patients with Follicular lymphoma (FL), 16 patients with Marginal zone B-cell lymphoma (MBZL), 10 patients with Primary Non-Hodgkin Lymphoma (Pl), 7 with T-Cell Lymphoma, 2 with Mucosa-associated lymphoid tissue lymphomas (MALT), and 5 other types.

Clinical and laboratory data, such as histopathological classification of the NHL, the Eastern Cooperative Oncology Group Scale of Performance Status (ECOG), International Prognostic Index (IPI), Ann Arbor stage, treatment response, five-year survival rate, Lactate dehydrogenase (LDH) levels and extranodal sites were collected and analyzed from the patients' medical records.

Genotyping Procedures

Two milliliters of fresh peripheral blood were collected from patients and controls, at the time of their routine blood collection, in sterile tubes with Ethylenediaminetetraacetic acid (EDTA), for DNA extraction. DNA was isolated using the Quick-gDNA MiniPrep kits (ZymoResearch, USA) and Wizard Genomic DNA Purification kits (Promega, Madison, WI, USA) according to the manufacturer´s instructions.

The DNA absorbance was verified by spectrophotometric quantification (BioSpectometer, Eppendorf, Germany). In order to analyze the genotypes of the mentioned SNPs, RFLP-PCR technique was performed using specific primers and FastDigest restriction enzymes, as previously reported [18, 23, 24].

Statistical Analysis

Data analysis was performed using GraphPad InStat software, MedCalc. Chi-square test and Fisher's exact test in order to compare the distribution of qualitative variables between cases and controls. Associations between genotypes, combined variant genotypes (homozygous and heterozygous), allele distribution among NHL patients and control groups were calculated as odds ratios (OR), recommended in case-control studies, with 95% confidence intervals (CI) and a significance level of 0.05.

Results

The distribution of allele's frequency and genotypes of the *CAT* C262T, *GPX1* Pro198Leu, and *MnSOD* Ala16Val among NHL patients and controls are shown in Table I.

No significant association was found between the presence of variant alleles of *CAT* C262T and *MnSOD* Ala16Val SNPs and NHL risk, but the variant homozygous genotype of *GPX1* Pro198Leu represents a risk factor for NHL development (p value = 0.04).

Although the number of cases analyzed is relatively low, we have studied the combined variant genotypes. The results for the combined *CAT* C262T, *GPX1* Pro198Leu and *MnSOD* Ala16Val genotypes and alleles among NHL patients and controls, are presented in Table II. In this case, no significant differences were observed between the presences of variant genotypes or variant alleles for all three investigated SNPs and NHL risk.

Furthermore, we evaluated the effect of these SNPs in detail, taking into account different risk factors such as gender, age, histological subtype of the lymphoma, survival rate, treatment response, LDH levels, and extranodal sites. Table III summarizes the clinical characteristics of NHL patients according to the genotypes. No differences were found regarding elevated LDH levels and variant genotypes/ alleles for the investigated SNPs (p value>0.05).

Table I. Distribution of CAT C262T, GPX1 Pro198Leu and MnSOD Ala16Val genotypes and alleles among NHL patients and controls

	NHL Patients	Controls	p value	OR	95%CI
CAT C262T					
CC	54	165	Reference		
CT	32	130	0.26	0.75	0.45-1.23
TT	5	20	0.80	0.76	0.27-2.13
CC+CT	37	150	0.28	0.75	0.46-1.21
Allele					
C	140	460	Reference		
T	42	170	0.33	0.81	0.55-1.19
GPX1 Pro198Leu					
Pro/Pro	2	26	Reference		
Pro/Leu	59	205	0.08	3.74	0.86-16.23
Leu/Leu	30	84	0.04	4.64	1.03-20.76
Pro/Leu+Leu/Leu	89	289	0.05	4.00	0.93-17.20
Allele					
Pro	63	257	Reference		
Leu	119	373	0.14	1.30	0.92-1.83
MnSOD Ala16Val					
Ala/Ala	7	33	Reference		
Ala/Val	64	208	0.54	1.45	0.61-3.43
Val/Val	20	74	0.81	1.27	0.49-3.3
Ala/Val+Val/Val	84	282	0.55	1.4	0.59-3.29
Allele					
Ala	78	274	Reference		
Val	104	356	0.93	1.02	0.73-1.43

Table II. Distribution of combined CAT C262T, GPX1 Pro198Leu and MnSOD Ala16Val genotypes and alleles among NHL patients and controls

	NHL Patients	Controls	p value	OR	95%CI
Combined genotypes					
CC+Pro/Pro+Ala/Ala	1	5	Reference		
CT+Pro/Leu+Ala/Val	12	83	0.57	0.72	0.07-6.73
TT+Leu/Leu+Val/Val	1	1	0.46	5	0.14-166.73
CT or TT+ Pro/Leu or Leu/Leu+ Ala/Val or Val/Val	37	128	1	1.44	0.16-12.76
Combined alleles					
C+Pro+Ala	12	68	Reference		
T+Leu+Val	14	58	0.52	1.36	0.586-3.191

In addition, we investigated the impact of these SNPs by the clinical manifestations such as Ann Arbor stage, ECOG or IPI. No associations were found between the presumed risk groups and variant genotypes of *CAT* C262T, *GPX1* Pro198Leu, and *MnSOD* Ala16Val gene polymorphisms (Table III).

Regarding the influence of allele's and genotypes in treatment response, no differences were noticed between NHL patients outcome and the investigated SNPs (p value >0.05) (Table III).

Moreover, we performed a comparison between different variant (heterozygous and homozygous) genotypes of *CAT* C262T, *GPX1* Pro198Leu, and *MnSOD* Ala16Val, and the survival rate of our patients. We found that two of the studied polymorphisms were associated with the survival rate thus: negative association regarding *MnSOD* Ala16Val, associated with a higher survival rate (p value = 0.031, OR = 0.15, CI 95% 0.028-0.078) and a positive association regarding *CAT* C262T, associated with a lower survival rate (p value = 0.025, OR = 3.763, CI 95% 1.208-11.721). The results for both heterozygous genotypes of *MnSOD* and *CAT* are statistically significant. While analyzing in detail, by allele frequency, both *MnSOD* 16Ala variant allele (p value = 0.33, OR = 0.73, CI95% 0.4-1.3) and *CAT* 262C variant allele (p value = 0.27, OR = 1.435, CI 95% 0.76-2.67) showed no association. The study was based on a relatively low number of carriers of the variant homozygous genotype, a fact that may have led to a non-statistically significant results in those cases. However, according to combined variant genotypes (heterozygous and homozygous- Dominant model), the results remained statistically significant, but only for *CAT* C262T polymorphism.

Life expectancy of NHL cases according to the genotypes is graphically represented in Figure 1. The five year overall survival rate is calculated and described with Kaplan Meier curves, and is estimated to be: *CAT* C262T (p value = 0.017) CC – 80%, CT – 43%, TT – 100%, *GPX1* Pro198Leu (p value = 0.704) Leu/Leu - 82%, Pro/Leu – 60%, Pro/Pro – 100%, *MnSOD* Ala16Val (p value < 0.0001) Ala/Ala 43% Val/Ala 70%, Val/Val 75%.

No differences were found regarding clinical and demographical characteristics of patients and *CAT*, *GPX1* and *MnSOD* variant genotypes (p value >0.05) (Table III).

Discussions

Important advances in the understanding of the molecular pathogenesis of malignant lymphomas have been made in the last few years. Several unforeseen compounds referring to the oncogenic signaling courses are studied in different trials in patients diagnosed with lymphoma [25]. The evidence towards the oxidative stress involvement as an important contributor to cancer, producing inflammation and DNA damage, is still standing [22].

Diminished *MnSOD* activity has been proved to be found in lymphoma tumors, as its increased activity induces apoptosis and suppresses tumorigenesis [22]. Furthermore, it has been showed that *MnSOD2* Ala16Val, *GPX1* Pro198Leu, and *CAT* C262T SNPs decrease the enzymatic activity, and by inducing DNA damage, they also produce a high risk for developing cancer [26,27].

In the present study, we investigated the *CAT* C262T, *GPX1* Pro198Leu and *MnSOD2* Ala16Val SNPs on NHL patients in order to evaluate the associations between the mentioned SNPs and NHL risk, clinical characteristics, treatment response and overall survival rate in a Romanian population, from the country's central region.

The *CAT* C262T polymorphism has been intensively studied because the allelic T variant is associated with a decreased enzyme activity [28], resulting in a high level of ROS which maintains the possibility of developing some types of cancer.

In a recent meta-analysis [17] of several studies regarding different cancer types, including hematological ones, it

Table III. Patients characteristics according to the CAT, GPX1 and MnSOD genotypes

	CAT C262T					GPX1 Pro198Leu					MnSOD Ala16Val				
Genotype / p value	**CC**	**CT**	**p**	**TT**	**p**	**Pro/Pro**	**Pro/Leu**	**p**	**Leu/Leu**	**p**	**Ala/Ala**	**Ala/Val**	**p**	**Val/Val**	**p**
No. patients	54	32		5		2	59		30		7	64		20	
Sex (men/women)	22/32	13/19	p>0.5*	2/3	p>0.5*	2/0	26/33	p>0.5*	9/21	p>0.5*	5/2	23/41	p>0.5*	9/11	p>0.5*
Age, median	58	68	p>0.5*	63	p>0.5*	59.5	58	p>0.5*	66.5	p>0.5*	70	60.5	p>0.5*	52	p>0.5*
Ann Arbor Stage															
Ann Arbor Stage 1,2 number of patients (%)	32(59.25)	18(56.25)	Ref.	2(40.00)	Ref.	1(50.00)	32(54.23)	Ref.	19(63.33)	Ref.	2(28.57)	38(59.37)	Ref.	12(60.00)	Ref.
Ann Arbor Stage 3,4 number of patients (%)	22(40.74)	14(43.75)	0.82	3(60.00)	1	1(50.00)	27(45.76)	1	11(36.66)	1	5(71.42)	26(40.62)	0.22	8(40.00)	0.208
LDH															
LDH < 280 UI number of patients (%)	26(48.14)	15(46.83)	Ref.	2(40.00)	Ref.	1(50.00)	26(44.06)	Ref.	16(53.33)	Ref.	5(71.42)	30(46.87)	Ref.	8(40.00)	Ref.
LDH > 280 UI number of patients (%)	28(51.85)	17(53.12)	1	3(60.00)	1	1(50)	33(55.93)	1	14(46.66)	1	2(28.57)	34(53.12)	0.26	12(60.00)	0.208
ECOG															
1.2 number of patients (%)	38(70.37)	21(65.62)	Ref.	2(40.00)	Ref.	1(50.00)	40(67.79)	Ref.	20(66.66)	Ref.	3(42.85)	44(68.75)	Ref.	14(70.00)	Ref.
3.4 number of patients (%)	16(29.62)	11(34.37)	0.81	3(60.00)	0.315	1(50.00)	19(32.20)	1	10(33.33)	1	4(57.14)	20(31.25)	0.216	6(30.00)	0.364
IPI															
0,1,2 number of patients (%)	26(48.14)	18(56.25)	Ref.	3(60.00)	Ref.	1(50.00)	31(52.54)	Ref.	15(50.00)	Ref.	2(28.57)	34(53.12)	Ref.	11(55.00)	Ref.
3,4,5 number of patients (%)	28(51.85)	14(43.75)	0.509	2(40.00)	0.670	1(50.00)	28(47.45)	1	15(50.00)	1	5(71.42)	30(46.87)	0.260	9(45.00)	0.384
Treatment response															
Resistant number of patients (%)	18(39.13)	18(58.06)	0.215	3(60.00)	0.532	1(50.00)	25(47.16)	1	13(48.14)	1	3(60.00)	31(51.66)	0.550	5(29.41)	1
Partial remission number of patients (%)	18(39.13)	9(29.03)	1	2(40.00)	0.540	1(50.00)	20(37.73)	1	8(29.62)	1	2(40.00)	16(26.66)	0.496	11(64.70)	1
Complete remission number of patients (%)	10(21.73)	4(12.90)	Ref.	0(0.00)	Ref.	0(0.00)	8(15.09)	Ref.	6(22.22)	Ref.	0(0.00)	13(21.66)	Ref.	1(5.88)	Ref.
Overall survival															
Survival number of patients (%)	39(86.66)	19(63.33)	Ref.	5(100.00)	Ref.	1(50.00)	40(75.47)	Ref.	22(84.61)	Ref.	3(42.85)	45(83.33)	Ref.	15(78.94)	Ref.
Death number of patients (%)	6(13.33)	11(36.66)	0.025	0(0.00)	1	1(50.00)	13(24.52)	1	4(15.38)	1	4(57.14)	9(16.66)	0.031	4(21.05)	0.149

*calculated for both, men and women; LDH- Lactate dehydrogenase; ECOG- the Eastern Cooperative Oncology Group Scale of Performance Status; IPI- International Prognostic Index

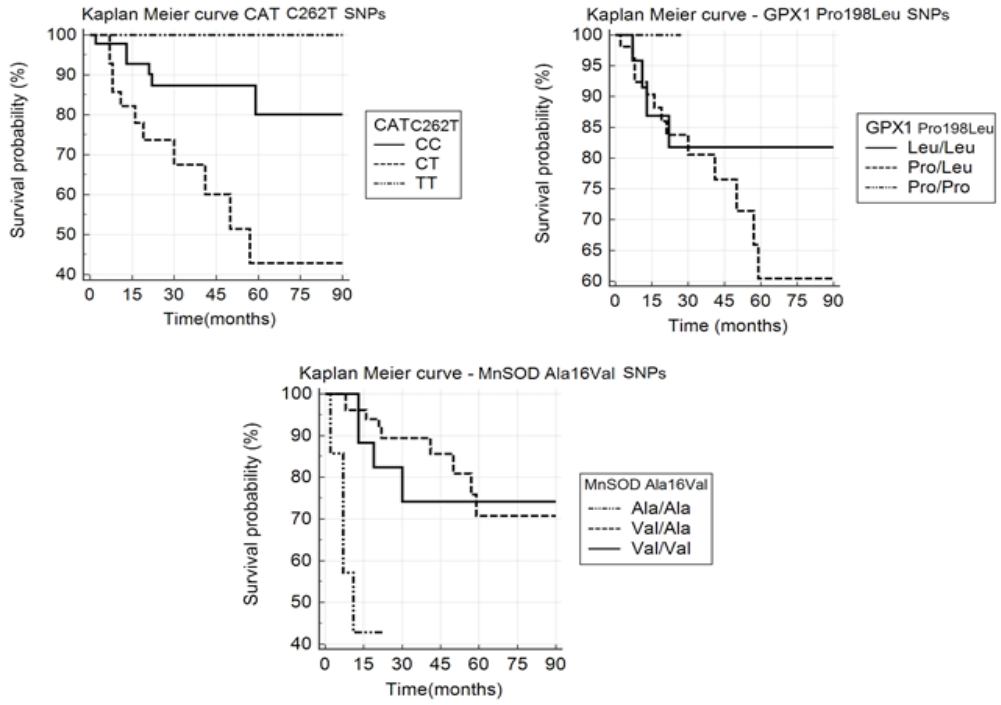

Fig. 1. Kaplan Meier survival curves of NHL patients according to the investigated CAT, GPX1 and MnSOD **gene polymorphisms**

was suggested that the *CAT* C262T polymorphism may be associated with cancer susceptibility, and even more, may be considered a potential tumor biomarker. Nevertheless, the *CAT* C262T polymorphism is not considered an accurate prognostic factor for cancer survival rate [17].

According to our results, the *CAT* C262T is not a risk factor for NHL development, but it was associated with lower overall survival rate in dominant model. However, the results should be interpreted with caution, due to the relatively small number of patients.

Additionally, well designed, multicenter studies are required in order to investigate the role of this functional polymorphism, which should lead to a better and more comprehensive interpretation of the associations between the *CAT* C262T polymorphism and NHL risk and the overall survival rate, preferable on a wider population.

Paz-y-Mino et al. revealed in their study that the association of *GPX1* Pro198Leu variant genotype with a decreased enzyme activity results in an increased risk of developing cancer in Caucasians [22]. Glutathione peroxidase has established its significant role as an antioxidant enzyme catalyzing the detoxification of hydrogen peroxide and being one of the most important enzymes discovered in humans [29].

In our study, we found an association between the variant homozygous genotype of *GPX1* Pro198Leu and the susceptibility for NHL, the mentioned SNP being a risk factor for NHL developing. We found no statistically significant differences between clinical parameters, treatment response or survival rate and the *GPX1* Pro198Leu polymorphism.

We found no differences in the distribution of the genotypes and alleles of *MnSOD2* Val16Ala among the two groups, but we highlighted a negative association of the heterozygous genotype and the survival rate, suggesting that the presence of this genotype may represent a protective factor, being associated with higher overall survival rate.

To our current knowledge, nowadays there is still no evidence supporting the role of these SNPs referring to the clinical characteristics as well as the five-year overall survival rate in the same treatment conditions and counting out other causes of death. Contrary to our findings, there are studies describing a significant association between *MnSOD* Val6Ala polymorphism and the risk of developing DLB-NHL [21]. A meta-analysis performed by Kang et al. [30], showed an association between the *MnSOD* Val16Ala and the NHL susceptibility and other types of cancer. In the current study, we discovered no association referring to this gene polymorphism and NHL subtypes (p value >0.05).

We found a similarity between the results of our analysis and a pooled analysis that investigated 2293 NHL cases and 3432 controls (from UK and USA) which found no association of this variant with the overall risk of developing NHL, but an association of the variant homozygous genotype with a decreased MZL risk was uncovered [10].

Superoxide dismutase has still an unclear role as its deficient activity has been highlighted in malignant lymphomas [31]. In some animal-based studies, it has been proved that a reduced *MnSOD* activity leads to DNA damage and increased cancer incidence [32]. For that manner, further studies are needed in order to clarify the role of this gene.

The limitations of our study include the relatively small cohort of lymphoma patients and its retrospective design. The described impact of two additional polymorphisms in the manganese superoxidase dismutase and catalase gene on the overall survival in lymphoma is limited by the heterogeneous group of lymphoma diagnoses. Another limita-

tion is represented by the lack of enzymatic level of catalase, glutathione peroxidase, superoxide dismutase.

Conclusions

In conclusion, the present study provides an evidence that the *GPX1* Pro198Leu gene polymorphism might be a risk factor for NHL in a Romanian population and that there is a possible association between *CAT* C262T gene polymorphism and a low five-year overall survival rate in NHL patients. Furthermore, *CAT* C262T and *MnSOD* Val16Ala gene polymorphisms appear not to be risk factors for the development of NHL.

Authors' contribution

Adriana Stela Cosma (Conceptualization; Methodology; Project administration)
Cristina Radu, Alexandra Moldovan (Data curation)
Alina Bogliș (Formal analysis)
George Andrei Crauciuc (Formal analysis)
Emőke Horváth (Data curation; Formal analysis; Validation)
Marcela Cândea (Data curation; Formal analysis; Validation)
Florin Tripon (Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Validation; Writing – review & editing)

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Shankland KR, Armitage JO, Hancock BW. Non-Hodgkin lymphoma. Lancet. 2012; 380(9844):848-857.
2. Armitage JO, Gascoyne RD, Lunning MA, Cavalli F. Non-Hodgkin lymphoma. Lancet. 2017; 390(10091):298-310.
3. Perry AM, Diebold J, Nathwani BN et al. Non-Hodgkin lymphoma in the Far East: review of 730 cases from the International non-Hodgkin Lymphoma Classification Project. Ann Hematol. 2016; 95(2):245-251.
4. Perry AM, Diebold J, Nathwani BN et al. Non-Hodgkin Lymphoma In The Developing World: Review Of 4539 Cases From The International Non-Hodgkin Lymphoma Classification Project. Haematologica. 2016; 101(10):1244-1250.
5. Bogliș A, Radu CG, Tripon F et al. XRCC1 Arg194Trp and Arg399Gln Polymorphisms and Risk of Non-Hodgkin Lymphoma in a Romanian Population. Rev Med Chir Soc Med Nat Iasi. 2016; 120(3):644-650.
6. Bogliș A, Crauciuc AG, Tripon F et al. No association between GSTT1, GSTM1 and GSTP1 gene polymorphism and risk of non-Hodgkin lymphoma in a population from Romania. International Journal of Innovation and Applied Studies. 2017; 19(1):1-8.
7. Dotlic S, Perry AM, Petrusevska G et al. Classification of non-Hodgkin lymphoma in South-eastern Europe: review of 632 cases from the international non-Hodgkin classification project. Br J Haematol. 2015; 171(3):366-372.
8. Moriya K, Tamura H, Nakamura K, Hosone M, Inokuchi K. A primary esophageal MALT lymphoma patient with Helicobacter pylori infection achieved complete remission after H. pylori eradication without anti-lymphoma treatment. Leuk Res Rep. 2017; 7:2-5.
9. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radic Biol Med. 2010; 49 (11):1603-1616.
10. Lightfoot TJ, Skibola CF, Smith AG et al. Polymorphisms in the oxidative stress genes, superoxide dismutase, glutathione peroxidase and catalase and risk of non-Hodgkin's lymphoma. Haematologica. 2006; 91(9):1222-1227.
11. Baecklund E, Iliadou A, Askling J et al. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. Arthritis Rheum. 2006; 54(3):692-701.
12. Callan MF. Epstein-Barr virus, arthritis, and the development of lymphoma in arthritis patients. Curr Opin Rheumatol. 2004; 16(4):399-405.
13. Ramos-Casals M, De Vita S, Tzioufas AG. Hepatitis C virus, Sjogren's syndrome and B-cell lymphoma: linking infection, autoimmunity and cancer. Autoimmun Rev. 2005; 4(1):8-15.
14. Laurent A, Nicco C, Chéreau C et al. Controlling tumor growth by modulating endogenous production of reactive oxygen species. Cancer Res. 2005; 65(3):948–956.
15. SNPedia database. Catalase gene. Avaiable at https://www.snpedia.com/index.php?title=Special%3ASearch&search=catalase&fulltext=1. Last time accessed on 27 January, 2019.
16. NCBI. Catalase gene. Available from http://ncbi.nlm.nih.gov/gene847. Last time accessed on 27 January, 2019.
17. Wang CD, Sun Y, Chen N et al. The Role of Catalase C262T Gene Polymorphism in the Susceptibility and Survival of Cancers. Sci Rep. 2016; 6:26973.
18. Bănescu C, Iancu M, Trifa AP et al. From Six Gene Polymorphisms of the Antioxidant System, Only GPX Pro198Leu and GSTP1 Ile105Val Modulate the Risk of Acute Myeloid Leukemia. Oxid Med Cell Longev. 2016; 6:1-10.
19. Farawela H, Khorshied M, Shaheen I et al. The association between hepatitis C virus infection, genetic polymorphisms of oxidative stress genes and B-cell non-Hodgkin's lymphoma risk in Egypt. Infect Genet Evol. 2012; 12(6):1189–1194.
20. Ekoue DN, Bera S, Ansong E et al. Allelic variations in MnSOD and GPx-1 affect metabolism, mitochondrial membrane potential and expression of signaling proteins. Proceedings of the AACR 107th Annual Meeting 2016; April 16-20; New Orleans, LA: Cancer Res; 2016; 76(14).
21. Wang SS, David S, Cerhan JR et al. Polymorphisms in oxidative stress genes and risk for non-Hodgkin lymphoma. Carcinogenesis. 2006; 27(9):1828-1834.
22. Paz-y-Miño C, Muñoz MJ, López-Cortés A et al. Frequency of polymorphisms Pro198Leu in GPX-1 gene and Ile58Thr in MnSOD gene in the altitude Ecuadorian population with bladder cancer. Oncol Res. 2010; 18(8):395–400.
23. Bănescu C, Trifa AP, Voidăzan S et al. CAT, GPX1, MnSOD, GSTM1, GSTT1, and GSTP1 genetic polymorphisms in chronic myeloid leukemia: a case-control study. Oxid Med Cell Longev. 2014; 2014: 875861.
24. Negovan A, Iancu M, Tripon F, Crauciuc A, Mocan S, Bănescu C. The CAT-262 C>T, MnSOD Ala16Val, GPX1 Pro198Leu Polymorphisms Related to Oxidative Stress and the Presence of Gastric Lesions. J Gastrointestin Liver Dis. 2018; 27(4):371-378.
25. Nogai H, Dörken B, Lenz G. Pathogenesis of Non-Hodgkin's Lymphoma. J Clin Oncol. 2011; 29(14):1803-1811.
26. Al-Alem U, Gann PH, Dahl J et al. Associations between functional polymorphisms in antioxidant defense genes and urinary oxidative stress biomarkers in healthy, premenopausal women. Genes Nutr. 2012; 7(2):191–195.
27. Yuzhalin AE, Kutikhin AG. Inherited variations in the SOD and GPX gene families and cancer risk. Free Radic Res. 2012; 46(5):581–599.
28. Ahn J, Nowell S, McCann SE et al. Associations between catalase phenotype and genotype: modification by epidemiologic factors. Cancer Epidemiol Biomarkers Prev. 2006; 15(6):1217-1222.
29. Skibola CF, Curry JD, Nieters A. Genetic Susceptibility to Lymphoma. Haematologica. 2007; 92(7):960-969.
30. Kang SW. Superoxide dismutase 2 gene and cancer risk: evidence from an updated meta-analysis. Int J Clin Exp Med. 2015; 8(9):14647-14655.
31. Bewick M, Coutie W, Tudhope GR. Superoxide dismutase, glutathione peroxidase and catalase in the red cells of patients with malignant lymphoma. Br J Haematol. 1987; 65(3):347–350.
32. Van Remmen H, Ikeno Y, Hamilton M et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiol Genomics. 2003; 16(1):29-37.

Acta Medica Marisiensis 2019;65(1):31

Errata

Table I (Performance of the 2 tests) from the research article „Effect of Food on the Pharmacokinetics of Gliclazide 60 mg Modified Release Tablet in Healthy Caucasian" published by Diana Pop et al. in Acta Medica Marisiensis 2018;64(4):163, was included in the text due to technical errors and should be removed, being part of another article published in the same issue.

Erratum for the article Optimization of a Density Gradient Centrifugation Protocol for Isolation of Peripheral Blood Mononuclear Cells published by Georgiana Mihaela Şerban et al. (DOI: 10.2478/amma-2018-0011) in Acta Medica Marisiensis 2018, Volume 64, Number 2

Correct header of Table II and legend of Figure 3 are listed below.
Figure 3 – column bar graph representing average variation of recovery rates with sample volume for a) ST; b) MB; d) HY. Average platelet removal is compared between MB and c) ST and e) HY. Sub-figure f) shows average viability for cell populations as given by HY protocol

Table II. Average numerical values from Phase-1

		PBMC recovery (%)			PLT removal (%)			PBMC purity* (%)		
		1ml	2ml	3ml	1ml	2ml	3ml	1ml	2ml	3ml
ST	E1	55.8	79.7	85.9	81.1	76.0	72.8	95.1	98.6	97.4
	E2	55.5	79.7	87.9	83.5	70.6	67.4	98.0	98.2	96.4
	Avg.	55.7	79.7	86.9	82.3	73.3	70.1	96.6	98.4	96.9
MB	E1	40.3	52.8	51.8	96.7	97.2	97.1	97.2	97.5	98.3
	E2	37.5	55.2	34.2	96.8	96.3	96.7	93.1	94.7	94.0
	Avg.	38.9	54.0	43.0	96.7	96.8	96.9	95.2	96.1	96.2

E1 – examiner no.1; E2 – examiner no.2; Avg. – average value; *PBMC purity accounts only for granulocyte/erythrocyte contamination (PLTs are treated separately)

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2. Georgiana Mihaela Șerban and Ion Bogdan Mănescu equally contributed to this work.

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