GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

TWO VIRUSES – CROSSING THE MILLENNIUM

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Rezumat

Teza de abilitare „**Two viruses – crossing the millennium”** își propune sintetizarea, conform indicațiilor CNATDCU, în 3 secțiuni, a direcțiile de dezvoltare profesională științifică și de cercetare pe care le-am exploatat până în momentul depunerii acesteia, proiectele de viitor și referințele bibliografice selectate din vasta literatură de specialitate.

Secțiunea I trece în revistă și face referire la două mari teme de cercetare pe care le-am avut în vedere de-a lungul întregii cariere. Este vorba de infecția HIV, care s-a constituit de-a lungul istoriei într-o veritabilă pandemie, cu dezvoltarea în timp a direcțiilor de cercetare privind clinica, cazurile deosebite prin gravitatea lor și intensitatea simptomatologiei, precum și aspectele psiho-emoționale frecvent legate de ceea ce am numit evoluția infectei HIV în „cohorta pediatrică”, a unor eșantioane de populație HIV infectate - adolescenții, etc.

Din nefericire pentru România, dar din fericire pentru devenirea mea profesională și înțelegerea fenomenului HIV, am cunoscut, evaluat și tratat pacienții din așa numita „cohortă pediatrică”, copii născuți la sfârșitul anilor 80 și începutul anilor 90 care și-au relevat statusul de HIV pozitiv în primii ani de viață. Monitorizarea și evaluarea psiho-emoțională a lor și a familiilor lor încă din perioada copilăriei, până la nivelul adultului ce își dorește integrare socio-profesională și o viață normală ca adult, cu aspirații spre familie și procreere, ne-a făcut să ajungem la un profund grad de înțelegere a fenomenului HIV în lume și în regiunea de nord-est a României.

Activitatea clinică și de cercetare s-au împletit în ideea extragerii unor concluzii pertinente și fructuoase pentru practician și pentru viitorii practicieni, a unor analize de caz în această patologie complexă, alături de suport psiho-emoțional din partea psihologului. Datorită terapiei HAART, infecția HIV s-a transformat dintr-o infecție acută cu o speranță de viață de câteva luni, într-o infecție cronică, pacientul seropozitiv având o speranță de viață egală cu cea a populației generale. Singura condiție este aderența și complianța la tratament, care se poate manifesta fluctuant de-a lungul istoriei personale a unui pacient infectat HIV, cu amprentă asupra statusului imuno-virusologic al acestuia, ce îl predispune la infecții oportuniste, carcinogenezeă sau altele.

Infecția HIV este o problemă globală, care trebuie abordată multidisciplinar, nu numai medical, dar și din punct de vedere al implicațiilor sociale majore, economice și psiho-emoționale, atât pentru individ, cât și pentru societate. Reacția de la individual la macrosocial față de această infecție, în termeni de stigmatizare, poate fi indicatorul unei sănătăți spirituale și a toleranței unei comunități.

În prezent, ne aflăm în fața celei de a 2 a pandemii, pe care am urmărit-o încă de la început, când la finalul anului 2019 au fost semnalate primele cazuri de insuficiență respiratorie acută în China, datorate unui nou coronavirus. La acel moment acele cazuri erau atât de departe din punct de vedere temporal și spațial încât am luat la cunoștință patologia provocată de acest nou virus ca un fapt științific. Rapiditatea și agresivitatea răspândirii pandemiei a luat prin surprindere mapamondul cu implicații nu numai medicale ci și economice, sociale și în termeni de noutate și cercetare științifică. Astfel, comunitatea medicală internațională a făcut un efort concentrat prin publicarea celor mai noi date legate de aspectele clinice, biologice și farmacologice ale acestei infecții.

În acest efort concentrat mi-am adus și eu contribuția, publicând date ce mi s-au părut relevante privind infecția SARS COV-2, cu implicații clinice, epidemiologice și de abord medical, inclusiv de management administrativ al bolii, specifice pentru zona noastră și pentru unitatea medicală în care lucrez.

Deși suntem la un an de evoluție a pandemiei, consider că aceasta va dezvălui în continuare multiple aspecte, atât clinice cât și în termeni de terapie și prevenție, pe viitor devenind o prezență permanentă. Preocuparea aceasta se reflectă și în grantul de cercetare cu Institutul Cantacuzino- I-MOVE, care este centrat pe evaluarea răspândirii virusului gripal în contextual pandemiei, două virusuri cu tropism respirator care se pare că au făcut și fac istorie.

Implicarea și asocierea bolilor cronice precum sunt diabetul zaharat și hipertensiunea arterială la pacienții COVID-19 pozitivi a fost o altă direcție de cercetare, fiind interesată de ceea ce se

semnalează peste tot în lume, aceștia fiind pacienții cu o evoluție infaustă și dezvoltând forme severe din care este selectată mortalitatea pacientului COVID-19 pozitiv.

Ca și infecția HIV, în timp, infecția cu noul coronavirus își va releva multiple fațete. În termeni de sănătate publică, marea deosebire între aceste două infecții virale este că, pentru COVID-19 există vaccin ce oferă protecție, ceea ce este cu mult mai puțin probabil în cazul HIV infecției, în condițiile fitnessului viral accentuat și al selectării sușelor de rezistență.

Cele 2 pandemii coexistă, au aliură diferită, una ce s-a dezvoltat lent pe parcursul a 3 decenii, alta pe parcursul unui singur an dar care, din punct de vedere al cercetătorului și clinicianului, reprezintă un univers fascinant cu multiple direcții de cercetare.

Secțiunea II prezintă direcțiile de dezvoltare a carierei științifice ulterioare ce sunt legate de patologia infecțioasă, dar în mod special, de aprofundare privind clinica, fiziopatogenia, terapia și rezultatele profilactizării infecției cu noul coronavirus, o linie de dezvoltare extrem de generoasă pentru specialistul infecționist.

Infecția HIV nu și-a relevat pe deplin întreaga paletă de problematici, mai ales în sensul noii terapii cu eliberare prelungită, cu administrare o dată pe lună, la care vom avea acces și de la care, sperăm o aderență și complianță crescută a pacienților, cu evitarea în timp a unei cazuistici frecvente, actualmente de patologie extremă și gravă, cum sunt cancerele generate de infecția HIV, tuberculoza, infecțiile cu mycobacterii atipice, pneumocistoze.

Alături de aceste două mari direcții de cercetare-dezvoltare, preocuparea pentru patologia infecțioasă va continua cu monitorizarea evoluției infecției cu Leptospira, o mai veche preocupare, cu infecțiile pediatrice, cum sunt meningitele și noile infecții pregnante clinic în ultima perioadă, din care fac parte borellioza și infecția cu *Clostridium difficile*.

Din marea cantitate de literatură de specialitate am selectat o serie de 392 articole care să susțină și ilustreze cât mai consecvent direcțiile de cercetare pe care le-am abordat de-a lungul unei cariere de peste 30 de ani legate de aceste 2 mari pandemii, datorate unor virusuri care încă își mai prezintă diversele fațete.

Abstract

The habilitation thesis called "**Two viruses – crossing the millennium"** is aiming to synthesize, in three sections, the professional, scientific and research activity, that I have explored until the moment of this thesis, and also future projects and bibliographic references selected from the scientific literature.

Section I summarizes two important research topics that I have taken into consideration throughout the entire career. One of the areas that I have approached is the HIV infection, that became, throughout the history, a genuine pandemic and involved multiple research areas. Also, the psychosocial aspect of this disease is frequently linked to what we know as the evolution of HIV infection in the "pediatric cohort" from Romania, in a specific category of HIV infected patients-adolescents.

Unfortunately, in Romania, throughout my career, I have seen, diagnosed and treated young patients, that are part of the "pediatric cohort", are born at the end of the eighties and have been diagnosed with HIV in the first years of life. The active monitoring and psychological evaluation of these patients and their families, from childhood up until the adulthood, when the HIV positive patient wants socio professional integration and a normal adult life, with the intention of having a family, brought us closer to understanding the HIV phenomena in the North-Eastern area of Romania and the rest of the world.

Clinical and research activity were closely interlinked in the idea of drawing relevant and fruitful conclusions, for the practitioners and future practitioners, and presented as case studies for this complex pathology, that also required psycho-emotional support. Initially, HIV infection was considered an acute infection with a life expectancy of only a few months. Through the instituted therapy, known as HAART therapy, this infection became a chronic infection, with a life expectancy equal to that of the general population. The only condition is adherence and compliance to the treatment, which can fluctuate throughout the personal history of an HIV-infected patient that sometimes can leave a mark on the immunovirological status, making the HIV positive patient susceptible to opportunistic infections, carcinogenesis, etc.

HIV infection is a global problem, not only medically, but also with major social, economic and psycho-emotional implications, both for the seropositive patient and for society. The way the HIV-positive patient and society sees this disease, can be an indicator of a spiritual health and tolerance of a community.

Unfortunately, in our lifetime we have seen the evolution of the second pandemic, since it`s first records, when the reports from late 2019 talked about cases of acute respiratory failure in China due to a new coronavirus, which at that time, were so far apart, and we became aware of it only as a scientific fact. The aggressive spreading of this new pandemic has taken the entire world by surprise, with not only medical implications, but also unimaginable economic and social ones. In terms of novelties and scientific research, the international medical community, was focused on making a concentrated effort to bringing the newest information regarding SARS COV-2 virus, by publishing the latest clinical and pharmacological findings for this infection. To this collective effort, I have also brought some contribution, by publishing data that seemed relevant to me about the SARS COV-2 infection, with epidemiological and clinical implications, including administrative management of this disease that are specific to our area and to my medical unit.

Although it has been a year since the start of the pandemic, I believe that this virus will continue to reveal its multiple faces, both clinically and in terms of therapy and prevention, and will become a permanent presence in the future. This concern is also reflected in the research grant conducted with Cantacuzino Institute called I-MOVE, which is focused on assessing the spread of influenza virus in the context of the pandemic, considering that these two viruses, with respiratory tropism made and are still making history.

The implications and association of chronic diseases, such as diabetes mellitus and hypertension have been another research direction which was approached, considering that all the reports, from around the world, were showing that these patients were having a severe evolution.

Like the HIV infection, over time, the infection with the new coronavirus will reveal its different faces. In terms of public health, the big difference between the HIV infection and the SARS COV-2 infection is that, at this time, there are vaccines that are offering protection against COVID-19, which is less probable with HIV, considering its viral fitness and also its possibility to select resistant strains.

These two pandemics are coexisting and have different forms. One that slowly developed over the course of three decades and one that developed in only one year. From the researchers and clinicians point of view, these two pandemics are a fascinating universe with multiple development possibilities.

Section II presents the future development directions that are linked to infectious diseases pathology, but in particular related to in-depth study regarding the clinical presentation, pathophysiology, therapy and the results of prophylaxis of infection with the new coronavirus, which are extremely generous lines of development for the infectious diseases specialist.

HIV infection has not yet fully revealed its complete range of problems, especially in the therapeutic direction with prolonged release that can be administered only once a month, to which we will have access and from which we expect an increased adherence and compliance. This new therapy can prevent, in time, the overgrow of severe and extreme cases that right now are slightly rare, like cancers generated as complication to HIV infection, tuberculosis, atypical mycobacterium infections and *Pneumocistis* infections.

Alongside these two major directions of research and development, the interest for infectious diseases pathology will continue with the monitoring of *Leptospira* infection, which is an older preoccupation, and also with pediatric infectious diseases, such as meningitis and with new clinically significant infections including borreliosis and *Clostridium difficile* infection.

From the vast medical literature, I selected 392 articles that present, as consistently as possible, the research directions I have approached during a career of over 30 years.

SECTION I. ACADEMIC, PROFESSIONAL AND SCIENTIFIC ACHIEVEMENTS

Personal background

The profession of doctor intertwined with the didactic activity, and more specific being a teacher for the new generation and my interest regarding scientific research, are the elements that represented the core of my professional activity. This manner of looking at the daily professional and academic activity is specific to a university teacher that tries at every moment to surpass itself portraying the "Grigore T. Popa" University of Medicine and Pharmacy vision. The university offered a fertile field that stood at the base of developing my skills regarding scientific research and of course, me becoming an infectious diseases doctor and teacher.

Professional activity

I graduated from "Grigore T. Popa" University of Medicine and Pharmacy of Iași in 1986, and in September 1990 I finished the first internship from Romania, that made me chose the infectious diseases field. From that moment onward, a continuous effort was directed towards self-improvement in my professional field, that materialized in the title of specialist doctor in infectious diseases. Five years later, I became a consultant doctor (MD) in the same specialty.

A turning point for my professional activity, and also a personal goal, was the fact that in 1996 I started working as a teaching assistant, and in 2001, I presented the doctoral thesis "**Bacterial meningitis - therapeutic novelties**" (Meningite bacteriene – noutăți terapeutice) that was coordinated by Prof. Dr. Ștefan Dimitriu.

With a short break of 11 months, during which I worked as a Primary Care Physician in Hârlau Hospital, my professional activity took place in "Sf. Paracheva" Clinical Hospital of Infectious Diseases Iași.

In the same timeframe, prior to preparation of doctoral thesis, in 1999 I was the beneficiary of a Tempus scholarship in Bruxelles, achieved through competition, and classes trough ARPAC in Amsterdam. I am also a member of International AIDS Society, Balkan Medical Society and Romanian Society for the Study of Chemotherapics. My training as a medical doctor and professor with valences of researcher was bound by participating in accredited international courses in Europe and USA.

Scientific activities

As the years passed by, the activity of publishing scientific research and observations from our clinic was extremely successful. Until now I have published 15 ISI articles as main author and 6 articles as co-author, and over 90 BDI articles.

In the current context, the multidisciplinary approach of the patient is very important, so I worked closelly with colleagues from the Faculty of Dentistry, in writting scientific papers that highlighted the importance of the infectious diseases pathology in the complex stomatognathic system.

Another academic achievement is the development of the Good practice guidelines in antibiotic therapy, which had 7 editions added annually, with the latest literature data regarding antibiotic therapy. Also, I have worked closely with my colleagues in developing explanatory materials trough case presentation, papers that were later materialized in sequential handbooks regarding the approach of the patient with infectious disease in the outpatient department.

I have to mention that I collaborated to create a national good practice guideline for the modern era of antibiotic therapy, a guide that was edited by Prof. Dr. Cepoi. Paving over a decade of experience in managing a hospital alongside with Prof. Dr. Carmen Dorobat I have written a handbook regarding the management of an Infectious diseases hospital in the SARS COV-2 era.

The papers that I have published cover topics such as rare diseases (leptospirosis), but also very common diseases such as *Borellia burgdorferi* infection, that is becoming a common finding in our area.

One of my particular passion was the infectious pathology related to a viral infection, that always raises questions for the researches around the world. This individual interest refers to HIV infection and is related to my clinical activity in the Regional HIV / AIDS Center. The multitude of complications related to HIV infection, from *Mycobacterium tuberculosis* infections to HIV related cancer has been materialized in publications in ISI and BDI journals and also oral presentations as a guest speaker and posters at various symposia, conferences and congresses, both national and international. I also have to mention the fact that I have moderated different sections at over 50 conferences or I was the invited guest speaker in symposiums.

Considering the fact that the HIV infection became a chronic illness, where the patient has to be regularly evaluated and followed-up by the doctor, throughout his entire life, there was a constant interest in the psychosocial and psychoemotional aspects of the HIV infected children, young and old adults. These concerns were materialized through my daily activity and also in my scientific activities.

The results are more visible when they take form as activity for a defined participation in a scientific grant with other collegues. Taking this into account, I want to mention that I was part of a team of international specialists, with different area of expertise, from human and veterinary medicine from Italy, Slovenia, Lithuania, Bosnia &Herzegovina, Macedonia and Croatia. The grant was called "*Zoonoses Online Education*" and was carried out over the course of two years. It was a succesful collaboration between universities of human and veterinary profile to raise awareness, increase knowledge and understanding of current and probable future public health significant infectious disease transmitted from animals to humans.

I have also been a member of the project "*The East European Network of Excellence for Research and Development in Chronic Diseases CHRONEX-RD*", MIS ETC Code 1840 conducted in Iași by "Grigore T. Popa" University of Medicine and Pharmacy and also, a project in collaboration with "Iuliu Haţieganu" University of Medicine and Pharmacy from Cluj and Rochester University from United States of America named "*Implementation of a user centered platform to stimulate practice based HIV/AIDS research and informatics training in Romania*".

Regarding my international training, I graduated multiple *Iversity* courses that are part of Springer Nature conducted by specialists from from "*Global Labor University"* which include: "*Post Corona Starts Now", "Clinical Management of HIV", "The Science of Global Health Implementation", "Health and Climate change"*.

Beyond the infectious disease field, I was interested in alleviating the suffering of the patient, who is beyond therapeutic resources, and I have obtained accreditation in palliative medicine after I graduated the training conducted by "Casa Speranței" in Brașov City.

- Introduction in palliative care - 20.01.-01.02.2016
    - Transylvania University, Faculty of Medicine, Braşov
    - Hospice "Casa Speranţei" Braşov
    - Palliative Medicine Studies Center
- Financing palliative care services – 16.06.2016 – 17.06.2016
    - Hospice "Casa Speranţei" Braşov
    - Palliative Medicine Studies Center, Brașov

These activities intertwined with the teaching, with multiple courses and case presentations for students and junior doctors regarding this fascinating world of infectious diseases.

Being engaged in a didactic activity that implicated courses and practical lesions for foreign students, a natural thing was to write, alongside my colleagues, papers in English and French, so that we could facilitate the learning of infectious diseases for students. We also developed the first book on tropical diseases, which was edited in our clinic.

I believe that the infectious diseases as a field, combines the informations gathered over time from various disciplines, which include virology, microbiology, clinical pediatrics, internal medicine, pharmacology and reveals their expression in a complex approach of the patient.

The new millennium brought a new pathogen, now known as the SARS COV-2 pandemic. Even though the *Coronaviridae* family was known to cause upper respiratory infections at all ages, the third millennium brought this class of viruses to our attention with the appearance of MERS infection in Asia Minor, which had a limited spread at that time, and caused acute respiratory failure and death.

At the end of 2019 we had a rapid evolving pandemic, which spread globally in a few months, with an increased number of deaths that surpassed 2.5 million and afects all age groups, especially people with comorbidities and the elderly.

In the face of such a novelty, from a clinical, virological, therapeutic and prophylactic point of view, the medical, pharmacological and the research community has quickly mobilized for trying to stop this pandemic.

I was also looking to contribute to this global effort, on the one hand by treating COVID-19 patients, and on the other hand by publishing our observations from this pandemic year that are in agreement with the scientific literature.

My scientific observation materialized in papers that were published in BDI and ISI journals, and also, helped me organize the first Romanian infectious diseases conference regarding the COVID-19 pathology (of which I also was the chairman). Scientific courses, symposiums, conferences, books that cover this topic (including the medical management of the COVID-19 patients) were some of the activities that I carried out in this pandemic year.

Unfortunately, the Coronovirus is still a current problem, and right now, humanity is in the process of accumulating scientific data regarding all aspects of this new infection. Probably, as it happened with HIV infection, we will continue to coexist with this virus.

Everything that I mention so far is an image of my medical, educational and scientific activity that is still continuing and started over 35 years ago. I have to mention again the 26 years of working as a teacher and a researcher in “Grigore T. Popa” University of Medicine and Pharmacy Iași.

CHAPTER I. SARS COV-2 INFECTION

I.1 STATE OF THE ART

The end of 2019 brought in Asia, most exactly in Wuhan area from China, a new acute respiratory syndrome which causes severe acute respiratory failure with rapid progression and probable origin in the fish market from the same area. The ethiological agent was later proven to be a *Coronavirus,* initially named Coronavirus-2019 (nCov) and later CoV-2.

The SARS COV-2 pandemic, which started at the end of 2019, brought to our attention other three viruses from the *Coronaviridae* family that already produced epidemics in the beginning of the third millennium (SARS COV-1, MERS-CoV). We also learned from the past, that these viruses are highly pathogenic for humankind and have no specific treatment.

The first cases of the now known SARS COV-2 infection were identified in December 2019. On the 21st of December 2019, the World Health Organization (WHO) takes notice of the first suspect cases.

The medical community takes notice of the virus which originated in China, but the world was taken by surprise by the rapid spread, especially in Europe. Initially, the most affected states, were Italy and later France and Spain. The virus crossed the Atlantic to Brazil and the United States of America, where it caused millions of deaths.

The virus has an ARN structure with approximately 30kb length. Regarding its replication, studies have shown that a Papain-Like cysteine protease (PLpro, NSP3) is essential for multiplication, which makes this protease a target for specific antiviral drugs. Studies performed regarding PLpro showed that two inhibitors from the molecular level, VIR250 and VIR 250, can inhibit the ubiquitin chain and can provide a structural basis for the observed substrate specificity profiles [Rut W et al., 2020].

Two independent groups of scientists, form China and USA (United States of America), proved that a Spike protein, located on the surface of the virus, has affinity for angiotensin-converting enzyme-2 (ACE2) and is recognized as the main mechanism of entering the cell [Letko M et al., 2020; Zhou P et al., 2020].

The animal reservoir seems to be a venomous snake that was sold in animal market from China. The A clade, trough natural genetic recombination has genetic material from SARS (severe acute respiratory syndrome) virus that is found in bats. Some scientists are skeptic regarding this phylogenetic chain and demonstrate that snakes can`t have coronavirus and only mammals and birds can be infected [Callaway E et al., 2020]. The most plausible theory seems to be that the vector for transmission are bats, mainly because the SARS COV-2 virus has a 96% of similarity with the coronavirus genome found in bats [Zhou P et al., 2020].

General presentation

Structure

The SARS-COV-2 is part of the *Coronaviridae* family alongside with SARS-COV and MERS (Middle-East respiratory Syndrome) – CoV. It has a single-stranded RNA genome [Zhou P et al., 2020]. The coronavirus invasion of the target cells is mediated by a transmembrane spike glycoprotein (S) that has two subunits: S1, for binding to the host cell receptors, and S2, for the fusion process with the host cell membrane. These subunits will remain in a prefusion conformation after specific protease cleavage, with the distal S1 subunit comprising the receptor-binding domains (RBD), specifically involved in recognition of human angiotensin-converting enzyme 2 (ACE2) [Gui M et al., 2017; Calina D et al., 2020]. The S protein will be further cleaved by host proteases and activated for the membrane fusion. Due to the peripheral location of S proteins, they are the main target for neutralizing antibodies and for new developing therapies.

The ACE2 is a functional receptor for the SARS COV-2 and has a ubiquitous distribution into the human body, but its expression is higher in the nasal mucosa, lung parenchyma, and gastrointestinal tract [Kirchdoerfer RN et al., 2020]. Some studies suggest a possible link between smoking and enhanced expression of ACE2 receptors [Kabbani N et al., 2020], thus, smoking could be a risk factor that increases the susceptibility of the patient to contact the new coronavirus.

SARS-Cov-2 is a highly transmissible virus, with an incubation period of approximately 14 days, with a median time of 4-5 days from exposure to the onset of the symptoms [Guan WJ et al., 2020; Li Q et al., 2020; Lauer SA et al., 2020]. It has been suggested that the viral transmission is through the droplets, direct contact, contact with an infected individual, fecal, oral, and body fluid [Ceccarelli M et al., 2020; Zhang W et al., 2020; Lai CC et al., 2020].

Pathogenesis of SARS COV-2 virus

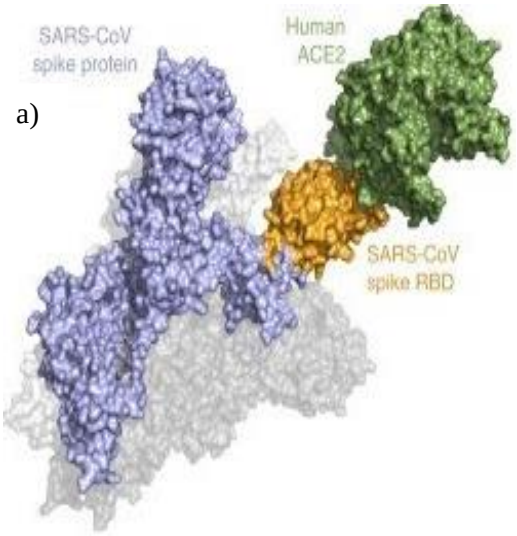
The medical scientific community, which was implicated in the research, was committed to understanding the pathogenesis of the infection as soon as the first cases were reported in Wuhan, China at the end of December 2019. The Chinese Center of Disease control estimated, at the beginning of the pandemic, that approximately 80% of the cases are going to be medium, 15% are going to progress to severe pneumonias with ARDS (acute respiratory distress syndrome) and 5% would be severe and associated with sepsis and MSOF (multiple systems organ failure). The pathogenesis of the evolution from medium to severe cases is not completely clear. Reviewing the literature, the pathophysiological model proposed by the progression of SARS COV-2 infection is represented in figure 1 [Letko et al., 2020].

Structurally, the virus is composed from 4 glycoproteins:

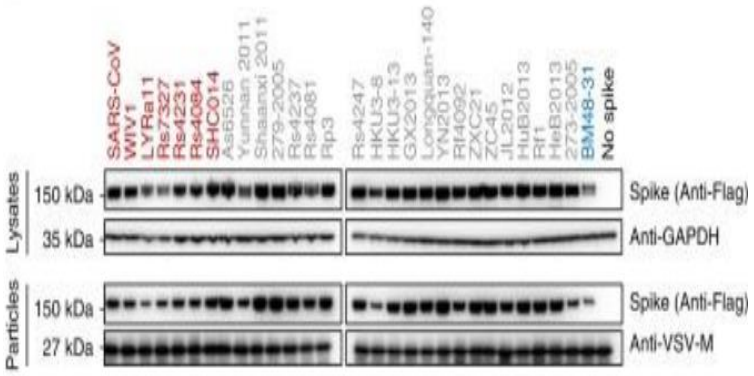
- Spike (S);
- Membrane (M);
- Envelope (E);
- Nucleocapsid (N);

The S protein is responsible for the viral binding and entry of the virus into the host cell. It has been found that ACE2, the human angiotensin 2 conversion enzyme, is a CoV-2 entry receptor. ACE2 receptor expression is present in various tissues, not only in the lungs, but also in the small intestine, kidney, thyroid, testis and adipose tissue.

a)



b)



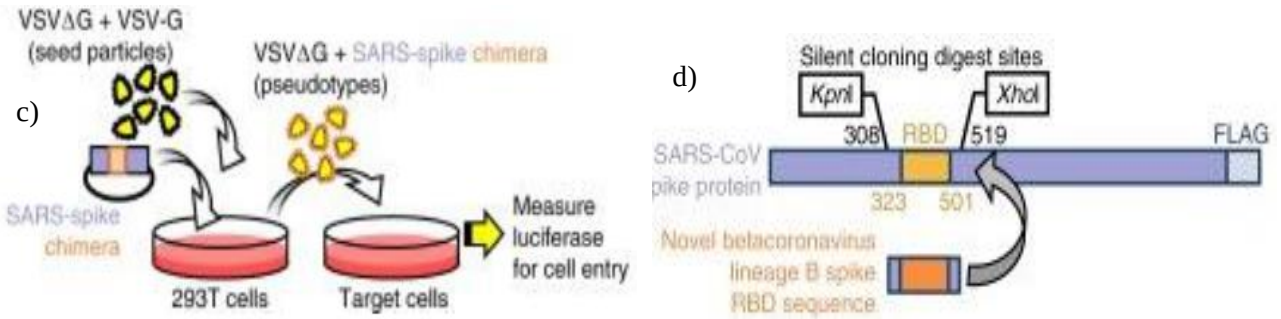


Fig.1 Betacoronavirus lineage B entry with human ACE2
a) Betacoronaviruses, including SARS-CoV, interact with the host-cell receptor via the RBD in spike; b) Western blot of producer cell lysates and concentrate reporter particles. The labels along the top show the origin of the RBD in the SARS-CoV spike protein; c) Outline of the experimental workflow; d) Engineered silent mutations in SARS spike facilitated replacement of the RBD sequence. SARS spike amino acid numbers are indicated in black for the silent cloning sites and orange for the RBD.

After the attachment of the virus, the next step is the fusion, mechanism that is already known from other type of viruses (eg. HIV). Simple cell binding is incapable of viral replication, nor is it capable of facilitating fusion. This phase requires the presence of a transmembrane protease or splitting due to host cell proteases.

Hoffman was the first to demonstrate that the S protein, in connection with transmembrane protease 2 (TmprSS2) which can be substituted with cathepsin B / L, is essential for the entry of SARS COV-2 into the cell [Hoffmann M et al., 2020].

As opposed to other coronaviruses, CoV-2 has a furin-like cleavage related to the S1 protein, located on the S1 and S2 subunits [Coutard B et al., 2020]. Inhibition or blocking of this process is a possible target for the molecules that can be used in therapy. This mechanism is unique to CoV-2 and has not been described in other coronaviruses. It is considered an adaptive mechanism of viruses, in order to use the reproductive features of the host cell for its own benefit. Next, the nucleocapsid remains in the cytoplasm of the host cell with replication of the viral genome. Vesicles with the new virions are transported into the cytoplasm, where they fuse with the membrane and are released and infect other cells [Walls AC el al., 2020].

Further studies are needed to determine the exact mechanism of TMPRSS2 protein S cleavage, as well as elucidating the contribution that the membrane protease in S-protein cleavage has in viral penetration. At the same time, the first line of the immune response is activated [Chen IY et al., 2019].

Other mechanisms are also involved, such as TLRS (toll-like receptors), recognized by alveolar macrophages and endothelial cells by inducing transcription of proinflammatory cytokines like NF (nuclear factor)-Kβ and activating IFN (Interferon) regulatory factors through type 1 immune-mediated response.

High levels of IL (interleukin) -1β in COVID-19 suggest local inflammation and produce systemic manifestations [Huang C et al., 2020].

The host respondes to these processes by secreting proinflammatory cytokines and chemokines such as IL-6, monocytes chemotactic S protein and IFN-gamma induced by UP-10 protein (Figure 2) [Bohn MK et al., 2020]. Dendritic cells and pulmonary macrophages activate LyT (T lymphocytes) followed by the elimination of the infected alveolar cells [Huang C et al., 2020].

The spike-antibodies are part of the antibodies produced in order to provide protection against SARS COV-2.

In summary, the immune response to the cytokine release and interferon activation is followed by SARS COV-2 clearance from the lungs. The infection can progress to severe disease due to immune response irregularities (Figure 3) [Zohar T et al., 2020].

Cytokine storm

Elevated concentrations of pro-inflammatory chemokines and pro-inflammatory cytokines were found to be statistically correlated with disease severity and increased mortality. Elevated levels of IL-6,2,7,10 and granulocyte growth-stimulating factors, IP-10, IFN-gamma, macrophage 1- alpha protein - MIP1a and TNF (tumor necrosis factor) were detected. This suggests a combined T-helper type 1 and 2 immune response [Tay MZ et al., 2020].

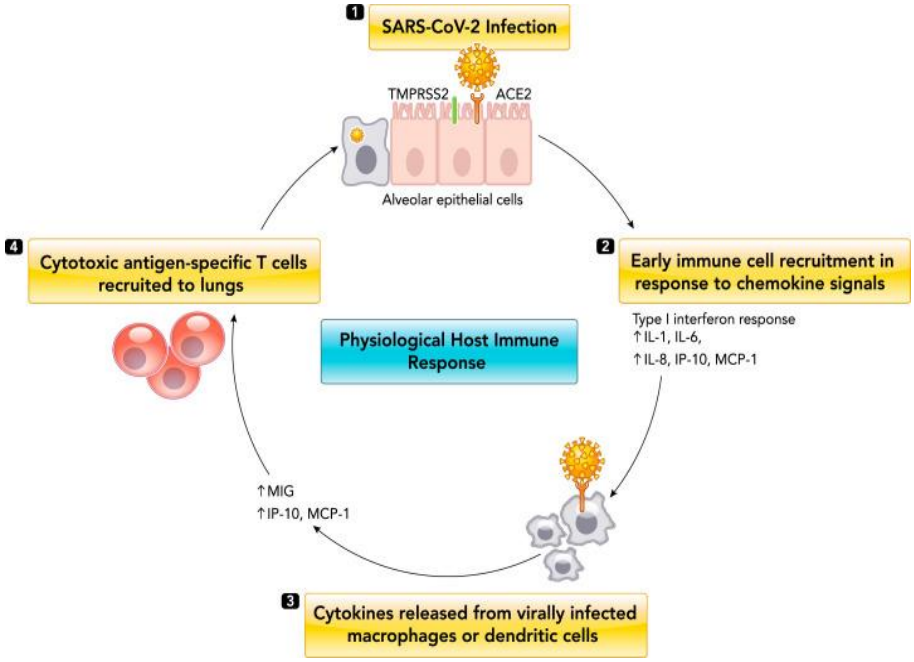


Fig.2 Immune response of the host

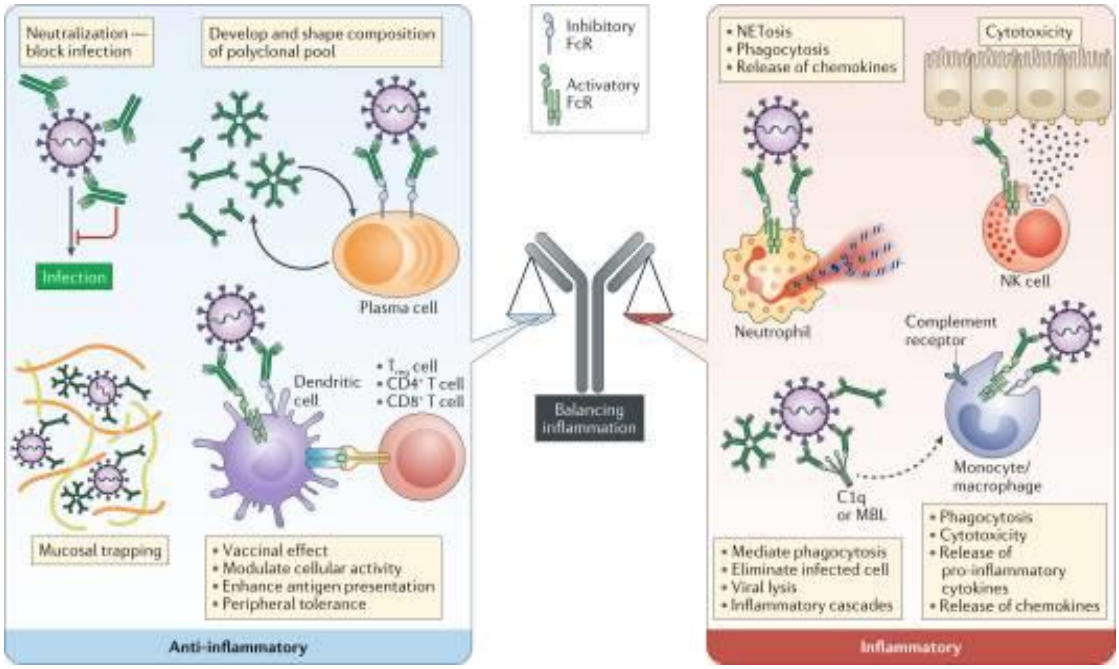


Fig.3 Antibody functions and their contributions to inflammation

IL-6 may become a target for molecules used in treatment and is associated with strong evidence of disease progression. Studies show that IL-6 over 55 picograms / ml is associated with a bad outcome and levels over 80 picograms / ml with increased mortality rates [Aziz M et al., 2020].

It has also been shown that high levels of inflammatory markers, such as C-reactive protein, ferritin, leukopenia with neutrophilia, PMN (polymorphonuclears) ratio are a good indicator for the severty of the disease and can guide our mamagement [Liu Y., 2020].

The mechanism for progressive lymphopenia remains unclear, probably due to TNF-alpha-mediated apoptosis or directly by cytopathic effect, eventually leading to hyperinflammation.

Patients with severe disease have been shown to have a higher percentage of antibodies, but it is unclear whether this is due to exposure to a higher amount of virus or is dependent on antibody growth (ADE = antibody dependent enhancement).

Genetic evidence of sever cases and increased mortality

Recently, researchers have shownd a link between the HLA system and the amplitude of the immune response or the overreaction of it. This is not specific only to SARS COV-2 cytokin storm but also to VVZ (varicella-zoster virus), EBV (Epstein-Barr virus), HH7 (Human Herpesvirus), human polyoma that uses residual amino acids for the seroreactivity [Kachuri L et al., 2020].

The ACE1 and ACE2 haplotypes seem be a plausible explanation for the reason that SARS-COV-2 affects some ethnic groups more, namely people with European ancestry, rather than Asians [Gemmati D et al., 2020].

Further research is needed for solid evidence of genetic determinism in the evolution to severity or fatality of the disease.

Coagulation and cardiovascular complication

It has been shown that in addition to the cytokine storm, with the involvement of inflammatory factors, coagulation disorders also play an important role in the pathogenesis of COVID-19. Tang N had found in more than 71% of hospitalized patients coagulation disorders with disseminated intravascular coagulation (DIC) in varying degrees [Tang N et al., 2020].

The autopsies performed showed, in 87% of cases, microthrombi in the small vessels with alveolar extravasation and endothelial damage suggesting "a state of hypercoagulability" in severe Covid-19 [Carsana L et al., 2020].

This aspect is closely related to the release of cytokines and inflammation as a means of defending the host against aggression. Lung tissue damage, mediated by complement as well as vascular microthrombosis were observed in severe COVID-19, the procoagulant state being associated with the inflammatory effect of cytokines in the vascular endothelium.

Thrombosis can be linked with increased D-dimers, fibrinogen and IL-6 and also the involvement and activation of monocytes. The association of PMN tough endothelial activation factor with cytokine-releasing accelerates thrombus formation.

In fact, the inflammation and coagulation factor are intertwined from a therapeutic point of view, requiring both prevention and treatment.

Cardiac involvement and heart failure have troponin levels as markers in relation to the increase of CRP (C reactive protein), IL-6 and Ferritin. The release of cytokines has a maladaptive role to the heart muscle fiber with a direct role in heart damage. Some studies show that there are patients who present initially with cardiac symptoms, with signs of myocarditis or myocardopathy attributed to hypoxia and respiratory failure. There is little evidence of direct viral action on the myocardial fiber - just over 1/3 of the necropsy evidence reveals viral RNA in the myocardium [Carsana L et al., 2020].

Extrapulmonary damage

Gastrointestinal manifestations mainly include diarrhea, more common in children, alongside with nausea, vomiting and abdominal pain. This might be explained by the fact that there is virological evidence of direct CoV 2 damage to the enterocyte.

In patients with increased ALAT (Alaninaminotransferase) and ASAT (Aspartate aminotransferase), a statistically significant correlation was demonstrated in those who will develop severe pneumonia with different pathophysiological explanations [Huang C et al., 2020].

Although hepatocytes do not increase ACE expression, studies have shown that in cholangiocytes there is an increased level of ACE2 which can explain the increased GGT (gama-glutamil transpeptidase), bilirubinemia and FAL (alkaline phosphatases). Wang's studies show that in hepatocytes there is an abundance of viral particles [Wang Y et al., 2020].

Another possible mechanism of ALAT growth could be the toxic one, due to medication, but changes in this enzyme were also observed at admission, before any treatment was given.

The most plausible mechanism of liver damage appears to be cellular mediation of LyT, IL-6 playing a role in the expression of amyloid A in serum CRP and fibrinogen. Wang also found that the exocrine pancreas was affected by the alteration of the biochemical value with the involvement of amylase and lipase, but without a bad outcome for the patient.

COVID-19 induces complications in patients with diabetes associated with immune dysfunction, vasculopathy and hypercoagulation [Drucker DJ et al., 2020].

Kidney damage

The renal impairment ranges from moderate or minor proteinuria, with a slight increase in creatine, to acute kidney failure. Data from the literature shows that renal impairment varies, with the mention that sometimes extracorporeal dialysis is required.

The pathophysiological mechanism is considered multifactorial and implies direct mechanisms in which ACE2 receptors present in the kidneys have a direct action to the tubular epithelium and podocytes fact proven in postmortem samples [Su H et al., 2020]. The direct damage is done at the level of mitochondria with the increase of inflammation, of the vascular permeability mediated by cytokines.

Neurological damage

Neurological damage was less studied, but data retrieved showed a percentage that varies, according to studies between 36-45.5% in patients with severe infection. Neurological manifestations include headache, confusion, epilepsy, ataxia, anosmia, hyposmia, ageusia, Guillain-Barre syndrome.

Anosmia and ageusia have been postulated as symptoms of direct damage to the olfactory epithelium, bulb, and olfactory neurons by alteration of the ACE2 signal. Virions were found in the CSF, the mechanism behind the neurological manifestations not being well elucidated.

Pediatric patients

Out of a group of over 2000 children suspected or confirmed with COVID-19 in China, 5% had dyspnea and hypoxemia and only 0.6% progressed to ARDS [Dong Y et al., 2020].

In Europe, a multicenter study on children confirmed with RT-PCR reported that only 4% of the patients required hospitalization in ICU (intensive care unit) and mechanical ventilation and 3% inotropic support [Götzinger F et al., 2020].

In both Asia and Europe, the clinical profile of the disease in pediatric patient was mild and moderate. Laboratory data are slightly different compared with the adult population with the CRP level, procalcitonin and LDH (lactatdehydrogenase) beeing altered only in severe forms of COVID-19. Lymphopenia has not been found, although this is surprising, given that this is common in adult patients with severe disease.

Kawasaki syndrome and toxic shock syndrome are associated in a small number of cases. In children, IL-10 and increased IL-6 appear to be characteristic of severe disease fact that was presented by Cheung EW that performed a study on a small cohort in New York [Cheung EW et al., 2020].
CDC defined that the following are severity criteria in pediatric patients

- Increase of CRP;
- Increase of Ferritin;

- Lymphocytopenia with neutrophilia;
- Hypoalbuminemia;
- Thrombocytopenia;
- Anemia;
- Increased D-dimers;
- Increased fibrinogen;
- Increased levels of troponin and natriuretic peptides from the brain have been observed in the majority of patients.

Further studies are needed to elucidate the mechanisms that provide protection against COVID-19 and the factors that predispose to multisystem inflammatory disorder in children (MIS-C) [Cheung EW et al., 2020].

Epidemiology of SARS COV-2 infection

Currently, in Europe, before the discovery of the new circulating strain in England (B.1.1.7) and South Africa (501.V2) the main management was represented by the RRR Strategy (Reduce-Relax-Repeat), which meant reducing the level of infection, followed by relaxation of anti-spreading measures with their repetition in the areas where it was required [Kupferschmidt K et al., 2020].

From an epidemiological point of view, before the development of the vaccine, 3 important strategies of controlling the infection were used: physical distancing, masks and lockdowns, to which massive testing was added.

Beginning of the vaccination for the general population gives hope of stopping this pandemic with major medical, social and economic implications.

From the epidemiological point of view, in the determinism of CoV-2 infection there are several factors that contribute to the spread of the virus, thus creating a pandemic (Figure 4).

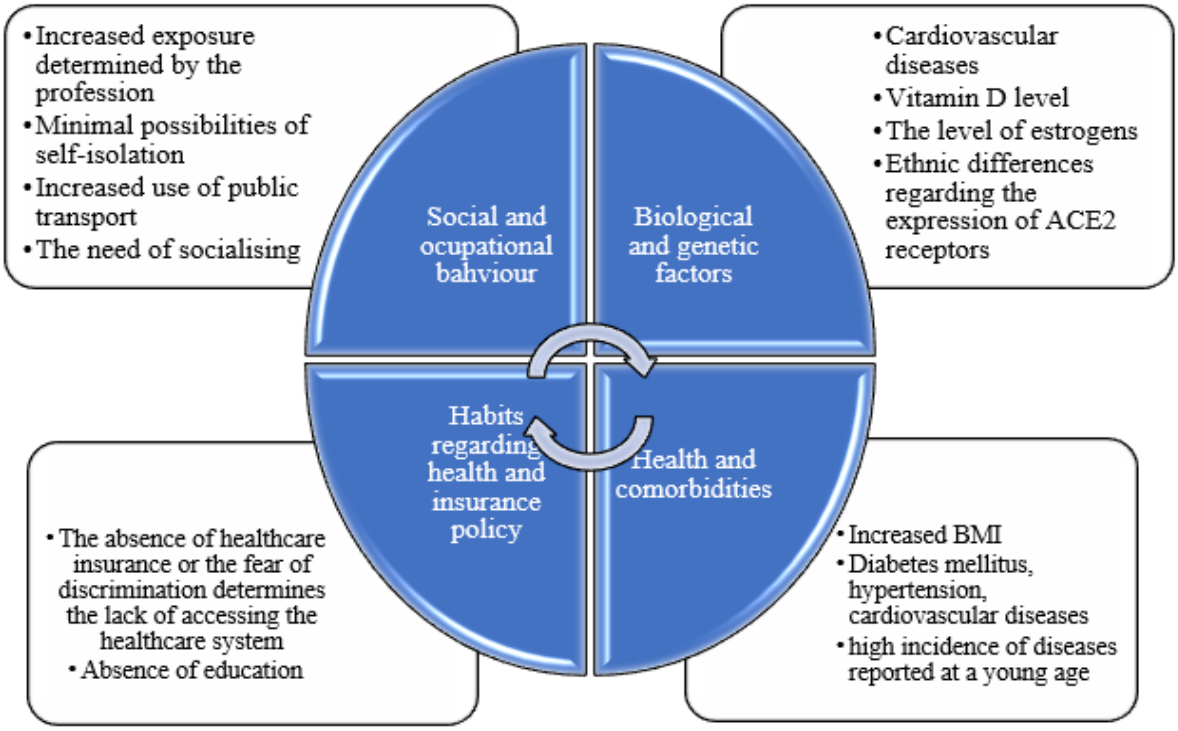


Fig.4 Factors that could increase or accelerate the pandemic

After highlighting these factors and reducig those that could accelerate the pandemic, the next step to consider is:

- Ensuring that all citizens are included in the national pandemic response plan;
- Research facilities for better understanding the diseases;

- Apropriate funds for eradicating inequality and discrimination;
- Promoting a healthcare system with universal coverage.

European Committee of Social Rights is stating that *„ COVID -19 is a brutal reminder of the importance of ensuring lasting progress with respect to social rights and universal public health services*." [https://www.coe.int/en/web/european-social-charter/social-rights-in-times-of-pandemic].

One of the epidemiological aspects that concerned us, in the context of an airborne disease is aerosolization and transmission through air conditioning devices in the hot period of the year, by similarity with Legionellosis. For this particular reason we published a review on the literature regarding the involvement of air conditioners in the transmission of this infection with serious health implications.

Pregnant woman

Assessing the risk of developing a severe outcome in pregnant women is important for the mother and fetus. Many studies show that the risk is equal to that of women that are not pregnant at fertile age, opposed to other respiratory viruses (e.g. influenza virus). Pregnant women are more often hospitalized than women of the same age, but it is difficult to say, outside of in-depth studies whether the hospitalization is related to COVID-19 or the pregnancy [Fenizia C et al., 2020].

Although the virus has been found in the placenta, it is not transmitted vertically. Further studies are needed for each trimester of pregnancy.

Andrea G. Edlow and collaborators analyzed the outcome of a group of 107 pregnant women, of which 37 were SARS COV-2 positive [Edlow AG et al., 2020]. The conclusion of the study was that there is a low probability of vertical transmission of the virus but also for the antibodies against it. Therefore, the newborn from a SARS COV-2 positive mother must be carefully followed-up and all maneuvers should be performed with a mask and taking into account a rigorous hygiene.

Zaigham M conducted a meta-analysis on 18 studies regarding SARS COV-2 positive mothers and studied clinical manifestations, maternal and perinatal outcomes and also included data regarding vertical transmission. His reports show that pregnant women had presented in the third trimester with the following symptoms: fever (68%) and coughing (34%). Regarding the paraclinical data, lymphocytopenia was found in 59% of cases, with elevated C-reactive protein in 70% of the patients. The majority of women (91%) delivered by cesarean section. Three maternal intensive care unit admissions were noted and no maternal deaths. One neonatal death and one intrauterine death were also reported [Zaigham M et al., 2020].

Questions and future research directions

Taking into account the data that we currently have, it is obvious that the virus can cause hypoxia, systemic stress involving inflammatory factors, thrombosis and inflammatory response and in worst cases MSOF.

Age seems to be an important risk factor and this is why most of the severe cases were seen in the elderly population. For children, the clinical forms are usually mild. The data suggests that in Cov 2, the immune response is dependent on age, with a worse prognosis due to the disproportion of ACE2 receptors as well as specific comorbidities. According to statistics, associated pathology is more common in men (hypertension, diabetes, cardiovascular diseases to which are added metabolic diseases (e.g. obesity) which leads to bad outcomes.

Studies for the implication of genetics in the outcome of this disease are still at the beginning but it seems that the HLA system and even the sanguine ABO groups are important for the outcome, which dictates a specific profile of clinical reactivity.

Risk factors for the progression of the diseases are not yet clearly identified but they are important to know, because it could help with stratification of the population at risk. The aim of the therapy is to allow antiviral attack and it has to limit the exacerbated immune response and inflammation. An adequate pathophysiological response is the key to a good evolution of COVID-19.

Another recent challenge which arose with this complex disease are the new circulating clades that first appeared in London and SE England, with a 70% higher degree of infectivity compared to the already known Wuhan strain. Researchers presented data that proves that the circulation of this new strain has been present since September and, at the time of writing (December 21st 2020) there is no answer to the question regarding the speed of spreading, but many questions arise considering that strains have been reported in both the Netherlands and Australia. At present, the main concern is that the London strain will dominate (N501Y) [WHO, 2020].

The question is whether the virus has acquired resistance as a result of a natural process of gene transfer or whether the initial massive therapeutic action with antivirals that the studies have shown to have no effect on it, have not generated the current situation (Oseltamivir, Lopinavir Ritonavir).

In the near future, vaccination that already started around the world will bring new scientific data, but at the present date, the challenge seems to come from the new circulating strains first found in December 2020.

Given the viral fitness and the emergence of resistant mutations, we expect that in the near future more and more variants will be reported, with different characteristics (infectivity, transmissibility, and resistance to antivirals).

In mid-January 2021, several variants of the SARS COV-2 virus were identified in the world:
- In the United Kingdom, a new variant called B.1.1.7 has emerged with an unusually large number of mutations [https://www.who.int/csr/don/21-december-2020-sars-cov2-variant-united-kingdom/en/];
- In South Africa, another variant called 1.351 has emerged independently of the variant detected in the UK;
- In Brazil, a variant called P.1 emerged and was identified in four travelers from Brazil [https://www.cdc.gov/coronavirus/2019-ncov/transmission/variant.html].

HIV care and COVID-19

Considering that we are facing two pandemics at the same time (SARS COV-2 and HIV), we must recognize the impact that the COVID-19 infection has on HIV-positive patients. This new pandemic has created barriers for HIV positive patients, making access to medication more difficult and limited their access to the health care system. Thus, these patients have seen pauses in their normal care. They were not able to have regular check-ups which involved periodic clinical, biological, viro-immunological testing, psychological and treatment evaluations [Kalichman SC et al., 2020].

In the north-east area of Romania, physicians and clinical psychologists have noticed an increase in adherence and compliance for ARV therapy with the patients no longer missing any doses, thus not havin any extra doses left.

Discussions with the HIV-positive patients showed that they had a real fear of dying, which was caused by the uncertainties of the future accessibility to ARV therapy and health care services in the context of the COVID-19 pandemic.

Diagnosis

The diagnosis of the SARS COV-2 infection is structured on 3 pillars:

A. Epidemiological context

The epidemiological context is obvious, considering that we are in the midst of a SARS COV-2 pandemic. History taking can reveal the lack of surface, hand hygiene, the lack or incorrect use of a mask or the lack of airborne prophylaxis, especially in the context of contact with a SARS COV-2 positive person. Overcrowding of classrooms, cinemas, as well as family events with an increased number of people are all factors that predispose to the outbreak of the COVID-19 infection. Social distancing measures and partial or total lock-down have been imposed and are still trying to cut off the transmission of the SARS COV-2 infection.

Currently, at the end of February - beginning of March 2021, the international community is concerned about the presence of the Kent strains (UK), the South African and Brazilian ones, which tend to become dominant. It is currently unknown if they are covered by the vaccination that started in December 2020.

B. Clinical diagnosis

The most typical symptoms experienced by the patients were fever (83-99%), cough (59-82%), fatigue (44-70%), anorexia (40-84%), shortness of breath (31-40%), sputum production (28-33%) and myalgias (11-35%) [Chen N et al., 2020; Huang C et al., 2020; Wang D et al., 2020]. Less common symptoms reported include headache, confusion, rhinorrhea, sore throat, hemoptysis, vomiting, and diarrhea (<10%) [Huang C et al., 2020; Wang D et al., 2020]. Sometimes behavioral disorders that range from negativity up to coma in very rare cases. Children can present with maculo-papular rashes similar to Kawasaki syndrome though that were rarely reported.

In the one-year experience I had managing COVID-19 patients, I also treatead several cases that showed erythematous rashes. A patient presented with Steven Johnson syndrome, considered one of the clinical signs of the disease, and which yielded to local, general, and symptomatic therapy.

C. Laboratory and imaging

The most frequent findings in the laboratory workup were leukopenia with mononucleosic like syndrome, in association with elevated CRP, D-dimer, IL-6, ferritin and LDH increased by hundreds of times.

The CT imaging can show “ground-glass” opacities, multifocal, predominantly peripheral or bilateral attenuation which are the most common images noticed in COVID-19 patients.

Treatment

Since the beginning of the SARS COV-2 pandemic, multiple off-label therapeutic regimens have been tried.

Initially, given the fact that the virus is part of the *Coronaviridae* family and is airborne transmited, this pandemic was assimilated with the flu pandemic and it was assumed that the new virus would respond to treatment with Oseltamivir, which later proved to be false.

Hydroxychloroquine (HQ) is a prescription medication used to treat malaria and autoimmune conditions like lupus and rheumatoid arthritis. The drug gained attention when early reports suggested it might benefit COVID-19 patients. These reports were based on preliminary studies in cells and small studies of COVID-19 patients that lacked control groups. Those studies, that were presented in February 2020, have shown that Hydroxychloroquine, known as Plaquenil in Romania, may have an immunomodulatory effect. Over time, large cohort studies have shown that this drug is not efficient in patients receiving oxygen therapy [Mahévas M et al., 2020].

Studies published in November 2020 showed that HQ did not decrease overall mortality, compared to the control group [Hong TS et al., 2020].

Regarding HQ, it can still be used in selected cases, but at present, this molecule has been left in the background once Umifenovir, Favipiravir and Remdesivir started being used and proved to be an efficent treatment.

Lopinavir / Ritonavir has been proposed as a treatment for COVID-19 on the basis of in vitro activity, preclinical studies, and observational studiesis. The drug is a protease inhibitor boosted molecule, used in ARV therapy. It was widely used in the first 5-6 months of the pandemic at a dose of 4 tablets per day in adults. In the second half of 2020 studies showed that this molecule has no effect and was withdrawn from protocols.

Shown in Table I is the protocol of treatment used in Romania up until July 2020 [https://www. cnscbt.ro/ index.php/legislatie_cov/1617-protocolul-de-tratament-covid-19/file].

Table I - Treatment protocol of SARS COV-2 Positive patients in Romania up to July 2020

Severity of the diseases	Recommended Treatment	Dose/day	Recommended time	Adverse reactions
Asymptomatic	NO			
Mild – outpatient	Acetaminophen Other symptomatic medication	3 x 500mg/day Children – 3 x 10mg/kgc/day/dose Standard dose	According to evolution of the patient	Hepatotoxicity if the dose and the time of treatment is exceeded
Mild – inpatient	Hydroxychloroquine* If not possible Lopinavir/Ritonavir (Kaletra)	2 x 400mg/day in day 1, then 2 x 200mg/day Children 5mg/kgc/day every 12 hours 2 x 400/100 mg/day Children 2 x 300/75 mg/ m^2 /day	6-7 days 5-7 days	Rhythm / conduction impairment Diarrhea (40.9%), Nausea (40.9%), Stomatitis (18.2%) Anemia (45.0%) Leucopenia (40.0%)
Medium-Pneumonia without severity criteria	Hydroxychloroquine* + Lopinavir/Ritonavir** (Kaletra)	2 x 400mg/day in day 1, then 2 x 200mg/day Children 5mg/kgc/day every 12 hours 2 x 400/100 mg/day Children 2 x 300/75 mg/ m^2 /day	5 days 10-14 days	The administration should be in the same time with food or with milk
Severe	Hydroxychloroquine* + Remdesivir Or Lopinavir/Ritonavir, if Remdesivir is unavailable (up until the moment is obtained) ±Tocilizumab (patients with cytokine storm*** and/or MODS)	2 x 400mg/day in day 1, then 2 x 200mg/day Children 5mg/kgc/day every 12 hours 200 mg/day in day 1 then 100mg/day Children under 40 kg: 5 mg/kgc/day in day 1, then 2,5 mg/kgc/day 8mg/kgc, maximum 800mg lent iv in adult (12mg/kgc in children under 30kg)	5-20 days 10 days 1-3 doses at minimum 8 hours interval	-

* Daily EKG for QT evaluation; Contraindication: QT>500msec; risk-benefit evaluation in case of pregnant woman; ** Substitution of lopinavir/ritonavir with darunavir/cobicistat in association with hydroxychloroquine in patients with cardiac diseases that have a risk of arrhythmias trough QT elongation; Hemophagocytic lymphohistiocytosis

In November 2020 the Ministry of health published the treatment protocol based on the severity of the disease as followes – Table II [http://legislatie.just.ro/Public /DetaliiDocument/234074].

Table II - Indication of treatment according to severity of disease – November 2020

Severity of the symptoms	Recommended Treatment	Recommended time
Asymptomatic	NO	-
Mild –inpatient	One of the available antivirals; Anticoagulant Prophylaxis only for patients that are not already on anticoagulant medication for other pathologies	According to evolution of the patient
Medium- Pneumonia without severity criteria	Antivirals should be administered as soon as possible Anticoagulants - prophylaxis or therapy Dexamethasone (or metilprednisolone) 10 days	According to evolution of the patient
Severe	Antivirals (with debatable clinical role after 12-14 day from the onset of symptoms); the epidemiological indication is maintained) + anticoagulant - prophylaxis or therapy + dexamethasone (corticoid), 10 day According to evolution of the patient + immunomodulators selected cases Selective indication: convalescent plasma, antibiotic	According to evolution of the patient

Umifenovir and Favipiravir

Patients with mild symptoms can be managed at home using symptomatic medication. The moderate forms of the disease can initially be treated with Umifenovir and/or Favipiravir with oral administration. Umifenovir is not licensed for use in the EU area. Adults can benefit from treatment with Favipiravir which is initially administered as a loding dose of 1.6mgBD (twice a day) in the first day followed by 600mg/BD without major adverse effects. We have to mention that Favipriravir is not given to patients of fertile age.

Remdesivir

This molecule is a broad-spectrum antiviral nucleotide prodrug that has been clinically evaluated in Ebola virus infected patients and has recently received emergency use authorization (EUA) for treatment of COVID-19. Remdesivir has been biochemically shown to inhibit the activity of EBOV large (L) RNA-dependent RNA polymerase (RdRp) as a nonobligate delayed chain terminator [E.P. Tchesnokov et al., 2020).

Different studies showed that Remdesivir decreased the number of hospitalization days at 11 days versus 15 days for placebo, improved the mortality rate to 8% versus 11% in the placebo group, with a P = 0.059 [Beigel JH et al., 2020; Ko WC et al., 2020]. It also been shown to reduce IL-6 levels [Beigel JH et al., 2020]. The study included 1,062 randomly selected patients, of whom 541 received placebo treatment.

We want to mention the SIMPLE study, which showed that there was no time difference in the recovery of the patients, between those that recieved five or a ten days course of Remdesivir treatment. The SIMPLE study included 397 randomized patients who did NOT require mechanical ventilation and were divided in two groups. One received a five-day course of treatment and the other a ten days course of Remdesivir treatment. The limitation of this study was the fact were compared d with each other, without having a placebo group, so that a definitive conclusion can not be drawn [Goldman et al., 2020].

The WHO Solidarity Trial Consortium-funded the SOLIDARITY study, which was conducted in 30 countries and included 405 hospitals, with a total of 11,330 adults, that received Remdesivir, Hydroxychloroquine, Lopinavir/Ritonavir, and interferon beta-1a. The findings of the study were that "*Remdesivir, Hydroxychloroquine, Lopinavir, and Interferon regimens had little or*

no effect on hospitalized patients with Covid-19, as indicated by overall mortality, initiation of ventilation, and duration of hospital stay." [WHO Solidarity Trial Consortium, 2020].

In November 2020 Umifenovir, Favipiravir and Remdesivir were suggested as treatment for the SARS COV-2 infection, thus being included by the national authorities in the management protocol for COVID-19 – Table III [http://legislatie.just.ro/Public/DetaliiDocument/234074].

Table III - Drugs with antiviral effect suggested as treatment for COVID-19

Drug	Dose	Duration	Adverse effects
Remdesivir	200 mg/day in day 1, followed by 100 mg/day; Children below 40 kg: 5 mg/kgc/day in day 1, followed by 2,5 mg/kgc/day	5 days (10 days on ECMO or OTI patients)	Hepatic cytolysis, Phlebitis, Constipation, Nausea
Umifenovir	3 x 200 mg/day	10 days	
Favipiravir	1.600 every 12 hours in day 1, followed by 600 every 12 hours; 1.800 mg every 12 hours in day 1 followed by 800 mg every 12 hours *	10-14 days	Teratogenic effect # Hyperuricemia (5%) ## Diarrhea (4,8%) ##
Hydroxychloroquine**	2 x 400 mg/day in day one (2 x 2 tb/day), followed by 2 x 200 mg/day (2 x 1 tb/ day) Children 5 mg/kgc/ day every 12 hours	5-7 days	Rhythm / conduction impairment

* For this dose, the toxicity of Favipiravir is not studied enough. **Daily EKG for QT evaluation; Contraindication: QT>500msec; risk-benefit evaluation in case of pregnant woman # It is used only together with contraceptives at fertile age patients ## The rate of adverse effects comes from studies that were conducted with lower doses

Tociluziumab

This molecule is a monoclonal antibody that binds to the IL-6 receptor. FDA approved Tocilizumab as a treatment in giant cell rheumatoid arthritis and for cytokine release syndrome after CAR (chimeric antigen receptor)-T-cell treatment.

Considering that, in COVID-19 the cytokine storm is a proven fact, the idea of using Tocilizumab came from translating the cytokine storm from this viral disease, to the cytokine storm produced by CAR -T-cell treatment and saturating IL-6 receptors as in rheumatoid arthritis [Sciascia S et al., 2020].

In Italy, a prospective multicenter study, on 63 patients with severe form of disease, showed that the administration of Tocilizumab lead to an improvement in oxygenation, with a rapid decrease in CRP. Observational studies in the Americas showed an improved at 30-day mortality in 68 US centers [Gupta S et al., 2020].

A double-blind, placebo-controlled study in 67 centers from Canada, France, Germany, etc. included 450 severely ill subjects and was commissioned and led by Roche. This study did not show any benefit for mortality at 28 days, the most important conclusion of the study was that Tocilizumab sould not be routinely recommended [Rilinger J et al., 2020].

Tocilizumab does not yet have FDA approval in the US and the European Drug Approval Commission have been used as extreme measures as off label treatment in case of severe acute respiratory failure and MODS.

Another important trial was COVACTA, published in September 2020, which included 400 hospitalized patients with severe COVID-19 pneumonia that received Tocilizumab or placebo. At day 28 the clinical status and mortality rate were not different between the 2 groups. Of course, these findings raised many questions, as the results can not be interpreted as unitary, as there are currently no obvious scientifically supported data on the benefit of using IL-6, especially in the circumstances mentioned, so that for now the best use remains unclear [Rubin EJ et al., 2020).

Other immunomodulators

Supportive studies have been conducted by the pharmaceutical industry to highlight the role of IL-6 inhibitors in the treatment of COVID-19. The data was published in the New England Journal of Medicine in February 2021. The first trial, that included 800 adults, who required respiratory and cardiovascular support in the ICU and were randomized in the first 24 hours in two groups. One that received IL-6 inhibitors (Tocilizumab or Sarilumab) and the other one standard of care. The median number of ICU support days for 21 days of hospitalization in these patients was calculated, and it was noticed that the patients that received IL-6 inhibitor had a faster improvement with 10 days average compared to standard of care. Also, mortality was lower, at 9% (27 vs. 36) for patients with IL-6 inhibitors than standard treatment [REMAP-CAP Investigators 2021].

The BLAZE-1 study is a randomized phase 2/3 trial that was conducted in 49 US centers and included ambulatory patients (N = 613) who tested positive for SARS COV-2 infection and had one or more mild to moderate symptoms. The study assessed the effect of Bamlanivimab as monotherapy or in combination with Etesevimab on viral load in patients with mild to moderate COVID-19. The study concluded that, among nonhospitalized patients with mild to moderate COVID-19 illness, treatment with Bamlanivimab and Etesevimab, compared with placebo, was associated with a statistically significant reduction in SARS COV-2 viral load at day 11. No significant difference in viral load reduction was observed for Bamlanivimab monotherapy [Gottlieb RL et al., 2020].

Ministry of Health also published guidelines for administration of the immunomodulator medication – Table IV [http://legislatie.just.ro/Public/DetaliiDocument/234074].

Table IV – Immunomodulatory medication suggested as treatment for COVID-19

Drug	Dose	Duration	Frequent adverse reactions
Dexamethasone (alternative – metilprednisolone)	Anti-inflammatory: 6-8 mg iv/day Immunosuppressive: 16 mg/day (24 mg/day on obese patients)	10 days	Irritation of digestive mucosa, uncontrolled diabetes
Tocilizumab	8 mg/kg (maximum 800 mg per administration)	1-3 given at 8-12 hours interval	Reactivation of some infection: tuberculosis, chronic hepatitis with VHB, herpetic infection, hepatic impairment up to hepatic failure, intestinal perforation, hypercholesterolemia
Anakinra	200-400 mg/day initial, then 100 mg/ day	7-10 days	Hepatic impairment
Convalescent Plasma	200-400 ml	Once only	Acute respiratory disfunction (TRALI), posttransfusion overload, allergic reactions
In trial with favorable preliminary results: siltuximab, baricitinib			

I.2 NEUROLOGICAL IMPAIRMENT IN SARS COV-2 INFECTION

I.2.1 Introduction

SARS-Cov-2 is a highly transmissible virus, with an incubation period of approximately 14 days, with a median time of 4-5 days from exposure to symptoms onset [Guan WJ et al., 2020; Li Q et al., 2020; Lauer SA et al., 2020]. It has been stipulated that the viral transmission is through the droplets, direct contact, contact with an infected individual, fecal, oral, and body fluid routes [Ceccarelli M et al., 2020; Zhang W et al., 2020; Lai CC et al., 2020].

Anosmia (loss of smell), ageusia (loss of taste), acrosyndroms (cutaneous manifestations due to vasomotor disturbances), and skin rashes were reported as symptoms of the SARS COV-2 infection, but more data are needed to better understand their role in the clinical picture [Giacomelli A et al., 2020; Recalcati S et al., 2020; Stanca HT et al., 2020]. An increasing volume of empirical data indicates that anosmia, hyposmia, ageusia, and dysgeusia may be possible symptoms of the SARS COV-2 infection, regardless of, or alongside, classic symptoms.

My interest in this field is reflected by the following paper:
1. Tanasa IA, **Manciuc Carmen**, Carauleanu A, Navolan DB, Bohiltea RE, Nemescu D. Anosmia and ageusia associated with coronavirus infection (COVID-19) - what is known? Exp Ther Med. 2020;**20** (3):2344-2347 – **Corresponding author**

I.2.2 Aim of the study

This report summarizes the evidence regarding the association of anosmia and ageusia with the SARS COV-2 infection. It also aims to describe these manifestations particularly in the clinical picture of symptomatic patients.

I.2.3 Material and methods

A literature search was conducted in MEDLINE, EMBASE, and Cochrane databases using the following keywords, including synonyms, and all the possible combinations of them: anosmia, ageusia, SARS COV-2, COVID-19, coronavirus. The studies that were included were those that documented symptoms associated with SARS COV-2 infection (up to May 5, 2020).

I.2.4 Results

Viral upper respiratory tract infections frequently determine olfactory and taste disfunction. Coronaviruses are the main determinants of the common cold, along with other viruses such as rhinoviruses, influenza, and parainfluenza. Therefore, it is no surprise that the SARS COV-2 virus would determine smell alteration in some infected patients.

Klopfenstein conducted a retrospective observational study, in which he reported that 54 patients (47%) with a confirmed SARS COV-2 infection developed anosmia, 4.4 (±1.9) days after infection onset, and in 38% (22/52) of the cases it was the third symptom to manifest. The mean duration of anosmia was 8.9 (±6.3) days, and one patient had not recovered at the end of the follow-up (after 28 days). Anosmia was associated with dysgeusia in 85% of cases (n=46) [Klopfenstein et al., 2020].

A retrospective case series in three hospitals in Wuhan, China published by Mao which included 214 patients confirmed with SARS COV-2 infection, evaluated the presence of neurological symptoms (central, peripheral) and musculoskeletal symptoms. As for the peripheral symptoms (8.9%), the authors reported that the most common were hypogeusia and hyposmia with 5.6 and 5.1%, respectively [Mao et al., 2019].

Although currently there are no guidelines that recommend testing people with symptoms such as anosmia or ageusia, some authors suggest testing and isolation for those who experience these

symptoms alongside with hyposmia and dysgeusia in the absence of other explanatory conditions [Vaira LA et la., 2020; Vaira LA et al., 2020; Salzano G, et la., 2020].

Recent studies suggested that the different variants of the virus could determine the extent of susceptibility and clinical recovery for the infected patient, so different patients may have a polymorphic clinical presentation [Lechien JR et la., 2020].

Li and his team demonstrated that some ACE2 variants might prevent the attachement with SARS COV-2 S-protein. However, little data is currently available, and more research could lead to a better understanding of this topic [Li et al., 2020]. On the other hand, Cao Y demonstrated the genetic polymorphisms of the ACE2 receptor and the specific prevalence in the European and Asian populations suggesting the possibility of different clinical courses for these patients [Cao Y et al., 2020].

Pathophysiology of anosmia and ageusia

Few studies described the pathophysiological mechanisms of the olfactory and gustatory dysfunction in the SARS COV-2 infection. Available data indicate the virus has a neural spread and a cytopathic effect on the neurons. It affects mainly neurons in the cortex and hypothalamus [Gu J et al., 2020].

Some authors reported three mechanisms for anosmia in COVID-19 patients: i) local infection of support cells and vascular pericytes in the nose and olfactory bulb that may affect the function of bipolar neurons or mitral cells; ii) damage to support cells in the sensory epithelium that may indirectly influence the signaling pathway from sensory neurons to the brain; and iii) damage to sustentacular cells and Bowman's gland cells that could lead to diffuse morphological damage to the olfactory sensory epithelium and altering of smell perception [Plasschaert LW et al., 2020; Bihun CG et al., 2020].

In the scientific literature, two syndromes with different outcomes were described. One is the olfactory cleft syndrome, which involves a conductive loss due to mucosal obstruction of the olfactory cleft. The other one is the post-viral anosmia syndrome, which implies a neural loss, due to the destruction of the olfactory sensory neurons [Trotier D et al., 2020]. The first syndrome has a relatively rapid resolution of anosmia, while the second one can be associated with a persistent olfactory deficit.

Netland detected residue of coronavirus after 60 h after exposure to SARS COV-2 in the olfactory bulb and after four days, its dissemination to the pyriform cortex and dorsal nucleus of the rafe. This aspect suggests a rapid viral propagation to the brain structures [Netland J et al., 2020].

Recent studies showed that SARS COV-2 could be detected in saliva and that it is possible to measure the viral load in this fluid [Xu H et al., 2020; To KK et al., 2020].

Two scientists studied the cellular distribution of taste cells and ACE2 receptor distribution and found that the percentage of ACE2 positive cells was higher in taste cells, which indicates that SARS-CoV-2 might invade them and lead to ageusia in patients [Xu H et al., 2020]. The virus may bind to the sialic acid receptors and occupy and accelerate the degradation of the gustatory particles, leading to a decrease in taste sensation [Pushpass RG et al., 2020].

Diagnostic tools

Medical imaging and neuropathology studies evaluated, in the infected patients, the changes in the olfactory bulb, cranial nerves, and brain. Thus, magnetic resonance (MR) imaging of the olfactory bulb can be used to evaluate patients with anosmia. The main MR findings in anosmia, secondary to upper respiratory tract infection, were a smallar olfactory bulb and tract volume, which can correlate with the olfactory function [Held P et al., 2020].

Anamnesis and personal reports of anosmia and ageusia are also necessary. We can use questionnaires for clinical diagnosis and follow-up in anosmia or ageusia patients. In a prospective study, Lechien evaluated anosmia and ageusia using questionnaires based on smell and taste component of the National Health and Nutrition Examination Survey, and the short version of the Questionnaire of Olfactory Disorders-Negative Statements (sQOD-NS). The authors found a substantially higher prevalence of olfactory and gustatory dysfunction in European COVID-19

patients. More, the olfactory disorder may appear before the rest of the complaints [Lechien et al., 2020].

Bagheri conducted a cross-sectional study in which 10,069 participants responded to an online questionnaire that evaluated the sense of smell and taste. The results indicated a significant correlation between anosmia and SARS COV-2 infection, decreased taste sensation, and decreased quality of life [Bagheri et al., 2020].

Treatment for anosmia and ageusia

French Society of Otolaryngology recomands that patients avoid corticosteroids for the treatment of the SARS-CoV-2 infection [Russell B et al., 2020].

The relative risk seems to be reduced in the case of intranasal steroids use, in this case, the main concern beeing the exacerbation of an upper respiratory tract viral infection [Stenner M et al., 2020].

Lechien reported that the most frequently used treatments for olfactory dysfunction in SARS-CoV-2 infected patients were nasal saline irrigations, followed by nasal and oral corticosteroids. As for gustatory dysfunction, clinicians used L-carnitine or trace elements and vitamins [Lechien et al., 2020].

Smell training is a simple, safe, and readily available method, in the context of social distancing, for smell recovery in different forms of anosmia [Hummel T et al., 2020; Damm M et al., 2020].

Expected recovery

Scarce data reported in the literature suggest that most patients will achieve smell recovery up to 14 days after resolving the disease.

In the study conducted by Lechien, 67.8% of the anosmic patients recovered olfactory function within the first eight days following the disease, and it seems that, at least, 25.5% of the patients recovered both olfactory and gustatory functions throughout the two weeks after the resolution of general symptoms [Lechien et al., 2020].

Conclusions

Further research is needed to demonstrate the association between anosmia and ageusia with the SARS COV-2 infection, the clinical manifestations determined by variants of ACE2 receptor, and recovery rates of olfactory and gustative dysfunction, and specific treatment protocols of these manifestations.

Anosmia and ageusia are symptoms that require further investigation during a clinical examination, due to increasing evidence of their association with the new coronavirus. Careful screening and prevention must be offered to avoid nosocomial and community infection.

Related articles and books

1. Miftode E, Luca C. **Carmen Manciuc**, Andrei Vata, Ioana Hunea, Larisa Miftode, Aida Badescu, Olivia Dorneanu. COVID-19: a course through stormy waters *Rev Med Chir Soc Med Nat Iasi* 2020; **124:** 351–62.

2. Filip-Ciubotaru F. **Carmen Manciuc**, Gabriela Stoleriu, Liliana Foia. NADPH oxidase: structure and activation mechanisms (review) Note I. *Rev Med Chir Soc Med Nat Iasi* 2016; **120:** 119–23.

3.**Carmen Corcaci**, V. Luca, T. Turcu, D. Mihalache, C. Dorobat, O. Fecioru Meningita cu Haemophilus inphluentzae- experienta Clinicii de Boli Infectioase Iasi in perioada 1984-2001. *Rev Med Chir Soc Med Nat Iasi* 2002; **2**:384-52

4. Carmen Mihaela Dorobat, **Carmen Doina Manciuc** – Management medical si administrativ al pacientului cu SARS COV-2- Editura Tehnopres 2020, ISBN- 978-606-687-412-0

I.3. THE PROGNOSIS OF PATIENTS WITH SARS COV-2 INFECTION AND METABOLIC DISEASES

I.3.1 Introduction

As the SARS COV-2 pandemic unfolded, diabetes mellitus (DM) was already considered one of the main causes of morbidity and mortality throughout the world. The pathophysiology of DM implies a plethora of macrovascular and microvascular complications that have an impact on the patient's survival [Williams R et al., 2020].

There are multiple studies that show the apparent association between diabetes mellitus, acute respiratory distress syndrome and increased mortality [Guan WJ et al., 2020; Wu Z et al., 2020; Yang X et al., 2020; Wu C et al.,2020]. There could be multiple explanations for this hypothesized association between DM as the underlying disease and the increased severity of SARS COV-2 infection. One is represented by the fact that natural immunity, which is the primary line of defense against SARS COV-2, is inevitably weakened in patients that have uncontrolled DM. The consequence of this is that the virus proliferates unrestricted within the host [Geerlings SE et al., 2020]. It has been demonstrated that even short-term hyperglycemia can briefly paralyze the natural immune system [Jafar N et al., 2020]. In addition, DM promotes an increases pro-inflammatory cytokine response, mainly involving interleukin (IL)-1, IL-6 and tumor-necrosis factor (TNF)-α, which further aggravates the prognosis of a SARS COV-2 infection [Onder G et al., 2020].

My interest in this field is reflected by the following paper:

1. **Manciuc Carmen**, Nemescu D, Vata A, Lacatusu GA. SARS-CoV-2 infection and diabetes mellitus: A North Eastern Romanian experience. *Exp Ther Med*. 2021;**21**(3):279.

1.3.2 Aim of the study

The study summarizes the experience of "Sf. Parascheva" Clinical Hospital of Infectious Diseases from Iași, Romania with patients that have an associated pathology diabetes mellitus. It also aims to present the observation regarding the CRP values and its correlation with the prognostic of the patients.

1.3.3 Material and methods

A retrospective observational study on confirmed SARS COV-2 patients, admitted to "Sf. Parascheva" Clinical Hospital of Infectious Diseases, Iași, Romania was conducted between March 4th (the first such admission) and June 30th (the writing of the article). The primary inclusion criteria of the patients in the present study were represented by confirmed SARS COV-2 infection tested by RT-PCR assay, which had as associated diagnosis type 1 or 2 DM. Patients that did not have a history of DM type 1 or 2 and/or had a negative result after RT-PCR testing were excluded from the present study. The following data were collected: Demographic data, medical history, clinical and paraclinical data, blood tests, administered treatment and outcome. The RT-PCR tests were performed by either a molecular biology hospital laboratory or other accredited laboratories from Iași county, Romania.

I.3.4 Results

From the beginning of the outbreak, a total of 1,080 patients were admitted to "Sf. Parascheva" Clinical Hospital of Infectious Diseases of Iași, Romania. Of these patients, 85 (7.87%) were known with DM, mostly type 2 (82 cases, 96.46%). Diabetic patients between 51-70 years old were the most affected by the SARS COV-2 infection, the mean age being 62 (Figure 5), and they presented in fairly equal proportions gender wise (42 were men, 49,41%).

Other associated pathologies were cardiovascular (hypertension, heart failure, and atrial fibrillation), neurological (stroke, epilepsy), neoplastic, renal (kidney failure with or without chronic dialysis) (Figure 6).

From a total of 1,080 patients admitted, for 78, with associated underlying diseases, intensive care unit therapy was required. Of these, 17 patients had DM as one of the underlying diseases. The majority of patients (64 cases), presented with oxygen desaturation (Sp02 <89%) and were dyspneic and polypneic, or they became hemodynamically unstable, with the arterial pressure level dropping below 85/50 mmHg. Fifty-nine patients ultimately required orotracheal intubation and mechanical ventilation due to multiple complications including aspiration bronchopneumonia, sepsis, and multiple organ dysfunction syndrome (MODS).

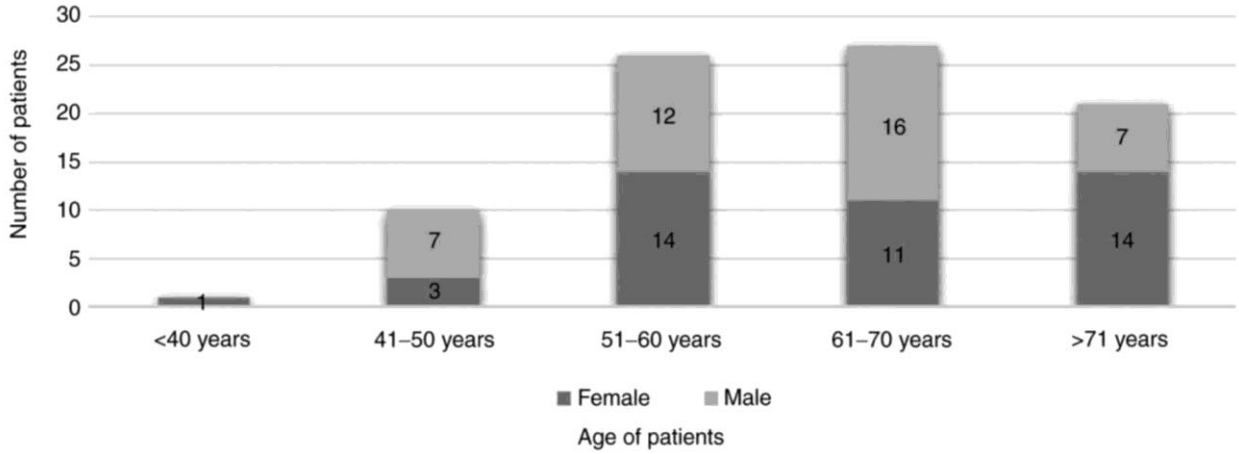


Fig.5 SARS COV-2 patient distribution by age and sex.

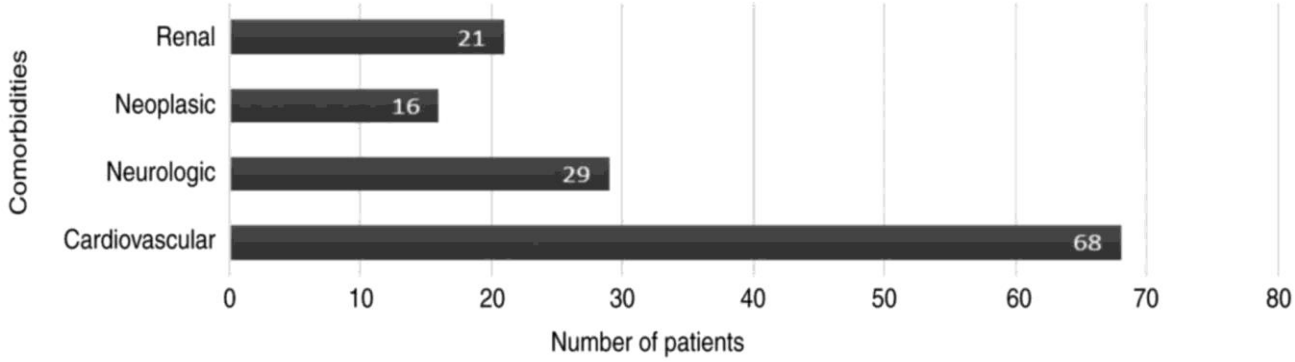


Fig.6 Other associated pathologies in SARS COV-2 DM patients. DM diabetes mellitus.

In addition, in all cases, chest CTs revealed lesions specific for the SARS COV-2 infection, described either as “ground-glass” opacities or “crazy paving” area patterns, linear densities or consolidations.

The treatment was given according to the international and national guidelines available at the time and included hydroxychloroquine (HCQ)/lopinavir + ritonavir/enoxaparin sodium/ Tocilizumab/antibiotherapy. Each patient received individualized treatment based on the clinical and paraclinical data, as well as taking into consideration medical interactions. During hospitalization, the management of associated cardiovascular, neurologic, neoplastic and renal comorbidities was ensured, alongside antibiotic treatment to address other types of infectious diseases whenever present, including urinary tract infections, sepsis, cellulitis, and *Clostridium difficile* infection. In addition, psychological therapy was performed upon request [Manciuc et al., 2014; Manciuc et al., 2016].

Regarding the paraclinical data of patients with DM, glycemic levels over 200 mg/dl were noticed in all the cases at admition and a median glycated hemoglobin level of 7.2% was calculated.

We studied two groups of patients admitted to the ICU. In group A we included patients without DM (61 patients) and in group B, patients with DM (17 patients). The fatality rate among the patients from group B was considerably higher (88,23%, 15 patients) than of those from group B (59.01%, 36 patients) (Figure 7).

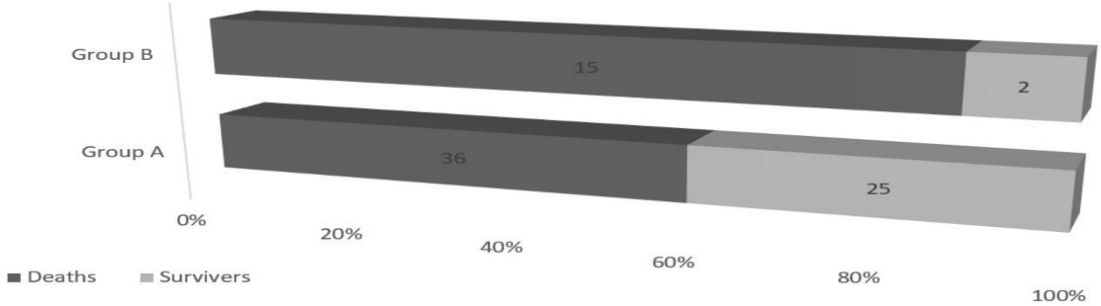


Fig.7 Distribution of the number of deaths and survivors with DM and without DM in SARS-CoV-2 patients admitted to the ICU. Group A, patients without DM; Group B, patients with DM.

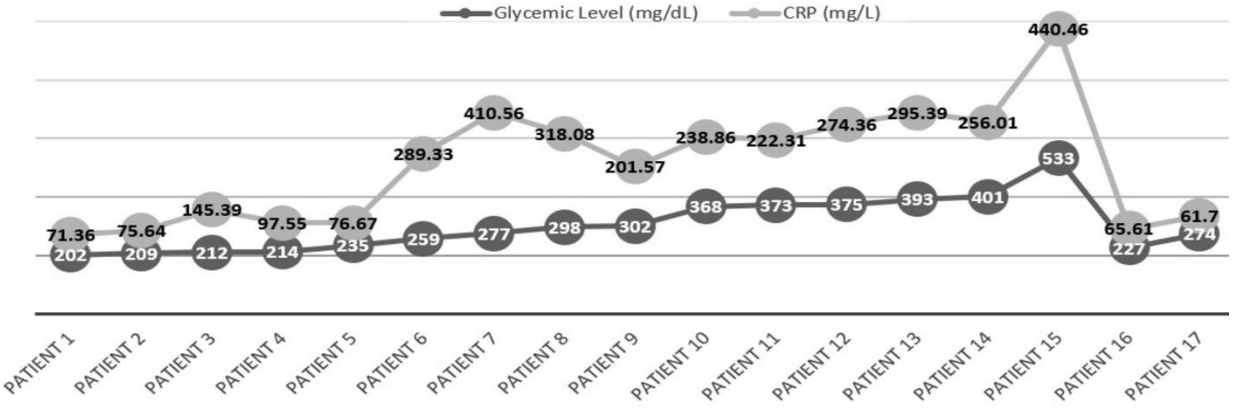


Fig.8 Association between the glycemic level and CRP in SARS-CoV-2 patients with DM admitted to the ICU.

Regarding the glycemic levels of the patients with DM admitted to the ICU correlated with the level of C reactive protein (CRP), it was noticed that CRP levels >70 mg/l were encountered in the patients that had a bad outcome. Patients admitted to the ICU with a CRP <70 mg/l (patient 16 and 17) survived and were later transferred to the clinic (Figure 8).

I.3.5 Discussion

The latest studies from the scientific literature have been revealing a noteworthy association between increased mortality and morbidity in patients with SARS COV-2 and advanced age, severe obesity (BMI ≥40 kg/m2), hypertension and DM as underlying diseases [Guan WJ et al., 2020; Yang X et al., 2020; Onder G et al., 2020; Centers for Disease Control and Prevention: National Diabetes Statistics Report, 2020; Yang J et al., 2020]. In the general population, the prevalence of DM is 8.5% [Sarwar N et al., 2020] and among the admitted patients considered for the present study it was 7.87%. In addition, the literature states that, elder patients are more affected by DM and often by other comorbidities, especially after the age of 60 [Chentli F et al., 2020; Davis JW et al., 2020; Tyrovolas S et al., 2020], a fact that can also be sustained by the present study in which >50% of the patients (56.47%) were >60 years old. If we take into consideration that hypertension, as well as cardiovascular disease are prevalent in DM patients, it is unclear whether DM independently contributes to this increased risk. However, diabetes has already been frequently reported to be associated with poor prognosis in other respiratory viral infections, mainly seasonal influenza, pandemic influenza A H1N1, SARS, and MERS [Hong KW et al., 2020; Schoen K et al., 2020].

In March 2020, an Italian health institute reported 2,003 patients that succumbed from SARS-

COV-2 infection [Istituto Superiore di Sanita, 2020]. Their median age was 80.5, which was considerably higher than the median age 67.84 of the patients included in the present study. In addition, the same study stated that the prevalence of diabetes was 35.5% and that 70% of the total number of patients were men [Kohio HP et al., 2013] while in our study gender differences were negligible (49.41% patients were men). Furthermore, in the largest case series reported by the Chinese Center for Disease Control and Prevention, performed on 72,314 cases of COVID-19, the patients with DM had a higher mortality (7.3% in DM vs. 2.3% overall) [Yang X et al., 2020].

Moutschen and Knapp acknowledge that poorly controlled diabetes inhibits lymphocyte proliferation, and also modifies the functions of neutrophils and monocytes/macrophages [Moutschen et al., 2020, Knapp et al., 2013]. Studies [Reading PC et al., 1988; Ilyas Ret al., 2011] performed in vitro demonstrated that pulmonary epithelial cells respond to high glucose levels by significantly facilitating the replication of influenza virus. This indicates that hyperglycemia may contribute to increased viral replication in vivo [Kohio HP et al., 2013], which can also apply to the case of the SARS COV-2 virus.

Furthermore, endothelial dysfunction and increased platelet aggregation have been associated with type 2 DM and insulin resistance. These are flaws which support the development of a hypercoagulable pro-thrombotic state [Dunn EJ et al., 2005]. Last but not least, patients with diabetes have also been revealed to have diminished forced vital capacity (FVC) and forced expiratory volume in one second (FEV1), which is associated with increased plasma glucose levels [Lange P et al., 1989]). This may explain the increased number of patients that required ICU therapy (42.35%) and also the increased fatality rate of the patients with underlying DM.

Multiple studies [Chen N et al., 2020; Chen T et al., 2020; Gao Y et al., 2020; Williams R et al., 2020) have determined the serum concentration of CRP in patients with COVID-19 and the results revealed that increased levels of CRP were observed in up to 86% of severe COVID-19 patients.

Increased levels of CRP were found among the patients with severe symptoms at the initial stage compared to patients who had mild symptoms [Chen T et al., 2020]. Luo observed in his study that patients who succumbed to COVID-19 had approximately 10-fold higher levels of CRP than the recovered patients [Luo X et al., 2020]. In the present study, the patients admitted to the ICU that succumbed had an increase of CRP value between 14-88-fold (71.36-440.46 mg/l).

I.3.6 Conclusions

In conclusion, uncontrolled DM appears to be a significant predictor of mortality, not only because of how it modifies the physiological mechanisms, but also by how it predisposes to multiple complications. However, even in the presence of advanced age and DM, 82.35% of the patients included in the study were cured. Careful assessment of the numerous components that contribute to poor prognosis of the patients with diabetes infected with SARS COV-2 virus may represent the best way to overcome the current situation.

Related articles

1. Iordan Ioana Florina**, Manciuc Carmen**. Double Problem – Clostridium Difficile and Diabetes. *J Phar Pharma* 2018; **6**: 169-173
2. Georgiana Alexandra Lacatusu, Ioana Hunea, Ioana Florina Iordan, Cristina Vasilescu, Oana Stamateanu, **Carmen Manciuc** - Risk Factors Associated with Recurrence of Clostridioides Difficile Infection. *Rev Med Chir Soc Med Nat Iasi* 2020; **124**: 200-206
3. Prisecariu LJ, Dorobăţ C**, Manciuc Carmen**, Nicolau I. Metabolic syndrome in HIV-infected patients with favorable response to antiretroviral therapy. *Rev Med Chir Soc Med Nat Iaşi*. 2011; **115**(3):776-780

I.4 IS SARS COV-2 INFECTION INFLUENCED BY ENVIRONMENTAL CHARACTERISTICS?

I.4.1 Introduction

Air conditioning devices are used on daily bases not only in personal homes, but also in healthcare facilities.

The pandemic caused by the SARS COV-2 was confirmed to have reached Romania on February 26, 2020. At the time of the writing the article, in our country, the peak of pandemic was reached in April 11th, when 523 cases were diagnosed (Figure 9) [https://www.worldometers.info/coronavirus/ country/romania/]. The fatality rate as June 1st was calculated at 6.50% and in the last week the median number of deaths was 12.85 (Figure 10).

Considering climate changes that showed a continuously increasing temperature in the last five years in the months of August (Figure 11) [http://www.meteoromania.ro/clima/monitorizare-climatica/], and also that fact that we do not have the certainty that SARS COV-2 virus will naturally stop its circulation we took into consideration that there is a possibility that, as the aviary flu (H5N1 flu), SARS COV-2 could also be transmitted in the warm months.

Even though we are on a descending curb, the number of total diagnosed patients keeps rising. Considering that summer is right around the corner, and the temperatures in the last five years are increasing, we ask ourselves if cooling air devices could contribute to the spread of COVID-19 by aerosolizing, even more so, that some of them are old and have defective cooling systems, where blue-green algae could develop and outbreaks can start.

We took into consideration the similarity of the COVID-19 transmission with that of the legionellosis, which is caused mainly by *Legionella pneumophila* and thought of the aerosol transmission for community acquired infection which is transmitted by the buildings cooling towers [Walser et al., 2014]. Aerosol transmission refers to the possibility that fine aerosol particles, called droplet nuclei, remain airborne for prolonged periods of time and involves particles that are <5 μm [WHO, 2020].

Legionella pnuemophila is a common cause for community and hospital-acquired pneumonia [Herwaldt et al., 2018].

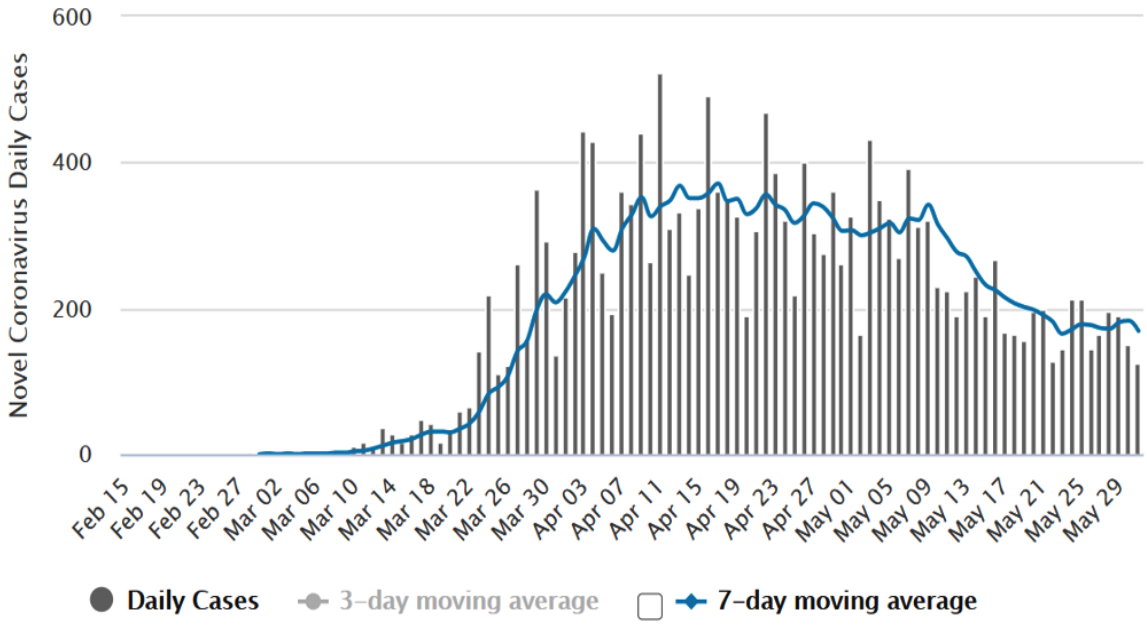


Fig.9 Daily cases of COVID-19 in Romania

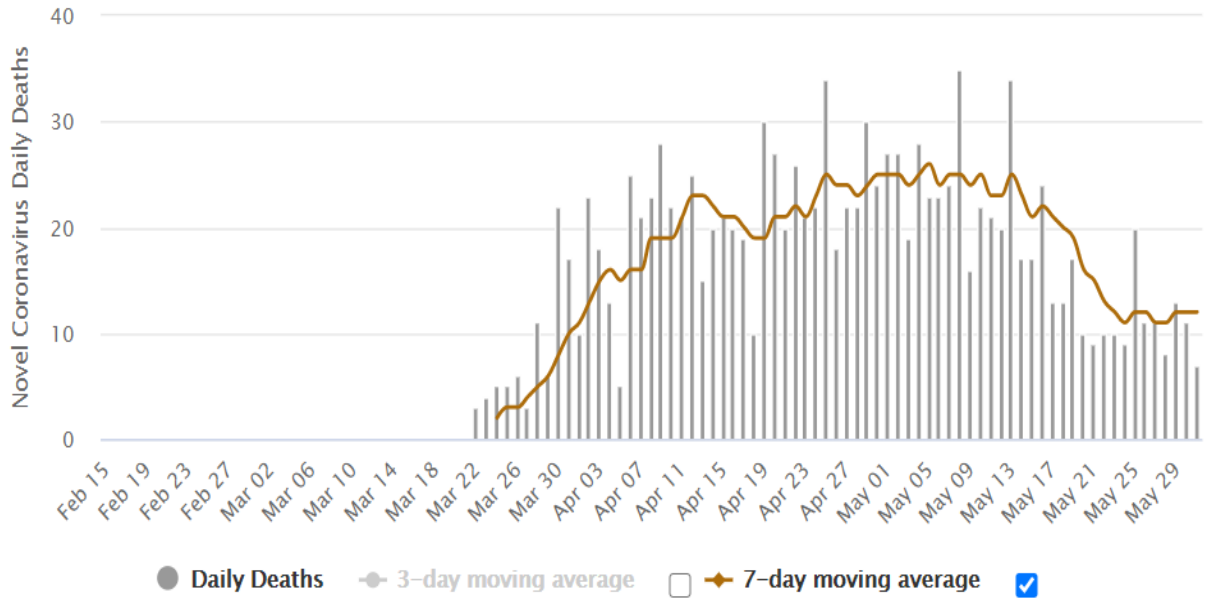


Fig.10 Daily deaths of COVID-19 in Romania

The main symptoms of the SARS COV-2 infection are fever, dry cough, fatigue and upper respiratory tract symptoms that include odynophagia, headaches, and myalgia. There are also reports of patients with gastrointestinal symptoms, including abdominal pain and diarrhea in children and adolescents. Patients with severe disease typically associate dyspnea and bilateral pulmonary infiltrates on chest imaging.

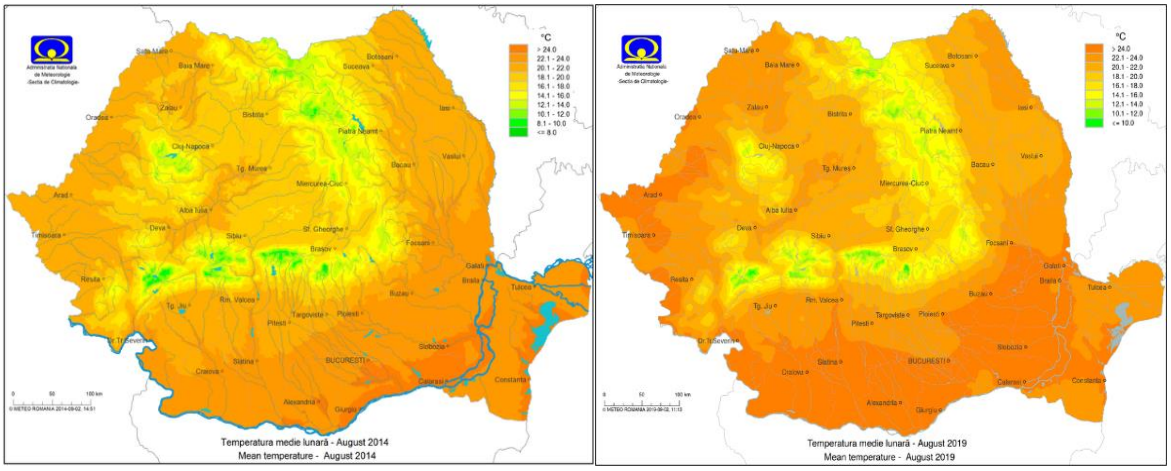


Fig.11 Temperatures in August 2014 vs. 2019

One of most severe complication of COVID-19 is the ARDS [Shi et al., 2020]. The clinical manifestations of *Legionella* infections are primarily respiratory. The most common presentation is that of an acute pneumonia, which varies in severity from mild illness to fatal multilobar pneumonia. [Manciuc et al., 2018]. Typically, patients have high, unremitting fever and cough, but do not produce much sputum. Extrapulmonary symptoms, such as headache, confusion, muscle aches and gastrointestinal disturbances, are also common [Winn et al., 2012; Manciuc et al., 2008].

Given the similarity of symptoms and also the fact that both can give pneumonia and ARDS, we sugest that there might be a parallel between the two diseases even though SARS COV-2 is a virus and *Legionella pneumophila* is a bacterium.

My interest in this field is reflected by the following paper:

1. Lacatusu GA, Vasilescu C, Mihai IF, Filip-Ciubotaru F, Vata A, **Manciuc Carmen**- COVID-19 And Air Conditioning - Is There an Environmental Link? *Environ Eng Manag J*. 2020;**19** (7);1255-1260

1.4.2 Aim of the study

Looking at the similarities between COVID-19 and *Legionella pneumophilla*, the aim of the study was to find if Heating, Ventilation and Air Conditioning (HVAC) Systems, including ductwork, to have a role in transmission of SARS COV-2 as is legionellosis.

I.4.3 Methods

A systematic literature reviews was conducted and PubMed/Medline, EMBASE and Google Scholar databases were used to identify potential articles. The following key-words: COVID-19, SARS COV-2, Air Conditioning, Ventilation, and HVAC were included in our search.

The study included any article that presented or excluded a link between the SARS COV-2 virus and air conditioning or ventilation systems. The articles that did not offer any information regarding the studied subject were excluded from the study.

I.4.4 Results

Poor ventilation in confined indoor spaces is associated with an increased transmission of respiratory infections [Knibbs et al., 2011]. There have been numerous COVID-19 transmission events associated with inclosed spaces, including some from presymptomatic cases [Rothe et al., 2020; Lu J et al., 2020].

Standard HVAC systems use filters that often have more than 1 micron pore diameter, and only in specific locations, such as intensive care units or isolation rooms have HEPA filters which are more efficient.

HVAC systems, which are used in healthcare facilities, are utilized as a primary measure for infection disease control by the diluting room air that is around the primary source. We looked at the idea that HVAC systems that are installed in healthcare facilities, may have a role in spreading a pathogen, and so, if they are not correctly used may contribute to the transmission of diseases as suggested in the past for SARS infection [Francisco PW et al., 2014; Li W et al., 2005; Qian H et al., 2018; Shajahan et al., 2019].

In the infection with SARS COV-2 virus, as in other diseases, there is a significant debate regarding the airborne transmissibility in the absence of physical contact and droplets [Pyankov OV et al., 2018]. Particles can have multiple trajectories, which are influenced by their size, environment conditions and also dissemination patterns. It is stated that for airborne transmitted infections, the particles transmission and floating time might be longer [Gameiro da Silva, 2020; Shajahan et al., 2019]. Studies have shownd that similar to MERS-CoV virus [Pyankov OV et al., 2018], the SARS-COV-2 virus has a viability in aerosols for at least three hours and also, the virus remains stable on stainless steel and plastic for up to 72 h which makes it plausible to have easy aerosol transmission [Shiu EYC et al., 2019]. It has been demonstrated that ventilation systems are a way of transmission/spreading infectious diseases such as measles, tuberculosis, chickenpox, influenza, smallpox and SARS [Li et al., 2007; Shiu et al., 2019].

One of the earliest studies addressing the way of transmission of the SARS COV-2 virus, which looked at certain indicators of airborne viral spread in a Wuhan hospital, where patients with COVID-19 were kept in isolation, viral RNA was still detected in areas of the hospital that it could only have reached through the atmosphere or the ventilation system [Liu Y et al., 2020].

Federation of European Heating, Ventilation and Air Conditioning associations (REHVA), published an updated guide regarding the possibility of spreading the virus, which is informing professionals on how to use and operate building services in workplaces, in order to prevent the spread of COVID-19. The recommendations addressed are mainly to stop air recirculation and to increase the inflow of outdoor air [Kurnitski et al., 2020].

Some authors assessed if airborne transmission is possible when HVAC systems are not adequately used and may contribute to the transmission of the virus. These conclusions were drawn after reviewing studies that described events from Japan [Correia et al., 2020], Germany [Correia et al., 2020; Rothe et al., 2020], and the Diamond Princess Cruise Ship [Shajahan et al., 2019; Zhang S et al., 2020]. The same study is suggesting the possibility of the virus spreading through air

conditioning and concluded that droplet transmission was prompted by air-conditioned ventilation, which lead to an outbreak in a restaurant in China [Correia et al., 2020; Lu J et al., 2020].

Scientist consider that the spread of the virus among patients may be facilitated in spaces with no HEPA filters, via common ducts [Shakoor et al., 2015]. Data regarding the spread of airborne infectious particles, quickly and evenly, were revealed, after studying different ventilation configurations in specific areas, such as operating theatres, using computational fluid dynamic modeling [Memarzadeh et al., 2002].

There is a theoretical possibility, that aerosolized particles, which contain the viral RNA and enter the ventilation ducts found in healthcare facilities, are expulsed through ventilation system into the environment, thus contributing to further sporadic cases. The same mechanism was found in aerosolisation of *Legionella pneumophila* [Yu ITS et al., 2004].

Another study, that assess the potential for transmission of the COVID-19 outbreak that unfolded aboard, the Diamond Princess Ship in January–February 2020, states that overall mean reproduction number in the confined setting reached values as high as ~11. This is higher than mean estimates reported from community-level transmission dynamics in China and Singapore in the range 1.1–7. It concluded that the main route for transmission was person-to-person but other routes should not be neglected, such as aerosol transmission via central air supply or drainage systems [Zhang W et al., 2020].

There are missing links in transmission and possible unknown indirect infection routes in many epidemiological studies. Pure aerosol transmission is denied by WHO but several pieces of evidence support this hypothesis [Correia et al., 2020].

I.4.4 Conclusions

There are no clear lines that can confirm if there is a link between aerosols produced by HVAC system and transmission of SARS COV-2 virus but it is important to follow this type of spreading as environmental contamination factor especially that literature showed that could be an important mean of transmitting as proved by legionellosis.

Further extensive studies can confirm or infirm, this type of spread, which will further help the battle against the pandemic.

Related articles

1. **Carmen Manciuc**, Carmen Dorobăţ, Constantin Luca, Mihai Nicu Leptospirosis: Clinical and environmental aspects of the Iaşi County. 2007; *Environ Eng Manag J* 2007; **6** (2):133-136

2. C. Luca, C. Dorobat, **Carmen Corcaci**, R. Scurtu, V. Luca Leptospiroza- aspecte clinico - biologice si terapeutice in 256 cazuri. *Rev Med Chir Soc Med Nat Iasi* 2002; **2**:352-56

3. **Doina Carmen Manciuc**, Ioana Florina Iordan, Anca Maria Adavidoaiei, Maria Alexandra Largu - Risks of leptospirosis linked to living and working environments. *Environ Eng Manag J* 2018; **17**(3): 1004-1006.

Instead of conclusions

Today, February 26th, 2021, we "celebrate" one year since the diagnosis of the first case of SARS COV-2 infection in Romania. The first case was identified in a 25-year-old man from Bucharest, who was cured. Unfortunately, he re-infected in November 2020, but he recovered again.

For our hospital, the 4th of March remains in our memory because of 2 unfortunate events. The first one is the earthquake of 1977 and the second one because on that date we hospitalized the first patient with SARS COV-2 infection in Iași. The infection was identified in a 71-year-old patient with multiple myeloma who presented with anosmia, ageusia, mental and neurological impairment with negativism and temporo-spatial disorientation. Later, he developed acute respiratory failure, which determined his transfer in the ICU. The patient was cured and discharged, being a success at that time.

On March 16th Romania started the first lockdown, which had as main purpose infection control. With the constant increase of cases, the only weapons against this virus at that time, were physical distancing, hygiene and mandatory use of a mask.

In October 2020 the 2nd wave followed, that brought a large number of deaths, with months were the ICU departments were insufficient, with over 1100 critic cases and increased number of deaths, compared to any other airborne infections, (e.g. influenza). The medical personal became overworked and started to struggle because of the physical and mental exhaustion, but continues to remain on duty. In March 2021 we face the 3rd wave of SARS COV-2 infection, and now the main threat are the new strains that seem to become dominant. The fact that we know about viral fitness of SARS COV-2 raises the question if there are similarity between SARS COV-2 and HIV, and the main concern is that SARS COV-2, as HIV, could develop resistance to already used antivirals.

First vaccines were produced by Pfizer and Moderna and requires a 2nd dose at 2, respectively at 28 days. Storage of these vaccines requires special conditions and are stable up to five days at low temperatures.

Regarding the side effects of the vaccine, the vast majority were mild and include pain at the injection site, chills, low grade fever etc.

On 22nd of February, Johnson & Johnson receives the approval as a single dose vaccine that in contrast with the first two already mentioned. It is much more stable and can be stored at fridge temperature.

There were countries that accelerated the vaccination program, because they wanted to waiver the lockdown measures and restart normal life, as was the case of Israel.

Romania also aligned with the efforts regarding vaccination. In our country, vaccination was designed in 3 stages and started in December 27th. In the first stage, the medical workers were vaccinated, in the second one the population over 65 of age and the patients with important comorbidities and in the last one the general population.

Right now, we are facing the third wave, that brings only uncertainties and questions. The main concerns are:

1. Is the vaccine capable of protecting against new strains?
2. Do we have enough therapeutic arsenal? Will it be compromised in the future as it happened with the first drugs used in HIV treatment?
3. Will this disease become a chronic one?

And multiple other questions keep arising.

CHAPTER II. HIV INFECTION

II.1 STATE OF THE ART

Medical knowledge regarding the HIV (Human immunodeficiency virus) infection has shown that in over 70% of cases there is transmission between species (human to animal) by crossing the genetic barrier from primates to homo-sapiens. The most plausible routes for this cross-species transmission, which initially involved simian retroviruses, is the exposure of humans to infected blood or tissue. Looking back, the first diagnosis of the HIV-1 infection was in 1959 in Kinshasa, Congo from a serum specimen [De Cock et al., 2008].

Retroviruses are a large family of enveloped viruses which can infect all classes of vertebrates. The coding of the genetic information being performed by RNA. The characteristic feature of this family is represented by the way of replication (Figure 12) [Coffin JM, 1997], which includes as a fundamental step the revers transcription into the double-stranded DNA of the RNA virion followed by the integration into the cell genome. This opposite direction transmission from RNA to DNA is also linked to the name (Retroviruses) and this contradicts an old law of molecular biology.

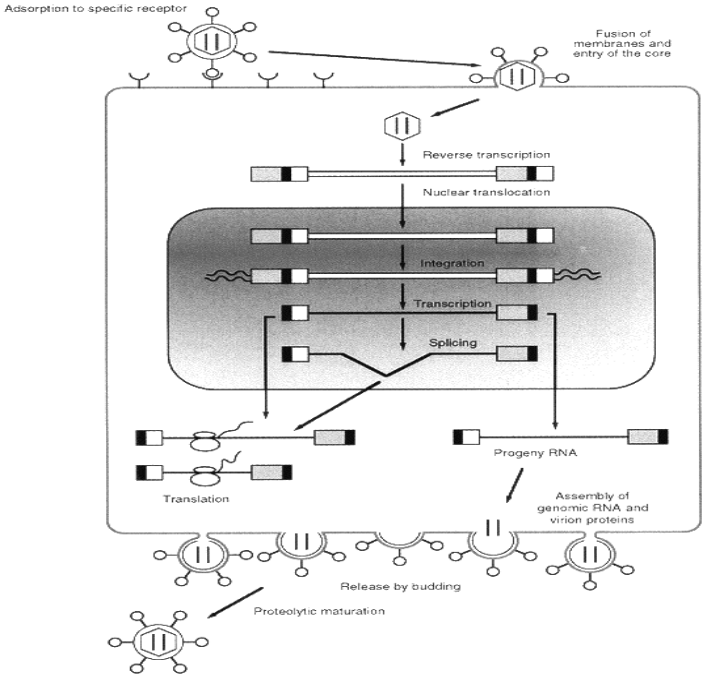


Fig.12 Retroviral replication cycle

Retroviridae family includes:

- *Oncoviridae;*
- *Lentiviridae;*
- *Spumaviridae;*

The oncoviruses are type-1 lymphotropic viruses (HTLV-1) which have been associated with adult T-cell.

Lentiviruses are a subfamily of retroviruses that have as a main characteristic a long incubation period, between the moment of infection until the clinical manifestation of the disease. The most widely known virus that is a part of this family is HIV.

Spumaviruses cause immunodeficiency in cats and by this time, no cross-species infection to humans was founded.

The understanding of retroviruses had revolutionized the way we perceive molecular carcinogenesis, making a great impact on molecular genetics.

The discovery of the way that retroviruses modfy the structure and the function of the host cell and how they activate specific genes that generate genetic recombination or mutation was an important breakthrough for the scientists.

One of the most important characteristics for retroviruses is the fact that they have an additional mechanism, which includes a malignant transformation, that leads to the determinism of an immunodeficiency status with dramatic consequences for the human that include the acquisition of opportunistic infection and carcinogenesis.

At the beginning of 1980, the medical world was facing a great challenge presented by the discovery of an infection with a retrovirus, which presented with problems regarding the short-term evolution. This infection was predisposing to major infections and the survival period after contacting the disease was up to 3 months.

The discovery of the virus was disputed between 2 great virologists Robert Gallo and Luc Montagnier, the last one receiving the Nobel prize for the discovery of the now known HIV.

The infection was described for the first time in 1981 in MSM (men who have sex with men) communities from USA (New York, San Francisco, Los Angeles) when a large number of young men presented with unusual opportunistic infections and rare malignancies. In the same year doctors diagnosed in these communities first cases of Kaposi's sarcoma and *Pneumocystis carinii* (now known as *Pneumocystis jiroveci*) pneumonia [Greene WC et al., 2018].

In 1983 a team of scientist from Pasteur Institute located in France, conducted by Doctor Luc Montagnier, reported that they have isolated a new T-lymphotropic virus, which was the outline of HIV-1, that conducted to the discovery of AIDS.

Regarding the treatment for this new virus, in 1987 Food and Drug Administration (FDA) approved as treatment for HIV the first drug that had a proven efficacy in the fight against HIV, which was Azidothimydina or Zidovudine (AZT) discovered in 1964 and used until then as antimycotic.

In December 1987, a total of 71715 cases of AIDS (Acquired immunodeficiency syndrome) were reported to World Health Organization (WHO) (over 40000 originated form USA). In the same year, WHO estimates that the total number of persons infected with HIV is around 3-5 million.

Scientists discovered that the SIV existed and infected primates for at least 32.000 years, which means that this virus has not been in contact with humans for thousands of years. Multiple theories were put forward regarding the crossing between species of the virus, but according to some data, it seems that the passage from primates to humans happened in Africa between 1924-1928 [Streinu Cercel A et al., 2018].

The epidemic extended until 1981, when the first patient was diagnosed. Until then, HIV spread in at least 5 continents which included North-America, South-America, Europe, Africa and Australia. During that time, the uncontrolled extension of the virus, which implied the immunodeficiency caused mainly HIV-1 virus, was encountered predominantly in West Africa.

In June 1981, after the discovery of *Pneumocystis carinii* (now known as *Pneumocystis jiroveci*) pneumonia in MSM communities, CSC Atlanta creates an operative group which had as main purpose the identification of the factors responsible for those new and severe opportunistic infections and also the main causes of malignancies with rapid and infaust evolution. Six months later, the first cases of AIDS were described on intravenous drugs users in Great Britain.

The team of doctors from Pasteur institute, lead by Dr. Luc Montagnier, reported, in 1983, that they have isolated a new virus, which may be the ethiological agent of AIDS. After a short period of time, Robert Gallo makes the same finding, but the paternity of discovery of the virus remains at Pasteur Institute.

Important personalities and non-profit organization, all over the world, implicated in the fight against AIDS. World renowned individuals from political life, like Princess Diana and Margaret Thatcher take a stand in the fight against AIDS. On the other hand, celebrities like Freddy Mercury and Magic Johnson were tested positive.

The first commercial HIV diagnostic test was available in 1985 and was detecting the antibodies from the blood.

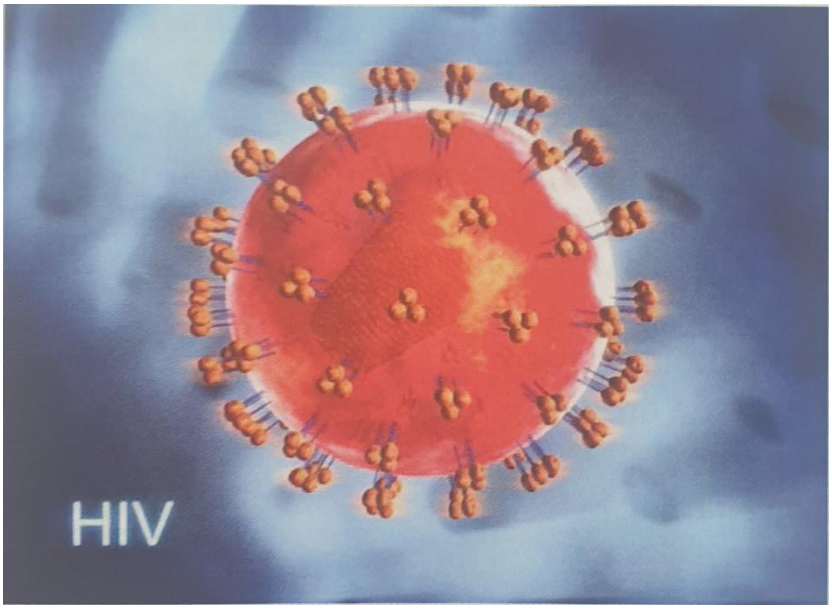


Fig.13 HIV-1 (the most common subtype) HIV-2 (dominant in West Africa)

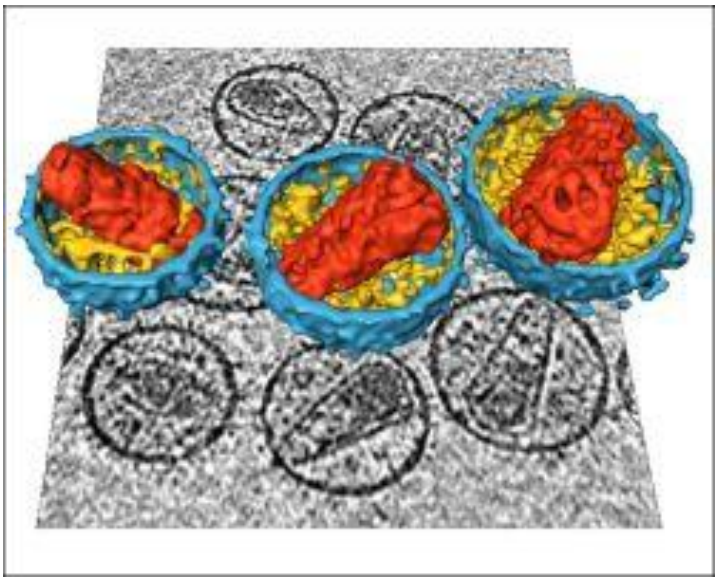


Fig.14 First 3D image of HIV

In 1992 WHO sets, as it`s primary objective, to offer the beginning of 2000, access to the population at risk from Africa and Asia to condoms, which were the most effective means of prevention for transmission of the HIV.

Unfortunately, in 1993 the first cases of AZT resistance of HIV were described and also, a new study (ACTCG 076) exhibits important data and proves the existence of mother to child transmission. The same study presents that AZT could reduce the risk of transmission if it is administered as prophylaxis.

At the beginning of 1995 WHO reports almost 18 million HIV infected adults and 1.5 million children from all around the world. Also, at the same time, WHO informs that over a million people are diagnosed with AIDS.

Over time, multiple molecules have proven their efficacy, especially considering that scientists were understanding more and more the physiopathogenic mechanism of HIV and his way of attack over LyT. The studies showed that the association of different antivirals, which blocked the key points where the virus could enter the cell, had a huge success and represented the first step to the now HAART (highly active antiretroviral therapy).

The infection spreads all around the world, even though authorities, doctors and researchers have been working together towards stopping the diseases. Is has become a pandemic with dramatic numbers in some areas (in 2003 Botswana declares a 38,8% prevalence of HIV infection, the highest from the world).

World's billionaires, like Bill Gates and Warren Buffet, were involved in the fight against AIDS and help the production of coformulation 3 types of ARV (antiretrovirals) drugs in one pill – called Atipla, which increased the adherence and compliance to treatment of HIV positive patients.

World leaders like Barack Obama promised, in their electoral campaign, an extensive program of support and medical access for HIV positive patients. Also, the Catholic Church, trough Papa Benedict is implicating in the fight against HIV urging on the use of condoms.

From the discovery of the virus, until now, this disease has presented, not only from the medical point of view, but also the economic, social and psychological one. At present, the psychological aspect is one of the most pressing one, considering not only the individual or couple psychology, but also the society point of view, which has a tendency to stigmatizes and isolate the HIV positive individual.

Since the discovery of the virus, one of the primary interests was the production of a vaccine, which even today, seems to be impossible, because of the continuous mutation that the virus expresses.

The history of HIV infection is still ongoing, being written under our own eyes. Up until this day, is still considered a pandemic that affects the quality of life and implicates socio-political and medical factors which require support from the society and also global efforts in stopping it.

History of HIV in Romania

In 1989, hundreds of children were diagnosed with HIV in Romania, and according to statistics from 1990, more than half of HIV infected children from the world originated from Romania. The main cause of this exponential increased number of cases in the 1990 were the major errors in the medical procedures, not only in outpatient departaments, but also in the primary health care facilities, specialized units and wards, especially those that were treating pediatric patients.

Regarding the transmission of HIV infection, we have to mention that the extension of the virus was almost exclusively by transfusion and the use of incorrect sterilized materials.

The introduction of the virus in Romania was performed by men that travelled in the affected areas. The transmission of the disease between the adult populations was predominantly through sexual contact (herosexual or bisexual). The first case of a HIV infected adult in Romania was diagnosed at a MSM person, which was a night train attendant.

At present, in our country, the infection affects all ages (from adolescents to elder people). Also, we want to highlight the increased numbers of female infected patients, which are of fertile age, because they raise an issue from the perspective of mother to-child transmission and therefore, without an appropriate prophylaxis can increase the number of HIV infected newborns.

In Romania, the infection has a particularity, considering that it was acquired as a nosocomial infection at the end of the XIX century and aimed especially the institutionalized children, which were born between 1989-1990. The lack of decent living conditions and the absence of equipment that could efficiently sterilize medical ustensiles, together with the absence of single use materials, lead to the biggest medical drama of that generation, which at present is known as the "pediatric cohort" [Kozinetz et al., 2001].

In the last decade of the XX century, over 90% of the cases of HIV infected patients in Romania were children under the age of 14.

These patients were infected in the first year of life, and the first questions were raised in 1990, when children presented with severe infections and different clinic-biologic statuses (suggestive for HIV infection) which later was recognized by the literature. These presentations of the disease brought the attention to an immunosuppression status which was unexplained at that moment. Those were:

- Hemorrhagic varicella;

- Varricelised shingles;
- Systemic salmonellosis etc.;

The HIV infected children were born between 1997 – 1990 and came from orphanages predominantlly located in Bucharest and Constanta [Serban IG et al., 2014].

After the discovery of the human immunodeficiency virus and also correlated with the new democratic regime that replaced communism, important figures from the medical and academic community drew attention to this pathology and gave a definition of HIV infection for these children.

At the beginning of the 1990, some data estimated that the total number of HIV infected pediatric patients was between 10.000-12.000 children. The evolution of the disease in these patients had depended a lot on the availability, on the market, of medication that offered new molecules. Romania soon joined the newest therapeutic guidelines. Through this and also because of a good adherence and compliance to the treatment, those children are now young adults which had a normal life and have families and children of their own.

For a better monitorization, the patients were divided in regional centers, according to their area of residence, where a doctor kept track of their clinical, biological and viro-immunological evolution and also their adherence and compliance to the medication [Buzducea et al., 2010].

Support groups, foundation and non-governmental organization were soon set up. They upported the fight against HIV, and some of them are still today the primary line of support.

The approach for a possible HIV positive patient or of an already diagnosed patient implies a multidisciplinary team. This includes an infectious disease doctor, a psychologist, an epidemiologist and a social worker. The main reason for the necessity of this small army of people is represented not only by the medical aspects of the disease, but also because of the socio-economic one.

Through the national program, patients have access to HAART therapy, which include latest generation molecules, and offers the possibility to create a personalized therapeutic profile according to the viro-immunological and resistance profile of every patient [Streinu-Cercel et al., 2019].

This type of holistic approach ensured long term survival of more than 50% of the HIV infected patients born between 1987-1990, also known as the “pediatric cohort”, patients that up until these days are still followed up according to protocol.

Unfortunately, Romania has seen a rise in the other means of transmission, which are represented by the use of intravenous drugs and sexual transmission, and left behind the iatrogenic path, which was the primary rout up until 1990.

Regarding the vertical transmission, up until now, we have a good control in the entire country, mainly because we acceded to standard chemoprophylaxis of the new-born and specific therapy in association with a very close-up monitoring of HIV infected mother by the infectious disease and obstetrics specialists.

The future of HIV infection treatment and HIV infected patients will bring, on one hand, an easier integration of HIV positive patients, with a minimal discrimination and social bullying, and on the other hand, an easier ARV therapy which incorporated new co-formulated drugs, which are easier to administer. This will increase the level of adherence and compliance, especially if eventually, one dose per week/month will be enough.

HIV epidemiology

The main route of the HIV transmission is represented by horizontal transmission, when the virus is exposed to mucosal surfaces (generally from unprotected sexual contact) or from percutaneous inoculation trough tattoos, piercings, or directly from infected needles that are used by intravenous drug users. Another way that HIV can be contacted, is represented by transfusion of HIV infected blood, which right now is almost impossible, taking into account that the donated blood is very well controlled.

Vertical transmission, refers to the transmission of the virus from the infected mother to her child, which usually happens in the perinatal period. Multiple maternal criteria, that include a low CD4+ lymphocyte count, advanced clinical stage and a high viral load, were implicated in increasing

the risk for vertical transmission. Also, breastfeeding is a risk factor for transmission of the HIV infection. This is not because the breastmilk contains a lower viral load, but mainly because in the course of breastfeeding the newborn can hurt the areola and by extension, the newborn enters in direct contact with the infected blood of his mother.

HIV pathophysiology

The human immunodeficiency virus is grouped to the genus *Lentivirus* that is part of the *Retroviridae* family, subfamily *Orthoretrovirinae*. According to genetic characteristics and taking into account the differences between the viral antigens, HIV is classified into type 1 (with a world-wide distribution) and type 2 (encountered especially in the occidental Africa).

HIV targets T lymphocyte (LyT) and the CD4 receptors that are presented by LyT alongside CCR4 and CCR5 receptors.

The multiplication of HIV in LyT follows different steps:

1. Recognition of CD4 positive cells and attachment to CD4 receptors on the host cell;
2. Fusion of the virus with cellular membranes that allows the translocation of the viral capsid into cytoplasm;
3. Reverse transcription – mediated by reverse transcriptase (RT) that allows HIV (which is an ARN virus) to replicate by transcribing the single-strand HIV RNA genome into DNA;
4. Viral DNA is integrated in the host cell genome with the help of integrase which inserts at random the proviral genome into the host cell genome;
5. When the integration is complete, host cell genome produces new components and molecules of RNA of proviral genome that are synthetized and assembled in cellular cytoplasm that is replicated alongside with the host genome in cellular division.

There are three main types of genes that code the proteic precursors *env*, *gag*, *pol* together with other regulatory genes.

The viral replication has as its main center of multiplication in the lymph nodes, with an exponential production rate through all the natural production, starting from the stage of acute infection until AIDS.

Before the discovery of the ubiquitin proteosome system, the protein breakdown of the cells was mainly linked by lysosomes.

First studies that focused on intracellular modification and are the pillars of ubiquitin-proteosome system were published at the beginning of 8th decade of the XX century. In 2004 jointly to Aaron Ciechanover, Avram Hershko and Irwin Rose received the Nobel prize for the discovery of ubiquitin-mediated protein degradation.

In 1975, findings from electronic microscopy proved the circular structure of the proteosome, information that crystallography could not sustain up until 1994 [Löwe et al., 1995].

In 2018 the first proteosome 26 S human holoenzyme anatomic structure, part of a poroteosomic complex was highlighted, which helped understand the degradation mechanism of human 26S [Dong Y et al., 2020].

These processes which happens at a cellular level are important and are part of the proteolysis process, in which the beta subunit of the particle 20S is involved, in apoptosis, by increasing the response of the ubuiquine, E1, E2, E3 enzymes to cellular stress.

The ubiquitin proteosome system in the HIV infection might have the same importance and this system could be used as idea in future scientific research.

The idea started from the fact that the ubiquitin proteosome system is implicated in the physiopathology of plurilateral diseases such as Parkinson and Alzheimer and also in carcinogenesis that could be influenced by HIV infection. The first proteosome inhibitor was created by big pharmacy corporation and was used as chemotherapy agent in multiple myeloma [Fisher RI et al., 2016].

Clinic of HIV

In the beginning, the HIV infection was considered an acute infection with infaust prognostic, but after the use of ARV therapy it became a chronical illness. HAART therapy brought a new perspective regarding the survival rate for HIV positive patients that can have a life expectancy equal to that of the general population, if they adhere and are compliant to the treatment.

The natural history of the HIV infection according to clinical presentation, has 3 stages:

1. HIV acute infection – usually develops between 2-4 weeks from the moment of contact with the virus. Clinically, the symptoms are nonspecific with flu-like symptoms, accompanied by headaches, fever, dysphagia and non-specific cutaneous eruption. This association of non-specific symptoms is called cute retroviral syndrome.

Serologically this stage represents the moment of seroconversion, when the organism develops antibodies against HIV. During this timeframe, the viraemia is very high, which means that the patient is extremely contagious.

2. 2nd stage – clinical latency

During this stage, the patient is asymptomatic, but the destruction of LyT-CD4 continues at cellular level. This stage can last up to 8-10 years, when HIV infection becomes chronic, and the multiplication of virus continues.

3. **3rd stage – Symptomatic chronic stage**

This stage is associated with the development of signs and symptom of the HIV infection that includes weight loss, fatigue, generalized adenopathy, cough, severe diarrhea, recurrent bacterial infection, opportunistic infections and carcinogenesis.

In this phase, the viral load, CD4 count and the presence of any opportunistic infection (eg. Tuberculosis) can be indicators of AIDS. At this point, CD4 count is under 200cel/mmc and the presence of any opportunistic infection (which may include tuberculosis) places the patient in AIDS stage.

Stages of HIV

CDC Atlanta classification of the HIV stages (Table V) is one of the most useful instruments, not only from the clinical point of view but also is applicable by public health professionals and used in disease reporting and surveillance, epidemiologic studies, prevention and control activities, and public health policy and planning [Institute of Medicine, 1988].

Table V - HIV infection stage, based on age-specific CD4+T-lymphocyte count or CD4+ T-lymphocyte percentage of total lymphocytes

	Age on date of CD4 T-lymphocyte test					
	<1 year		1—5 years		6 years through adult	
Stage*	Cells/μL	%	Cells/μL	%	Cells/μL	%
1	≥1,500	≥34	≥1,000	≥30	≥500	≥26
2	750—1,499	26—33	500—999	22—29	200—499	14—25
3	<750	<26	<500	<22	<200	<14

Tuberculosis

HIV infection represents a major risk factor for the progression of latent infection with *Mycobacterium tuberculosis* trough active tuberculosis.

The depletion of CD4 lymphocytes induced by immunosuppression caused by HIV conducts to a defective response of the organism against *Mycobacterium tuberculosis*. Active tuberculosis (TB) has an annual development rate between 5-12% on seropositive individuals with records of TB.

Mycobacterium tuberculosis infection, in all of its forms, (pulmonary, extrapulmonary or neuromeningeal) represents one of the most frequent opportunistic infection of HIV positive patients. In the North-East area of Romania, this complication is monitored in HIV-AIDS regional center from Iași which attends patients from all the 6 counties of Moldavia.

High viral load and low CD4 count, alongside with non-adherence and non-compliance of patients to the therapy, predisposed patients to emergence of TB, that always is one of the concerns. Prevention of terminal stages of AIDS and increasing the life expectancy of patients that attended the center through careful assessment was a constant focus for the medical professionals.

A dark moment for the medical history of our country, was the period between 1987 to1990, when a lot of children born were abandoned in hospitals. The majority of them presented hospitalism, malnutrition, rickets, and sever carential anemia. Because of this complication, some of the children required blood transfusions. We have to mention that in that time frame there was no rigorous control from the perspective of contamination with the less known Hepatitis B virus, or unknown viruses at that time like Hepatitis C virus and HIV. Over the course of time these children, that later became to be known as the "pediatric cohort", were diagnosed in the early stages of life (often between ages 5-6) with HIV infection acquired as healthcare associated infection.

Initially, the clinical presentation of the disease at these children was represented by severe dystrophies that could not be corrected under treatment, followed by recurrent pneumonias and bronchopneumonia, diarrheas caused by *Salmonella spp.*, sepsis with meningeal location, childhood diseases with severe presentation (e.g. Hemorrhagic varicella), and last but not least, infectious diseases that were supposed to be encountered in adults, like varicelloid herpes zoster. These severe forms of disease pointed to the fact that the children were severely immunosuppressed and raised multiple questions for medical specialists (Figure 15) [Mardarescu et al., 2019].

The initial reports approximated that over 12.000 children were iatrogenic infected with HIV and at the same time, the people responsible with managing the disastrous effects were proposing ambitious goals which include:

- Free latest generation ARV medication for the affected children;
- Increasing the life expectancy of those children with long term survival.

This goal was partially achieved, and in 2015 half of the patients monitored by the HIV-AIDS regional center form Iași were part of the "pediatric cohort". These children, are now young adults with the longest survival rate from Europe, being "poliexperimented" regarding every molecule used in the ARV therapy, and receiving up to 16 different therapeutic regimens.

The primary pillars for the survival of these patients were:

- Commitment of medical team;
- Access to ARV therapy;
- Multidisciplinary teams which included, alongside the medical professional, psychologists and social workers that created support groups and performed individual counselling. This helped increase the adherence and compliance of the patients to ARV therapy.

In the early phases, the medication of these children was administered by their carers, so the adherence and compliance were maximum. Once these children started growing, they realized the fact they have to take daily medication. This made them feel different from their friends and colleagues. At that moment, which coincide with the moment of puberty, they demanded explanation which was not entirely sufficient for all of them, so a part of them denied their disease, and rebelled against the protocol, with therapeutic abandonment, not only as a social manifesto but also as a personal manifesto to show their rebel personality.

This is how the first cases of immunosuppression caused by non-adherence and non-compliance started to appear, fact that later conducted to occurrence of Tuberculosis, including the neuro-meningeal one.

On the other hand, at that time, the incidence of tuberculosis in Romania was a national problem, with one of the highest incidence rates (23.3 at 100.000 persons) from Europe. Also, in the same time the first Multidrug resistant Tuberculosis cases were diagnosed, fact that made a huge impact over the

patients with already diagnosed TB. A small part of patients diagnosed with MDR (multidrug resistant) TB were patients that were already in AIDS stage.

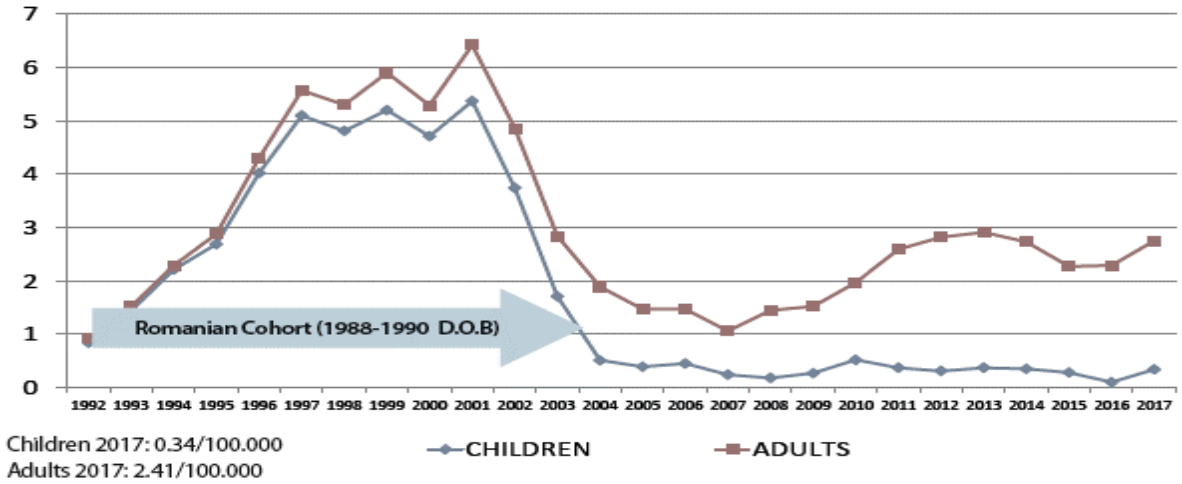


Fig.15 HIV Incidence Rate in Adults and Children Between 1985-2017

Mycobacterium tuberculosis infection has a long period of treatment, high potential to reactivate which can be related to the socio-economic status and requires a level of understating the necessity of adherence and compliance to treatment, not only for short periods but also for long term treatment, if required, for recovery in relapses.

The increased number of daily tablets necessary for treating TB infection adds up to the already high number of drugs used in controlling HIV infection fact that could, at that time, predispose the HIV positive patient to become non-adherent.

Tuberculosis treatment requires over 40 days of medication, with multiple follow-ups form the infectious disease specialist, who has to evaluate the clinical and paraclinical data. This includes the level of CD4 and viremia, in association with a pneumological consult that has to assess the clinical and radiological findings so it can evaluate the lung damage.

Tuberculosis Meningitis

TB meningitis (TBM) represents another clinical presentation of *Mycobacterium tuberculosis* infection, that is in fact a meningoencephalitis determined not only by Koch bacillus but also by atypical mycobacterium.

We emphasize that the evolution of TBM depends of an early diagnosis and a fast therapy to minimize the complications that are produced by encephalitis which creates cerebral hypoxemia and tuberculomas.

Traditionally TBM is suspected in patients that have a history of pulmonary tuberculosis or have a clear epidemiological context – poverty, low socio-economic status, living with a person that is diagnosed or is a suspect of TB. When a TBM case is diagnosed, epidemiological inquiries in the outbreak area can determine the source of infection and primary case followed by the close contacts or secondary cases form the community.

Considering that TBM is a severe pathology with a 100% mortality rate if not treated we considered a proper subject to investigate and draw attention of the medical community regarding this rare but serious pathology.

Usually, TBM has an insidious evolution in contrast with bacterial meningitis, and has a non-specific presentation that includes low-grade fever, fatigue, headaches, misconduct, encephalic signature up to comma. In presence of these symptoms, the infectious diseases doctor soul ALLWAYS take into account the possibility of TBM even if initially the patients have specific meningeal syndrome which later is accompanied by the clinical presentation that we previous mentioned.

The gold standard for diagnosing any type of meningitis is lumbar puncture, that offers valuable information for the infectious diseases specialist.

One of the fastest methods for an undeniable diagnosis of TBM is represented by PCR (Polymerase chain reaction) for *Mycobacterium tuberculosis*, which offers the result in the first 24 hours. The biggest advantage that this method offers is that once the result from PCR is positive there are no doubts regarding the ethiological agent of meningitis. Thus, the patient can start a specific tuberculostatic treatment from the first day of hospitalization. This gives the patient the highest chance of survival and to have the lowest neurological and psychological sequelae, compared with the times when diagnosis by PCR was not available.

This method is not always within the reach of all laboratory hospitals, especially ones not located in a university hospital, so the final diagnosis is based on the biochemical modification of CSF and cultures performed on special growth medium for Koch bacillus. Considering this, we have to mention that even in specialized laboratories the results take up to 21 days for the cultures and even then, the rate of a positive result is up to 20% of the time.

In the presented context, I have considered necessary the evaluation of a parameter that indicates the TBM infection. We considered all data that we gathered and in association with our clinical experience and laboratory parameter it seems that the most accurate element to suggest a TB etiology is the low level of glycorrhachia.

Regarding the complete diagnosis, we have to take into consideration an epidemiological context that can be found if we conduct a correct anamnesis which reveals the primary contact. To this, we have to associate the clinical, biological, and laboratory data that sustain the radiological evidence of TB images.

We highlight the importance of lumbar puncture and CSF (Cerebrospinal fluid) examination, performed after a CT scan which can show modification starting from discreet edema up to hydrocephalus with encephalic compression and ventricular dilatation.

The CSF in a TBM, is slightly hypertensive, clear, with a variable number of elements per cubic milliliter (mmc), but no more than 500. The main type of cells in CSF are lymphocytes which that are found at a value up to 90%. Sometimes, in the first days there is a discordance regarding the precent of cells with a predominance of neutrophils fact that can mislead the clinician. These variations oblige the clinician to repeat the lumbar puncture in 48-72 hours precisely to show the migration of neutrophils to lymphocytes.

Regarding the biochemistry of CSF, we can find:

- Increased level of albuminorachia, that initially exceeds 1 gram per liter, but in evolution, can increase up to a few grams per liter;
- Low level of chloride;
- Low level of glucose, that is much mor important compared with the one in bacterial meningitis;

Taking this data into consideration we want to draw attention to the medical community upon this verry important biochemical exam which ca be a good indicator of TBM.

Basically, considering that except PCR, which is not widely available, we do not have fast methods that can prove the ethiological agent, in association with a poor clinical presentation of the diseases that can mislead the specialist and produce confusion the biochemistry of CSF has to be the first indicative of TBM. If the diagnosis of TBM is delayed or even worse, not suspected, the prognosis of the patient is bad and can lead to death. When TBM is suspected, the clinician has the obligation to start the quadruple association tuberculostatic treatment within the first 10 days from the moment of onset.

Tuberculosis meningitis on HIV patients

In HIV infected patients the prognostic is more severe compared to an immunocompetent person, especially considering that, by default, *Mycobacterium tuberculosis* can cause generalized tuberculosis in a HIV positive patient.

The HIV positive patient has to be evaluated according to the WHO indication at least every 6 months or whenever necessary. An important feature of the infectious diseases doctor that is also trainedin HIV infection is the fact that he has to have a special psychological profile. He does not only have to create a doctor patient bond, but also empathize with his patient, wich will ensure the follow-up of the patient without harming his feelings.

This quadruple therapy of tuberculostatic, has to be initiated as soon as the suspicion of TBM arises, even if there is no PCR test to certify the infection.

If TBM is determined by atypical *Mycobacterium,* or multidrug resistant *Mycobacterium tuberculosis* the treatment can last up to 18 months.

If there are major contraindication for the first line of therapy for TB, either from the beginning, or which appeared over the course of treatment, the second line therapy is the one that is used. An important fact to mention is that all second line therapeutic agents are bacteriostatic.

If a HIV positive patient is diagnosed with TBM the first line tuberculostatic therapy is initiated for 10 days. The ARV therapy will start after another 10 days because we have to take into consideration the immune reconstruction syndrome, which can be fatal to a HIV positive patient because of its clinical presentation.

From the beginning, the use of corticosteroids, like Dexamethasone, for up to 21 days, is important. It has a dual role, one to minimize the inflammation of the haemato-meningeal barrier and the second one is the prevention of the immune reconstruction syndrome.

ARV treatment has to be chosen carefully, mainly because of the multiple interaction between first line tuberculostatic and some ARV. We insist over the differential diagnosis of TBM with decapitated bacterial meningitis, carcinomatous meningitis and viral meningitis, which are usually with a clear CSF.

Toxoplasmosis

In the HIV infection the differential diagnosis can also be made with herpetic meningitis and cerebral toxoplasmosis.

We were interested in the incidence of toxoplasmosis in pregnant woman, mainly because we wanted to have a better understanding, considering the consequences of this disease can have on the fetus, especially if the female becomes infected in the first trimester of the pregnancy.

Toxoplasmosis in HIV positive patients

In the HIV positive patient that is of fertile age, the evaluation regarding *T. gondii* infection at the moment of presentation, or at the moment when she expresses the intent to have a baby, is a must because toxoplasmosis is the most common central nervous system infection in patients with the acquired immunodeficiency syndrome who are not receiving appropriate prophylaxis. This is especially true considering that the literature highlights that cross contamination with other *T. gondii* strains can produce reinfection of the already immunocompromised organism, which can have catastrophic consequences on the fetus [Pomares C et al., 2018].

HIV opportunistic infection and sepsis

The most common opportunistic infections associated with HIV are:

- Herpes Zoster with severe presentation – generalized or varicelised;
- Herpes simplex – including the encephalitic syndrome;
- Recurrent candidiasis systemic or generalized or with *Candida krusei;*
- *Pneumocystis jiroveci* pneumonia with acute respiratory failure;
- Cerebral toxoplasmosis;
- Atypical Mycobacterium infection and *Mycobacterium tuberculosis;*
- Coinfection with Hepatitis B, C and delta virus.

Carcinogenesis

Another aspect of AIDS is represented by the sever depletion of CD4 Ly and one of the most common presentation is Kaposi sarcoma.

It seems that fragments from the viral genome of Herpes simplex 7 virus are an important factor in the development of the Kaposi sarcoma.

The main characteristic of HIV patients from Romania in 90`s decade is the fact that at that time the majority of them were children. They were diagnosed after they presented with severe forms of childhood diseases, or repeated infections, which drew attention to the possibility that they might be immunosuppressed.

The pediatric patients came from orphanages and presented with hospitalism, and they were infected trough contamination caused inappropriate use of non-sterile needles and syringes. These patients were malnourished, with growth deficit and were diagnosed with systemic salmonellosis that included meningeal presentation, pneumonias and bronchopneumonia. Most of these children were born between 1988-1990 and they were called the "pediatric cohort", which included over 12.000 cases.

Over time this cohort became biologically exhausted, caused by non-adherence and non-compliance, that produced an important immunosuppression that left them with no resources to fight against the latent infection (e.g. *Cryptococcosis neoformans* meningitis, TBM) and carcinogenesis.

In the meantime, the profile of the HIV positive patient changed, and now, the main characteristic of them is the fact they live their entire life with HIV and become poliexperimented. This means that they received all the therapeutic regimens, in the order of discovery, up until present, when they are the beneficiary of HAART therapy, which gives them the possibility to take up to 4 molecules in one tablet.

On a different note, these patients have the highest rate of survival and are now 30 years old young adults, that have passed through all of the challenges of adolescence, and are at their moment in life when they want to have a family and children of their own.

Despite pharmacological and scientific advances, at this moment, the number of patients from the HIV/AIDS regional center Iași, Romania, that make-up the "pediatric cohort" is equal to that of the patients that became infected through sexual contact or drug use.

Another category of patients is the late presenters that refused to come to a clinical evaluation or ignored the fact that they presented with symptoms that could indicate HIV infection. This is the case for some patients that were diagnosed after they had indicative signs of carcinogenesis.

Oncogenesis starts when the patients are immunosuppressed and often, they have a value of CD4 Ly that is under 200 cells per mmc. Some studies show that immunohistochemistry is positive for viral fragment of different herpetic virus (Table VI) that include *Herpes simplex* 7 and 8 [Rewane A et al., 2020; Luppi M. et al., 1996; Munawwar A et al., 2016].

The *Herpesviridae* family is a large family, that at the end of the XX century drew attention to new representatives that were implicated in carcinogenesis, lymphoma, nasopharyngeal cancer which is sustained by strong scientific evidences [Luppi M et al., 1996].

„*HHV-6 was isolated from the peripheral blood of patients with lymphomas and a possible role for this beta-herpesvirus in Hodgkin's disease and in angioimmunoblastic lymphadenopathy (AILD) has emerged from serological and molecular studies. HHV-7, a beta-herpesvirus genetically close to HHV-6, has not yet been found in a human disease but it utilizes CD4 as a receptor on the lymphocyte surface. Only partial HHV-8 genomic sequences have been identified so far, suggesting a genetic homology with members of the gamma-herpesvirus family, including EBV. HHV-8 sequences have been identified for the first time in all forms of Kaposi's sarcoma as well as in a variety of lymphoid disorders, including body-cavity-based non-Hodgkin's lymphomas, Castleman's disease, AILD and a type of HIV-negative reactive lymphadenopathy with peculiar histologic features*. „ [Luppi M et al., 1996].

Table VI - Mechanisms of interactions between human immunodeficiency virus-1 and coinfecting human herpesviruses

Mechanisms	Human herpesvirus
Immunoactivation	HSV, CMV, EBV
HIV-1 LTR transactivation	HSV, CMV, EBV, HHV-6, HHV-8
CD4, CCR5 or CXCR4 downregulation	HHV-6, HHV-7
Expression of virokines and viroceptors	CMV, HHV-6, HHV-7
Modulation of cytokine signaling	EBV
Inhibition of apoptosis	EBV, CMV
Aberrant activation of autologous complements	HHV-6, HHV-6
MHC downregulation	CMV, HHV-6, HHV-7

HSV: Herpes simplex virus, CMV: *Cytomegalovirus*, EBV: Epstein Barr virus, HHV: Human herpes virus, LTR: long terminal repeat, MHC: Major histocompatibility complex

Even though there is a big possibility that Kaposi sarcoma and non-Hodgkin lymphoma are pathologies that can suggest the HIV positive status, most of the times they present at HIV positive patients which are non-adherent and non-compliant to the ARV therapy. The main mechanism is represented by the integration of the viral genome into the host genome, which produces irregularities that later enables the growth process with the development of tumors, and in advanced stages, metastasis.

Kaposi's sarcoma occurs more frequently in men, generally in MSM people, and is clinically presented as a red lenticular spot, sometimes exceeding the skin`s surface by neoformation of vessels. A particular aspect is that it can appear on the oral mucosa, but also at the level of pulmonary epithelium and genital mucosa. These patients have a CD4 value under 50/mm^3, which means a marked degree of immunosuppression. Ulcerations may occur in the compression areas (interdigital area of the lower limb).

Weight loss and the appearance of these lenticular spots on the skin, can often be attributed to intense physical and intellectual activities or, even if the patient knows his HIV positive status, he did not follow his treatment, with the abandonment of it, for at least 6 months to one year. In their view, because they deny their HIV positive status, they think that the problem does not exist.

Another possibility is that a non-diagnosed HIV patient, or a MSM patient with risky sexual behavior (which do not take into consideration that fact that they could be HIV positive), presents with deterioration of the general health, with marked fatigue which determines the patient to present with lesions that can suggest HIV infection.

Regarding other types of neoplasia that HIV positive patient are at risk of developing, we have to mention the case of a woman who abandoned the treatment and later developed cervical carcinoma. It required total hysterectomy with bilateral anexectomy. After the resumption of ARV therapy, the patient restored her old immunologic status and did not develop metastases.

In these cases, as was the situation for our patients, this disease appeared in people with a high socioeconomic status and a high level of education, similar to those reported in literature in people that lived a bohemian life, the so-called HIV of white collars.

In this context, the patient's approach must be in cooperation with a clinical psychologist, mainly because the psychologist’s contribution is essential. Succesive counseling sessions are required for optimal adherence and compliance to therapy, in order to achieve viro-imunologic success and social re-integration. For newly diagnosed patients it is important to have good support to facilitate their re-intregation into society. They have to make changes to their daily routines, which sometimes cand be hard. The triangle formed by the doctor, the patient and the psychologist, working

as a team towards a common goal, is the key to a successful management of the disease and should be a model included in every center that manages HIV.

Treatment

In the treatment of HIV, UNAIDS [Joint United Nations Programme on HIV/AIDS, Prevention gap report] has a precise plan in the fight against HIV/AIDS in USA and UK. The plan is called 90-90-90 and had the following goals:

- By 2020, 90% of all people living with HIV will know their HIV status;
- By 2020, 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy;
- By 2020, 90% of all people receiving antiretroviral therapy will have viral suppression;

This plan aims to diagnose HIV infection as soon as possible after its acquisition, as well as rapid access to HIV therapy. The most important thing is that the treatment is chosen correctly and protect the HIV-positive population from the risk of complication, like opportunistic infection, the development of neoplasms, etc (Figure 16) [Centers for Disease Control and Prevention. Ending the HIV epidemic. A plan for America].

The notion of risk refers not only to medical risks, but also to socio-professional ones, so that these patients are not excluded from society and experience rejection, bullying and the impossibility of being hired.

In UK the target of this plan is to diagnose 92% of the cases, 90% to receive treatment, and 87% to obtain viral load suppression (Figure 17) [O'Halloran C et al., 2019].

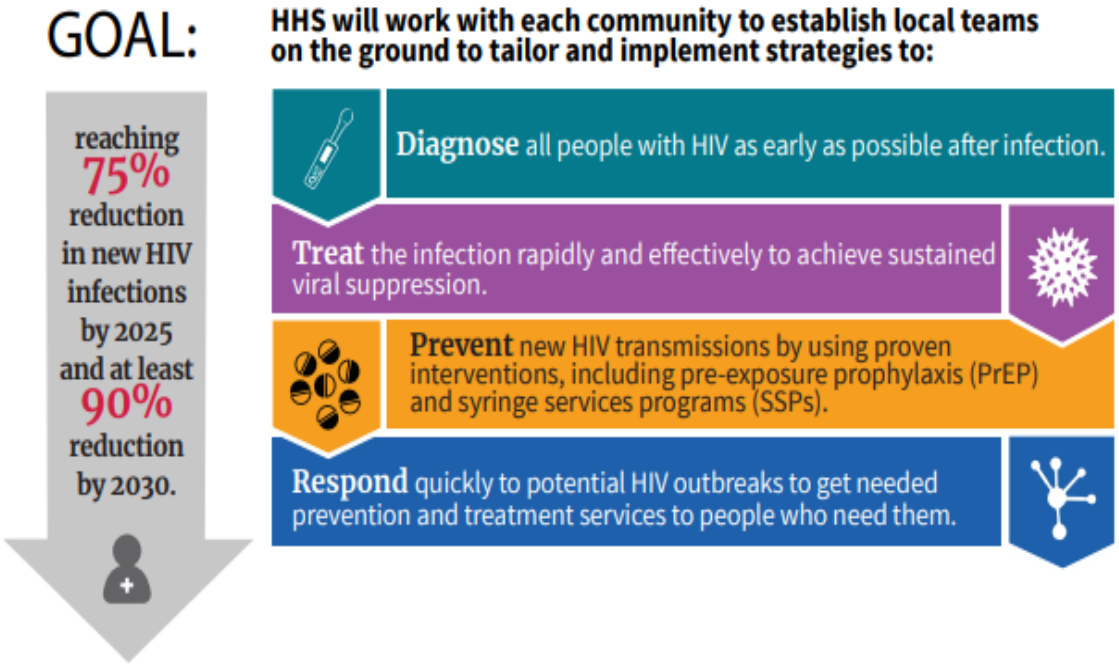


Fig.16 Ending the HIV Epidemic: A Plan for America

Regardless of the age group at which HIV infection is diagnosed, starting from the beginning of 2009, and observing the number of patients over a decade, it was found that there is an increase from 65,000 close to 95,000 of patients that receive medical care, so we can conclude that their share of treated patients has increased by about 1/3 from 2009 to 2018. Even the patients older than 65 years old had an increase of share between the patients that receives medical care. The most constant age group remains the adults between 35 to 49 years old (Figure 18) [O'Halloran C et al., 2019].

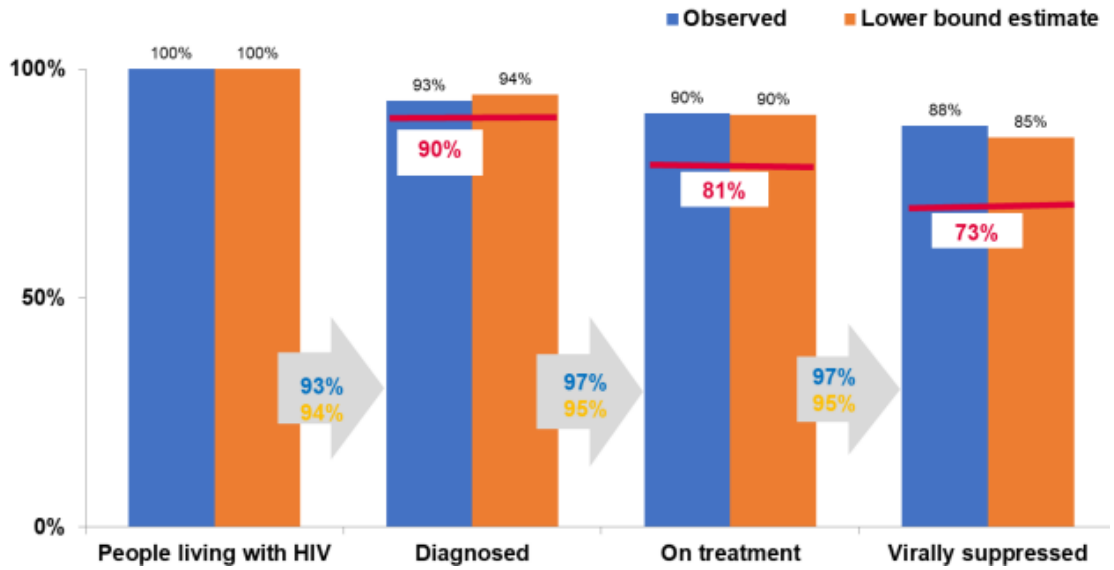


Fig.17 Comparison of the UNAIDS target outcomes for observed and lower bound scenarios

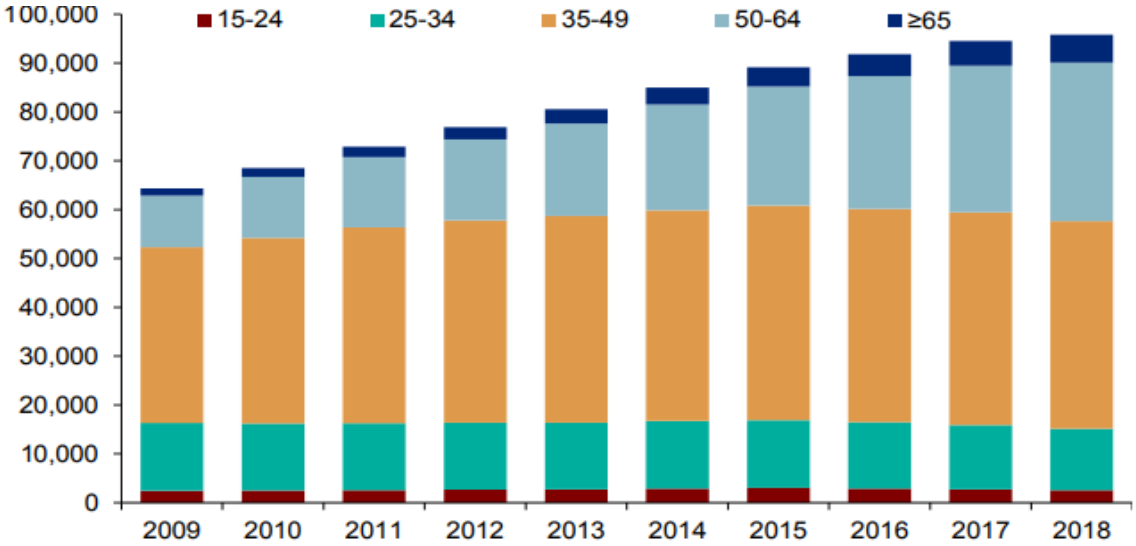


Fig.18 People diagnosed with HIV receiving care, by age group: UK, 2009 to 2018

From figure 19 [O'Halloran C et al., 2019], we can observe a significant difference between those who access various health services. The most common health services related to reproductive health are more frequently accessed by men compared to women, by white people compared to those of color and by the age group 15-49 years. Also, from figure 20, we can observe that patients with a Ly CD4 level grater then 350, are more frequently accessing the health services, probably due to increased adherence and compliance to therapy fact that makes them a more constant presence in the doctor's office, compared with those that have value of CD4 count below 350.

Special groups and HIV infection - mothers, newborns, children and adolescence

WHO estimates that 50% of new diagnosed HIV infections worldwide are in young people between the ages of 15-24, with an increased in the number of children that become infected at birth and are growing with an HIV-positive status.

Over 5 million children are from developed countries, and the most concerning thing is that around 5,000-6,000 young people become HIV -positive on a daily basis, with a predominance of newly diagnosed cases in high-income countries [UNAIDS Inter-agency Task Team on Young People - Preventing HIV/AIDS in young people].

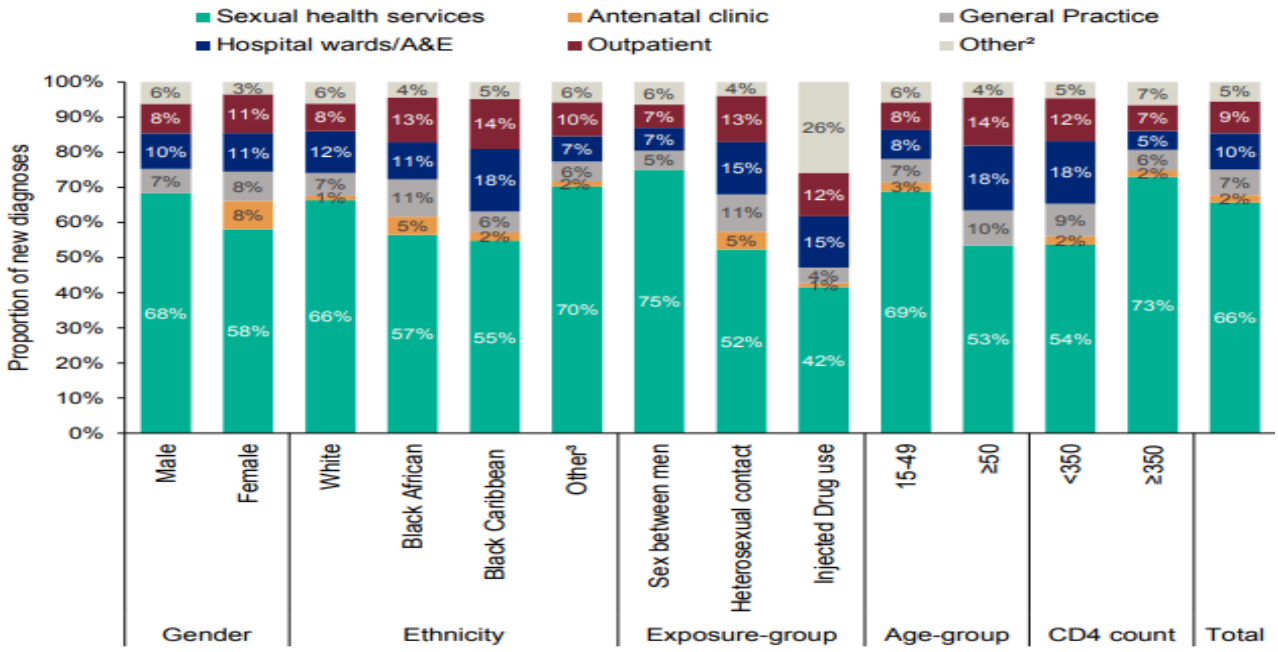


Fig.19 Setting of first positive test among adults newly diagnosed with HIV by population group: UK, 2018

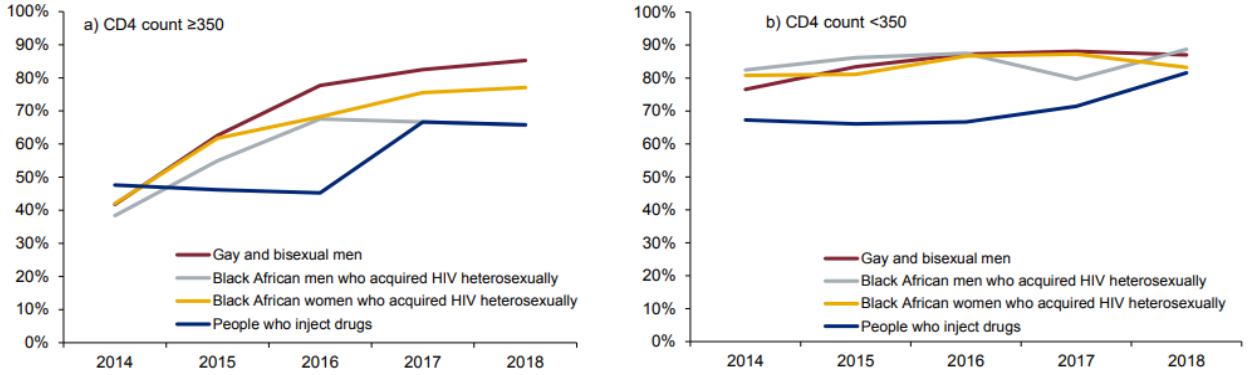


Fig.20 Proportion initiating treatment within 91 days by CD4 status: UK, 2014 to 2018

There is a dedicated department created within the WHO - Department of Maternal, Newborn, Child and Adolescent, that has a strong connection with the HIV lucrative department and together, they create guidelines and protocols for every country, according to their specific.

It is stipulated that the young population should have access to HIV testing services, as well as socio-psychological and professional support for those who have contacted the disease.

The reports of these organizations embody evidence-based recommendations, with guidelines for health policies, management and health programs to achieve the UN (United Nations) goals of the HIV pandemic and youth.

They aim to detect prevalence and identify vulnerability in the young population, increase access to information and services, supervise the intervention programs in schools, health services, media, etc. Those that are responsible to conduct and carry out these intervention programs operate as follows:

- **Steady** – no programs are implemented yet, longer time to evaluate is necessary;
- **Ready** – careful and paucimannose implementation;
- **Go** – large scale implementation.

World-renowned universities, such as Johns Hopkins University, have been involved in the support program for low-income countries (e.g. Uganda) where there are large communities of young people living with HIV.

„*The UNAIDS 2011–2015 Strategy: Getting to Zero, the UNAIDS Joint Action for Results: Outcome Framework 2009–2011, and the UNAIDS Business Case 2009–2011 for the priority area on young people present an opportunity to focus on and scale up effective programs for young people, and to create links between partners involved in the response*." [UNAIDS 2011–2015 Strategy. Getting to zero].

Over time, health policies and the implementation of programs, for a long period of time, seem to have paid off, with UNAIDS publicizing the declining prevalence of HIV infection among young people and adolescents in low-income countries.

„*A trend analysis in HIV prevalence among young people in 2008 revealed that HIV prevalence declined in 15 of the 21 countries most affected by the HIV epidemic. Ten countries showed a statistically significant decline of 25% or more in HIV prevalence that occurred among young pregnant women or men in either urban or rural areas or both. These countries include Botswana, Côte d'Ivoire, Ethiopia, Kenya, Malawi, Namibia, South Africa, the United Republic of Tanzania, Zambia and Zimbabwe. The other five countries (Burundi, Lesotho, Rwanda, Bahamas, Haiti) had declines of more than 25%. These findings suggest that there may be an overall decline in HIV incidence*" [Securing The Future Today Synthesis of Strategic Information on HIV and Young People].

A big part of these special groups, that are HIV infected, is represented by children, that now are adults and were infected in the early stages of life, making up the "pediatric cohort" of Romania. This is why we consider that there is need for a certain medical, social and psychological approach to this age group of infected patients. The importance of studying the interaction between these variables is strongly reflected in the improvement of everyday practices in medical and care institutions, as well as in the social perception of HIV infected people (although this is a field in which education is continuous). Informing people about certain issues that have been, until recently, considered "taboo", can be a step in combating discrimination, which would enable the young people from this category to effectively be part of society and to have a normal social, professional, and emotional development.

New horizons of treatment and prevention

1. Long-Acting Oral Treatment – Currently there are molecules approved that are administered once a week, orally. This molecule is a nucleoside reverse transcriptase inhibitor in combination with Doravirin and is called Islatavir. It is also administered as treatment and for pre-exposure (PrEP);
2. Injectable compounds intramuscular/subcutaneous (i.m/s.c);
   - GS-6207 HIV Capsid Inhibitor (S.c 12 weeks or 6 months);
   - Rilpivirina combination with Cabotegravir;
   - Cabotegravir + Integraza inhibitor (CAB);
3. Implants;
4. Infusions - With neutralizing antibodies that are still in clinical trials [Orkin C et al., 2020].

For this type of therapy, researchers expect much greater adherence and compliance than classic HAART medication, because they are only conditioned by the administration of an injection at 3-6 months according to a predetermined plan. This is the most promising method for managing and prevention of the HIV infection.

Of course, we have to take into consideration the side effects, and the fact that they mut be well known by the prescribing physician and psychologist mainly because they are the ones that have to explain them to the patients. Also, the staff administering the therapy must have good knowlage of the possible side effects

Another important aspect for the scientists is to determine which patients are eligible for the treatment and to study, over time, the viro-immunological efficacy and the cost-effectiveness.

Islatavir, which is a nucleoside reverse transcriptase inhibitor, has an active half-life of 80 to 130 hours. In phase three studies, which included the administration of the compound to humans, results showed that, a single oral dose of less than 0.5 suppressed HIV RNA for more than 7 days.

Side effects consisted in minor digestive reaction, like nausea, vomiting, which can also be associated with diarrhea. Weight gain was noticed after week 24, fact that could be benefic for patients who want to gain weight, but also a minus for people who want to control their weight and whose self-esteem may decrease.

Another drug which is currently in trial is a molecule that addresses subcutaneous administration with long-term inhibition of the viral capsid, and is a pioneer for it`s class. The drug is called Lenacapavir and is a first-class capsid inhibitor.

Regarding PrEP prophylaxis, the administration of molecules with increased half-life can be considered, especially because these new molecules are oral agents and can be administered once a week.

The risk of HIV-positive patients to become infected with CoV-2 and vaccination against it

When the SARS COV-2 pandemic hit, there was no knowledge regarding the degree of exposure and vulnerability to the infection with this new virus for HIV-positive patients and whether they have different reactivity, compared to the general population. With the world's medical scientific focus on the CoV-2 infection, there has been evidence that this second viral infection in HIV-positive patients with a low value of CD4 and VL may have a reserved prognostic.

One of the first studies conducted by Blanco in UK, regarding the mortality of HIV-positive patients infected with COVID-19, showed that immunosuppressed seropositive patients had a higher mortality rate compared to that of the general population. This data is sustained by a statistically significant p ($p = 0.08$). The limitation of the study was the fact that it did not take into account the level of CD4, the viral load or the ARV therapy [Blanco JL et al., 2020].

A second study, which took into account age, gender and comorbidities, such as metabolic disorders, or the presence of certain addictions, like smoking, found that most HIV-positive patients who died had significant associated pathologies and vices (the patients were obese and were smokers). The cohirt of patients was small with only 33 subjects. The conclusions of the study were that extensive information is needed on these issues [Härter G et al., 2020].

Bhaskaran and Tesariero showed that people with HIV and comorbidities have a higher risk of mortality than the general population [Bhaskaran K et al., 2021; Tesoriero et al., 2020). „*People living with HIV had higher risk of COVID-19 death than those without HIV after adjusting for age and sex: hazard ratio (HR) 2·90 (95% CI 1·96–4·30; p<0·0001*)" [Bhaskaran K et al., 2021].

„*PLWDH experienced poorer COVID-related outcomes relative to non-PLWDH (people living with diagnosed HIV), with 1-in-522 PLWDH dying with COVID-19, seemingly driven by higher rates of severe disease requiring hospitalization*." [Tesoriero et al., 2020].

The main reason why we consider these studies important is the fact that most HIV positive patients are located in Africa, and from these studies we ca draw a pertinent conclusion regarding the link between HIV and SARS COV-2. We can suggest that this could apply to the entire African continent, which has a low socio-economic status and by extent implicates the metabolic diseases.

Etienne shows that about 51,000 PLWH (people living with HIV) live in Paris. The COVID-19 pandemic also affected this group, with 1% of them requiring hospital admission, with medium to severe forms of the disease [Etienne N et al., 2020].

He identifies risk factors *„such as age, male sex, metabolic disorders, cardiovascular and chronic respiratory diseases, while the role of immunosuppression remains unclear.*

Those who developed severe or critical disease had more frequently metabolic disorders ($P = 0.004$*), hypertension (*$P = 0.002$*) and/or renal insufficiency (*$P = 0.006$*).*

By multivariate analysis, increased age, male sex and being from sub-Saharan Africa origin were independently associated with severe and critical forms of COVID-19 in PLWH. Furthermore, the presence of a metabolic disorder (obesity and/or diabetes) was independently associated with the occurrence of severe and critical forms. There was no significant difference (for data prior COVID-19), between severe/critical disease and moderate/minor disease for $CD4^+$ *T cell count, proportion*

of patients with HIV RNA less than 40copies/ml or receiving a protease inhibitor-based treatment" [Etienne N et al., 2020].

The study concluded that boosted protease inhibitors had no protective effect on patients already receiving this treatment. This confirms the suspicion that the product Lopinavir/ Ritonavir (Kaletra) is not effective against the SARS COV-2 infection.

With this new informations, the question that arises is whether it is still necessary to maintain the ARV therapy in COVID- 19 infection. The answer of the international medical society is that there is no reason to use these molecules in the therapy for SARS COV-2, this association being reserved only for HIV infection.

Bouth Boulle, who studied patients from South Africa and Hoffmann, who evaluated 175 patients from 3 European countries (Spain, Italy, Germany), came to the same conclusion, that the prognosis of HIV infected patients is more severe if they have a low level CD4 (below 300 cells / mmc or even below 200mmc) [Boulle A et al., 2020; Hoffmann C et al.,2020].

„*While our findings may over-estimate HIV- and tuberculosis-associated COVID-19 mortality risks due to residual confounding, both HIV and current tuberculosis were independently associated with increased COVID-19 mortality. The associations between age, sex and other comorbidities and COVID-19 mortality were similar to other settings"* [Boulle A et al., 2020].

„*The early data of 175 PLWH from three countries revealed cellular immune deficiency as a possible risk factor for severe SARS-CoV-2 infection. Mortality was 4% and the only factor associated with mortality was a low CD4 T-cell nadir. Although there was no evidence of any direct therapeutic effect of tenofovir (mainly used as TAF) and HIV PIs, data strongly argue against any interruption or delayed initiation of ART in the current pandemic, in order to prevent even moderate cellular immune deficiency*" [Hoffmann C et al., 2020].

The same authors also emphasized that the risk for severe disease increases with age and gender (male can present a more severe form of COVID-19). Chronic diseases such as hypertension, chronic lung and heart disease, obesity and diabetes also play an important role. In conclusion, patients with uncontrolled therapeutic immunosuppression of HIV infection and associated chronic diseases have a higher risk of mortality from COVID-19.

The issue of therapy was also discussed, in the sense that certain therapeutic combinations may decrease the risk of COVID-19. Del Amo J, on a Spanish cohort of over 77,000 cases, showed that patients who received the non-nucleoside reverse transcriptase inhibitor (NRTI) had a higher risk of being hospitalized with COVID-19 than those who received TDF / FTC. However, all studies are unanimous in estimating that no having ARV therapy or/and a low CD4 value (<200 cell/mmc) are high risk factors for COVID-19 infection [Del Amo J et al., 2020].

„*Given the structural similarity with lopinavir, darunavir is a potentially effective treatment against SARS-CoV-2 and is currently under investigation in phase III clinical trials. However, with these clinical reports, we provide preliminary evidence that darunavir, at least at the currently adopted dosage of 800 mg, did not prevent SARS-CoV-2 infection in people living with HIV and, at least in one case, did not protect from the worsening of respiratory function*" [Riva A et al., 2020].

A very large study on TDF + FTC in association with a lower dose of HQ used in pre-exposure prophylaxis in health care workers is underway in Latin America and Spain. This included 4,000 participants between the ages of 18 and 70. It had 4 arms, one of which was placebo and used:

1. Tenofovir Disoproxil Fumarate 245 mg/Emtricitabine 200 mg + Placebo of Hydroxychloroquine 200 mg;
2. Hydroxychloroquine 200 mg + Placebo of Tenofovir Disoproxil Fumarate 245 mg/Emtricitabine 200 mg;
3. Tenofovir Disoproxil Fumarate 245 mg/Emtricitabine 200 mg + Hydroxychloroquine 200 mg
4. Placebo.

The inclusion criteria in the study were:

- Participants, after receiving appropriate information on the study design, objectives, possible risks and acknowledging that they have the right to withdraw the consent at any time, sing the informed consent;
- Male or female aged 18-70years;
- Health care workers in public or private hospitals in areas of risk of SARS COV-2 transmission;
- No previous diagnosis of SARS-CoV-2 (COVID-19) infection plus no symptoms compatible with SARS COV-2 since 1st of March 2020 until the date of enrolment in the study;
- Understanding of the aim of the study and, therefore, acknowledging they have not been on any drug aiming at pre-exposure prophylaxis against SARS COV-2 since 1st of March 2020. This also includes PrEP for HIV;
- Negative pregnancy test during the previous 7 days to start treatments or more than 2 years after menopause;
- Women of reproductive age and their partners should commit to use and highly effective contraceptive method (double barrier, hormonal contraception), during the study period and until 6 months after the last dose of treatment.

The Exclusion Criteria:

- Having symptoms suggestive of COVID-19 infection;
- HIV infection;
- Active hepatitis B infection;
- Renal failure with estimated glomerular filtration rate (GFR) < 60 ml/min) and patients on Hemodialysis;
- Osteoporosis;
- Myasthenia gravis;
- Pre-existent maculopathy;
- Retinitis pigmentosa;
- Bradycardia < 50bpm;
- Weight < 40kg;
- Participant with any immunosuppressive condition or hematological disease;
- Have taken any medications such as PrEP against SARS COV-2 from March 1, 2020 until trial entry (also includes PrEP for HIV);
- Treatment with drugs that may prolong QT in the last month before randomization for more than 7 days including: azithromycin, chlorpromazine, cisapride, clarithromycin, domperidone, droperidol, erythromycin, halofantrine, haloperidol, lumefantrine, mefloquine, methadone, pentamidine, procainamide, quinidine, quinine, sotalol, sparfloxacin, thioridazine, amiodarone;
- Breastfeeding;
- Known allergy to any of the medication used in this trial.

Up until now, there is no evidence that preexposure prophylaxis can have an effect over the SARS COV-2 infection [https://clinicaltrials.gov/ct2/show/NCT04334928].

A future therapy could be represented by CCR5 receptor inhibitors and uses a humanized monoclonal antibody called PRO140 or Leronlimab. A study performed on 10 patients showed the benefit of increasing the CD4/CD8 ratio, with the concomitant decrease in SARS COV-2 viremia, along with the improvement of the clinical condition and implicitly of prognosis [Patterson BK et al., 2020].

II.2 HIV TESTING AND SCREENING PRACTICES OF HEALTH CARE PROVIDERS

II.2.1 Introduction

If a person screens positive for HIV, the WHO recommends that s/he receive health education and counseling services and then be retested before starting ART [World Health Organization., 2015].

Once on ART, chances of acquiring an HIV-related illness are greatly reduced and quality of life improves [ECD: HIV testing., 2010]. HIV can still be transmitted, however, many studies have shown that once a person knows his/her status, s/he reduces their risky behaviors, improving consistent condom usage, and not sharing needles [ECD: HIV testing., 2010]. Thus, screening and testing not only leads to increases longevity and quality of life for PLWH, but also decreases the odds of transmission through the reduction of risky behaviors and through the consistent use of ART.

Furthermore, if someone screens HIV negative, there still are positive public health benefits, since testing is an opportunity to provide educational materials about condoms, sharing needles, and pre-exposure prophylaxis (PrEP), and to take action to implement safe sexual behaviors [WHO: Consolidated guidelines on HIV testing service., 2015].

Unfortunately, the ECDC observes that there is, generally, complex perceptions of one's risk, so people at high risk may not seem testing in the first place [ECD: HIV testing.,2010]. A further barrier to testing is also fear that a positive diagnosis will lead to stigma [ECD: HIV testing., 2010]. Providers can also form a barrier to offering HIV testing if they do not feel comfortable discussing the subject with their patients.

Only 4% of the 10,000 children diagnosed in the 1990s were estimated to have transmitted vertically, indicating that the contaminated syringes were largely responsible for the spread of HIV in Romania [Kozinetz CA et al., 2001].

Subsequent diagnosis and treatment for this large cohort of children (now in their mid-20s) has been inconsistent and incomplete, resulting in non-continuous treatment, poor access, and stigmatization [Kozinetz CA et al., 2001; UNAIDS: Country Progress Report, 2015]. Thus, this cohort is at the center of Romania's HIV/AIDS epidemic, characterized by uneven screening complicated by fear and stigma.

In 2014 Romania approved a 2014-2020 National Public Health Strategy that included strategic objectives related to the AIDS response. However, the budget estimations are limited and HIV prevention are affected by the lack of funding [UNAIDS: Country Progress Report, 2015]. HIV testing is performed in hospitals or private clinical specialized centers as a differential diagnosis, not as a routine testing, one of the reasons the HIV infection is diagnosed very late during the evolution [Rutã S et al., 2008; Baylor International Pediatric AIDS Initiative, 2019].

My interest in this field is reflected by the following paper:

1. Barbosu CM, Radulescu A. **Manciuc C**, Muir E, Levandowski BA, Dye T. Attitudes, practices, and priority of HIV screening and testing among clinical providers in Transylvania and Moldavia, Romania. *BMC Health Serv Res* 2019; **19:** 970.

II.2.2 Aim of the study

The purpose of this analysis is to examine HIV testing and screening practices of health care providers in two areas of Romania: Transylvania and Moldavia.

II.2.3 Material and methods

We conducted an electronic survey of clinical providers (physicians, residents, nurses, others) in Transylvania and Moldavia, two regions of Romania. The instrument was an adaptation of the New York State Department of Health AIDS Institute's Clinical Education Initiative's Assessment, responsive to locally-stated priorities and streamlined to better understand clinical and training priorities in Romania [Barbosu CM el al., 2017]. Participants (physicians, nurses, dentists, psychologists, and social workers) were identified through a snowball/referral process originated in the HIV clinical hubs in Cluj-Napoca (Transylvania) and lasi (Moldavia). A REDCap (Research Electronic Data Capture) [Harris PA et al., 2009] survey, using a cloud-based system, was deployed through sharing an invitation with a link to the survey to potential participants. The health care providers were asked if they routinely provide HIV screening and testing to their patients 13-18, 19-30, and over 30 years old. The instrument included a range of multiple answer questions about services they provide, their training interests and needs, their position, geographic location, and institutional type.

Analysis

If respondents did not provide HIV screening and testing to any segment, they were classified as "Not Provide HIV Screening and Testing," the primary endpoint for these analyses. Data were reduced to reflect "Physician" and "Non-physician" respondents, and "Hospital" and "Non- Hospital" respondents. Data was analyzed using Pearson Chi-square analysis and forward stepwise logistic regression [Hosmer DW et al., 2004]. Logistic regression generated Odds Ratios (OR) to reflect the magnitude of association between the relevant variables, with 95% confidence interval (95% CI) indicating statistical range. Data missing the primary end- point ("Not Provide HIV Screening and Testing") were excluded from analysis. SPSS 24.0 (IBM Corporation, 2016) was used for all quantitative analyses.

Ethical review

The project was approved by both "luliu Hatieganu" University of Medicine and Pharmacy, Cluj- Napoca (UMF- Cluj) and "Grigore T. Popa" University of Medicine and Pharmacy, lasi (UMF lasi) and by the University of Rochester's Research Subjects Review Board. The project was performed in accordance with the ethical standards contained within the 1964 Declaration of Helsinki and its later amendments.

II.2.4 Results

Overall, 125 participants responded to the assessment. Two-thirds (62.9%) lived in Transylvania, three-fourths (75%) work in hospitals and the vast majority (81.5%) were physicians (Table VII). Participants from rural areas were less likely (though not significantly) to offer HIV screening and testing than their urban counterparts. Furthermore, no differences in HIV screening and testing were noted by whether or not the respondent was a physician, nor in which region (Transylvania or Moldavia) the respondent was located.

Specialized organizations that provide HIV treatment were more likely to also offer screening and testing ($p > 0.001$), as were organizations that had written policy in place around HIV screening. Organizations with written policies on post- exposure prophylaxis (PEP) were also more likely to offer HIV screening. Indeed, the odds ratio remains statistically significant between having institutional policies on HIV screening, testing and offering screening and testing to all segments of the population, even after controlling for hospital setting (Adjusted OR: 11.1; 95% CI: 3.0, 41.8). The relationship between having institutional policies in place for HIV testing and screening was significantly associated with the likelihood that screening is offered to all population segments in both a hospital ($p < .05$) and a non-hospital ($p < .001$) setting (data not shown).

Table VII - HIV Screening and Testing by Selected Institutional and Respondent Characteristics, Transylvania and Moldavia Clinical Provider Assessment, 2017

	Total	No, Do Not Offer HIV Screening/ Testing	Yes, Offer HIV Screening/ Testing	Odds Ratio	
Characteristic	n (%)	n (%)	n (%)	OR (9S%Cl)	p-value
Hospital-based	93 (75.0)	33 (35.5)	60 (64.5)	Referent	0.027
Non-hospital based	31 (25.0)	18 (58.1)	13 (41.9)	2.5 (1.1, 5.8)	-
Transylvania	78 (62.9)	18 (39.1)	28 (60.9)	Referent	-
Moldavia	46 (37.1)	33 (42.3)	45 (57.7)	1.1 (0.5,2.4)	0.728
Urban	117 (94.4)	46 (39.3)	71 (60.7)	Referent	Urban
Rural	7 (5.6)	5 (71.4)	2 (28.)	39(0.7, 20.7)	0.095
Physician Provider	101 (81.5)	41 (40.6)	60 (39.4)	Referent	-
Non-physician Provider	23 (18.5)	10 (43.5)	13 (56.5)	1.1(0.5, 2.1)	0.800
Organization provides treatment por HIV patients	60 (48.4)	16 (25.0)	48 (75.0)	Referent	-
Organization does not provide treatment for HIV patients	64 (51.6)	35 (58.3)	25 (41.7)	4.2 (2.0, 9.0)	<.001
Organization has a written policy on testing for HIV	103 (83.1)	33 (32.0)	70 (68.0)	Referent	-
Organization does not have a written policy on testing for HIV	21 (16.9)	18 (85.7)	3 (14.3)	12.7(3.5,46.3)	<.001
Organization has a written policy on Post-Exposure Prophylaxis (PEP)	72 (58.1)	20 (27.8)	52 (72.2)	Referent	-
Organization does not have a written policy on Post-Exposure Prophylaxis (PEP)	52 (41.9)	31 (59.6)	21 (40.4)	3.8(1.8, 8.2)	<.001

As shown in Table VIII, 40.8% of the clinical care providers did not offer HIV screening and testing to at least one segment of the population. Participants working in a hospital setting were significantly more likely to offer HIV screening and testing to all segments compared to workers form a non-hospital setting (58.1% v. 35.5%, respectively; p .05). Hospital-based participants were significantly more likely to offer HIV screening and testing to 13—18-year-old than their non-hospital-based counterparts ($p < .05$). Though hospital-based participants were also more likely to screen patients from other age groups than were non-hospital-based participants, these differences were not statistically significant.

Table VIII - HIV Screening and Testing by Institutional Type, Transylvania and Moldavia Clinical Provider Assessment, 2017

	Total n (%)	Hospital (n %)	Non-hospital n (%)	Hospital OR (95% Cl)	p-value
Provides HIV screening to:					
13-18 years old	77 (61.6)	63 (67.7)	14 (45.2)	2.6 (1.1, 5.9)	0.026
19-30 years old	92 (73.6)	71 (76.3)	21 (65.6)	1.7 (0.7, 4.0)	0.244
Over 30 years old	91 (72.8)	71 (76.3)	12(62.5)	1.9 (0.8, 4.6)	0.137
Does not provide HIV testing to one or more groups	51 (40.8)	33 (35.5)	18 (58.1)	2.5 (1.1, 5.8)	0.027

Shown in Table IX are the most common reasons for not offering HIV screening and testing. The main reasons were that the participants didn't feel that screening was their responsibility (33.3%) and that the institutions where they worked did not require screening (33.3%). Some participants also suggested that they would like more training regarding HIV screening and testing (11.8%). They also implied that there were other barriers in their organizations that prevented HIV screening and testing. Respondents mentioned that more training on HIV screening and testing (37.3%) and also on how to talk to patients about HIV (31.4%) should be priorities, as well as providing more justification on why testing is important (27.5%).

When asked about future training interests, 38.4% of providers were "somewhat interested" in more learning about HIV screening and testing, and 56.0% of responders indicated they were "very interested" in such training (data not shown).

Table IX - Reasons for not offering HIV screening and testing and preferences for training and intervention, Transylvania and Moldavia Clinical Provider Assessment, 2017

Reason indicated for not screening and testing (among participants indicating they did not screen at least one group)	n (%) (n = 51)
It is not my responsibility	17 (33.3)
I don't think all my patients are at risk	4 (7.8)
I feel uncomfortable asking some patients	3 (5.9)
I don't think there is a good reason to screen	1 (2.0)
I need training in HIV testing	6 (11.8)
The organization I work for does not require that I do it	17 (33.3)
There are barriers in my organization that prevent me from doing so	6 (11.8)
What would help you recommend HIV testing to all your patients?	
More justification for why it is important	14 (27.5)
Training in HIV testing	19 (37.3)
Training in how to ask patients	16 (31.4)

II.2.5 Discussion

Screening and testing for HIV is the cornerstone for ending the epidemic worldwide [WHO, Consolidated guidelines on HIV testing services, 2015]. The WHO and UNAIDS promote universal access to knowledge of the HIV status [Organization WHO. Statement on HIV testing and Counselling, 2012]. Early detection prolongs life, prevents transmission, and reduces avoidable morbidity and mortality [Centers for Disease Control and Prevention. Revised guidelines for HIV

counseling, testing, and referral, 2001]. In the context of an unusual and atypical HIV/ AIDS epidemic in Romania, a large proportion of health care providers indicated that they do not routinely test and screen for HIV. Promoting greater access to knowledge of HIV status for Romanians will require deliberate policies and programs aimed at reducing the gaps that currently exists, that miss identification of PLWH and result in late presentation for care of people with advanced disease.

This study used snowball sampling to identify as many HIV providers as possible, in two large Romanian cities. While this sampling strategy is limited to only obtaining the perspectives of those who were named, three authors are current or former HIV clinicians in Romania. Due to the small community of HIV clinicians and advance planning of obtaining clinician lists and email addresses, we feel this study was distributed to the majority of HIV clinicians in both cities. The results are subject to non-response bias.

This assessment indicates that screening, within the hospital systems, occurs more frequently than in the non- hospital settings. Promoting HIV screening within the acute medical care environment of the hospital is an opportunity to identify PLWH, perhaps secondary to other purposes for their hospital engagement. This hospital-level screening identifies PLWH and links them with care, while also helping to prevent occupational exposure to HIV for health workers within that setting.

A perhaps wider opportunity, however, exists with expanding HIV screening and testing efforts beyond the hospital, into primary care settings, especially in rural towns and villages. HIV screening is not common in these areas, and given Romania's unique experience with a cohort of HIV-exposed children now living as adults [Hersh BS et al., 1993], screening efforts in rural areas could be an important element of prevention. Non-hospital sites of care seem particularly reluctant to screen teenagers; addressing the potential of this population to be exposed to HIV and remain undetected until late AIDS stage could form an important element of prevention for Romania.

Among the 23 European Union countries, Romania has more than 60% of late diagnosis (CD4 cell count < 350/mm^3) suggesting that early infection is poorly diagnosed and evaluated [ECDC - HIV/ AIDS Surveillance in Europe, 2018]. The new approaches of testing outside of conventional health facilities (hospitals) delivered within communities by trained medical staff and promoting individual testing as home sampling and self- testing should be considered [Rutã S et al., 2008].

The ECDC/WHO 2018 report shows that the new HIV diagnosis in Romania were heterosexual, MSM and IDU (intravenous drug users) transmitted, and more than 60% were diagnosed late (CD4 cell count < 350/mm3) compared with the EU/EEA average of 49% late diagnosis Control [ECDC - HIV/ AIDS Surveillance in Europe, 2018]. In fact, a shocking 37% of IDUs are diagnosed with a CD4 cell count < 100/mm3. Thus, while Romania has achieved the first 90% goal, it is not surprising that it is falling short of 90% for meeting treatment needs, since those being diagnosed late cannot receive as effective treatment [Hersh BS et al., 1993]. This suggests that achieving 90% of people knowing their status requires that people also need to know their status early, in order to progress towards the next 90% goal. Consequently, the new approaches of testing outside of hospitals and conventional health facilities delivered in rural areas and within communities by trained medical staff, and promoting individual self-testing as home sampling should be considered, as in Romania there are no policy or guidelines related to community-based testing or self-testing [Rutã S et al., 2008; HIV Testing in Europe - Dublin Declaration Report, 2017].

Further, specialized institutions that offer HIV treatment and have institutional policies in place on HIV screening and testing and also on PrEP, are more likely to offer screening and testing to general population. Having institutional policies in place has been demonstrated elsewhere as an effective strategy for promoting HIV testing and screening [Deblonde J et al., 2010], being considered best practice [Centers for Disease Control and Prevention. Revised guidelines for HIV counseling, testing, and referral, 2001]. A substantial proportion of the survey's respondents, who do not currently offer HIV screening and testing, indicated that their institutions do not require the implementation of

such procedures, and that they do not feel it was their responsibility, or believed there were other institutional challenges that limited their ability to screen and test for HIV.

Almost all providers indicated they were at least somewhat interested in more training around HIV screening and testing, and a substantial portion of providers who did not currently offer routine HIV screening and testing to at least one of the segmented populations indicated that more training on HIV screening and testing, more rationale for offering screening and testing, and training on how to talk with patients about HIV would help them recommend HIV screening to all their patients. These providers are also currently using social media platforms, and were interested in using social media technology for clinical learning and support [Manciuc C et al., 2018]. Such training has been demonstrated elsewhere as an effective component of enhancing HIV screening, and could form a component of local and regional targeted continuing medical education, integration with medical training, and also in training of ancillary personnel [Centers for Disease Control and Prevention. High-impact HIV prevention, 2011].

Despite much progress, rates of HIV testing in Europe remain low. For example, in Romania, HIV testing performed to TB patients is only 3.4%, testing in pregnant women is 39.2% and testing by request is 52%. Also, criminalization remains a barrier to providing testing in some countries, including Romania [HIV Testing in Europe - Dublin Declaration Report 2017].

II.2.6 Conclusion

Providing targeted training to clinicians around HIV screening and testing along with assistance to institutions for developing policies around HIV- related screening, testing and care could help improve identification of PLWH before their disease progresses to an advanced stage. In Romania's unique experience with an atypical HIV epidemiology and progression, such medical trainings could form an important part of the plan to end the epidemic by 2020.

Related articles

1. **Manciuc Carmen**, Nicu M, Largu MA, Filip-Ciubotaru F, Dorobăţ C. - Sustainable development and key health trends: case study of dislipidemy in HIV patients. *Environ Eng Manag J* , 2010, 9(4):495-503
2. Largu A. **Carmen Manciuc**, A. Vâţă, Cristina Nicolau, L. Prisacaru, Florina Filip Ciubotaru, Cătălina Luca, Carmen-Mihaela Dorobăţ. Dyadic adjustment in HIV sero-concordant and sero-discordant couples. *Rev Med Chir Soc Med Nat Iasi* 2012; **116:** 718–21.
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5. Vâţă, A., **Manciuc Carmen**, Nicolau, C., Prisăcariu, L., Vâţă, L.G., Dorobăţ, C. Late Diagnosis of HIV Infection in Iaşi County - Frequency, Associated Factors, Therapeutic Options. *Ther Phar Cl Tox.* 2011; **15:** 18–22.

II.3 NEW TECHNOLOGY AND HIV CARE

II.3.1 Introduction

Online information is omnipresent and social media is pervasive, allowing unlimited professional networking, sharing of opinions, professional education and training, and dissemination of knowledge [Ventola CL., 2014]. Medical professionals are using the variety of social media outlets to improve patient-provider communication, supplement their professional development, provide and receive peer support through networking capabilities, and contribute to public health prevention and service [Moorhead SA et al., 2013; George DR et al., 2013]. Indeed, greater than 90% of physicians reported using social media outlets. Regular use of Facebook accounts by physicians was estimated to have grown from 13% to 47% in 2011 [George DR et al., 2013; Von Muhlen Met al., 2012].

UNAIDS has established that 'universal access to HIV prevention, treatment, care and support services to be as ubiquitous as mobile phone coverage, and that the way to achieve this goal is by "harnessing technology" like using mobile health interventions [UNAIDS. Feature story telecom: tools connecting the world and communicating about HIV]. Already this goal is being realized through information and communication technologies (ICTs), like the internet and social media that are used in many health scenarios, including ending the HIV/AIDS epidemic [UNAIDS. Meeting report: Ending the AIDS epidemic by 2030; McNab C et al., 2009; Cao B et al., 2017; Coursaris C et al., 2009; Henwood R et al., 2016; Al-Surimi et al., 2017]. Consequently, the internet and social media are valuable tools for targeting people at risk of HIV/AIDS, enhancing support and care for people living with HIV/AIDS, and training clinical providers to better care for their HIV/AIDS patients. Romania, with very strong and widespread digital technologies and increasing connectivity, could experience substantial improvement in it`s AIDS epidemic with the adoption of ICTs [UNAIDS. Meeting report: Ending the AIDS epidemic by 2030; Akamai., 2016].

Identifying the social media outlets used by the target audience is a critical first step to ensuring the information flows directly to consumers and is subsequently utilized to address public health concerns [McNab et al., 2009; Chew F et al., 2004]. Therefore, the purpose of this study was to determine how Romanian clinical care providers interact with specific social media platforms, in order to better understand the potential of the current ICT infrastructure. As information is exchanged online, social support groups and training modules have showen to be effective [Henwood R et al., 2016; Al-Surimi et al., 2017; Alsobayel H., 2016; Attai DJ et al., 2015; Chang LW et al., 2012].

My interest in this field is reflected by the following paper:

1. **Manciuc C**, Levandowski BA, Muir E, Radulescu A, Barbosu M, Dye TD. Access to digital and social media among Romanian HIV/AIDS clinical providers. *Glob Health Action* 2018; **11:** 1513445.

II.3.2Aim of the study

The study intends to determine the current social media practices, specifically in Romania, as a foundation for a future collaborative construction of a new digital platform.

II.3.3 Material and methods

An online survey was conducted among Romanian HIV/AIDS clinical care providers, in order to determine their current medical education and capacity-building needs regarding HIV/ AIDS. Romanian HIV/AIDS clinical care providers were recruited, using snowball sampling and referral from the Cluj-Napoca and Iași HIV clinical hubs in the Transylvania and Moldavia regions of Romania, respectively. Two authors (CM and AR) sent clinical providers in their professional networks an email with a link to an online survey in REDCap, which was available for 12 days in July

2017. Email recipients were encouraged to forward the link to their collegues who were HIV/ AIDS clinical care providers.

The needs assessment survey was based on the New York State AIDS Institute Clinical Education Initiative's domestic assessment of HIV/AIDS training needs, and updated to reflect the Romanian context. Participants were asked to indicate the frequency of their use of media sources using a five-point Likert scale (never, rarely, several times per month, several times per week, daily). Social and online sources included Facebook, Instagram, LinkedIn, WhatsApp, Skype, and other.

Sparse demographic variables were collected, such as primary occupation, practice setting, urban or rural location, and geographic regions of Transylvania and Moldavia. Descriptive statistics were used to assess frequencies. Bivariate analyses were conducted using two- way ANOVAs, two-tailed Pearson Correlations, and Pearson chi-square tests to ascertain relationships between variables, testing each of the demographic variables (primary occupation, practice setting, urban/rural, and Transylvania/Moldavia) against each of the social media platforms. Statistics were calculated using SPSS 24.0.

II.3.4 Results

One hundred twenty-five Romanian clinical care providers completed the online survey. Out of these, 82% were physicians, 74% worked in hospitals, and 94% were in urban settings, with 63% located in Transylvania and 37% in Moldavia. Overall, Facebook and WhatsApp were the social media sites used most frequently, with 74% and 63% reporting daily or weekly use, respectively, followed by Skype (20% used daily or weekly). Skype was used more frequently than Instagram and LinkedIn (41%, 21% and 20% reporting monthly use, respectively) which were rarely used ($p < .05$). Over half of the respondents reported never using Instagram or LinkedIn (Figure 21).

Bivariate analyses were conducted comparing social media platforms with the demographic characteristics described above, with no significant associations found. Bivariate analyses were conducted, comparing social media platforms with other social media platforms, finding that users of one social media platform were significantly more likely to use at least one other platform. Facebook users were significantly more likely to use Instagram ($p = .009$) and WhatsApp ($p = .02$). LinkedIn users were significantly more likely to use Instagram ($p < .001$), WhatsApp ($p = .03$) and Skype ($p = .01$).

Bivariate analyses were conducted comparing never using any social media type or daily use of each social media type to the sociodemographic characteristics described above (Table X). The HIV/ AIDS clinical workforce living in Transylvania and Moldavia differed in their use of social media: about one-fifth (18.7%) of Transylvanians never used Facebook, compared to only 4.5% of Moldavians ($p = .02$). Moldavians are more likely to use Facebook daily (79.5% vs. 57.3%, $p = .01$, respectively), but also more likely to use Instagram daily (22.5% vs 7%, $p = .02$, respectively).

The HIV/AIDS clinical workforce who were physicians, compared to other specialties differed in their use of Facebook as a platform: while Facebook is the most used social media platform in this study, 16.3% of physicians never used Facebook (compared to 0% of non-physicians, $p = .04$). That said, 64.3% of physicians used Facebook daily (compared to 71.4% of non-physicians, $p = .4$). Almost half (46.7%) of providers used WhatsApp daily, with non-physicians being significantly more likely to be daily users (66.7% vs. 42.4%, $p = .04$). Four percent of physicians reported daily use of LinkedIn, while 0% of non-physicians reported daily use ($p = .55$).

II.3.5 Discussion

Social media is a standard component of contemporary communications, both inside and outside of the workplace. This needs assessment identifies critical information about reaching the Romanian HIV/AIDS workforce, engagement with whom is necessary to make progress on difficult and complex HIV-related problems in the country. Many studies have been conducted among

physicians regarding their use of the internet to access such resources, which show that social media use is commonly utilized to link physicians to a variety of online professional resources. These include clinical guidelines, peer-reviewed medical and resource journals, and other resources such as continuing medical education courses provided both through live and online training [Moorhead SA et al., 2013; Ventola CL et al., 2014; Chew F et al., 2004; Bennett NL et al., 2004].

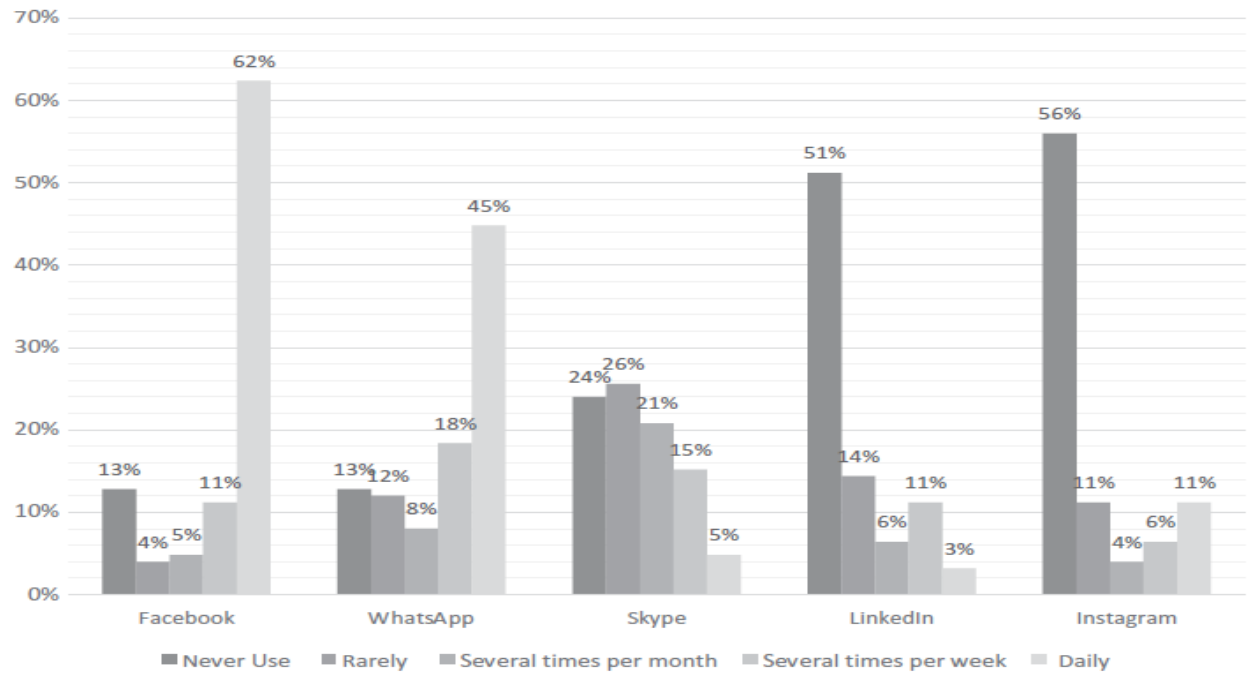

Fig.21 Frequency of social media platform use among Romanian HIV/AIDS clinical providers (n =125),2017

Table X - Social media usage and preference, HIV/AIDS Clinical Providers, Romania, 2017

	Transylvania	Moldavia	MD	Not MD[a]	Hospital	Not Hospital[b]
Facebook						
Mean Usage[c] (mean, SD)	2.9 (1.6)	3.6 (1.0)[d]	3.0 (1.5)	3.5 (1.0)	3.2 (1.5)	3.0 (1.4)
Daily Usage (%, n)	57.3 (43)	79.5 (35)[d]	64.3 (63)	71.4 (15)	67.8 (59)	59.4 (19)
Never Use (%, n)	18.7 (14)	4.5 (2)[d]	16.3 (16)	0.0 (0)[d]	13.8 (12)	12.5 (4)
WhatsApp						
Mean Usage[c] (mean, SD)	2.8 (1.5)	2.6 (1.5)	2.7 (1.5)	3.1 (1.4)	2.8 (1.5)	2.7 (1.5)
Daily Usage (%, n)	50.6 (39)	39.5 (17)	42.4 (42)	66.7 (14)[d]	47.3 (43)	44.8 (13)
Never Use (%, n)	13.0 (10)	14.0 (6)	15.2 (15)	4.8 (1)	14.3 (13)	10.3 (3)
Skype						
Mean Usage[c] (mean, SD)	1.4 (1.1)	1.6 (1.3)	1.5 (1.2)	1.2 (1.3)	1.6 (1.3)	1.1 (0.9)
Daily Usage (%, n)	2.8 (2)	9.8 (4)	4.2 (4)	11.1 (2)	7.0 (6)	0.0 (0)
Never Use (%, n)	26.4 (19)	26.8 (11)	24.2 (23)	38.9 (7)	25.6 (22)	29.6 (8)
LinkedIn						
Mean Usage[c] (mean, SD)	0.7 (1.1)	1.2 (1.4)	0.92 (1.3)	0.40 (0.7)	0.9 (1.3)	0.7 (0.9)
Daily Usage (%, n)	1.4 (1)	7.7 (3)	4.3 (4)	0.0 (0)	4.9 (4)	0.0 (0)
Never Use (%, n)	65.2 (45)	48.7 (19)	57.0 (53)	73.3 (11)	62.2 (51)	50.0 (13)
Instagram						
Mean Usage[c] (mean, SD)	0.8 (1.3)	1.2 (1.7)	0.87 (1.4)	1.3 (1.6)	0.94 (1.5)	0.93 (1.4)
Daily Usage (%, n)	7.0 (5)	22.5 (9)[d]	12.1 (11)	15.0 (3)	13.4 (11)	10.3 (3)
Never Use (%, n)	67.6 (48)	55.0 (22)	64.8 (59)	55.0 (11)	63.4 (52)	62.1 (18)

MD = medical doctor; SD = standard deviation
[a] Primary professional occupations included pharmacists, nurses and social workers.
[b] Primary practice settings included OBGYN clinics and private practices
[c] Variable measured on a Likert scale where 0 = Never Use; 1 = Rarely; 2 = Monthly; 3 = Weekly; 4 = Daily;
[d] $P < .05$

Social media benefits offer the opportunity for real-time responses at a relatively low cost, information sharing, increased accessibility and peer support [Moorhead SA et al., 2013]. This research corroborates our findings, showing that Romanian physicians were using social media resources, such as Facebook and WhatsApp, daily, suggesting that these platforms can be used for connecting Romanian physicians and provide medical education resources. As over two-thirds of non-physicians are using Facebook and WhatsApp daily, these platforms are a relatively easy way to gain access to guidelines, journals and other resources related to HIV prevention and treatment.

Our findings showed that HIV/AIDS clinical providers in Moldavia were more likely to use Facebook and Instagram daily, compared to those from Transylvania. This was a surprising finding, as Transylvania is considered to be the most technologically advanced region in Romania, housing many national and international technology businesses. We hypothesize that Moldavian providers are more curious in exploring online resources for medical education, and note that this finding deserves further investigation.

This study identified that Facebook and WhatsApp are clearly highly used and offer great potential for reaching clinicians with engagement, training opportunities, clinical communication, and support. Providers can collaborate and gain social support using social media, such as Facebook groups, WhatsApp chats, and Skype calls to exchange advice, ask questions, and address concerns [Coursaris C et al., 2009]. A Facebook group could be easy to access and use, offering differing levels of anonymity to protect both clinicians and the patients they serve [Bennett NL et al., 2004; Jaganath D et al., 2012; Longfield K et al., 2007; UNAIDS. Romania Country. Progress Report on AIDS, 2016]. Such a group could provide information and emotional support to enhance provider knowledge, deconstruct stigmatic myths, and compare methods of patient care, including validation and encouragement of proper, effective, ethical methods.

II.3.6 Conclusions

Social media offers important resources and opportunities that can help bridge the digital gap. Extending international collaboration, engagement, and training to social media platforms can help provide needed access to training and communication that can help clinicians who are otherwise somewhat removed from such opportunities. Regular social media use can be beneficial to physically isolated Romanian care providers. They can benefit from their peers' experiences with HIV/AIDS in ways that would reduce HIV/AIDS stigma surrounding identification and treatment. These findings support the use of social media platforms to connect HIV/AIDS care providers in Romania, which may also be applicable in other low resource settings.

Related articles

1. **Manciuc Carmen**, Nicu M, Largu MA, Filip-Ciubotaru F, Dorobăț C. - Sustainable development and key health trends: case study of dislipidemy in HIV patients. *Environmental Engineering and Management Journal*, 2010; **9**(4):495-503

2. **Manciuc Carmen**, Dorobat C, Danaila C, Largu MA. Lymphoma in an HIV-positive patient. *Rev Med Chir Soc Med Nat Iasi* 2015; **119:** 97–100

II.4 THE PREVALENCE OF PARASITIC INFECTION IN PREGNANT WOMAN

II.4.1 Introduction

Toxoplasmosis is a zoonotic infection caused by the obligate intracellular apicomplexan parasite *Toxoplasma gondii* (*T. gondii*) [Iemmi T et al., 2020; Ybañez RHD et al., 2020]. *T. gondii* can infect both birds and mammalians [Duong HD et al., 2020]. However, the only hosts where the parasite's sexual reproduction takes places are the members of the Felidae family: Both domestic and wild cats [English ED et al., 2019].

Scientific reports showed that around one third of the global population is infected with *T. gondii* [English ED et al., 2019]. The contamination could occur by ingestion of sporulated oocysts from contaminated soil, water or food [Foroutan M. et al., 2019]. Ingestion of raw or undercooked meat of the animals that care the pathogen is the primary source of infection (Foroutan M. et al., 2019). Vertical transmission from infected mother to fetus is also possible [Olariu TR et al., 2019], as well as transmission via blood products and graft tissue [Sanchez-Petitto G et al., 2020]. A large European study found that traveling outside Europe and the United States of America or Canada is also considered a risk factor predictive of acute infection in pregnant women, besides the ingestion of undercooked meat (lamb, beef, game), contaminated water or contact with contaminated soil [Cook AJ et al., 2000].

Congenital toxoplasmosis is caused by transplacentar infection of the fetus. This could determine a spontaneous abortion, fetal demise, intrauterine growth restriction, hydrocephalus, encephalitis, neurological, ocular or auditive diseases, inflammation or cardiovascular diseases [Olariu TR et al., 2019, Khan K et al., 2018]. Also, infection in pregnancy could be associated with preterm birth [Freeman K et al., 2015]. The risk of vertical transmission is correlated with the gestational age at the time of infection. It is approximately 15% at 13, 44% at 26, and 71% at 36 weeks of pregnancy [Thiébaut R et al., 2007]. Even if the risk of fetal infection is directly correlated with gestational age, the severity of fetal damage inversely correlates with gestational age, at the time the infection occurs [Kravetz J., 2013].

The global incidence of congenital toxoplasmosis is approximately 190,000 cases each year with a rate of 1.5 cases/1,000 births. The incidence varies in different regions from 0.5-1.6/1,000 births in Europe, 0.4-3.4/1,000 births in America to 2.0-2.4/1,000 births in Africa [Torgerson PR et al., 2013; Robert-Gangneux F et al., 2012].

The global prevalence of toxoplasmosis is estimated between 25-30% [Robert-Gangneux F et al., 2012; English ED et al., 2019]. Within low prevalence areas (between 10-30%) are: North America, North of Europe, South-East Asia and Sahelian Africa [Torgerson PR et al., 2013]. Areas with a moderate seroprevalence (30-50%) are Central and South of Europe [Torgerson PR et al., 2013]. High seroprevalence areas are Latin America and Tropical Africa [Torgerson PR et al., 2013; Robert-Gangneux F et al., 2012].

Childbearing and pregnant women from different geographical zones showed different seroprevalence, between 9% in the United Kingdom [Nash JQ et al., 2005] and 48.7% in Bruxelles, Belgium [Breugelmans M et al., 2004). In 2015 in Romania, a study by Olariu showed a seroprevalence of 57.6% in women of childbearing age [Olariu TR et al., 2008].

My interest in this field is reflected by the following paper:

1. Motoi S, Navolan DB, Malita D, **Manciuc Carmen** et al. A decreasing trend in toxoplasma gondii seroprevalence among pregnant women in Romania - results of a large-scale study. *Exp Ther Med* 2020; **20:** 3536–40. **Corresponding author**

II.4.2 Aim of the study

The aim of our study was to analyse the dynamics of *T. gondii* seroprevalence during a 10-year period and to correlate it with age and demographic features of the pregnant women.

II.4.3 Material and methods

Study design, settings and patients

A cross-sectional study involving 6,889 adult pregnant women was performed in Timisoara, Romania in two successive time-frames: i) 2008-2010 (group 1: 1,457 patients at City Universitary Emergency Hospital, Timisoara, Romania); and ii) 2015-2018 (group 2: 5,432 patients at "Bioclinica" Laboratory SRL, Timisoara, Romania). Patients were enrolled according to a consecutive-case population base. We collected data for each patient, namely age and area of residence.

Ethical issues

Approval from the Institutional Board of the "Victor Babeș" University of Medicine and Pharmacy (Timisoara, Romania; approval no. 848/06.04.2011) was obtained to perform this study. The present study meets the ethical guidelines, including adherence to the legal requirements of the study country. Informed consent was obtained from each patient.

Serological testing

The IgG- anti-*T. gondii* antibodies titer was determined by the immune-chemiluminescence method using an Immulite One Machine (Diagnostic Products Corporation) and commercial tests (Siemens Healthcare Diagnostics Products) for group 1 (2008-2010) and by chemiluminescent microparticle immunoassay (CMIA) method using an Architect i1000SR engine (Abbott) and commercial tests (Abbott) for group 2 (2015-2018). According to the cut-off values we stratified patients into two categories: Those with positive test values of IgG antibodies; and those with negative or inconclusive IgG test values.

Statistical analysis

Data was stored in the Astraia database (Astraia Software GmbH) and Microsoft Office Excel (Microsoft Corporation). Instat GraphPad Prism Software (GraphPad Software, Inc.) was used for statistical analysis. The results are presented as medians (interquartile ranges). Mann-Whitney-U, respectively Fisher's exact tests (proportions) were used to assess the differences between groups. Cochran-Armitage test (Chi-square test for trend) was used to evaluate the association between seroprevalence rate and age of pregnant women. $P<0.05$ was considered to indicate a statistically significant difference.

II.4.4 Results

Demographic characteristics of pregnant women

Among women from group 1, 71.15% (1,051/1,457) declared to be from urban and 27.85% (406/1,457) from a rural area, while of women in group 2, 65.19% (3,541/5,432) were from urban and 34.11% (1,891/5,432) from rural areas (Table XI).

Table XI - Demographic characteristics of participants

Characteristics	Group 1 (2008-2010) n=1,457	Group 2 (2015-2018) n=5,432
Area of residence [n (%)]		
Urban	1,051 (71.15)	3,541 (65.19)
Rural	406 (27.87)	1,891 (34.11)
Age (years)	28(6)[a]	29(7)[a]

[i] [a]P<0.001.

Pregnant women from group 1 (2008-2010) were younger than those from the group 2 (2015-2018): 28 years (n=6) vs. 29 years (n=7), (P<0.001; Table XI).

Seroprevalence results

Of the 1,457 women included in group 1, 638 (43.79%) tested positive, 812 (55.73%) negative and 7 (0.48%) inconclusive. Of the 5,432 women included in group 2, 2,108 (38.81%) tested positive, 3,210 (59.09%) negative and 114 (2.10%) inconclusive for the presence of IgG-anti-*T. gondii* antibodies.

Women from urban areas showed lower seroprevalence rates compared to those from rural areas: Group 1 (2008-2010) 40.53 vs. 52.22% (P<0.001) or group 2 (2015-2018) 34.85 vs. 46.22% (P<0.001; Table XII).

Table XII - Seroprevalence to *Toxoplasma gondii* according to the area of residence and age of pregnant women

Variables	Group 1: 2008-2010, n=1,457	Group 2: 2015-2018, n=5,432	P-value
Overall seroprevalence	638 (43.79%)	2,108 (38.81%)	<0.001
Area of residence			
Urban	426/1,051 (40.53%)	1,234/3,541 (34.85%)	<0.001
Rural	212/406 (52.22%)	874/1,891 (46.22%)	<0.05
P-value	<0.001	<0.001	
Age interval, years			
18-26	216/527 (40.99%)	606/1,595 (37.99%)	
27-35	346/836 (44.98%)	1,172/3,078 (38.07%)	
≥36	46/94 (48.94%)	330/759 (43.47%)	
P-value	0.074	0.035	

A decreasing trend was found of IgG-anti-*T. gondii* seroprevalence in pregnant women. In the first tested period the seroprevalence was found to be 43.79% compared with 38.81% in the second tested period (P<0.001). This trend was observed in pregnant women from both urban (40.53 vs. 34.85%; p<0.001) and rural (52.22 vs. 46.22%; p=0.02) areas (Table XII).

We found an increasing tendency of seroprevalence among older pregnant women. Thus, women older than 36 years had a higher seroprevalence rate compared to women aged 27-35 years or younger than 26 years. The difference was observed in both groups, however a significant value was found only in the second group (P=0.035), while in the first group the significance was borderline (P=0.074; Table XII).

II.4.5 Discussion

We found that 62.3% from group 1 and 56.26% from group 2 were susceptible to a primary *T. gondii* infection in our region. In low seroprevalence regions the risk of contracting an infection is low, but if a contamination occurs, the risk of primary infection is high. On the contrary, in high prevalence regions, the risk for a pregnant woman to contract a primary infection is low because the majority of pregnant women are seropositive. In the context of iatrogenic immunosuppression or of a disease, reinfection may occur. Also, contamination with other *T. gondii* strains may lead to reinfection in an immunocompetent organism [Pomares C et al., 2018].

The global *T. gondii* seroprevalence in the general population is approximately 25-30% [English ED et al., 2019; Robert-Gangneux F et al., 2012]. Studies from the United States of America (USA) showed that the prevalence in the USA has continued to decline from 14 -23% (1988-1994),

to 9% (1999-2004) and 6% (2009-2010) [Jones JL et al., 2014; Centers for Disease Control and Prevention. Parasites-Toxoplasmosis]. Research from other regions showed a general seroprevalence of approximately 44% in France [Berger F et al., 2009], between 51 - 72% in Latin America and between 5 - 35% in Asia, China and Korea [Rorman E et al., 2006].

Data on childbearing age women showed a seroprevalence of 9% in Great Britain (Nash JQ et al.,2005), between 16 - 29% in Spain [Ramos JM et al., 2011], approximately 24% in North of Portugal (Lopes AP et al.,2012), 29.1% in Croatia [Vilibic-Cavlek T et al., 2011], 44% in France [Villena I et al., 2010] and 19% in Italy [Pinto B et al., 2012]. Other studies showed a seroprevalence of 9.1% in 2009 in the USA [Jones JL et al., 2014]. Thus, showed that pregnant women from our region fit in a moderate level of seroprevalence. However, in Europe only women from France showed a higher seroprevalence [Villena I et al., 2010].

A study conducted by Olariu et al in 2008 showed a seroprevalence of 57.6% in women of childbearing age [Pappas G et al., 2009]. Our results showed a decresing trend in the seroprevalence among pregnant women from our region from 43.72% (2008-2010) to 38.70% (2016-2018). Likewise, a decrease in the seroprevalence rate was found in the USA from 14.9% (1988-1994), to 11.1% (2004) and to 9.1% (2009-2010) [Jones JL et al., 2014]. Studies by Villena et al reported in France a decrease in seroprevalence in pregnant women from 66% (1980), to 54% (1995) and 44% (2003) [Villena I et al., 2010]. Similarly, researchers from Netherlands showed a decline in seroprevalence in pregnant women from 32.5% (1995-1996) to 18.5% (2006-2007) (Hofhuis A et al., 2011).

Data on seroprevalence from many other European countries showed a similar decreasing trend: Austria [from 48% (1970) to 31-35% at present] [Edelhofer R et al., 2010; Sagel U et al., 2011], Italy - from 31% (2001) to 21% in (2005) [Robert-Gangneux F et al., 2012], Portugal - from 31.4% (2005) to 24.4% (2010) [Pinto B et al., 2011], and Poland - from 41.8% (2004) to 37.8% (2012) [Nowakowska D et al., 2014].

The decrease of seroprevalence was found in both women from rural (52.22 vs. 46.22%) and urban areas (40.53% vs. 34.85%) and could be explained by an improvement of socioeconomic and hygiene condition over the last ten years.

Our study showed a higher *T. gondii* seroprevalence in pregnant women from rural areas compared with women from urban areas, in both groups: Group 1 (52.22 vs. 40.53%) and group 2 (40.53 vs. 34.85%). Such results are in line with a previous study by Olariu et al which highlited the same trends in women (76 vs. 55.3%) [Olariu TR et al., 2015] and in children (18.4 vs. 14.7%) [Căpraru ID et al., 2019].

Other countries also reported higher seroprevalence rates in people from rural areas compared with urban ones: Mexico 32 vs. 12% [Alvarado-Esquivel C et al., 2013] or Argentina 36.4 vs. 26.8% [Rivera EM et al., 2019]. This variation can be attributed to the lower socio-economic status in some rural areas compared with urban ones, as well as to the less easy access to some services, such as running water. An epidemiological profile with an increased risk of seropositivity for *T. gondii* is represented by a lower educational status, a lower economic level, without a job outside the household, and people living in a household without running water resources. These risk factors are more common in rural and suburban areas, with poor socioeconomic conditions and more frequent contact with animals, which may explain the higher seroprevalence [Belluco S et al., 2016; Mareze M et al., 2019].

Our results confirm the studies of Vilibic-Cavlek in Croatia and Olariu in Romania, showing an age-related increase in seroprevalence [Vilibic-Cavlek et al., 2011; Olariu TR et al., 2015].

It should be noted that a limitation of the study could be the fact that the two institutions in which the patients were tested might be slightly different in accessibility. The clinical hospital is a public institution while the “Bioclinica” Laboratory is a private institution. This might, theoretically, indicate a different social structure of the patients tested in the two institutions/groups: 2008-2010

and 2015-2018. However, we consider that this aspect could be negligible because in both institutions the tests were done at a similar cost for the patient. Future studies are needed in order to confirm this decreasing trend in seroprevalence for *T. gondii*. We include screening for TORCH agents along with first and second trimester screening in antenatal care program [Navolan D et al., 2017; Ionescu CA et al., 2017; Vladareanu S et al., 2017; Nicolov M et al., 2017].

II.4.6 Conclusions

In conclusion, in the Western Region of Romania *T. gondii* seroprevalence in pregnant women has declined from 43.79 to 38.81% in the past ten years. This trend was found in both urban (40.53 vs. 34.85%) and rural areas (52.22 vs. 46.22%). A higher seroprevalence rate was found in rural areas compared to urban ones. In addition, we found an increasing tendency of seroprevalence related to the age of pregnant women.

Related articles

1. **Carmen Manciuc,** Nicolau C, Vata A, et al. Prophylactic antiretroviral treatment in new-born infants from HIV-positive mothers in 2012 to 2015, for the North-Eastern part of Romania. *J Int AIDS Soc* 2016; **19:** 17.
2. Vasilescu C, Lacatusu GA, Stefan M, **Manciuc C**. Infection and Pregnancy - The Experience Of The Hospital Of Infectious Diseases Iasi In 2018. *Rev Med Chir Soc Med Nat Iasi* 2020; **124:** 247–59.
3. **Manciuc Carmen**, Nicolau C, Vâţă A, Prisăcariu LJ, Matei D, Boghian A, Largu A, Dorobăţ C Mother to child HIV transmission in the north-east of Romania. *Ther Pharm Clin Tox*. 2010; **14** :300-301

Books

1. **Manciuc Carmen** -Infectii oportuniste-in Abord interdisciplinar in infectia cu HIV, Iasi, Editura Tehnopres, ISBN 978-606-687-354-3, p262-346
2. **Carmen Manciuc,** Cristina Nicolau- Categorii speciale de pacienti in infectia cu HIV- in Abord interdisciplinar in infectia cu HIV, Iasi, Editura Tehnopres, ISBN 978-606-687-354-3, p 381-404
3. Liviu Miron , Lavinia Ciuca, Dumitru Acatrinei Olimpia Iacob, Larisa Ivanescu Constantin Roman, Raluca Mandru, **Doina Carmen Manciuc et. all** - Dirofilariosis/Dirofilarioza- Guide of main parasitic diseases transmitted from non-human animals to humans- dirofialiosis in humans and animals, Iasi, Editura Ion Ionescu De La Brad, ISBN 978-973-147-313-0
4. Liviu Miron , Lavinia Ciuca, Dumitru Acatrinei Olimpia Iacob, Larisa Ivanescu Constantin Roman, Raluca Mandru, **Doina Carmen Manciuc et.al -** Malaria-guide of main parasitic diseases transmitted from non-human animals to humans Editura Ion Ionescu De La Brad, ISBN 978-973-147-315-4-0

II.5. PSYCHOLOGICAL ASPECTS OF INSTITUTIONALISED HIV POSITIVE CHILDREN FROM "PEDIATRIC COHORT" IN ROMANIA

II.5.1. Introduction

The environment is a key issue in determining health, since it is estimated to account for almost 20% of all deaths in the WHO European Region [WHO. Environment and health, 2017]. The imbalanced sharing of people's exposure to – and potentially of disease resulting from – environmental conditions is powerfully correlated to a variety of determinants, which can refer to socio-demographic issues or particular sensitive human categories.

Involvements of decision makers to undertake environmental health variations should be based on an appraisal of the extent of these health impacts and on the identification of population groups that are most exposed or most vulnerable to environmental risks [WHO, 2012].

Nowadays, it seems that at least one research and policy dimension remains to be investigated: the correlation between HIV/AIDS and the environment. This meeting point of HIV/AIDS and the environment generally could affect millions of people [Hunter LM, 2007; Manciuc et al., 2010].

The HIV/AIDS patient has a number of features that must be dealt with in a specific manner. Clinical practice has shown that most HIV/AIDS patients suffer from an inferiority complex due to their physical appearance that has been altered by the side effects of some antiretroviral drugs [Manciuc et al., 2009]. The impact of physical changes experienced by patients is significant in terms of emotional anxiety, depression, and stress. Physical incapacity, weakness, and being rejected by peers can cause low self-confidence.

In Romania, more than two thirds of the HIV-positive population is made up of young people that have been infected in childhood, through unscreened blood transfusions [Buzducea et al., 2010; Lazăr et al., 2008]. An impressive number of them were abandoned in orphanages, because of their parents ignorance, poor living conditions or other disease related disability. The rest remained with the family, where they had two distinct types of attitudes: acceptance and support or blame and stigma - depending on the level of education of the parents [Buzducea, 1997].

The main characteristics of an institutionalized child, whether abandoned at birth or in the first 4 years of life, are mental retardation, failure to establish deep relationships with other people, numbness of emotional reactions, aggression, and low self-confidence, increased antisocial impulses, closely linked to emotional problems. Even in relatively well-integrated and apparently balanced children, there is a diminished development and expression of feelings, as well as difficulties in establishing social contacts [Dumitrana M, 1998].

Another frequently encountered aspect is the tendency for young HIV- positive institutionalized youth to have aspirations and make plans that are inconsistent with their personal capacities, thus setting themselves up to fail. Also, the fear of overcoming their social status, the fear of real or imaginary social stigma, the lack of confidence in one's self, as well as the attitude some people have towards them, all contribute to a negative self-image and underestimation [Close K, 2007].

Prejudices about children/young people with HIV from orphanages arise from stereotypes that may or may not have a real basis. They operate on the principle of generalization by which group characteristics are assigned to every member, undifferentiated, a simplistic cliché of appreciation and categorization [Usaci et al., 2003].

My interest in this field is reflected by the following paper:

1. **Manciuc C**. Largu, M.A. - The Impact of the Institutionalized Environment on the Psycho - Emotional Development of the HIV-positive Youth. *Environ Eng Manag J* 2014; **13:** 2897–903.

II.5.2 Aim of the study

The purpose of the present research was to highlight the impact that two major aspects HIV/AIDS status and family background (growing up in a family or an orphanage) induces on the individual self-esteem.

II.5.3 Material and method

We evaluated 93 young people aged between 14 and 25 years, over a period of 12 months, from January to December 2013. We divided the subjects into four different groups. A number of 25 subjects in the experimental group 1 (HIV infected individuals from families) were randomly selected from patients admitted to the department of HIV/AIDS in the Infectious Diseases Hospital lasi, Romania. The 19 subjects in the experimental group 2 (HIV infected people from placement centers) were selected from the "Gulliver" Hospice Type Placement Center.

The experimental group 3 (uninfected persons coming from families) consisted of 25 randomly selected people from the following institutions:

- "Al. I. Cuza" University of lasi, Romania, from the Department of Sociology Sport and Physical Education, Geography and Geology;
- "Gheorghe Asachi" Technical University of lasi, Romania, from the Departments of Civil Engineering, Electronics and Telecommunications;
- "George Enescu" University of Arts, lasi, Romania, Department of Interior Design and Decorative Arts;
- "Gheorghe Asachi" Technical High school, lasi, Romania;
- "George Calinescu" School no. 39, lasi, Romania.

Subjects in the experimental group 4 (uninfected institutionalized persons) were a total of 25, and were recruited from the Placement Center, lasi, Romania. We evoked two independent variables the person's HIV status (positive/negative) and the person's growing-up environment (family/placement center). Our goal was to evaluate their individual and cumulated impact on the dependent variable - self-esteem (Table XIII).

Table XIII - Variables and experimental groups in the study

Independent variable 1 / Independent variable 2		HIV status	
		HIV +	HIV -
Growing-up environment	Family	Experimental Group 1	Experimental Group 3
	Placement Center	Experimental Group 2	Experimental Group 4
Dependent variable		Self esteem	

To measure self-esteem, we used The Self- Esteem Inventory (SSI), developed by the Center for Applied Psychology in France, based on a study of the origins, implications and correlations of self- esteem. The instrument was designed to measure self-esteem on four levels: social, family, personal and professional (or school). There were 58 items that describe feelings, opinions and reactions. Most items come from the Dymond Rogers scale.

There are two forms - school and adult — for which compiling the inventory is the same:

- The overall scale: 26 items (1, 3, 4, 7, 10, 12, 13, 15, 18, 19, 24, 25, 27, 30, 31, 38, 39, 43, 47, 48, 51, 55, 56, 57);
- Social scale: 8 items (5, 8, 14, 21, 28, 40, 49, 52);
- Family scale: 8 items (6, 9, 11, 16, 20, 22, 29,44);

- Work / school scale: 8 items (2, 17, 23, 33, 37,42, 46, 54);
- Lying scale: 8 items (26, 32, 36, 41, 45, 50, 53,58).

For the purpose of our study, we used the school form. All the subjects completed the questionnaire individually. In group 1, 2 and 4 the psychologist was sometimes asked to read the questions aloud and to explain their meaning, depending on the education level of the persons.

II.5.4 Results

Study sample

The study evaluated 93 young people, with a mean age of 20.48 year (Figure 22); 33 were male (35.48%) and 60 females (64.51%). Forty-nine came from a family environment (52.6%) and forty-four from placement centers (47.3%). As for the level of education, more than half (53.7%) of the young people were attending or had finished high school. In 25.8% of cases education was limited to primary schooling (4-8 grades), and we reported two cases of home-schooling. Out of all the young people 18.27% had a higher education, attending university or post-high school classes.

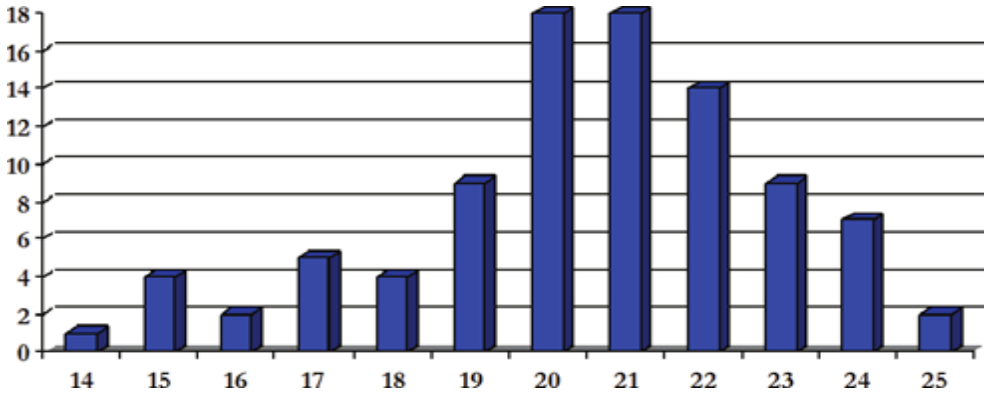

Fig.22 Distribution of subjects according to age

Impact of the HIV status and growing-up environment on the Overall Self-Esteem

There are significant differences between the results obtained by the institutionalized youth and the ones growing up in families, on the Overall Self-Esteem. The independent variable "growing-up environment" affected the subjects' results on the dependent variable Overall Self-Esteem, as following:

- HIV positive youth from placement centers had a significantly lower overall self-esteem than HIV positive youth form families ($p = 0.001 < 0.05$);
- HIV negative youth from placement centers also had a significantly lower overall self-esteem than HIV negative youth form families (p - 0.002 0.05).

Regarding the impact of the second independent variable, HIV status:

- comparing youth from placement centers, HIV positives had a significantly lower overall self-esteem score than HIV negatives (p - 0.008 0.05);
- comparing youth growing up in families, HIV positives had a significantly lower overall self-esteem score than HIV negatives ($p = 0.004 < 0.05$).

After performing a Test of Between- Subjects Effects specific to SPSS, we noticed that, even though each of the independent variables had an impact on the subject's level of overall self-esteem, there was no cumulated impact ($p = 0.380 > 0.1$).

Impact of the HIV status and growing-up environment on the Social Self-Esteem

There are significant differences between the results obtained by the institutionalized youth and the ones growing up in families, on the Social Self-Esteem. The independent variable "growing-up environment" affected the subjects' results on the dependent variable Social Self-Esteem, as following:

- there was no significant difference between HIV positive youth growing up in placement centers and families, regarding social self-esteem (p = 0.278 > 0.1);
- HIV negative youth from placement centers had a significantly lower social self-esteem than HIV positive youth form families (p - 0.001 < 0.05).

Regarding the impact of the second independent variable, "HIV status":

- comparing youth from placement centers, there was no significant difference between HIV positives and HIV negatives regarding social self-esteem (p - 0.744 > 0.05);
- comparing youth growing up in families, HIV positives had a significantly lower social self-esteem score than HIV negatives tp = 0.01l< 0.05);
- There is also a cumulated effect of both independent variables' interaction on the subjects' social self-esteem (p = 0.011 < 0.05).

Impact of the HIV status and growing-up environment on the Family Self-Esteem

There are significant differences between the results obtained by the institutionalized youth and the ones growing up in families, on the Family Self-Esteem. The independent variable "growing-up environment" affected the subjects' results on the dependent variable Social Self-Esteem, as following:

- HIV positive youth from placement centers had a significantly lower family self-esteem than HIV positive youth form families (p = 0.000 < 0.05);
- there was no significant difference between HIV negative youth growing up in placement centers and families, regarding family self-esteem (p = 0.030 < 0.05).

Regarding the impact of the second independent variable, "HIV status":

- comparing youth from placement centers, HIV positives had a significantly lower family self-esteem than HIV negatives (p - 0.003 < 0.05);
- comparing youth growing up in families, there were no significant differences between HIV positives and HIV negatives regarding family self-esteem score (p - 0.030 > 0.1).

Impact of the HIV status and growing-up environment on the School Self-Esteem

There are significant differences between the results obtained by the institutionalized youth and the ones growing up in families, on the School Self-Esteem. The independent variable "growing-up environment" does not influence the results on the dependent variable "school self-esteem", in the sense that there are no significant differences between the results of subjects from orphanages and those from families in the variable (p = 0.120 > 0.05 for HIV+; p= 0.344 > 0.05 for HIV-).

However, the independent variable "HIV status" does influence the results on the dependent variable "school self-esteem", in the sense that there are significant differences between the results of infected and uninfected subjects. Comparing youth from placement centers, HIV positives had a significantly lower school self-esteem than HIV negatives (p = 0.009 < 0.05). The same can be said when comparing youth growing up in families. HIV positives had a significantly lower school self-esteem than HIV negatives (p = 0.043 < 0.05).

II.5.5 Discussions

After analyzing the results, we concluded that HIV positive young people have a lower level of overall self-esteem compared with uninfected youth. Both young people from families and from placement centers recorded significantly lower scores in cases of HIV infection than in the absence of disease. Family support is the main factor influencing family self-esteem, as there are no significant differences between positive and negative youth that grew up in a family environment. For young people from placement centers, there are differences in scores, lower averages having been obtained by HIV- positive youth.

These results regarding family self-esteem can be explained by analyzing the main causes of child abandonment in lasi and the importance of maintained family ties. In the case of HIV-negative youth from the "Ion Holban" Placement Center, their parents decision to give them up as babies was

mainly motivated by poor living conditions, the inability to ensure a decent living, and rarely because of certain neuro and/or locomotive disabilities.

For the HIV-positive youth living in the "Gulliver" Hospice Type Placement Center, the situation was different. Most of them have been abandoned prior to their diagnosis, the main reason being their parents ignorance and fear in the face of the unknown and threatening disease that was HIV/AIDS. These very different situations determined the young people's attitudes towards keeping in touch with their family, and also shaped positive or negative emotions about them. Since self-esteem refers to the perception of their competence to cope [Branden N, 2001], parental attitude is, in the case of HIV positive children, the main factor that shapes and strengthens negative beliefs about their ability to maintain significant relationships with their parents, being loved and accepted.

In the case of school self-esteem, young people living with HIV/AIDS recorded low scores, regardless of their growing-up environment. This can be explained by two specific characteristics of the infection. First of all, poor administration of the antiretroviral treatment allows the virus to penetrate into the central nervous system and affect the brain structures responsible for attention, memory, and focus on intellectual tasks [Dorobăț et al., 2008].

The students recorded poor school performance, which caused them to create a negative image of themselves. Secondly, the HIV-positive child faces discrimination ever since the beginning of school [Wigfield et al., 1994]. Parents or caregivers are required to inform the school doctor or nurse [Astarastoae et al., 2000], which not always respect confidentiality, especially in rural areas, where gossip is the main means of circulating information. Thus, children are stigmatized by teachers who can convey their attitude to other children; this creates a tense learning environment in which good school results fail to appear. In rural communities, where there is sometimes a lack of proper medical education and scientific understanding, children are often forced to drop out of school due to such extreme discriminatory manifestations.

Our results also show that, regardless of their HIV status, young people growing up in placement centers have a lower overall self-esteem. Differences emerged regarding social self-esteem, where the tendency was manifested only in HIV- negative persons. HIV-positive youth, however, recorded similar levels of social self-esteem, with no significant differences between subjects growing up in different environments.

Social self-esteem emerges by comparison with persons of the same age, by interacting with them, by dealing with various situations involving interpersonal relations and solving them [Baumeister, 2003; Leary 1990]. However, HIV-infected young people often wear the stigma of their disease in these social interactions. This stigma is either imposed by society, or perceived as such by its members, or it is felt only by the individual, that reacts in line with expectations derived from it.

II.5.6 Conclusions, limitations and perspectives

The most important limitation of this study was the instrument used to measure self-esteem the Self Esteem Inventory (SSI). Even though we have used SSI in the school form, practice has shown us that a number of items (It) were difficult to understand by the subjects. These items contain negations that are difficult to answer (for example It 1. "Generally, I do not worry"), or some neologisms (for example, in Romanian It 22 "agasat" (annoyed), It 17- "stingher" (lonely), It 56 – "a intreprinde" (to undertake)). Given the intellectual or school level of some subjects, they had difficulty in relating to these items. To overcome this limit, we opted for reading aloud the questions for the majority of subjects.

Another limitation derives from the tendency subjects have to lie. This trend was measured by the Lying Scale. There was a greater tendency of presenting oneself in a favorable light in the case of youth from placement centers, whether HIV positive or not, due to a possible desire to please, to be accepted etc.

Research conducted with HIV-negative subjects from various categories, has shown self-esteem to be relatively stable over time. Measurements taken in our research have evaluated the self-esteem in a single moment. However, given the specific topic discussed, and the major changes that occur in the subjects' lives at different stages of development, we believe that longitudinal studies are necessary, in order to observe self-esteem at different points in the development of children, adolescents and young people to adult stage.

Related articles

1. **Manciuc Carmen,** Nicolau C, Prisăcariu LJ, Dorobăţ C. Palliative care in AIDS cases *Ther Phar Cl Tox.* 2010; **15:** 100–02.
2. Largu MA, Dorobat C, Astarastoae V, **Manciuc Carmen**. The psycho-emotional profile of the HIV positive naive patients. *Rev Med Chil* 2014; **118:** 733–38.
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6. **Manciuc Carmen**, Largu MA, Vâţă A, Nicolau C, Prisăcariu L, Stoica D, Dorobăţ C. The quality of life in HIV/AIDS patients in Iaşi Romania. *Rev Med Chir Soc Med Nat Iasi* 2011; **115:** 1214–18.
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Books

1. Largu Maria Alexandra, **Manciuc Doina Carmen**, Dorobăţ Carmen - Adolescence - A New Multilevel Approach on the HIV/AIDS Patient in IntechOpen - Global Bioethics Perspective for Human Survival Open access book ISBN: 978-953-307-537-2
2. **Carmen Manciuc**, Alexandra Lacatusu, Cristina Vasilescu, Alexandra Largu – The psychological impact on families of departed patients with infectious diseases in IntechOpen – Bioethics in Medicine and Society, Open access book ISBN: 978-1-83881-178-5

II.6 COINFECTION OF VIRUSES

II.6.1 Introduction

Hepatitis A and E are common, acute, self-limited viral infections produced by hepatitis A virus (HAV) and hepatitis E virus (HEV), generally acquired through the fecal-oral route, via either person-to-person contact or ingestion of contaminated food or water [Gossner CM et al., 2015; Pavio N et al., 2015]. A rare form of transmission is through blood transfusion, in the case of contaminated blood or when the donor is in the viremic prodromal phase of the infection, which is more frequently encountered in the transmission of hepatitis B or C [Stănculeț N et al., 2012].

HAV infection affects 120 million people annually worldwide, especially children [Dalton HR et al., 2014]. By contrast, World Health Organization estimated that HEV causes 20 million new infections annually and over 55,000 deaths [World Health Organization. Hepatitis E Fact sheet, 2016]. Currently, HEV infection represents a worldwide public health issue [Ditah I et al., 2014], Netherlands reported a 5-fold increased incidence of HEV infections in 2014 compared to previous years, while the number of cases in Germany went up 40-fold in the last 10 years [Aspinall EJ et al., 2017, Faber M et al., 2018].

Situated in southeastern Europe, Romania is part of this globalization process due to a growing number of foreign visitors and international food trade. Romania has an intermediate endemicity of HAV infection [Istrate A et al., 2020].

Hepatitis are common among patients with human immunodeficiency virus infection because of shared routes of viral transmission. Coinfection is associated with high morbidity and mortality in the absence of proper clinical management, making identification of these cases crucial.

My interest in this field is reflected by the following paper:

1. Mihai IF, **Manciuc C**, Hunea IM, et al. Enterically transmitted hepatitis in the third millennium in northeastern Romania. *Exp Ther Med* 2021; **21:** 274 – **Corresponding author**

II.6.2 Aim of the study

In this context, the aim of the present study was to evaluate the most recent epidemiological, clinical, biological and therapeutic data concerning HAV and HEV infections based on clinical practice at “Sf. Parascheva” Clinic Hospital of Infectious Diseases (Iași, Romania).

II.6.3 Material and methods

Studied patients

This was a retrospective analysis of hospital-based medical records of patients with a diagnosis of hepatitis A and hepatitis E viral infections hospitalized between 2018 and 2019 in “Sf. Parascheva” Clinic Hospital of Infectious Diseases from Iași. The inclusion criteria for the study consisted of in-patients with a diagnosis of HAV and HEV infections confirmed by blood tests and serological tests. Patients with hepatic disease (other than HAV or HEV) were excluded from the study.

Data collection

The following data were collected: demographic data, medical and medication history, clinical data, blood and urine tests, serological tests, treatment administered and outcome. All blood and urine tests were performed by the central hospital’s laboratory. The District Public Health Directorate, Iași, Romania, performed the serology tests (IgM HVA and IgM HVE, respectively).

Statistical analysis

Only cases with a complete dataset were included in the statistical analysis. Data were analyzed by descriptive statistics, where applicable. Correlation between demographic parameters, clinical data and outcome was performed using Pearson test in XLSTAT version 2019 software. Kendall's Tau correlation coefficients were calculated [Gust ID., 1992]. Statistical analysis was performed using Statistical Software for Excel (XLSTAT) version 2019.

II.6.4 Results

Our analysis consisted of 272 HAV and HEV infected patients with complete dataset, of which 98.9% were HAV infections and only 1.1% were HEV infection cases. There were no recorded cases of HAV and HEV coinfection. Patients were hospitalized at "Sf. Parascheva" Clinic of Infectious Diseases in Iași between 2018 and 2019.

Patients characteristics

A wide range of age was noted for the HAV-infected patients (between 11 months and 47 years), but as expected, most patients were aged 8-15 years (53% cases) (Figure 23).

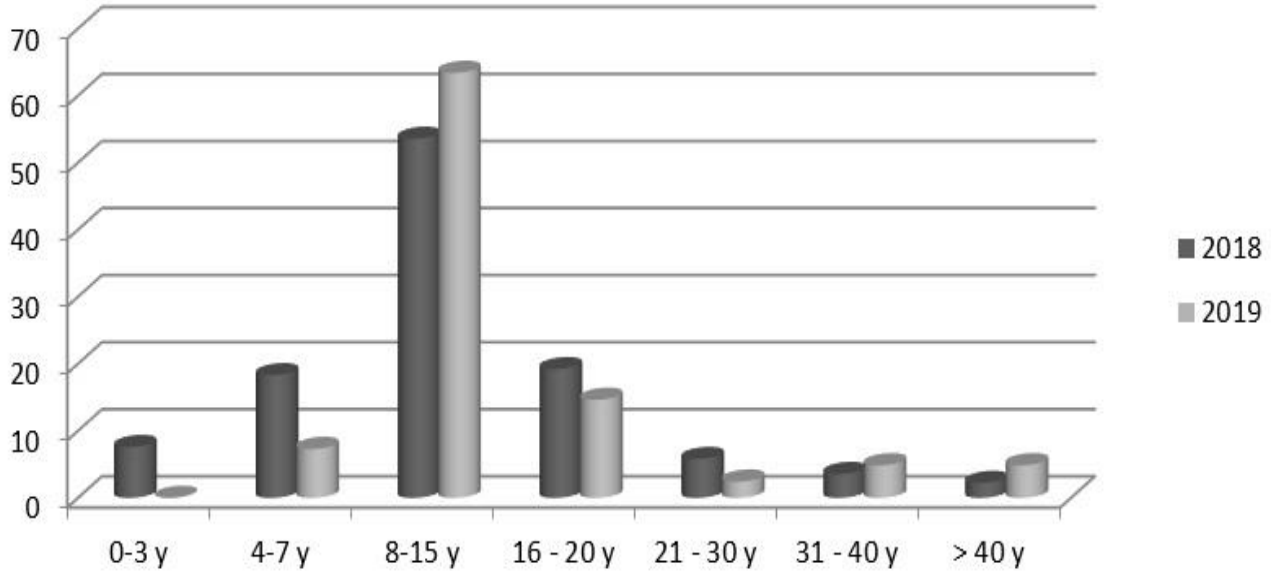

Fig.23 Distribution of HAV infections by age and year.

Most of the patients were male (53.9% cases) with epidemiological context of outbreaks of HAV infections from rural area (87.73% cases) (Table XIV).

Table - XIV Patients characteristics

Characteristics	HAV infection	HEV infection
	Number / Percentage	Number / Percentage
Mean age ± St dev (years)	13.31 ± 8.56	54.33 ± 3.06
Male / female ratio	145(53.9%) / 124(46.1%)	3 (100%)/0
Residence area – rural	236 (87.73%)	2 (66.67%)
Symptomatology	168 (62.45%)	3 (100%)
Comorbidities	12 (4.46%)	2 (66.67%)

Comorbidities were recorded in 12 cases (6.3%) of HAV infections, the majority represented by digestive disorders (41.66%, 5 cases). Hypertension (24.99%, 3 cases), chronic peripheral venous insufficiency (8.33%, 1 case), chronic kidney disease (8.33%, 1 case), urinary infection (8.33%, 1 case) and oncological pathology (8.33%, 1 case) were also noted in this group of patients (Figure 24).

Regarding the HEV infection, only three male patients with a mean age of 54.3 years were identified in the hospital database (Table XIV). None of the patients had international travel history within the last 2 months, and only one patient described previous contact with farm animals and possible contaminated water.

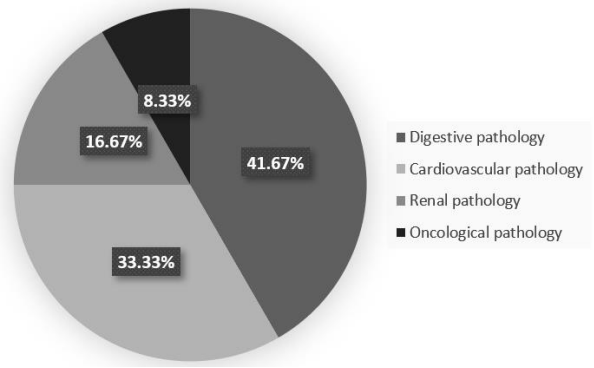

Fig.24 HAV-infected patients distribution by comorbidities (%).

Clinical findings

Regarding the onset symptomatology, most of the HAV infected patients (57.24%, 154 cases) had digestive disturbances (abdominal pain, nausea) and/or jaundice (52.78%, 142 cases) at clinical examination. Flu-like onset was present in 31 patients (11.52%), fatigue and neurasthenia in 17 patients (6.31%) and pruritus was found in 7 patients (2.6%) (Figure 25).

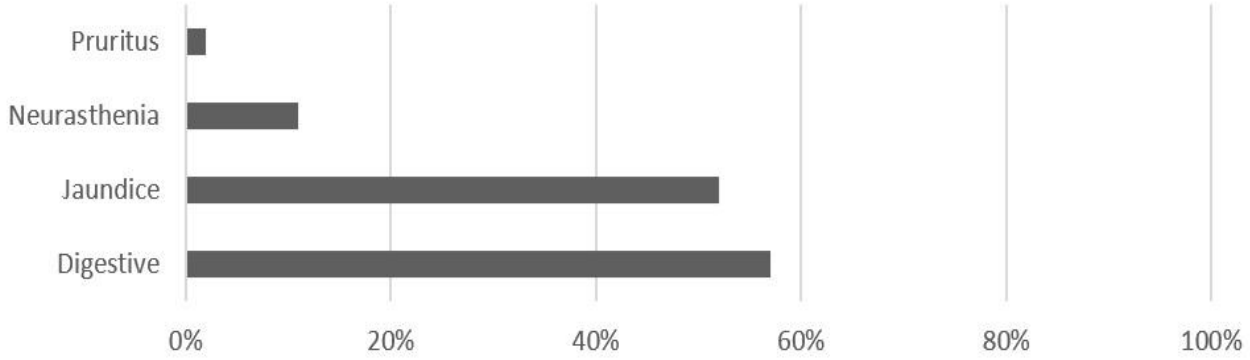

Fig.25 Distribution of symptoms in the HAV study group.

Regarding the HEV cases, the patients described a typical onset of infection with digestive symptoms (nausea, vomiting, abdominal pain) in 2 cases or with flu-like associated with somnolence and asthenia in the third case.

Laboratory findings

In the HAV cases, biological investigations revealed cholestasis in 59.65% of the cases. Based on the intensity of cholestasis syndrome, it was found that most patients had the moderate form of the disease, with a slight significant difference between adults (67.19%) (Figure 26) and children (54.43% cases) (Figure 27).

During an average hospitalization period of 12 days (maximum period of 36 days), the evolution was favorable with a mean decrease of 83% for alanine transaminase (ALT) and 60% for bilirubin.

In HEV, jaundice with significant cytolysis (transaminases increased up to 100-fold) and moderate cholestatic syndrome were noted in all 3 cases.

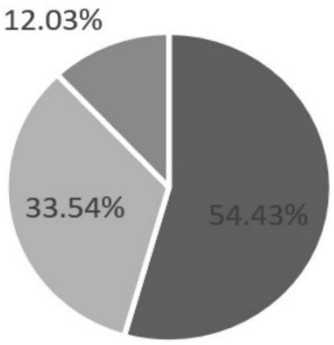

Fig.26 Severity of HAV in the adults

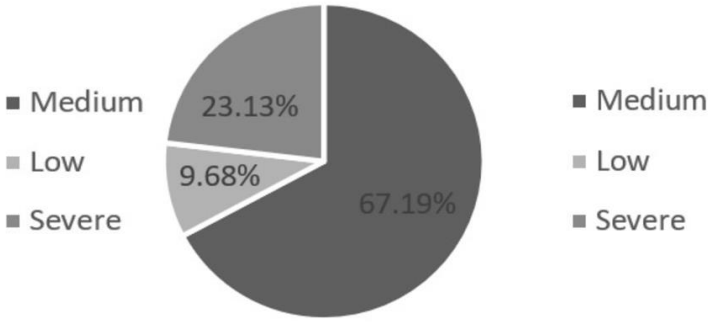

Fig.27 Severity of HAV in the children (0-18 years)

HEV infection had a self-limited evolution under hepatoprotective treatment, with jaundice remission and significant decrease in cytolysis and cholestasis in up to 16 days of hospitalization (Figure 28). Extrahepatic manifestations were not identified in all 3 cases and associated disorders (diabetes mellitus, hypertension, pre-renal failure) were not aggravated by viral infection.

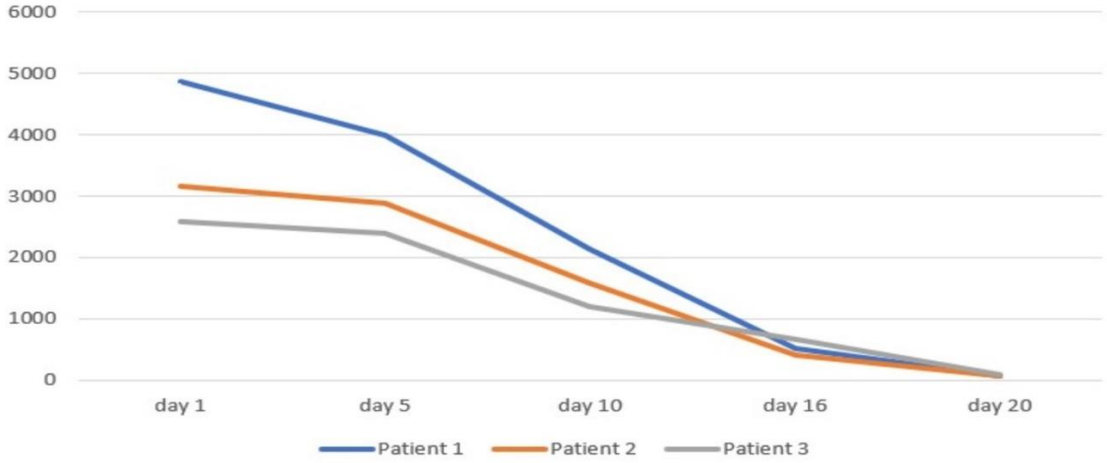

Fig.28 Evolution of cytolysis (TGP, glutamic pyruvate transaminase) in all 3 cases with HEV

Evolution

After hospital isolation, diet, strict hygiene, hepatoprotective treatment and Isoprinosine administration (in 2.5% of cases), over 95% of the HAV infected patients showed a favorable evolution, with only 5 cases of relapse in the first 3 months post-first infection.

There was a weak association between the duration of hospitalization and age (Kendall's tau = 0.198; Figure 29) but a strong correlation with the severity of liver dysfunction (Kendall's tau = 0.297, Figure 30).

The cases diagnosed with HEV, found in adult men, had a favorable evolution, with a hospital stay of about 2 weeks.

II.6.5 Discussion

In this retrospective study, we evaluated the epidemiological and clinical-biological evolution of patients with HAV and HEV infections hospitalized at "Sf. Parascheva" Clinic Hospital of Infectious Diseases in Iași in northeastern Romania. Only laboratory-confirmed cases with clinical criteria were included. Of the total number of patients hospitalized in our clinic during the analyzed period, 272 cases were included in our study. The majority of these cases originated from rural areas with poor sanitary conditions and/or bad hygienic habits. Moreover, in some cases with HAV disease, a familial aggregation was noted.

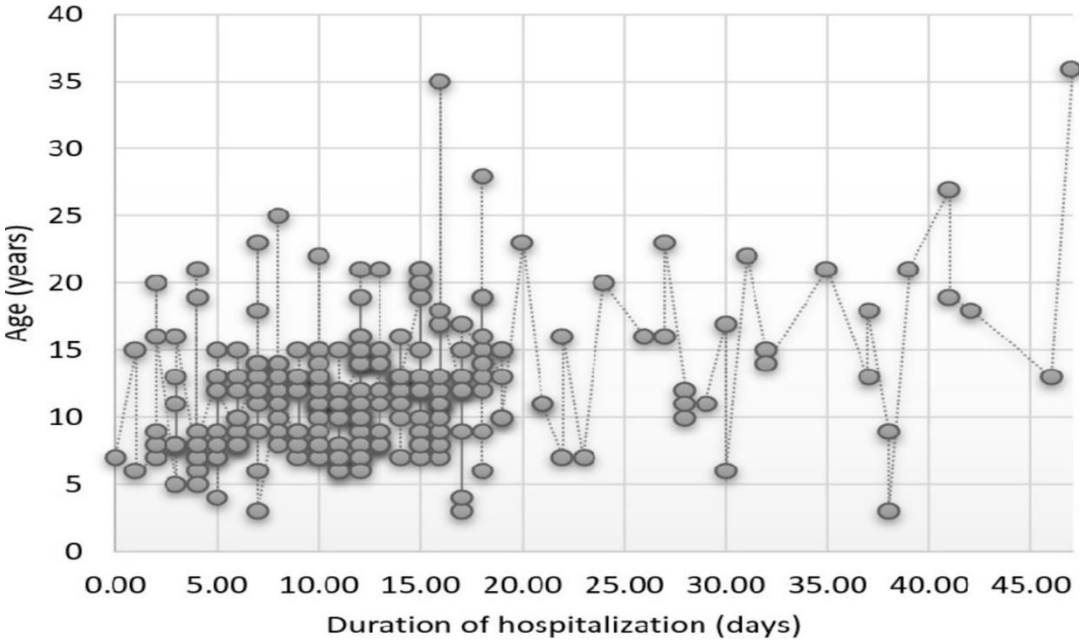

Fig.29 Correlation between age and duration of hospitalization (days).

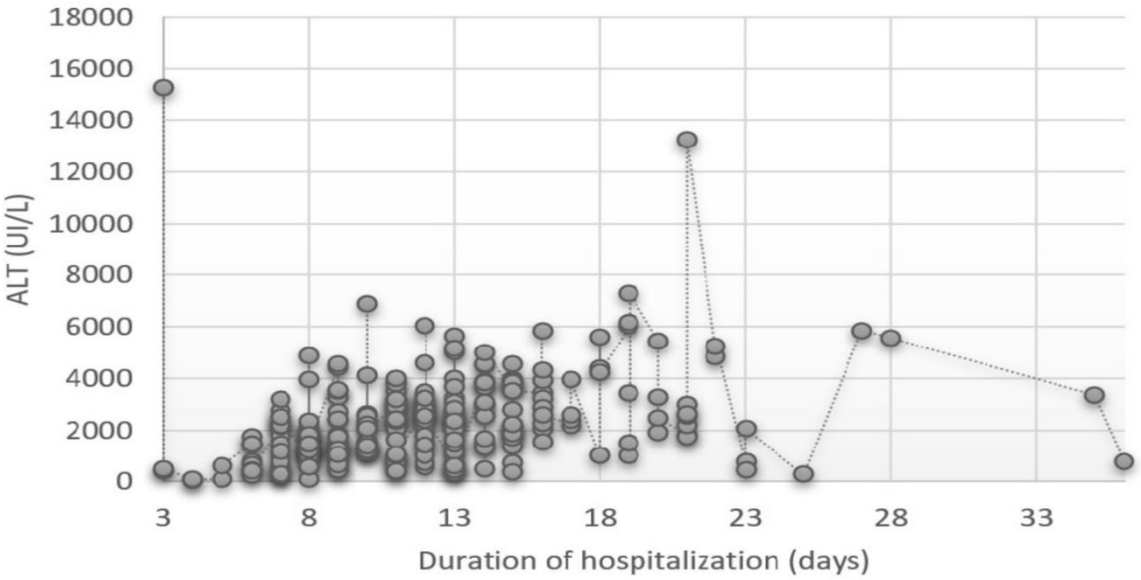

Fig.30 Correlation between severity of cytolysis (ALT) and duration of hospitalization (days)

Hepatitis A infection occurs worldwide, sporadically or in an epidemic form. Globally, an estimated 1.4 million cases occur each year [World Health Organization. Global Alert and Response Hepatitis A, 2007]. The US reported an increase in the incidence of HAV by 294% during 2016–2018 compared to 2013-2015 and in 2017, more than 650 people in the state of California were infected with hepatitis A (including 417 hospitalizations and 21 deaths) [California Department of Public Health. Hepatitis A, 2020].

International outbreaks have occurred via importation of contaminated food from areas where HAV is endemic [Wheeler C et al., 2005]. In some circumstances, seemingly sporadic occurrences may reflect cases from geographically distant outbreaks. In one report, for example, 213 cases of hepatitis A were detected in 23 schools in Michigan and 29 cases in 13 schools in Maine; all were related to contaminated frozen strawberries from a common source [Hutin YJ et al., 1999].

The epidemiological characteristics of HAV disease recorded in our study are in line with other Southeastern European countries [Mrzljak A et al., 2019; Tsankova GS et al., 2020]. Tsankova found in Bulgaria recurrent outbreaks with time and geographic fluctuations. Similar with our data, male gender and children aged less than 9 years are the main risk factors associated with spread of the disease [Tsankova GS et al., 2020].

Adult patients appear to be more frequently affected in the last few years, since we found 9% of cases in 2018 and almost 15% cases aged over 25 years. Even though we have noted an increase in age of HAV-infected patients during this period, the incidence of this infection is uncommon in patients over 50 years. When the serological tests for HAV infection in three male patients aged over 50 years were negative, we took into consideration HEV infection.

In a recent study performed in northwestern Romania, the mean age of HEV-infected patients was 50.6 years compared to 39.1 years for HAV infection [Teshale EH et al., 2010]. The diagnosis was based on clinical and serological IgM anti-HEV tests. We have to mention that only three other cases were confirmed in our clinic in the last 11 years and is not a frequent cause of viral hepatitis in our country.

The cases of HEV previously reported, diagnosed and treated in the infectious diseases clinic in Iași were not numerous, but, nevertheless, recent studies conducted in our region found a seroprevalence of 32.5% in the general population, which indicates the fact that most cases are possibly related to zoonotic transmission [Riveiro-Barciela M et al., 2015] similar to the way of transmission of leptospirosis [Manciuc DC et al., 2018, Manciuc C et al., 2007). Other studies show a seroprevalence rate of IgG HEV antibodies up to 17% in Romania, comparable to other east European countries (e.g. 20.9% in Bulgaria, 15% in Serbia) [Guerra JADAA et al., 2017].

In 2014, research on the evidence of HAE infection in pigs and humans in the northeastern part of Romania showed a seroprevalence of 17.14% (12/70) and 12.82% (10/78), in the tested human sera. The authors also reported that HEV infections were found in middle-aged adults exposed to the virus through contact with pig farms [Kamar N et al., 2012; Aniţă A et al., 2014].

The genotype 4 of HEV is the most frequently detected in the European area and is involved especially in cases of zoonotic transmission, including undercooked deer meat, wild boar meat, pig liver sausage, and internal organs of animals [Murrison LB et al., 2017, Li TC et al., 2005]. Information regarding other possible routes of transmission (e.g. blood transfusions, organ donors, vertical transmission) are limited in our region [Teshale E H et al., 2011].

The risk of infection is increased in travelers in endemic areas [Khuroo MS et al., 1995]. However, we could not identify a clear epidemiological context for our HEV-infected patients. Most patients with acute HEV are asymptomatic or mildly symptomatic, but severe cases were noted in middle age male or elderly patients, especially with comorbidities. The HEV infection is often associated with extrahepatic manifestation, including central nervous system (CNS) disorders, acute pancreatitis, glomerulonephritis or hematological abnormalities [Hewitt PE et al., 2014; Kim JH et al., 2014; Colson P et al., 2010] which can also cause psychological distress and may require the intervention of a psychologist [Manciuc C et al., 2014; Manciuc C et al., 2016].

II.6.6 Conclusions

In conclusion, moderate cases with only hepatic involvement were found in our hospital. The number of cases of HEV infection identified in our hospital was extremely low compared to HAV infections. One reason is that an HAV infection is a nationally notifiable infectious disease that must be reported from all medical specialists to the District Public Health directorates, when detected. By contrast, HEV infections remain underevaluated in humans in our region. In the meantime, studies performed in farm and wild animals confirm that HEV is a ubiquitously pathogen in swine, in northeastern Romania, and is a potential reservoir for HEV-associated infection.

HEV infection is a new threat to global public health, with fatal outcome in sporadic cases. Even in light of this fact, the evaluation within Europe is still uncertain due to limitations in surveillance systems, differences between diagnosis tests and lack of information.
Although the seroprevalence of HEV infection is increasing in Romania, the use of different methods for the diagnosis of hepatitis and the increased incidence of HEV infection among asymptomatic patients, can allow this pathology to go undiagnosed.

Related articles

1. Plesca C. **Carmen Manciuc**, Egidia Miftode, Cătălina Luca, Aida Bădescu, Olivia Dorneanu, Luminița Smaranda Iancu. Predictive value of laboratory markers in HIV-positive patient diagnosed with severe sepsis. *Revista de Chimie* 2018; **69:** 241–47
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9. Vâță A, Luca MC, **Manciuc Carmen**, Nicolau C, Vâță LG, Dorobăț CM - Changes in the clinical and epidemiological profile of acute hepatitis B in the Infectious diseases hospital of Iasi in the last 15 years. *Revista Română de Boli Infecţioase* 2013; **16** :96-100

Chapters in books

1. Cristina Vasilescu, Maria Ioana Hunea, Cătălina Mihaela Luca, M. Hurmuzache, Alexandra Lăcătuşu, C. Eva, **Carmen Manciuc**- Deplasarea morbidităţii prin hepatita acută virală A spre vârsta adultului tânăr - analiza cazurilor din Spitalul Boli Infecţioase Iaşi – Provocarea teoriei in practica medicla curenta editia V- Editura „Gr. T. Popa" Iasi 2019 ISBN 978-606-544-607-6 p 362-367

II.7 NEUROLOGICAL DAMAGES OF HIV INFECTION RELATED TO OPPORTUNISTIC INFECTIONS

II.7.1 Introduction

World Health Organization reported that tuberculosis generates up to 10 million cases each year and 1.5 million deaths annually, and it is ranked together with the human immunodeficiency virus (HIV) as a foremost origin of death worldwide [Alvaro-Meca et al., 2016; Petruciolli et a1., 2016; Raviglione et al., 2016; WHO, 2015]. The coinfection TBM (tuberculosis meningitis) and HIV, if not treated as soon as possible is deadly.

New tools for diagnosis and new biomarkers become more and more essential to appraise both pathogen and host key elements of the answer to infection. Biomarkers that point out the start of effective treatment could enable progresses for alternative treatment approaches. The WHO End TB Strategy discussed in the Global Tuberculosis Report [WHO, 2017] is to "End the global TB epidemic". Taking 2015 year as baseline, the milestone and targets for ending the global TB epidemic (zero deaths, disease and suffering due to TB), are quantified in some indicators, as illustrated in Table XV. The graph on the incidence of tuberculosis and deaths required to achieve the milestones and targets are illustrated in Figure 31.

Table XV - The WHO End TB strategy and indicators

Indicators	Milestones (%)		Targets (%)	
	2020	2025	2030	2035
Percentage reduction in theabsolute number of TB deaths (compared with2015 baseline)	35	75	90	95
Percentage reduction in theTB incidence rate (compared with2015 baseline)	20	50	80	90

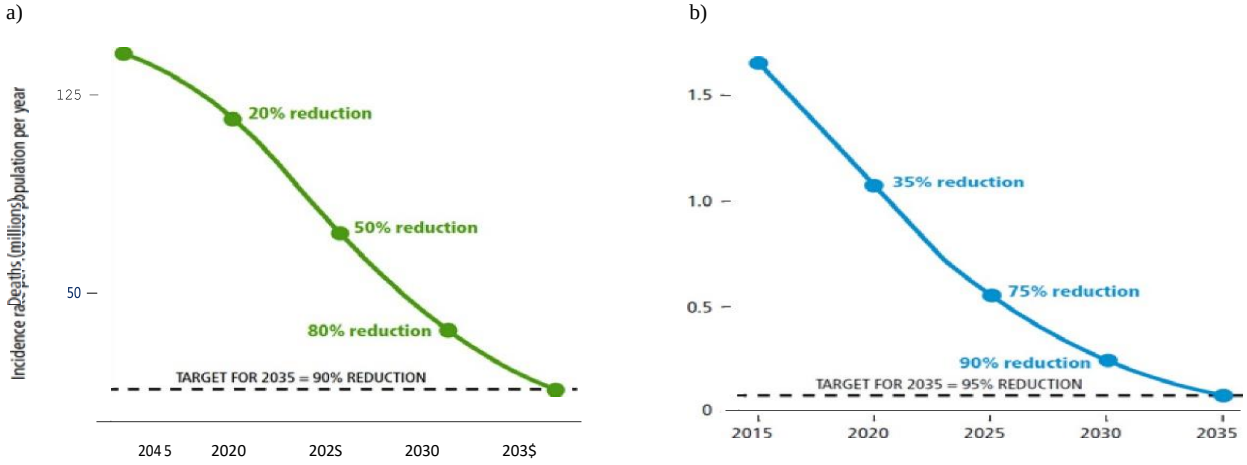

Fig.31 End TB Strategy incidence (a) and mortality (b) curves projected to reach during 2015-2035

My interest in this field is reflected by the following paper:

1. Cislariu SA, Lacatusu GA, Largu A, Iordan IF, Vata A. **Carmen Manciuc** - Changes in glucose levels – a predictive marker for an adequate environment aimed at *Mycobacterium tuberculosis* growth. *Environ Eng Manag J* 2018; **12:** 3007–11.

II.7.2 Aim of the study

In this paper, the issue of robust diagnosis of TB is addressed by presenting and discussing a case study, where the correlation of Mtb (*Mycobacterium tuberculosis*) presence and TB disease is performed via glycosylation level.

II.7.3 Case study

Case presentation

Epidemiological studies have showed that adults with diabetes have a significantly increased risk of developing active TB and it is estimated that globally 15% of TB cases are attributable to DM [Jeon CY er al., 2008; Lachmandas et al., 2015; Ruslami et al., 2010].

It is well-known that glucose is the largest energy source for brain cells, extracted from circulation, from capillary blood, with normal values in the cerebral-spinal fluid of 50-80 mg/dL, about 0.6-0.7 of the plasma concentrations.

In this case study we present the case of a 42- year-old urban patient, with no personal pathological history, athletic, risk-free, and with no contact with tuberculosis cases. He has been admitted in Infectious Diseases Clinical Hospital in lasi, Romania, between August 22nd, 2017 and September 30th, 2017. At admission, he presented with intense headache, fever chills, hypertension. On clinical examination, the patient was conscious, cooperative and without pathological changes in other systems. Prior to admission to the Infectious Diseases Department, the patient was evaluated in the neurology department, where it was revealed that no acute cranial-cerebral lesions occurred, and the appearance of the CT scan showed no abnormalities. From the paraclinical point of view, on admission, neutrophilia (71.4%) and mild thrombocytosis was noticed, and chest x-ray showed no abnormalities.

Diagnosis, evolution and therapy

Three days after admission, fever persisted, the headache got worse and patient developed photophobia, why the lumbar puncture was decided. In the microscopic examination of the cerebrospinal fluid (CSF), increased cellularity (124 cm/mm) with 15% polymorphonuclear and 85% lymphocytes was observed. Also, relevant are some of the biochemical results — low glycosylation (30 mg/dL) and albuminorheia with values of 0.63g/L (Table XVI).

Antibiotic therapy with CSF penetrating agents was started and and glucose level was well below the normal range, pre-treated bacterial meningitis was suspected and it was decided to administer dexamethasone to diminish the inflammatory process.

Evolution after 24 hours from the first lumbar puncture was not favorable, the patient suddenly developed diplopia. In conjunction with low glucose, this raised the suspicion of tuberculosis meningitis, despite not noticing a suggestive image for tuberculosis on chest x-ray, and not having any relevant epidemiological history.

Table XVI - Analysis results of the first lumbar puncture

Analysis	Results
Cellularity (ecn/mmc)	124
Polymorphonuclear neutrophils (%PMN)	15
Lymphocytes(%Ly)	58
Albumin (g/L)	0.63
Glucose (mg/dL)	30
Chlorine (g/L)	6.8
Others	Increased cellularity Pathological CSF glucose levels

It was thus decided to re-evaluate the CSF. The new sample showed an increased cellularity, but a lower level compared to the previous one (76 cc/mm), while the glucose level decreased to 16 mg/dL (Table XVII).

Table XVII - Analysis results of the second lumbar puncture

Analysis	Results
DNA Mycobacterium tuberculosis	Positive
Cellularity (ecn/mmc)	76
Polymorphonuclear neutrophils (%PMN)	32
Lymphocytes(%Ly)	64
Macrophages (%)	4
Glucose (mg/dL)	16
Others	Pathological CSF glucose levels

The lower glucose levels compared to the previous one (which was already low), proned us to performe a PCR (Polymerase Chain Reaction) for rapid detection of *Mycobacterium tuberculosis* DNA. The result came back positive, thus confirming the tuberculosis etiology. HIV serology was negative [Codina et al., 2011; Manciuc et a1., 2010; Manciuc et al., 2014]. Quadruple combination therapy (Etambutol, Pirazinamide, Isoniazid, and Rifampicin) was initiated [Hurmuzache et a1., 2017].

The condition of the patient remained the same even after 7 days of tuberculostatic therapy. A sudden hemiparesis was noticed on the left side of the mouth, diplopia got worse and fever persisted. A second HIV serology was negative [Costan et al., 2016; Teodor et al., 2013]. The patient was neurologically reexamined with magnetic nuclear imaging (MRI), and a new lumbar puncture was performed, targeting growing cellularity (370 ecn/mmc), but with DNA for *Mycobacterium tuberculosis* - undetectable. The MRI showed that cerebral edema and a slight triventricular dilation was noticed. Quadruple combination tuberculostatic treatment and steroidal anti-inflammatory therapy was continued. An improvement of the neurological symptoms was noticed over the next few days and the febrile syndrome remised [Nakao JH et al., 2016].

At discharge, lumbar puncture showed normal cellularity, with glucose levels still below the normal range, but higher than previous ones, MRI examination - within normal limits, and the motor deficit was minimal (Figure 32) [Incesu et al., 2015].

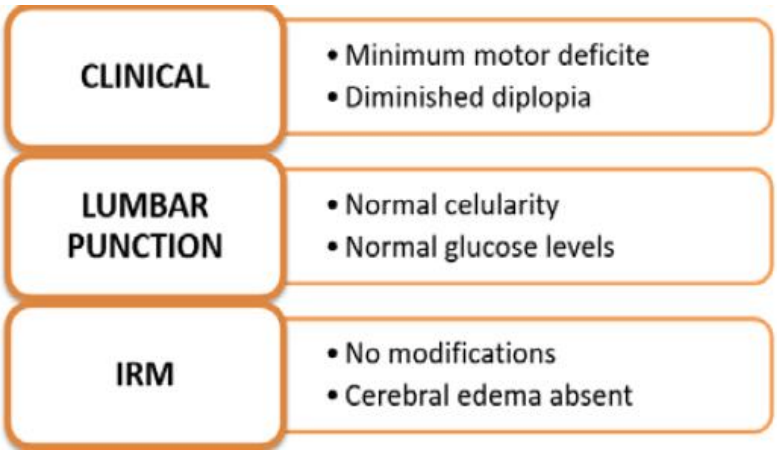

Fig.32 Clinical and paraclinical data at discharge

II.7.4 Conclusions

The biochemical analysis of the CSF, especially high protein rhinorrhasia and low glucose, in the absence of other pathological conditions, should be correlated with epidemiological, clinical and paraclinical data. In this case, low glucose in the CSF has created a favorable environment for the development of *Mycobacterium tuberculosis*.

For a diagnosis of *Mycobacterium tuberculosis* infection, positive cultures are ideal, but require a large amount of cerebral-spinal fluid, 30-50 mL and approximately 3-5 repeated lumbar punctures. The results are obtained in 2-6 weeks, thus the PCR is the most promising method of rapid identification of the Koch bacillus.

Tuberculosis meningitis often occurs in the absence of infection in other extra pulmonary sites and, although suspected in immunosuppressed patients with diabetes, alcohol consumption, HIV-positive or endemic TB, the disease also occurs in healthy and risk-free individuals and even the slightest suspicion should lead to specific investigations.

Related articles

1. Ioana Florina Mihai. Alexandra Largu, V. Dorobat, **Carmen Manciuc**- Modifying Cerebrospinal Fluid Constants for the Tuberculousis Etiology of a Meningitis Case. *Revista de Chimie* 2019; **70**: 3981-83.

2. **Manciuc Carmen**, Georgiana Alexandra Lacatusu, Ioana Florina Mihai, Cristina Vasilescu, Alexandra Largu, Florin Petrariu - Environmental and Clinical Context In Meningococcemia: Biochemical Modification Of Stiehm-Damrosch/Niklasson Severity Score - Prognostic Factor. *Environ Eng Manag J* 2020; **19**: 511-16.

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II.8 THE EVOLUTION OF BACTERIAL RESISTANCE AND THE SENSIBILITY TO LAST GENERATION ANTIMICROBIALS

II.8.1 Introduction

Urinary tract infections (UTIs) are among the most commonly reported diseases, occupying the fourth position of all healthcare-associated infections, representing the most commune infection that needs an antibiotic prescription [Vâță A et al., 2019]. According to the official guidelines and recommendations published by the US Centers for Disease Control and Prevention (CDC), multidrug-resistant organisms (MDRs) are predominantly bacteria, which are resistant to one or more classes of antimicrobial agents [Hertz F.B et al., 2016; Harrison et al., 1998]. The HIV positive patients are more likely to develop urinary tract infections due to the suppression of their immunity, so the resistance to antimicrobials could become a big problem.

The constant worldwide growing resistance of bacteria to antimicrobial drugs gives is a great concern for all medical practitioners, so it is important to limit the use of antibiotics only to situations where they are required. Limiting broad-spectrum empiric treatment to particular cases, administering the correct treatment according to the antibiogram, may contribute to slowing the continuously increase resistance of bacteria to antibiotics [Anand NI et al., 2011; Durante-Mangoni E et al., 2019; Eshetie S et al., 2015; Shaikh S et al., 2015; Tenney J et al., 2018].

Carbapenems, represented by Meropenem, Ertapenem, Doripenem, Imipenem/Cilastatin, have one of the broadest spectrums of activity over bacteria. This group of antibiotics should be used as a last resort for the treatment of severe or high-risk bacterial infections. Because of their potency, these antibiotics are considered "backup" for infections that are caused by MDR strains [Dorobăț C et al., 2011; ECDC: Stockholm, 2019; Gould CV et al., 2010].

Carbapenems represent the primary choice of treatment for severe infections with extended-spectrum beta-lactamases (ESBLs) producing bacteria [Vâță A et al., 2019; Dorobăț C et al., 2011]. Clinical data that compare the effectiveness of various compounds (imipenem/cilastatin, meropenem, ertapenem and, more recently, doripenem) are still limited [Anand NI et al., 2011; Dorobăț CM et al.,2012].

My interest in this field is reflected by the following paper:

1. **Manciuc Carmen,** Ioana Florina Mihai, Florina Filip-Ciubotaru, Georgiana Alexandra Lacatusu - Resistance Profile of Multidrug-Resistant Urinary Tract Infections and Their Susceptibility to Carbapenems. *Farmacia* 2020; **68:** 715–21.

II.8.2 Aim of the study

Regarding the current study, our concern focused on highlighting the susceptibility spectrum of urinary tract infections with multidrug-resistant bacteria to carbapenems and, at the same time, the resistance profile of these infections. Also, we took into consideration that the MDRs are resistant to at least 5 different antibiotics.

II.8.3 Material and methods

Study design and patients selection

In this context, we conducted an observational, retrospective study, using the hospital-based medical records of patients with a diagnosis of UTI hospitalized in the Infectious Diseases Clinical Hospital in Iași during January - June 2019. In the study, we included in-patients with microbiologically confirmed UTI defined by the growth of MDR bacteria $\geq 10^5$ UFC/L.

Extrapolating the definition of the term MDR mentioned above, we included in our study only urine cultures of patients that showed bacteria resistant to more than five antibiotics, regardless of the

class of origin. Thus, we have obtained 46 positive samples with multi-resistant germs. We collected information about: demographic data regarding the age, sex, area of residence (urban or rural), the presence of comorbidities or relapses of the current disease, the causes of urinary tract infections, laboratory data (urinalysis, urine culture), the presence of multidrug-resistant strains and the performed treatment. The antibiogram performed for the urine samples were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [Filip-Ciubotaru F et al., 2016; Gould CV et al., 2010; Manciuc C et al., 2016]. Two-tailed paired t-test was used to obtain statistical significance.

Isolation and identification of uropathogenic strains

The samples of urine were obtained by the clean-catch method or by briefly inserting a sterile catheter, through the urethra, into the bladder. The most commonly used criterion for defining significant bacteriuria is the presence of $\geqslant 10^5$ UFC/mL. The complete urinalysis involved three steps: 1. assessment of the color, cloudiness and concentration of the urine; 2. examination of the chemical composition of the urine using a test strip and 3. examination of the urine under a microscope to look for bacteria, cells and parts of cells. After seeding, incubation was done at 35-37°C for 18-24 hours.

The results were semi-quantitative and expressed as the number of UFC/mL [Filip-Ciubotaru F et al., 2016]. The urine samples were cultured on agar plates for growth, concentration, identification and isolation. Urine cultures containing bacteria exceeding the threshold of 10^5 UFC/mL are considered positive, whether we are talking about one or more isolated bacteria [Manciuc C et al., 2016].

Antibiotic sensitivity testing

For the complete diagnosis, the antimicrobial susceptibility testing (AST) was performed. AST is typically conducted as a phenotypic assay that measures bacterial growth in the presence of specific antimicrobial agents. AST takes an additional 24–48 hours and shows results for each pathogen–antimicrobial combination and is interpreted according to EUCAST guidelines and reported as sensitive, intermediate, or resistant [Gould CV et al., 2010; Hertz FB et al., 2016; Khawcharoenporn T et al., 2013]. The modified Kirby-Bauer disc diffusion method [Eshwarappa M et al., 2011; Gould CV et al., 2010] was used and the teste antibiotics were ampicillin (AMP), Augmentin (AUG), amikacin (AMK), nitrofurantoin (NTR), tobramycin (TBR), colistin (COL), carbapenems (CR), cotrimoxazole (CTX), fluoroquinolones (FLQ), cephalosporins II+III (CEF) and piperacillin/tazobactam (PIP-TAZO). Also, the multiple antibiotic resistance (MAR) index of each antibiotic was calculated.

Management of UTI

There were only two cases of uncomplicated urinary tract infection caused by *E. coli.* The average length of hospitalization was 9 days. Patients with uncomplicated urinary tract infection received treatment with antibiotics based on the 3rd generation of cephalosporins, and in complicated cases treatment with Nitrofurantoin (30.4% of cases), Carbapemene (45.6% of cases) and Colistin (10.8% of cases) was used. Complete remission of UTI was noted for all treatments.

II.8.4 Results

During the above-mentioned period, a total number of 782 patients were hospitalized in our clinic for suspicion of urinary tract infection (patients presented signs and symptoms of a UTI). Out of these, in 359 cases the urine cultures tested positive, out of which 46 with MDR germs (70 strains). These cases made the subject of our study.

Regarding the demographic characteristics of the patients, the mean age was 63 years, with age that varied from 12 to 93 years old. The majority of cases were encountered in females (60.8%) and in patients from an urban environment (65.2%). Clinical characteristics of patients were summarized in Table XVIII.

Table XVIII - Clinical characteristics of patients

CHARACTERISTICS	PATIENTS WITH MDR BACTERIURIA	
	No 46	% 100
Age (years) – median Range	63 12-93	
Female: male ratio	28:18	60.8: 39.2
Urban: rural ratio	30:16	65.2: 34.8
History of UTI	15	32.6
Underlying disease	43	93.4
Dysuria	40	86.9
Abdominal pain	46	100
Fever	36	78.2
Urgency	44	95.6
Intensive care unit	15	32.6
Antibiotic prophylaxis	5	10.8

The vast majority of the patients had multiple comorbidities (91.3%) as indicated in Table XIX. There were also cases reported in patients admitted in ICU (32.6%).

Table XIX - Risk factors frequency in the investigated patients with UTI

RISK FACTORS/ COMORBIDITIES	FREQUENCY (%)
Urinary catheter	43.5
Hypertension	28.2
Diabetes	15.2
Acute kidney failure	17.4
Chronic kidney disease	13

As showed in other studies as well, the main risk factor for our patients was catheterization, followed by hypertension and kidney failure [Eshwarappa M et al., 2011, Gould C.V et al., 2010, Khawcharoenporn T et al., 2013, Vâță A et al., 2019].

Analyzing the data found in the hospital-based medical records, that had a complete anamnesis performed on admission to the hospital we concluded that 15 (32.6%) patients had relapses of the disease, 41 (89.1%) patients were hospitalized for a different pathology and 38 (82.6%) came by transfer from other clinics (Figure 33).

From the 46 urine samples included in our study, in 4 samples three types of colonies were isolated (Figure 34), whereas, in the other 16 samples two types of bacteria were found (Figure 35). A total of 70 MDR strains were isolated.

After the data analysis, we concluded that the majority of urinary tract infections had *Escherichia coli* as a primary pathogen, followed closely by *Klebsiella spp.* The statistical parameters indicated a significant variation and a direct correlation between the number of cases and the pathogenic strains ($r = 0.9999$; $p < 0.05$, single tailed) (Figure 36).

In vitro testing showed different degrees of resistance to various classes of antibiotics, which underlines the importance of performing the antibiogram, in order to have a correct and complete therapy for these infections. Table XXI includes the results obtained for the resistance test for the most frequent pathogens detected in the investigated urine cultures.

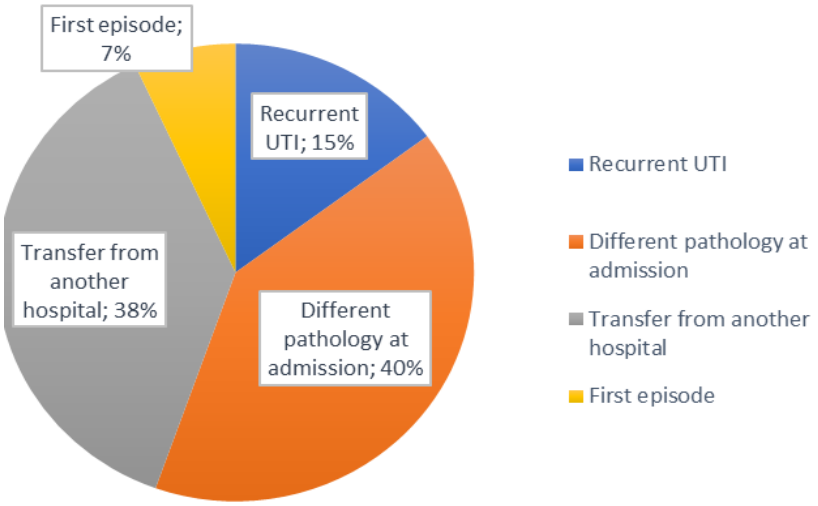

Fig.33 The prevalence of different types of UTI admitted in the clinic

Table XX - The spectrum of urinary tract infections in the study group

Organism	No of cases	Percentage
Escherichia coli	27	38.6
Klebsiella spp.	10	14.3
Klebsiella pneumoniae	9	12.8
Enterococcus spp.	7	10
Candida spp.	7	10
Enterococcus faecalis	4	5.7
Proteus mirabilis	3	4.2
Pseudomonas aeruginosa	3	4.2

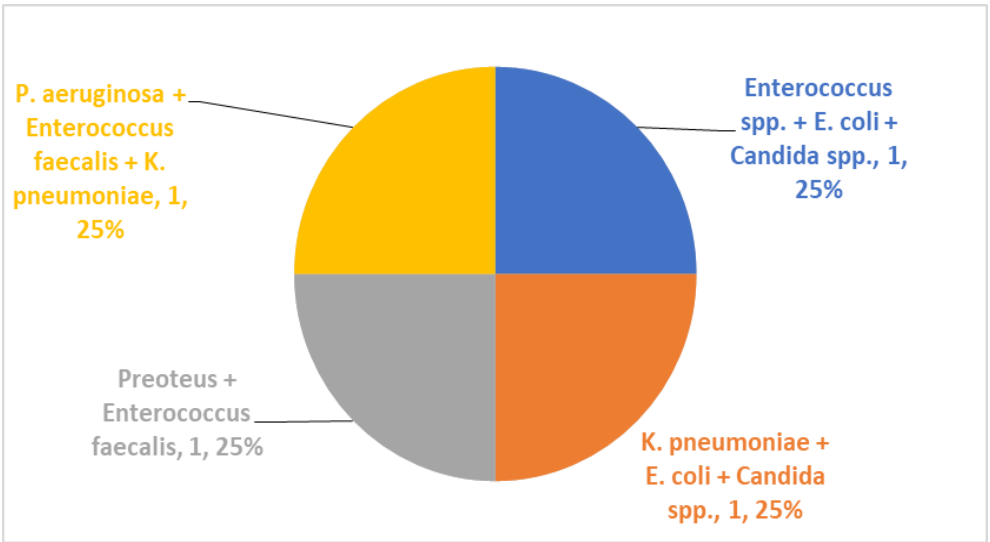

Fig.34 Types of isolated colonies with 4 different strains

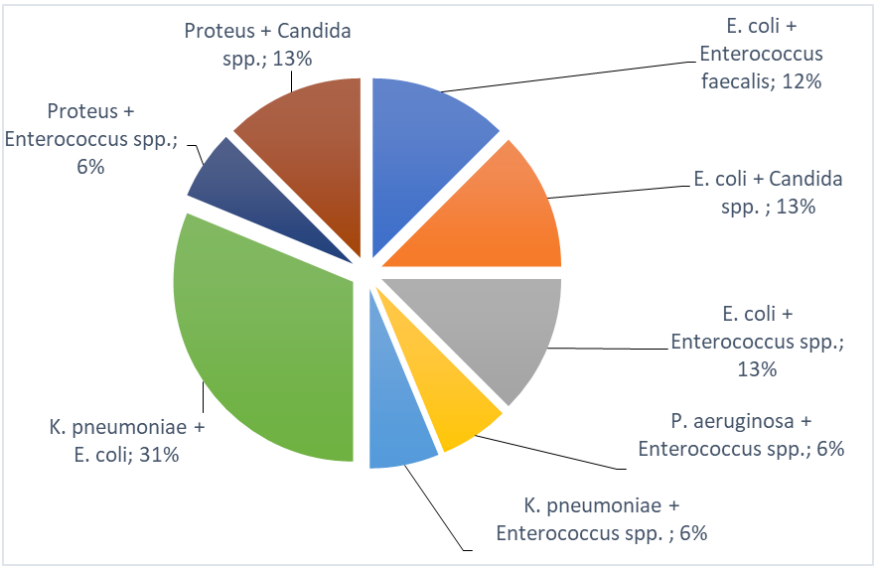

Fig. 35 Types of isolated colonies with 3 different strains

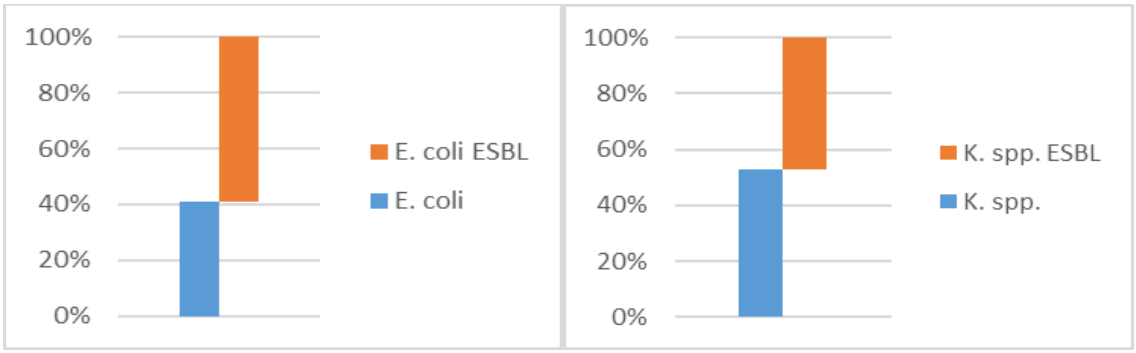

Fig. 36 The percentage of *E. coli/E. coli* ESBL and *Klebsiella spp./Klebsiella spp.* ESBL isolated strains

Table XXI - Antibiotic resistance table for *E. coli* and *Klebsiella spp*

Pathogen		**No. of isolates resistant to antibiotics/Total cases**									
		AMP	**FLQ**	**CEF**	**CTX**	**TBR**	**NTR**	**AUG**	**AMK**	**CR**	**COL**
E. coli	*cases / total*	27/27	24/27	25/27	17/27	5/27	3/27	15/27	5/27	0/27	0/27
	%	100	88.89	92.59	62.96	18.52	11.11	55.55	18.52	0	0
Klebsiella spp.	*cases / total*	12/19	19/19	9/19	0/19	8/19	12/19	19/19	9/19	5/19	0/19
	%	63.16	100	47.36	0	42.11	63.16	100	47.36	26.32	0

Also, figures 37 and 38 illustrate the degree of resistance (expressed as number of cases) to different classes of antibiotics tested for *Escherichia coli* and *Klebsiella spp.* isolated from urine samples.

When the MAR indices of the isolates was calculated, (Figure 39), the highest was found for FLQ (0.93) followed by AMP (0.84) indicating that these antibiotics were highly resistant among almost tested uropathogens.

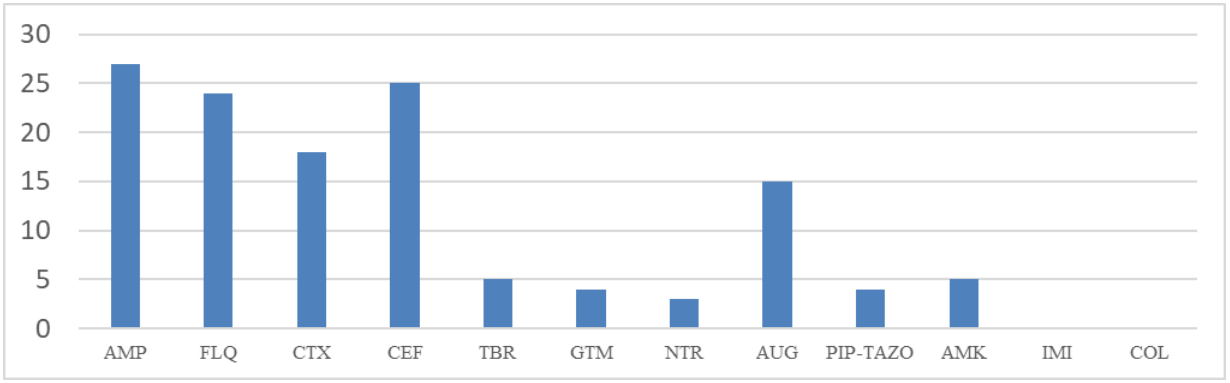

Fig.37 Antimicrobial resistance of *Escherichia coli* (no. of cases) AMP (ampicillin), FLQ (fluoroquinolones), CTX (cotrimoxazole), CEF (cephalosporins II+III), TBR (tobramycin), GTM (gentamycin), NTR (nitrofurantoin), AUG (Augmentin), PIP-TAZO (piperacillin/tazobactam), amikacin (AMK), COL (colistin), CR (carbapenems, either imipenem or meropenem).

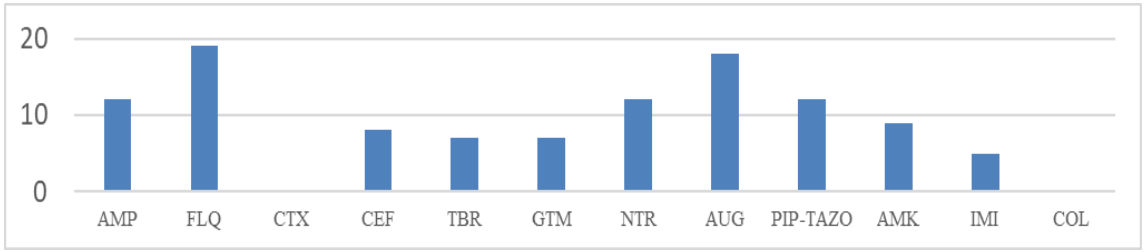

Fig.38 Antimicrobial resistance profile of *Klebsiella spp*. (no. of cases) AMP (ampicillin), FLQ (fluoroquinolones), CTX (cotrimoxazole), CEF (cefalosporins II+III), TBR (tobramycin), GTM (gentamycin), NTR (nitrofurantoin), AUG (augmentin), PIP-TAZO (piperacillin/tazobactam), amikacin (AMK), COL (colistin), CR (carbapenems, either imipenem or meropenem).

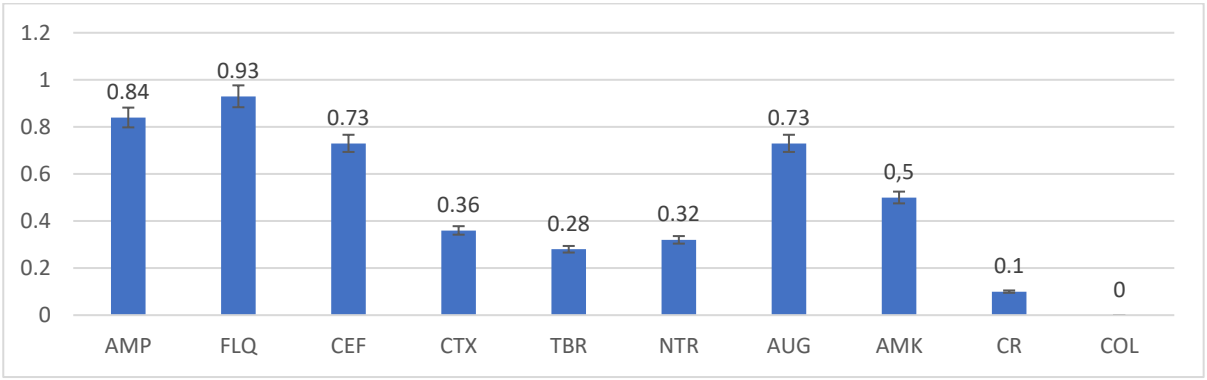

Fig.39 Overall resistance of all isolated *E. coli* and *Klebsiella spp*. uropathogens against tested antibiotics

As the data shows, we can see that the highest level of resistance for *E. coli* was obtained in the case of ampicillin. An alarming result was that *E. coli* and *Klebsiella spp.* had increased resistance to fluoroquinolones. In vitro, the strains isolated from *E. coli* maintained their sensitivity to Colistin and Imipenem, and in the case of *Klebsiella spp*., only sensitivity to Colistin was observed. Moreover, from the total of the 19 tested *Klebsiella spp*. strains, only five were carbapenem-resistant. The overview of the susceptibility to carbapenems (imipenem, meropenem and ertapenem) of the *Escherichia* and *Klebsiella* strains are shown in Figure 40.

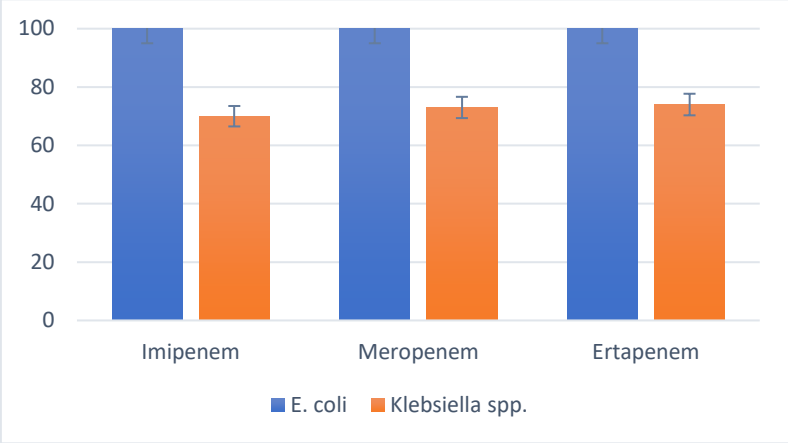

Fig.40 Percentage susceptibility to carbapenems
(Pearson correlation r=0.9999, p < 0.025, two-tailed)

II.8.5 Discussion

Various risk factors can increase the susceptibility of acquiring MDR bacteria that can cause UTI. As the results showed, elderly population, female gender, urinary catheterization, and multiple hospitalizations are the most encountered risk factors for the observed cases. Also, as the data suggested, associated diseases, such as diabetes and other diseases that impair the immune system can increase the risk of UTIs. Such results are confirmed also by the known literature [Anand NI et al., 2011; Dorneanu R et al., 2017; Dorobăț C. et al., 2011; Hertz FB et al., 2016]. Moreover, our results are in agreement with studies from other countries (India, Italy and UK), in which the most important and frequent bacterial strains identified in clinical isolates were *Escherichia coli* and *Klebsiella sp* [Durante-Mangoni E et al., 2019; Eshetie S et al., 2015; Eshwarappa M et al., 2011; Khawcharoenporn T et al., 2013; Toner L et al., 2016]. There are also slight differences in terms of the comorbidities (hypertension and acute kidney failure versus diabetes mellitus and recent urinary infections) that influence the occurrence of UTIs in our patients [Eshwarappa M et al., 2011]. Most probably, variations are influenced by the lifestyle and ethnical specificity.

It is known that previous use of antibiotics can influence the appearance of a urinary tract infection. All over the world, both in the hospital and in the outpatient setting, it is common to see the concurrent use of multiple drugs and also auto medication [Durante-Mangoni E et al., 2019; Eshetie S et al., 2015; Gupta K et al., 2011]. Such practices lead to long term risks, usually translated into a higher microbial resistance [Durante-Mangoni E et al., 2019; Eshetie S et al., 2015; Hertz FB et al., 2016].

Noteworthy is the fact that similar to other studies, most of the investigated clinical isolates were resistant to fluoroquinolones, while remaining susceptible to carbapenems [Toner L et al., 2016].

The patients with multiple recurrences had experienced depression linked to the gravity of the disease. In some cases, the intervention of the hospital psychologist was required. The role of this medical intervention is to minimize the burden of this disease [Khawcharoenporn T et al., 2013; Manciuc C et al., 2014].

Knowing the number of patients that came from different health-care centres or those that acquired a UTI during hospitalization for other diseases, we can speculate that the majority of UTIs with multidrug-resistant germs were acquired either as a healthcare-associated infection, or generated by the resistance of the bacteria that occurred after the multiple courses of antibiotic.

According to European Centre for Disease Prevention and Control, the susceptibility to carbapenems of different bacterial strains is constantly increasing, *K. pneumoniae*, percentages of carbapenem resistance ranging from 0% to more than 60%, data that are also sustained by the presented study in which 26.31% of *Klebsiella spp.* were carbapenem-resistant [Khawcharoenporn T

et al., 2013; Manciuc C et al., 2016]. For this reason, practitioners should opt for limiting their use only in complicated urinary tract infections [Manciuc C et al., 2014; Mihai IF et al., 2019]. Thus, there are concerns about the increased rate of resistance to carbapenems, especially given the fact that there are few therapeutic options for treating infections caused by carbapenem-resistant bacteria (such as *Klebsiella pneumoniae* and other carbapenem-resistant *Enterobacteriaceae*).

Limiting the use of invasive devices is another potentially important intervention for limiting the extension of carbapenem-resistant bacteria. Such strains have been identified from device-associated infections, particularly catheter-associated urinary tract infections [Gould CV et al., 2010].

The results of the study, in conjunction with the literature data, draws attention to the increased antibiotic resistance of microorganisms involved in the etiology of urinary tract infections, thus exhausting the treatment opportunities. To avoid therapeutic failure and minimize the spread of antibiotic-resistant bacteria, data regarding the natural and acquired resistance of uropathogenic organisms should be known.

II.8.6 Conclusions

According to the study, *Escherichia coli* dominated the ethiological spectrum of urinary infections, followed by *Enterococcus spp.*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Proteus mirabilis.* All strains of *Enterobacteriaceae* isolated showed various levels of antibiotic resistance, except for Colistin, while *in vitro* sensitivity was maintained. Overall, the susceptibility to carbapenems was high, preserving their value as a backup antibiotic in the therapy of these infections.

An increasing number of community infections with enterobacteria producing ESBL have been reported in the last two decades. In these cases, carbapenems were used. As such, resistant strains have emerged and thus a major public health problem occurred. In order to identify the correct treatment, to avoid therapeutic failure and to decrease the spread of antibiotic-resistant bacterial strains in communities, the therapy needs to be based on information. The most reliable information is provided by the epidemiological surveillance and the resistance patterns of uropathogenic bacteria. The results support the importance of monitoring the antibiotic resistance and equipping the bacteriology laboratories with modern means that allow the ethiological diagnosis and the early establishment of targeted therapy, on a scientific basis, of these infections.

Related articles

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SECTION II. FUTURE EVOLUTION AND DEVELOPMENT PLANS

In the future, my professional activity will focus on 3 elements of personal development:
- Scientific activity
- Clinical activity
- Teaching activity

PERSPECTIVES IN SCIENTIFIC ACTIVITY

Considering that I am a clinician by excellence, the scientific papers that I will be writing will have as a case source "Sf. Parascheva" Clinical Hospital of Infectious Diseases.

I will follow three major research directions, precisely to continue the old interests, the scientific activity being led by a red thread with the following interests:

1. SARS COV-2 infection – clinical, therapeutic and pathogenic updates

I believe that this new infection represents a challenge for everyone in the medical community, and has revealed only a small proportion of the true valences and strength, in terms of challenge to the international medical community. I will try to contribute with clinical trials and I will be open to participate in research grants that will reveal new perspectives regarding new molecules for the therapy of COVID-19 patients, as well as the prophylaxis of the disease with vaccines developed by renowned pharmaceutical companies.

This interest regarding airborne infections, such as influenza, in direct connection with the pandemic is a current concern and materialized in a research grant, of which I am the director, with the Cantacuzino Institute in Bucharest. "Sf Parascheva" Clinical Hospital of Infectious Diseases is one of the 2 hospitals in the country selected as a sentinel regarding co-infection between influenza viruses of any type and SARS COV-2 infected patients.

The study is called I-MOVE and uses a protocol for hospital-based study designed to measure 2020-2021 seasonal flu vaccine effectiveness against influenza laboratory confirmed SARI hospitalisation among the elderly in Romania.

The objectives of the study are the following:

a. Primary objective

The primary objective will be to measure, in EU/EEA MS, seasonal IVE against laboratory-confirmed influenza in elderly hospitalised SARI patients.

b. Secondary objectives

- To estimate seasonal IVE against laboratory-confirmed influenza requiring hospitalisation in elderly SARI patients:
  - in each of the participating study sites;
  - by risk group (e.g. specific chronic conditions);
  - by age group (65–79 years, 80+ years);
- To estimate the effect of statins on laboratory-confirmed influenza in elderly SARI patients requiring hospitalization;
- To identify vaccine types (e.g. adjuvanted vs. non-adjuvanted, groups of vaccines (split virion, subunit, adjuvanted, trivalent vs. quadrivalent)) and brands with different effectiveness;
- To understand the factors affecting IVE: duration of protection, the role of repeated seasonal vaccinations, the role of statins;
- To identify key influenza virus phenotypic or genotypic evolutions that could affect vaccine performances and estimate VE against specific clades.

The study will use a hospital-based test negative design (TND) case-control study in each of the two-participating hospital. Which are "Sf Parascheva" Clinical Hospital of Infectious Diseases, Iași and "Dr. Victor Babeș" Clinical and tropical Hospital of Infectious Diseases, București.

The study population will consist of all community-dwelling individuals aged 65 years and above hospitalized with SARI, with no contra-indication for influenza vaccination.

The study will begin when the seasonal influenza vaccine of the 2020-2021 season becomes available and the influenza season begins in Romania and will finish at the end of the influenza period (we estimate the study period from week 46/ 2020 to week 18/ 2021).

The influenza activity (season) is declared in Romania, in the week when more than 10% of tested samples are positive for the same subtype/variant of influenza virus.

The patient will **not** be enrolled in the study if she or he:

1. Is less than 65 years of age at the time of hospital admission;
2. Has a contraindication for influenza vaccine;
3. Was hospitalised < 48 hours prior to sari onset;
4. Had his/her sari onset ≥ 48 hours after admission at the hospital;
5. Is unwilling to participate or unable to communicate and give consent (the consent may also be given by her/his legal representative, or by specific consent procedures);
6. Is institutionalised at the time of symptoms onset (lives in a residence for people who require continual nursing care and have difficulty with the required activities of daily living);
7. Had a respiratory specimen taken ≥ 8 days after SARI onset;
8. Tested positive for any influenza virus in the current season before the onset of symptoms leading to the current hospitalization.

2. *Clostridioides difficile* infection

Another line of research explored with devotion and dedication before the pandemic was represented by the *Clostridioides difficile* infection, with all its aspects, which is on of the 21st century challenges for the infectious diseases specialist.

Ferring study comes as a natural continuation of these interest. The study is a randomised, double-blind, placebo-controlled phase 3 trial that aims to determine the efficacy and safety of an intestinal microbiota preparation for the prevention of recurrent *Clostridioides difficile* infection in adults.

a. Primary objective

- determine the efficacy of intestinal microbiota preparation as compared to placebo in preventing recurrence of *Clostridioides difficile* infection (CDI) in subjects with recurrent CDI (rCDI).

b. Secondary Objectives:

- To determine the sustained clinical response of intestinal microbiota preparation compared to placebo in subjects with rCDI;
- To determine the sustained impact on quality of life of intestinal microbiota preparation compared to placebo in subjects with rCDI;
- To determine the safety of intestinal microbiota preparation in subjects with rCDI.

The inclusion criteria in this study are :

Inclusion Criteria:

1. Signed informed consent obtained before any trial-related procedures;
2. Male or female subjects ≥18 years old at screening;

3. Medical record documentation of recurrent *Clostridioides difficile* infection (rCDI) per the trial definition that includes ≥1 recurrence after a primary *C. difficile* infection (CDI) episode with ≥1 completed round of standard of care antibiotic therapy;
4. Stool test positive for toxigenic *C. difficile* or *C. difficile* toxin for the current/presenting CDI episode, documented at the time of CDI diarrhoea;
5. Currently taking or have just received a prescription of a course of antibiotics to control CDI-related diarrhoea at screening;
6. Compliance to the antibiotic washout period (discontinuation of the antibiotics for a minimum of 1 day (≥24 hours) to a maximum of 3 days immediately before randomisation (assessed at baseline);
7. Completed antibiotic treatment for a minimum of 10 consecutive days, and a maximum of 30 calendar days before the antibiotic washout period (assessed at baseline);
8. Controlled CDI diarrhoea, defined as <3 unformed/loose stools/day (i.e., Bristol Stool Scale type 6-7) for the 2 consecutive days immediately before start of the antibiotic washout period and throughout the antibiotic washout period up to the morning of the baseline visit (assessed at baseline);
9. Female subjects should adhere to at least one of the following conditions throughout the trial period:
    a. Post-menopausal (women ≥45 years with no menstrual period for ≥12 months without an alternative medical cause) or
    b. Surgically sterile or
    c. Use of an adequate method of contraception (i.e., implants, injectables, hormonal intrauterine devices, combined hormonal contraceptives, having a vasectomised sexual partner, or total abstinence from heterosexual relations with no plans of becoming pregnant through insemination or in vitro fertilization).
10. Willing to comply with trial procedures, including attending scheduled visits and adherence to treatment.

3. The insert of medical graduates in the work medical field, through internships

This study, conducted by Prof. Dr. Norina Forna, comes as a wish of improving education and training systems for emploiment market of woung students.The study aims to facilitate the transition from education to work, and strengthening education and training systems and their quality, through mechanisms for anticipating skills, adapting curricula and creating and developing learning systems based on work, including dual learning and apprenticeship systems .

The general objective of the study is to facilitate the transition from school, to working life by participating in “on-the-job learning”, counseling and career guidance activities to improve the professional skills and aptitudes, of 360 students from the University of Medicine and Pharmacy in the licensing cycle - Faculty of Medicine and Faculty of Dentistry, in order to increase their capacity to enter successfully and sustainably into the labor market.

The objective of the study is to increase the number of graduates from the medical field who find a job, as a result of access to learning activities.

PERSPECTIVES IN ACADEMIC AND PROFESSIONAL ACTIVITY

In terms of clinical activity, infectious diseases specialty has proven its ability to be an ongoing challenge for any physician who has embraced this branch of medicine.

Over time, the satisfactions offered by the clinical part of this branch proved to be remarkable, since this sector of medicine offered me the satisfaction of treating patients with severe diseases, which initially seemed that had no chance, sometimes even without sequelae.

In this regard, I mention the epidemic of viral meningitis with West-Nile virus in 1997, the measles epidemic, the AH1N1 influenza virus pandemic.

The academic activity will be intertwined with the clinical and research activity in the desire to share with future generations from my 35 years of experience as a clinician and also the research that can be taken over and transferred to future generations.

I believe that the academic activity is a vocational one and can be deeply explored in the context of the specific activity that is sustained by the generous learning platform offered by "Grigore T. Popa" University of Medicine and Pharmacy.

The multilanguage sections of the university (Romanian, English and French) will offer me the option to explore the possibility of creating and editing materials in these languages, as well as develop practical and theoretical classes that will be updated according to the newest scientific data from the literature.

Last, but not least, the preoccupation for case presentations, study materials, as well as practical guides for different specialties doctors, such as pneumologist, general practitionars (GP) etc. and other type of medical staff, that includes nurses that graduated "Grigore T. Popa" University of medicine and Pharmacy from Iași will be future preoccupations that will continue the activities carried out so far.

One of my fondest activities, that is also a source of energy for me, is coordonating students and young doctors with their thesis. This does not only apply to the field of infectious diseases, but to other branches of medicine as well.

I aim to continue the work that I have started and this is highlighted by the papers and posters presented by junior doctors at various simposions (Spitalul de Recuperare Days, Arrhythmia forum, Reziderma, Conference of Infectious diseases pathology etc.).

SECTION III

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