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CUPRINS

CHEMICAL AGENTS AS ANTIMICROBIAL FACTORS

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ABSTRACT

A wide variety of chemicals can be used as antibacterial agents to prevent contamination. Antiseptics and disinfectants are widely used in hospitals and are an essential element in combating and preventing nosocomial infections. A wide variety of active chemicals or biocides are present in these products. Disinfectants and antiseptics affect bacteria in many ways. The widespread use of antiseptics and disinfectants has contributed to the development of microbial resistance, in particular cross-resistance to antibiotics. In medical practice, antimicrobial agents must be selected taking into account the specific microorganisms, pH, solubility, toxicity, organic material present and last but not least the cost. No ideal antimicrobial chemical exists, however the correct choice and proper use of current substances will result in an adequate level of disinfection. This paper aims to review the main antimicrobial chemical agents, the mechanisms by which they act and their importance in clinical practice.

Key words: antimicrobial chemicals, alcohols, halogens, aldehydes, quaternary ammonium compounds, silver.

INTRODUCTION

During life we are constantly exposed to a wide variety of germs. Last year, the public health emergency surrounding the COVID-19 pandemic highlighted the importance of good biosecurity measures and practices. To prevent the spread of infection, disinfection and hygiene habits are crucial, especially when the microorganism can persist and survive on surfaces. Contaminated surfaces have been called fomites and sometimes microorganisms can survive here even for months [1]. As a result, fomites serve as an important reservoir of pathogens and facilitate the transfer between hosts. To prevent contamination, a wide variety of chemicals can be used as antibacterial agents. At present there are various classifications of them but the vast majority take into account the mechanism of action by which these substances exert their bactericidal effect. Antiseptics and disinfectants are widely used in hospitals and are an essential element in combating and preventing nosocomial infections [2]. A wide variety of active chemicals or biocides are present in these products. Disinfectants and antiseptics affect bacteria in many ways. Those that lead to bacterial death are called bactericidal agents. Those that cause temporary growth inhibition are bacteriostatic agents. There is no single antimicrobial agent that is most effective in all situations. That is why different situations may require different agents. The increase in the potential for microbial contamination and the risk of infection has led to an increase in the use of antiseptics and disinfectants by the general public [3]. Despite this, knowledge about the mode of action of these agents is still limited. It is, however, well known that their spectrum of activity is wider than that of antibiotics. While antibiotics tend to have specific intracellular targets, antibacterial chemicals may have multiple targets. A number of techniques such as: lysis of intracellular constituents, examination by studying the disruption of cellular homeostasis, study of enzyme inhibition, electron transport and oxidative phosphorylation, interaction with macromolecules, examination biocides are used to study the mechanisms of action of antiseptics and disinfectants on microorganisms[4, 5]. The widespread use of antiseptics and disinfectants has contributed to the development of microbial resistance, in particular cross-resistance to antibiotics [2]. A number of factors affect the selection of the best agent for any given situation. Antimicrobial agents should be selected for specific micro-organisms and environmental conditions. Additional variables to consider when selecting an antimicrobial agent include: pH, solubility, toxicity, organic material present and last but not least cost. The present paper aims to review some of the antimicrobial chemical agents, the mechanisms by which they act and their importance in clinical practice.

1. Alcohols

The mechanism of action of alcohols is nonspecific, the bacteriostatic effect is explained by their ability to distort proteins. They are also solvents for lipids and as a result, can damage lipid complexes in the cell membrane. Cells will be lysed and cellular metabolism will be disrupted [2, 6]. This is supported since the

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1950s by specific studies that have shown the denaturation of the dehydrogenases of the bacterium Escherichia coli and the delayed growth of Enterobacter aerogenes, actions explained by inhibiting rapid cell division [7]. In addition to these mechanisms, alcohols are also known as dehydrating agents. Under certain conditions, the elimination of water from the cells caused by the action of alcohol has a bacteriostatic effect. This may explain the relative inefficiency of absolute alcohol on "dry" cells. In addition to these mechanisms, part of the effectiveness of alcohol in disinfecting surfaces can be attributed to its cleaning or detergent action which results in the mechanical removal of microorganisms. It has been observed that the general antimicrobial activity of alcohols increases with the length of the carbon chain, the maximum being reached at six carbon atoms [8]. As a result, the most effective are: ethanol, isopropanol and n-propanol. Alcohols have broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses and fungi, but have no sporicidal effect. However, it is known to inhibit the sporulation and germination of spores, an effect that is reversible [9]. Due to the lack of sporicidal activity, alcohols are not recommended for sterilization, but are widely used for disinfecting hard surfaces and skin antisepsis. Lower concentrations can also be used to potentiate the activity of other biocides. Among the mentioned alcohols, isopropyl alcohol is considered more effective against bacteria, while ethyl alcohol is more powerful against viruses [2]. However, this is dependent on both the concentrations of the active agent and the microorganism tested. Against S. aureus, E. faecium or P. aeruginosa, the bactericidal activity of ethyl alcohol appears to be 80% stronger than 95% [10].

Ethanol is also effective against various mycobacteria. Since 1947 it has been shown that ethanol at a concentration of 95% kills M. tuberculosis in sputum within 15 seconds, while a concentration of 70% required a contact time of 30 seconds and at 50% 60 seconds [11]. The same very effective bactericidal activity was observed with 70% ethanol against M. bovis [12]. In addition, ethanol has a wide bactericidal activity against most fungi, including yeasts and dermatophytes [12, 13, 14]. As for the activity of ethanol against viruses, it largely depends on the concentration of ethanol. As expected, higher concentrations of ethanol (95%) generally have better antiviral activity than low concentrations (60 to 80%), especially against non-encapsulated viruses [15]. Most non-encapsulated viruses such as poliovirus, astroviruses, feline calicivirus, rotaviruses and ecoviruses are also inactivated by ethanol [13, 16, 17, 18]. The hepatitis A virus is perhaps the only virus that is not completely inactivated in the presence of ethanol. Regarding encapsulated viruses, ethanol is active against vaccinia virus, influenza A virus, togaviruses, Newcastle disease virus, HIV, HBV and herpes simplex viruses [19, 20].

Isopropyl alcohol (isopropanol) has higher lipophilic properties than ethyl alcohol and is less active against hydrophilic viruses such as polio. The bactericidal activity of isopropanol starts at a concentration of 30% and increases with increasing concentration, but an interesting aspect is the reduction of bactericidal activity to 90% [8]. The same is true for the bactericidal activity of n-propanol. It appears that the bactericidal activity of isopropanol refers to 13 gram-positive species, 18 gram-negative species. Tuberculocidal activity of isopropanol was also tested and good results were observed at concentrations between 50 and 70% [8]. It has limited activity against non-encapsulated viruses. However, if the exposure time is prolonged, it has an effect against non-encapsulated viruses such as ecovirus (90% isopropanol for 10 minutes), feline calicivirus (50-70% isopropanol for 3 minutes) or adenovirus (50% isopropanol for 10 minutes). 10 minutes) [21]. Isopropanol has no sporicidal activity against B. subtilis and Clostridium novyi spores [8].

N-propanol has been discovered since 1904 and has been described as an alcohol with a very strong bactericidal effect starting from a concentration of 30% [22, 23]. The antimicrobial activity of n-propanol is thought to be similar to that of isopropanol [24]. In general, the antimicrobial activity of alcohols is significantly lower at concentrations below 50% and is optimal in the range of 60-90%. Ethanol has been the first recommended for hand disinfection since 1888 and the antimicrobial activity of isopropanol was first investigated in 1904 [23]. This was followed by many studies that supported the use of the two propanols for hand disinfection [8]. The following classification of bactericidal activity was established: n-propanol is stronger than isopropanol and isopropanol is stronger than ethanol. Also, the bactericidal activity is higher at a temperature between 30 and 40 degrees Celsius compared to the range of 20-30 [25].

2. Halogens

A halogen is any of the five elements (fluorine, chlorine, bromine, iodine and astatin) in group VII A of the periodic table. They exist as free diatomic molecules and form salt as compounds with sodium and most other metals. Chlorine or iodine are important halogens used as potent antimicrobial agents either in their free form or in the form of their compounds.

Chlorine and its compounds

Chlorine is most commonly used in gaseous form or as its compounds. Chlorine in gaseous form is difficult to handle unless special equipment is available for its distribution. Therefore, chlorine gas is used in large-scale operations. Many chlorine compounds are now available, which can be used more conveniently than free chlorine and which, under proper conditions of use, are as effective as free chlorine. Calcium and sodium chloride compounds in the form of calcium hypochlorite - Ca (OCl) 2 and sodium hypochlorite - NaOCl are used in small-scale operations. In addition to hypochlorites, there are other chlorine compounds in the chloramine group. Chloramines are more stable than hypochlorites in terms of prolonged chlorine release and are often used as disinfectants, sanitizers or antiseptics. Examples of chloramines are monochloramine (NH2Cl), chloramine-T and azochloramide. As a mechanism of action, it is known that chlorine acts on microbial cells through hypochlorous acid formed as a result of the reaction between free chlorine and water. However, hypochlorites and chloramines undergo hydrolysis, resulting in the formation of hypochlorous acid. The fate of the hypochlorous acid formed in each case is the same, it undergoes decomposition resulting in HCl and oxygen. Oxygen released during the decomposition of hypochlorous acid is a strong oxidizing agent. It acts on the cellular constituents of microorganisms and leads to their death. The destruction of microbial cells by chlorine and its compounds is achieved by direct effects on cell membranes and enzymes.

Iodine and its compounds

Iodine in its crystalline form is black-bluish with a metallic luster. It is poorly soluble in water, but slightly soluble in alcohol and aqueous solutions of potassium or sodium iodide. Although less reactive than chlorine, iodine is bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal, being an extremely effective bacterial agent, unique in that it is effective against all types of bacteria [26]. Iodine solutions are already used 150 years ago as antiseptics, being considered one of the oldest and most effective antimicrobial agents, traditionally used as "iodine tincture". Aqueous solutions are generally unstable, at least seven species of iodine are present in the solution. Of these, molecular iodine is primarily responsible for antimicrobial efficacy [26]. More recently, iodine has been combined with an organic compound to form iodophore. Iodophores are the so-called "iodine carriers" or "iodine-releasing agents". The most used are povidone-iodine and poloxamer-iodine both in antiseptics and as disinfectants. They are considered less active against certain fungi and spores [27]. The mode of action of iodine and its compounds is not clearly understood. Iodine, being an oxidizing agent, is considered to have the property of irreversibly oxidizing and thus inactivating essential metabolic compounds, such as proteins with sulfhydryl groups.

3. Aldehydes

Most low molecular weight aldehydes are antimicrobial. Two aldehydes, formaldehyde and glutaraldehyde, are the most effective and are most commonly used to kill spores, therefore they are sporicidal. As a mechanism of action, both commonly used aldehydes, formaldehyde and glutaraldehyde, are highly reactive molecules that combine easily with organic nitrogen compounds, such as nucleic acids and proteins, and inactivate them, probably by crosslinking and alkylating molecules. Inactivation of nucleic acids and proteins disrupts the function of cellular organs and, as a result, cells are killed.

Glutaraldehyde has a broad spectrum of activity against bacteria, spores, fungi and viruses. Studies on its mechanism of action have shown a strong adhesion of glutaraldehyde to the outer layers of microorganisms such as E. coli and Staphylococcus aureus, inhibition of the transport of gram-negative bacteria, inhibition of dehydrogenase and other enzymes, prevention of lysis induced by a number of substances in S. aureus and in E. coli and inhibition of RNA, DNA and protein synthesis [2, 28, 29]. Glutaraldehyde is more active at alkaline pH than acid. Because the external pH at the cell surface changes from acid to alkaline, a faster bactericidal effect is reached. The antiviral capacity of glutaraldehyde has also been demonstrated [30]. It reduces the activity of hepatitis B surface antigen (HBsAg) and interacts with lysine residues on the surface of hepatitis A virus (HAV) [2].

Formaldehyde

It is a monoaldehyde that exists as a free water-soluble gas. It is generally used clinically as a disinfectant and sterilizer with bactericidal, sporicidal and virucidal effect, but it works slower than glutaraldehyde [28, 31]. As a mechanism of action formaldehyde is an extremely reactive chemical that interacts with proteins, DNA and RNA in vitro. It has the ability to penetrate inside bacteria, and to modify HBs Ag and HBcAg of HBV [2].

4. Aromatic alcohols

Aromatic alcohols have effective antimicrobial properties for disinfection, even in the presence of biological fluids.

Phenol (C6H5OH) is an organic compound consisting of a benzene ring bearing a single hydroxy substituent. Phenol exerts antimicrobial activity against Gram-positive and negative bacteria, fungi and viruses, but is not as effective as a sporicide [1]. Although the specific mechanism of action of phenol derivatives is not clearly known, there is consensus that these compounds cause physical damage to the plasma membrane of the microbial cell. As a result, the content of essential cell metabolites drains causing cell damage with cell lysis, while acting as a protoplasmic poison that causes the cytoplasm to coagulate, with the death of microorganisms. The efficacy of triclosan (a phenolic derivative) was investigated in vitro and was found to have bacteriostatic effect at lower concentrations, and bactericidal activity at higher concentrations. The activity of triclosan is higher against gram-positive microorganisms than against gram-negative bacteria, especially P. aeruginosa, and the fungicidal activity is good, including yeasts and dermatophytes [8].

Hexachlorophenol is another bis-phenol whose mode of action has been investigated based on studies with Bacillus megatherium. It has been found to be bactericidal at 0 ° C, despite the fact that it causes minimal leakage through the bacterial membrane at this temperature. Despite the efficacy and broad spectrum of hexachlorophen, concerns about its toxicity, especially in newborns, lead to its limited use in antiseptic products [2].

Chloroxyleneol is another phenol used in antiseptic or disinfectant formulas. Chloroxylene is bactericidal against certain bacteria, while others are very resistant, for example P. aeruginosa. Surprisingly, its mechanism of action has been little studied despite its widespread use over time. Due to its phenolic nature, it would be expected to have an effect on microbial membranes.

In conclusion, commonly used phenolic compounds have antimicrobial efficacy against bacteria, fungi, viruses, including HIV. However, literature also reports that some phenolic disinfectants have a limited effect on Coxsackie B4, Enterovirus 11 and Polyovirus [32].

5. Quaternary ammonium compounds

Quaternary ammonium compounds are the most popular cationic detergents. They are characterized by a positively charged nitrogen and a long hydrophobic aliphatic chain. Quaternary ammonium compounds have a structure similar to ammonium chloride, but with some changes. The demand for these disinfectants has increased over the decades, in addition, their use is not limited only as a germicidal agent, but they have been widely used in a variety of industrial, agricultural, clinical and consumer applications [33]. As mechanism of action, it has been shown that microbicidal activity is due to their adhesion to acidic proteins or phospholipids in the membrane, which leads to the formation of so-called hydrophilic voids. Denaturation of essential cellular proteins will increase the permeability of the membrane and ultimately cause the destruction of the microbial cell. In addition, it seems that they are also involved in inactivating energy production and can be linked to DNA [34]. As a result, they are used as solid bactericidal agents, especially against

Gram-positive bacteria and against encapsulated viruses (eg herpes simplex, adenovirus, vaccinia), not being sporicidal, tuberculocidal or with an effect on hydrophilic viruses. Basically, they are commonly used in sanitizing non-critical surfaces, such as floors, furniture and walls. The scientific literature reports the effectiveness of some quaternary ammonium compounds in removing and / or inactivating S. aureus and P. aeruginosa from the computer keyboard. Moreover, a recent paper by Brown et al. [35] demonstrated that the microbial reduction activity following their application on glass, continues for a long time and in humid conditions. A number of advantages of quaternary ammonium compounds can be listed, such as: high stability, low color, no odor and relatively low toxicity, as opposed to phenols and chlorine-based bleaches.

6. Chlorhexidine

Chlorhexidine is a cationic biguanide and its quality as an antimicrobial agent was established in 1954 [8]. It exists as acetate (diacetate), gluconate and hydrochloride salts and is a biocide commonly used in antiseptic products, especially for hand washing and oral products, but also as a disinfectant and preservative. It has a wide spectrum of action and is well tolerated by the skin, rarely causing irritation. A large number of studies have been dedicated to elucidating the antimicrobial mechanism of action of this important substance [2]. Thus, it was noted that chlorhexidine gluconate is rapidly absorbed by bacteria such as E. coli and S. aureus and that this depended on its concentration and pH. The outer layer of the microbial cell deteriorates, though insufficiently to induce lysis or cell death, but then the agent crosses the cell wall - probably by passive diffusion - and subsequently attacks the cytoplasmic bacterial membrane, followed by leakage of intracellular constituents and microbial death. High concentrations of chlorhexidine cause coagulation of intracellular constituents. The antimicrobial activity of chlorhexidine depends on its concentration. Thus, at lower concentrations, chlorhexidine has a bacteriostatic effect against most gram-positive bacteria, on many gram-negative bacteria and bacterial spores. At very high concentrations, a bactericidal effect can be expected, as well as activity against yeasts [8]. In liquid soap, chlorhexidine usually has a concentration of 4% and has a bactericidal activity against Gram-negative and Gram-positive bacteria [36]. In a number of comparative studies, chlorhexidine suspension (4%) was less effective against MRSA than against methicillin-resistant S. aureus [8]. Chlorhexidine does not inactivate non-encapsulated viruses such as rotavirus, hepatitis A virus or poliovirus, this is explained by its activity limited to nucleic acid or the outer shell. It seems that the latter is a more important target [2]. And compared to dermatophytes, such as Trichophyton mentagrophytes, chlorhexidine (1.5%) has no activity. Chlorhexidine testing in clinical practice demonstrated in a study of 52 volunteers who washed their hands with a 4% chlorhexidine liquid soap 24 times a day for 5 days, a significant decrease in the number of resident bacteria of skin compared to a batch that was washed with classic soap [8]. Another study in which hands were artificially contaminated with MRSA liquid chlorhexidine soap proved to be as effective as plain soap. A similar result has been reported after contamination of the hands with S. aureus [37]. In conclusion, chlorhexidine (2-4%) has a good activity against most vegetative bacteria, yeasts and encapsulated viruses but limited activity against mycobacteria, dermatophytes and non-encapsulated viruses. Washing your hands with a chlorhexidine soap can reduce the number of bacteria.

7. Heavy metals and their compounds

Most heavy metals and heavy metal compounds or metal salts have some degree of toxicity to microorganisms. For many years, heavy metal ions have been used as germicides, but more recently, they have been replaced by other less toxic and more effective heavy metals and their compounds. The most toxic heavy metals are mercury, silver and copper, and the least toxic are sodium and potassium. Mode of action: heavy metals and metallic compounds combine with proteins, often with their sulfhydryl (SH) groups and inactivate them. The high concentration of metal salts, especially those of mercury, silver and copper, coagulates cellular proteins that lead to damage or death of the microbial cell. Metal salts can also precipitate and in high concentrations can cause the death of a microbial cell. Silver compounds have been used to prevent burn infections and some eye infections. It appears that silver salts and other heavy metals such as copper act by binding to functional groups of fungal enzymes. The mechanism is similar in the case of microbes, silver ions bind to enzymes important for microbial activity, causing their inhibition [2]. In addition to its effects on enzymes, silver ions produce other changes in microorganisms. It has been demonstrated that silver nitrate is able to cause a marked inhibition of Cryptococcus neoformans growth by depositing in the vacuoles of this fungus and in its cell wall in the form of granules [38]. Silver ions inhibit cell division and damage the cell lining and contents of P. aeruginosa. Bacterial cells increase in size and there are structural abnormalities of all cellular components. In addition, the interaction of silver ions with nucleic acids has been noted, preferably with DNA bases rather than phosphate groups, although the significance of this in terms of its lethal action is unclear [39]. Silver sulfadiazine is a combination of two antibacterial agents, silver and sulfadiazine. The question was asked which of the two compounds is due to the antibacterial effect or whether it occurs as a result of the interaction of the two. Silver sulfadiazine has a broad spectrum of action and unlike silver nitrate, it causes damage to the membrane of sensitive bacteria, it binds to cellular components, including DNA. Bacterial inhibition is likely to occur when silver binds to sufficient base pairs in the DNA helix, thereby managing to inhibit transcription. The complete mechanism of action of silver sulfadiazine has not yet been elucidated [40].

In conclusion, the perfect antimicrobial chemical probably does not yet exist, but we have the opportunity to make the right choice and proper use of current chemicals, so as to avoid both the increase in antimicrobial resistance and environmental problems. Thus, a deep knowledge of the antimicrobial agent together with the type of surface on which it is to be applied, would result in an adequate level of disinfection.

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AGENȚII CHIMICI CA FACTORI ANTIMICROBIENI

REZUMAT
O mare varietate de substante chimice pot fi folosite ca agenti antibacterieni in vederea prevenii contaminarii. Antisepticele și dezinfectanții sunt folosiți pe scară largă în spitale si reprezinta un element esențial de combatere si prevenire a infecțiilor nosocomiale. In aceste produse sunt prezenti o mare varietate de agenți chimici activi sau biocide. Dezinfectanții și antisepticele afectează bacteriile în multe feluri. Utilizarea pe scară largă a antisepticelor și a produselor dezinfectante a contribuit la dezvoltarea rezistenței microbiene, în special a rezistenței încrucișate la antibiotice. In practica medicala, agenții antimicrobieni trebuie selectați având în vedere microorganismele specifice, pH-ul, solubilitatea, toxicitatea, materialul organic prezent și nu in ultimul rand costul. Agentul chimic antimicrobian ideal probabil ca nu exista, dar alegerea corectă și utilizarea adecvată a substanțelor actuale, va avea drept rezultat un nivel adecvat de dezinfecție. Aceasta lucrare isi propune o trecere in revista a principalilor agentii chimici antimicrobieni, a mecanismelor prin care ei actioneaza si a importantei lor in practica clinica.

Cuvinte cheie: agenții chimici antimicrobieni, alcooli, halogeni, aldehide, compusi cuaternari de amoniu, argint.

EVALUATION OF THE ABILITY OF SOME MULTIDRUG-RESISTANT BACTERIAL STRAINS TO FORM BIOFILMS

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ABSTRACT

The crystal violet (CV) based microtitre plate assay is one of the most widely used method in screening for biofilm forming bacterial strains or in evaluating the efficacy of antibiofilm products. Despite the modifications made over the time in order to increase CV staining method's accuracy, this assay still shares the issue of ''edge effect'', phenomenon due mainly to evaporation of the liquid from the peripheral wells. This allows the planktonic cells to stick to the walls, which in turn binds the CV dye and gives a false result as biofilm biomass, thus increasing the rate of experimental errors. The present study aimed to improve the accuracy of the crystal violet staining assay, by reducing the ''edge effect'' through certain modifications that involved either changing the incubation conditions, by using a climate chamber instead of a classic incubator or the working technique, by filling the peripheral wells from lines A, B, G, H and columns 1, 2, 11 and 12 of a 96 well microtiter plate with sterile distiled water. Results showed that by filling the peripheral wells with water, the „edge effect" is reduced and the OD values are relatively homogene with lower standard deviations, when compared with incubating in a classic incubator and in the climate chamber ($p < .05$). Therefore, this improvement to crystal violet based microtiter plate assay has shown to give minimum variability in our results, mainly when the biofilm assay requires long incubation time involving more than 24 h.

Key words: biofilms, multidrug-resistant bacteria, bacterial strains, edge effect

INTRODUCTION

Biofilms are microbial communities attached to different surfaces wherein bacterial cells are embedded in a self-produced extracellular polymeric matrix [1]. Biofilms cause significant problems in both medical (e.g. device-related infections) and non-medical settings (e.g. food processing environments, drinking water distribution systems) [2, 3, 4].

Over the years, a broad range of *in vitro* models have been described for the study of biofilm formation and developement [5]. Two methods are currently used for the phenotypic identification of biofilm-producing bacteria, namely the Congo red agar test [6] and the microtiter plate assay [7]. While the Congo red agar test is mainly used for the identification of biofilm-producing staphylococci, the microtiter plate method is suitable for any bacterial specie of interest. The first method consists in the property of biofilm-producing staphylococci to bind the Congo red dye and develop black colonies on the red agar, while the non-biofilm-forming strains apear as red-coloured colonies [4]. Also, Congo red staining is largely used to identify the amyloid fibres-producing bacteria. Amyloids are found in almost all natural biofilms. These fibres contribute to bacterial aggregation and to protective role of biofilms, serving also as virulence factors [8, 9]. One of the most intensively studied biofilm-associated amyloids are curli fibres in *E. coli* and *Salmonella enterica*, which also occur in other enterics [10]. In *E. coli*, curli fibres are co-regulated with pEtN-cellulose [11] and in many strains are produced below 30°C only, suggesting a major role in environmental biofilms [12]. However, curli fibres and/or pEtN-cellulose can also be produced at 37°C by certain commensal or pathogenic *E. coli* strains [13]. In the human intestine, curli fibres promote inflammation and act as a virulence factor and can even trigger autoimmunity [9, 14, 15].

The microtiter plate assay was developed to replace the test tube assay, the first method used for macroscopic assessment of bacterial biofilms on the surface of plastic tubes [16]. These technique produces quantitative results based on the assessment of matrix and both living and dead cells (biofilm biomass tests), of viable cells (viability tests) and of matrix components, based on specific staining (matrix quantification

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tests) [17].

The crystal violet (CV) based microtiter plate assay is an indirect method of biofilm biomass quantification in the entire well [7, 18]. CV is a basic dye, which binds to negatively charged surface molecules and polysaccharides in the extracellular matrix, as well as to both living and dead cells [19, 20]. Despite the modifications made in order to increase CV staining method's accuracy [18], this assay still shares the issue of ''edge effect''. The ''edge effect'' is due mainly to two reasons; first, the peripheral wells are more ventilated thus can provide more oxigen for bacterial growth. Secondly, when incubated in normal incubators for more than 24 h, water evaporates quickly from peripheral wells. This phenomenon allows the planktonic cells to stick to the walls, which in turn binds the CV dye and gives a false result as biofilm biomass [21].

The present study aimed to improve the accuracy of the crystal violet staining assay, by reducing the ''edge effect'' through certain improvisations that involved either changing the incubation conditions or the working technique.

MATERIALS AND METHODS

Multi-drug resistant *Escherichia coli* strains CO1165, CP7921, DK0336, CU0181, DM3527, CO1453 and *Staphylococcus aureus* strains CP7438, CO0587, CP7170, CP8150, CL9002 were overnight cultured on blood agar. One or two colonies from each culture were suspended in 10 ml of Brain Heart Infusion (BHI) and adjusted to 0.5 Mc Farland. Different numbers of wells from the 96 well microtiter plates were inoculated with 150 µL of bacterial suspension, depending on the applied protocol. Wells containing the same quantity of BHI served as blanks. For biofilm formation, the microtiter plates were incubated for 72 h at 37±0.5°C, in a classic incubator (protocol 1 – P1, 2 – P2 and 3 – P3) or in a climate chamber ensuring 95% relative humidity and a temperature of 37±2°C (protocol 4 – P4). In P1, P2 and P4, columns 1 and 12 remained uninoculated, column 2 and line H served for the blanks (17 wells) and the rest of the microtiter plate was inoculated with three stains (21 wells for each strain). In P3, in order to reduce evaporation, the peripheral wells corresponding to lines A, B and H and to columns 1 and 12, were filled with 200 µL physiological saline remaining 14 wells for the blanks and 12 wells for each of the three strains.

Following the incubation period, the supernatant was removed from each well and the wells were rinsed using 160 µL physiological saline and air-dried for several minutes. Subsequently, 160 µL of 1% CV solution was added to each well. After 10 minutes, the excess dye was removed and the plates were rinsed twice with 160 µL (P1 and P2), respectively thrice with 200 µL physiological saline (P3 and P4) and again air-dried for several minutes. The biofilm biomass bound CV was released by adding 160 µL of 96% ethanol. After 30 minutes the absorbance was measured at 540 nm (Tekan).

In protocol 1 and 2 there have been used the *E. coli* strains and in protocols 3 and 4, the *S. aureus* strains. The difference between protocol 1 and 2 consisted in the type of micropipette used; in protocol 1 (as well as in protocols 3 and 4) has been used a single channel micropipette while in protocol 2, a multichannel one, in order to be more time effective.

Each strain was incubated in duplicate because one of the microtiter plates served for swabing the surface of the weels after removing the supernatant and rinsing with physiological saline, in order to assess the viable cells adherent to the biofilm. The colony forming units per milliliter (CFU/ml) were determined after incubation at 37°C overnight, by serial dilution technique and plating on nutrient agar. Only the plates displaying between 25 and 300 colonies were taken into account in the final formula:

$$CFU/ml = \Sigma \text{ (no. of colnies x dilution factor) / no. of plates x volume of the inoculum}$$

Statistical analysis

The blank corrected absorbance values of *E. coli* and *S. aureus* strains which fitted in the interval mean ± 1 standard deviation were used for reporting biofilm production. After eliminating the outliers and the negative values obtained in several cases, the final means and standard deviations were recalculated.

To determine whether the number of viable bacteria in the biofilm (CFU/ml) had any influence on the total biofilm biomass (optical density OD), the Pearson correlation was applied.

In order to determine which of the four protocols provides the most homogeneous results, respectively increases the accuracy of the CV based microtiter plate assay, there have been taken into account the number (proportion) of the extreme values needed to be eliminated and the mean value of the standard deviations obtained in each protocol. To compare between the proportion of outliers and between the mean values of standard deviations in each protocol, there have been used the Chi-square test with Yates correction, respectively the Mann-Whitney U test. For each of the applied tests the level of significance was seted at $p < .05$.

RESULTS AND DISCUSSIONS

As shown in Tables I-IV and in Fig. 1, there were some differences between the protocols in which the same strains have been used (P1 vs. P2 and P3 vs. P4). Mack et al. (2000) proposed a blank corrected mean absorbance value of 0.1 in order to distinguish between weak and strong biofilm producing bacterial strains [22].

Table I. Mean, standard deviation, viable cell count and number of outliers, for the *E. coli* strains in *protocol 1*

	CO1165	CP7921	DK0336	CU0181	DM3527	CO1453	
Mean (OD)	0.115582	0.880945	0.371791	0.132527	0.029232	0.219874	TOTAL
SD	0.054273	0.083929	0.137623	0.019853	0.015438	0.046503	
CFU/ml	56 x 10^5	104 x 10^5	74 x 10^5	71 x 10^5	138 x 10^5	81 x 10^5	
No. of outliers	9	9	13	11	12	8	62
Total wells / strain	21	21	21	21	21	21	126

Table II. Mean, standard deviation, viable cell count and number of outliers, for the *E. coli* strains in *protocol 2*

	CO1165	**CP7921**	**DK0336**	**CU0181**	**DM3527**	**CO1453**	
Mean (OD)	0.363226	0.671408	1.26464	0.290696	0.054717	0.679466	**TOTAL**
SD	0.085919	0.107018	0.411332	0.045167	0.04611	0.282692	
CFU/ml	77.5 x 10^4	36.5 x 10^4	40 x 10^4	92 x 10^4	34 x 10^5	26 x 10^4	
No. of outliers	9	8	14	10	13	11	65
Total wells / strain	21	21	21	21	21	21	126

Table III. Mean, standard deviation, viable cell count and number of outliers, for the *S. aureus* strains in *protocol 3*

	CP7438	**CP7170**	**CP8150**	**CL9002**	**CO0587**	
Mean (OD)	0.283805	0.040724	0.408894	0.09568	0.336069	**TOTAL**
SD	0.023113	0.005345	0.053726	0.0397	0.028633	
CFU/ml	106,3 x 10^4	3 x 10^4	62,5 x 10^3	26 x 10^4	106 x 10^4	
No. of outliers	6	7	5	4	7	29
Total wells / strain	12	12	12	12	12	60

Table IV. Mean, standard deviation, viable cell count and number of outliers, for the *S. aureus* strains in *protocol 4*

	CP7438	**CP7170**	**CP8150**	**CL9002**	**CO0587**	
Mean (OD)	0.726884	0.201412	0.954736	0.208999	0.783432	**TOTAL**
SD	0.039399	0.069513	0.114076	0.069468	0.055975	
CFU/ml	70,5 x 10^4	45,5 x 10^3	130,5 x 10^3	17,2 x 10^4	2,8 x 10^4	
No. of outliers	12	11	12	10	8	53
Total wells / strain	21	21	21	21	21	105

In the case of *E. coli* strains, in both protocols (P1 and P2), four out of five strains were found to be strong biofilm producers and only one strain (DM3527) had an OD value lower than 0.1. Excepting strain CP7921, all the rest exhibited higher OD values in P2 comparative to P1 ($p > .05$), in the same incubation conditions and working procedure. The average of the standard deviations in P2 was almost three times higher than in P1, however the differences were statistically insignificant ($p > .05$) (Fig. 4). The higher variability of the results obtained in protocol 2 might be due to the usage of multichannel micropipette. Applying a highly concentrated CV dye (1%) with a multichannel micropipette requires a lot of attention and skill, so as not to splash the walls of the wells. If this occurs, rinsing the excess dye with the multichannel micropipette is not as effective as using the single channel one, because the first of them cannot be easily rotated so that the rinsing solution to reach on the entire surface of the wells.

In the case of *S. aureus* strains, in protocol 3, two out of four strains were found to be strong biofilm producers, while in

protocol 4 all the four strains had OD values higher than 0.1. All strains exhibited higher OD values in P4 comparative to P3 (p> .05). The average of the standard deviations in P4 was almost two and a half times higher than in P3, with statistically significant difference (p= .03) (Fig. 4).

Both P3 and P4 protocols were designed to reduce the „edge effect" and thus to increase the homogenity of the results. It is well known that the environmental conditions impacts biofilm formation and developement [23, 24]. Protocol 4 was designed to prevent evaporation from the peripheral wells by using a climatic chamber that maintained 95% humidity during the 72 h period. These equipments share the disadvantage of temperature oscillation within wider limits than classical incubators to maintain first of all constant humidity. In this particular case the temperature variations were of 2 degrees around 37°C. Many studies have reported the effect of factors, such as temperature and humidity changes, nutrient availability, oxygen level, pH and surface type, on the biofilm formation of pathogenic bacteria such as *S. aureus* [24]. However, the effect of temperature changes on the biofilm formation of *S. aureus* remains unclear and there are discrepancies between studies undertaken in this regard. Some have found that the biomass of *S. aureus* biofilms grown at 37°C was more important than those grown at 25°C on polystyrene [25, 26]. Other authors reported a higher cell count of the *S. aureus* biofilm at 25°C in contrast to that obtained at 37°C on stainless steel. Instead, Da Silva Meira et al. (2012) showed that there is no clear effect of the incubation temperature (7 and 28°C) on the biofilm formation of *S. aureus* [27]. However, in the present study a temperature variation between 35 and 39°C for a period of 72 h determined an increased biofilm biomass production and less homogen results comparative to incubating at 37±0.5°C, when quantificating *S. aureus* biofilm in 96-well microtitre plates.

Assessment of the relationship between the viable bacteria count in the biofilm (CFU/ml) and the biofilm biomass (OD) showed variable results between the protocols. Regarding the *E. coli* strains subjected to P1 and P2, in the first protocol there was no correlation between the two variables r(62)=0.1779, p> .05, while in the second protocol it was found a moderate negative correlation r(59) = -0.5955, p< .00001 between the viable bacteria count and the biofilm biomass (Fig. 2). Regarding the *S. aureus* strains subjected to P3 and P4, in protocol 3 it was found a weak positive correlation between the two variables r(29)=0.3125, p> .05, but without statistical significance, while in protocol 4 there was no correlation between the viable bacteria count and the biofilm biomass r(50)=0.1722, p> .05 (Fig. 3).

Figure 4 shows that regardless of the applied protocol, approximately half of the OD values were outliers as a result of not falling within a deviation from the normal distribution of the mean, with variations from 48.33% in protocol 3 up to 51.59%, in protocol 2. The differences between the proportion of the outliers in each protocol were not statistically significant X^2 (3, N = 417) = 0.2325, p = .9721.

As previously stated the primary cause for the „edge effect" phenomenon is evaporation [21]. To reduce excessive water loss and to maintain humidity during the incubation period, physiological saline has been added in the peripheral wells, in protocol 3. Results showed that by applying this protocol, the „edge effect" is reduced. As shown in Fig. 5, the OD values in protocol 3 are relatively homogene with reduced standard deviations, mainly when comparing with protocols 2 and 4 (p< .05). Moreover, protocol 3 is significantlly less time consuming than the rest of the protocols.

CONCLUSIONS

This modification to crystal violet based microtiter plate assay has shown to give minimum variability in our results and is mainly helpful when the biofilm assay requires long incubation time involving more than 24 h.

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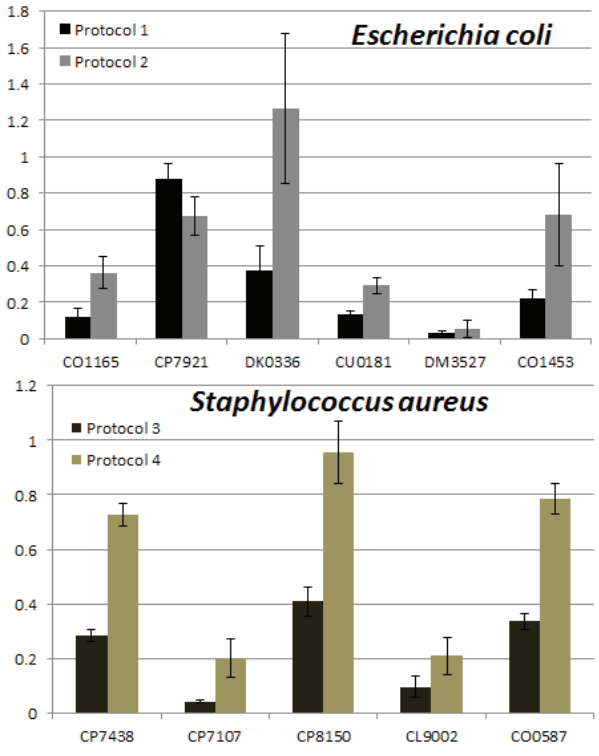

Fig. 1. Mean OD ± SD for all the bacterial strains subjected to different protocols

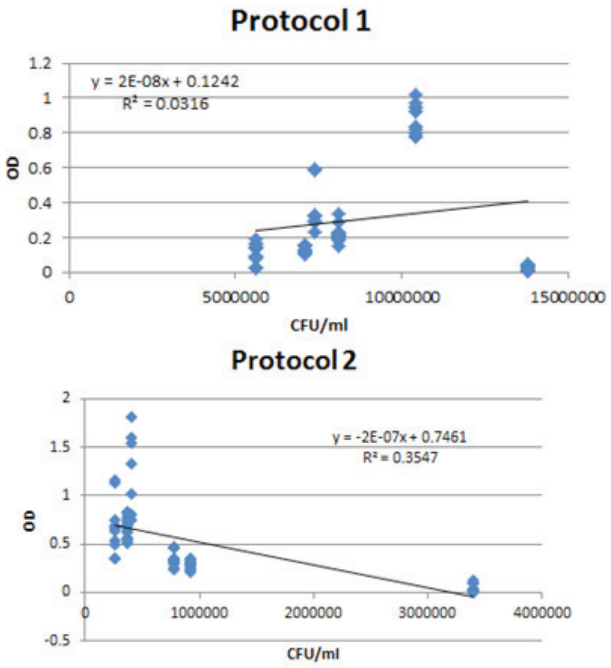

Fig. 2. Relationship between the number of viable bacteria in the biofilm (CFU/ml) and the biofilm biomass (OD), in *E. coli* strains: Protocol 1 – lack of correlation, Protocol 2 – moderate negative correlation

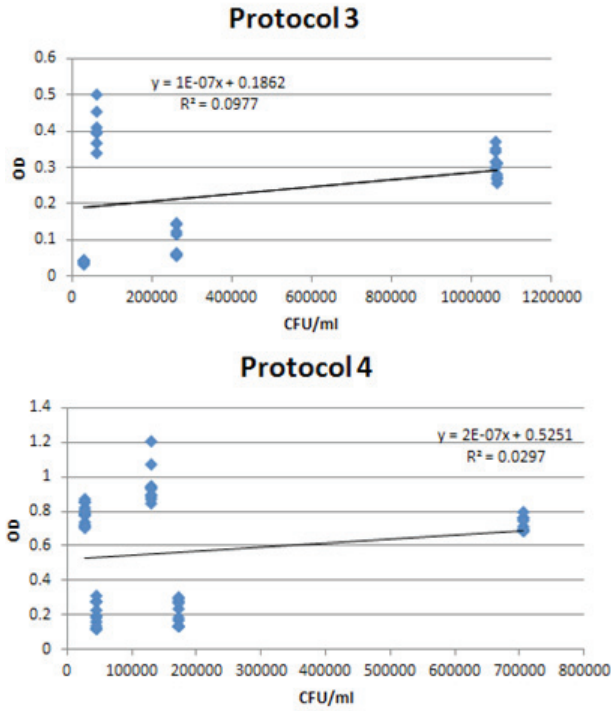

Fig. 3. Relationship between the number of viable bacteria in the biofilm (CFU/ml) and the biofilm biomass (OD), in *S. aureus* strains: Protocol 3 – weak positive correlation without statistical significance, Protocol 4 – lack of correlation

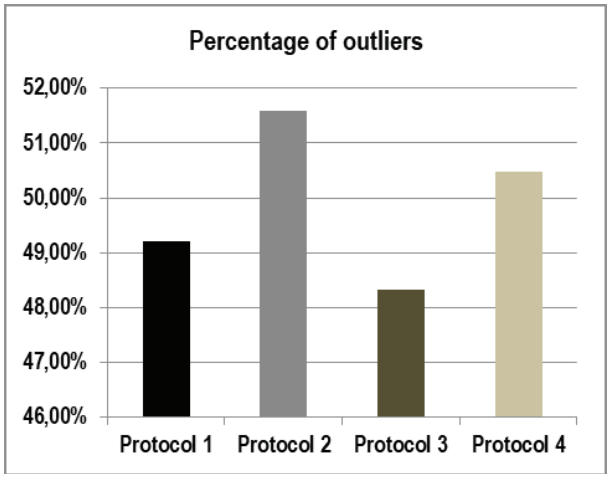

Fig. 4. Percentage of outliers that needed to be excluded due to not fitting in mean ± 1 SD

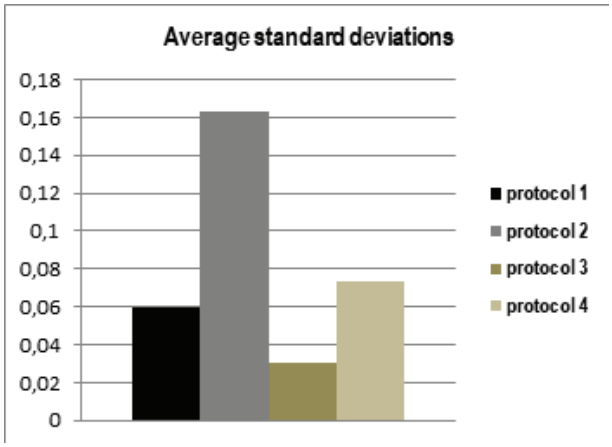

Fig. 5. Average values of the standard deviations in each protocol

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EVALUAREA CAPACITĂȚII UNOR TULPINI BACTERIENE MULTIREZISTENTE DE A FORMA BIOFILME

Rezumat:
Tehnica în plăci de microtitrare cu cristal violet este una dintre cele mai utilizate metode pentru evaluarea capacității tulpinilor bacteriene de a forma biofilme, precum și pentru testarea preparatelor care împiedică formarea acestora. În ciuda încercărilor de îmbunătățire întreprinse de-a lungul timpului, această metodă încă prezintă dezavantajul apariției „efectului de margine", fenomen datorat evaporării lichidului din godeurile marginale. Pierderea de lichid determină atașarea celulelor planctonice la pereții godeurilor, fixarea colorantului și astfel, interpretarea eronată ca și biofilm. Scopul prezentului studiu a fost de a încerca reducerea evaporării din godeurile marginale, prin modificări care vizează fie condițiile de incubare (incubarea într-o cameră climatică), fie tehnica de lucru, prin umplerea godeurilor de pe liniile A, B, G, H și coloanele 1, 2, 11 și 12 ale plăcii de microtitrare, cu apă distilată sterilă. S-a evidențiat o reducere a „efectului de margine" atunci când godeurile periferice au fost umplute cu apă, cu obținerea unor densități optice mai omogene și a unor deviații standard mai mici, comparativ cu rezultatele obținute în urma incubării în termostatul clasic și în camera climatică ($p < .05$). Astfel, îmbunătățirea adusă tehnicii de evidențiere a biofilmelor bacteriene în plăci de microtitrare, cu cristal violet, scade variabilitatea rezultatelor, în special atunci când este necesar un timp de incubare mai mare de 24 de ore.
Cuvinte cheie: biofilme, bacterii multirezistente, tulpini bacteriene, efect de margine

SPREAD AND PHYSIOLOGICAL ECOLOGY OF NONTUBERCULOUS MYCOBACTERIA

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ABSTRACT
Nontuberculous mycobacteria (NTMs) are ubiquitous microorganism, which are not only found in the natural environment (water, soil and dust), but also in the human environment. Due to their morphological and functional properties, they can survive in all natural and artificial environments. Water from distribution systems and sanitary installations is the ideal place for development and multiplication of NTMs, as well as the main transmission route of NTM from nature in the artificial system. NTMs form biofilms on the surface of household objects and the aerosolubilization and subsequent inhalation by humans is the main way of producing NTM lung disease. NTMs include saprophytic and opportunistic microorganisms. Most of them have a low pathogenicity, which is why it requires an affected host to produce disease.
Key words: NTMs, opportunistic microorganisms, physiological ecology

INTRODUCTION

The incidence and prevalence of lung disease caused by NTM (LD-NTM) are increasing and their impact on human health is a current topic of great interest [1]. Generally, the term nontuberculous mycobacteria (NTM) refers to species of mycobacteria othen than Mycobacterium tuberculosis and M. Leprae complex [2]. In the specialized literature, NTMs have been named for various names, including "environmental mycobacteria", "atypical mycobacteria" and "mycobacteria other than M. tuberculosis". In the past, they were considered minor pathogens, in relation to the pathology caused by Mycobacterium tuberculosis.

Currently, due to the increase in the percentage of immunocompromised hosts, in particular due to the increasing incidence of HIV/AIDS, NMT_S have emerged as important pathogens worldwide [3]. NTM infection mainly presents four distinct clinical diseases in humans*:* chronic pulmonary disease; disseminated disease in severely immunocompromised patients; skin and soft tissue for clinicians [4]. In addition, microbiological recurrence is common even after successful treatment infections; and superficial lymphadenitis, especially cervical lymphadenitis in children [5]. Pulmonary disease accounts for 80–90% of NTM-associated diseases [6].The diagnosis and treatment of NTM lung disease remain challenging, with substantially high reinfection rate.

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Table I. Classification of mycobacteria. [5]

Group	Species
Mycobacterium Tuberculosis Complex	*M. tuberculosis, M. bovis, M. africanum,M. canettii, M. caprae, M. pinnipedii, M. orygis, M. microti, M. mungi, M. suricattae*
Leprotic Mycobacteria	*M. leprae, M. lepromatosis*
Non-Tuberculous Mycobacteria	
• Slow Growing	*M. avium complex (M. avium, M. chimaera, M. intracellulare), M. haemophilum, M. gordonae, M. kansasii, M. simiae, M. marinum, M. malmoense, M. xenopi, M. ulcerans*
• Rapidly Growing	*M. abscessus group (M. abscessus, M. bolletii, M. massiliense), M. fortuitum group (M. fortuitum, M. peregrinum, M. porcinum), M. smegmatis, M. vaccae, M. mucogenicum*

Spread of NTMs

NTMs can be found anywhere in nature and at all altitudes. They grow best in soil, but are also present in water [7]. NTMs are represented by potentially pathogenic mycobacteria in humans and animals, but also by normally saprophytic mycobacteria, which are non-pathogenic or potentially pathogenic [8]. Potentially pathogenic mycobacteria are widespread throughout the wild and can become pathogenic under certain circumstances [9]. They are called opportunistic or occasionally pathogenic mycobacteria in order to differentiate them from the strictly pathogenic ones. Some of them are rarely (ex M malmoense) or never (M ulcerans) isolated in the environment, although the epidemiological profiles of the diseases generated by them indicate that they are present in nature [10]. Saprophytic or potentially pathogenic mycobacteria often refer to non-tuberculous mycobacteria [11]. They form homogeneous groups that are easy to identify in the laboratory. Although the clinical manifestations produced by these mycobacteria are similar to those produced by the Koch bacillus, they are considered atypical, because the etiological agent does not belong to the M tuberculosis complex [12]. People are constantly in contact with these mycobacteria, which they can inhale or ingest. As a result, a temporary or permanent colonization of the skin, respiratory and digestive tracts is performed[13]. The natural environment is considered to be the environment that was not created by humans or that is not normally subject to human pollution (urban water systems, urban rivers that flow into natural rivers or lakes) [14]. In these environments the distribution of mycobacteria is different. If the urban environment seems ***to be*** conducive to the development of mycobacteria (ex M terrae), the environment located along the water or savannah coasts seems ***to be*** conducive to the isolation of mycobacterial species of the MAC complex[15]. The distribution of mycobacteria in the environment is influenced by a number of factors, such as: the chemical composition and pH of the soil, as well as the temperature of the environment. Artificial environments (eg water sources, swimming pools) are colonized by another spectrum of mycobacteria, some of which appear to live only in man-made environments (eg M kansasii, M xenopi) [7]. Some of the mycobacteria (M marinum, M chelonae, M avium) are rare in the wild, but are common in artificial environments [16]. There is also a group of non-tuberculous mycobacteria that have not been identified in the environment (M haemophilum, M genavense), but epidemiological data on the diseases they cause suggest that they come from the environment. These mycobacteria require special growth media in vitro or grow poorly in laboratory conditions, which may explain why they have never been isolated in the environment [17].

Mycobacteria Ecology

The physiological ecology of a microorganism is defined by those physiological characteristics (features) of that microorganism, which are determinants of their ecology and, therefore, of the epidemiology of the diseases determined by it [18].

Intraspecies genomic variation of NTMs is a property of microorganisms that allows their phenotypic variability. Analysis of the mycobacterial genome revealed a number of genes responsible for encoding virulence factors. The virulence genes available in the chromosome provide flexibility for opportunistic pathogens to infect a host when conditions become favorable. The genetic elements responsible for these pathogenic traits can be incorporated into the stable chromosome or are transferred through mobile plasmids. Plasmids contain resistance, tolerance or persistence genes that have a beneficial potential for maintaining and spreading mycobacteria. Mycobacteria

incorporated plasmids independently and maintained the plasmids that helped each strain survive in its specific conditions. It is necessary in the future to give a major emphasis to the investigation of the genome of various NTM species in order to better understand the genetic elements and genomic diversity that may influence the ability of environmental mycobacteria to become opportunistic pathogens. Specifically, the identification of potential virulence factors, drug resistance genes, and plasmids using complete genome information sheds light on this area. Also, the study and identification of genomes for each species are necessary both for the investigation of the pathogenic potential of NTMs and for use in diagnostic methods [19].

Slow growth and the underlying metabolism of NTM are important characteristics that allow them to adapt to stressful environmental conditions [20] such as anaerobiosis [21], starvation [22], low pH [23], high temperature [24], osmotic stress [25], but also less sensitive to antimicrobial agents and disinfectants [26,27]. Thus, the adaptation of mycobacteria to these conditions is achieved by reducing the metabolism that protects the cell from harmful conditions together with the mycobacterial cell wall [28]. The slow growth of NTM is achieved by a small number of 16 S r RNA genes but also by limiting resources for essential cellular processes, as well as population growth that involves a lot of energy for the synthesis of long chain C60-C80 mycolic acids in the mycobacterial cell wall [29]. Protein synthesis, such as M spA in the case of M. smegmatis, improves nutrient absorption and increases growth rate [30]; this property is also found in the case of M. fortuitum but absent for M. tuberculosis [31].

The cell wall, made up of a glycoprotein layer, surrounded by a lipid membrane is the fundamental structural feature that ensures the resistance of mycobacteria to environmental factors. Due to the lipid-forming outer cell membrane, mycobacteria are hydrophobic microorganisms and relatively impermeable to substances that cross the membrane of these cells. Although the rates of transmembrane transport of nutrients are quite low compared to other microorganisms, which limits mycobacterial growth and replication, mycobacteria have been shown to be particularly resistant to a number of antimicrobial agents, especially ***the*** hydrophilic ones [32, 33, 34, 35]. The hydrophobic character of the outer membrane favors the attachment of mycobacteria on the surfaces, thus preventing the washing and dilution of these microorganisms inside the water systems (rivers and drinking water distribution systems). Also, hydrophobicity causes the concentration of environmental mycobacteria at the air-water interfaces, where the high concentration of organic compounds provides a good source of food for these microorganisms [36]. For this reason, the best sampling sites for environmental mycobacteria are water surfaces and monsters. The hydrophobic, waterproof outer lipid membrane must be thought of as a 2-faceted structure: on the one hand it limits mycobacterial growth, and on the other hand it offers a higher environmental resistance, which has the effect of occupying larger habitat areas [37]. In drinking water systems, disinfection (e.g. chlorination) reduces the number of most microorganisms, leaving mycobacteria resistant to these disinfectants, a larger habitat for nutrient consumption and attachment to surfaces in the absence of competition [37]. The persistence and growth of mycobacteria in drinking water distribution systems and sanitary facilities are favored by their attachment to surfaces, a phenomenon favored by the hydrophobicity of outer membranes. As a result, attached mycobacteria are less likely to be removed by washing with high water flows, and mycobacterial growth and biofilm formation lead even to higher levels of microbial resistance. On the other hand, the hydrophobicity of the cell surface of mycobacteria will generate cell aggregation, a rather annoying problem for researchers in laboratories, in the case of culture growth of mycobacteria. The increased hydrophobicity of the external surface of opportunistic mycobacteria favors the formation of mycobacterial biofilms [37].

Biofilm refers to a macromolecular aggregate composed of microorganisms, whose cells adhere to each other and also adhere to a surface [38,39]. Thus, the cells become adherent due to an extracellular matrix composed of substances of polymeric nature. Biofilm formation by Mycobacterium avium, which requires divalent cations, is higher when cells are in nutrient-rich and low-nutrient conditions and is inhibited by humic acid [39,40]. Mycobacterium smegmatis [41] and glycolipoproteins are required for biofilm formation in the case of M. avium on polyvinyl chloride (PVC), but not on plastic or glass surfaces [42]. A surprising finding was that M. avium mutants, deficient in biofilm formation, also showed deficiencies in the invasion of the cell epithelium [43]. In the future, it is particularly important that the interpretation of biofilm formation rates include a separate analysis of the surface adhesion stage and the analysis of the biofilm growth stage. Also, increased hydrophobicity of mycobacteria is a determinant of aerosolization.

Aerosolization is the process by which mycobacteria are transferred from water to air, thus favoring the inhalation of these microorganisms in the human respiratory tree. Mycobacterial cells, with hydrophobic outer membranes, attach to the walls of air bubbles that rise from the water column and when the bubbles reach the surface, they break and form a crater whose walls are enriched with mycobacteria. Following the disappearance of the crater, multiple very small drops of water appear, which are thrown at heights of 10-20 cm. These drops have a concentration of mycobacteria over 10,000 times higher than in deep water [18]. In this regard, an enrichment factor with mycobacteria in discarded drops has been described, which varies depending on the species, strain and type of mycobacterial colony. Also, this enrichment factor is closely correlated with the hydrophobicity of the mycobacterial cell surface [44]. Some of the droplets formed are so small that they are able to penetrate the bronchi and alveoli of the lungs [18]. In the future, it is necessary to find better strategies for collecting mycobacteria from water tanks. In this regard, it is recommended that mycobacteria be harvested either from the collection of aerosolized droplets [45], surface microlayers [46] or fractional water samples [47].

Resistance to amoebae is an important feature of non-tuberculous mycobacteria [48]. This parasitic relationship may

explain the occurrence of human infections. Both microorganisms have the same habitat, namely insufficiently treated waters. In the last 2 decades, numerous community infections and outbreaks of healthcare associated (nosocomial) infections following surgeries and endoscopic interventions caused by non-tuberculous mycobacteria have been described. [49, 50, 51, 52, 53]. There are studies that have shown the colonization of drinking water, even in hospitals, with vegetative forms of amoebae, but also with non-tuberculous mycobacteria [54]. Due to the particular structure of the cell wall, environmental mycobacteria are resistant in the cytoplasm of living amoebae [55]. This feature makes it possible for the mycobacterium phagocytosed by the amoeba to be found in the wall structure of the amoeba cyst, realizing what are called pathogenic trophozoites [56]. These practical and scientific observations have led to the development of a pathogenic ameobiantrophozoite model, which acts as a "Trojan horse" and protects environmental non-tuberculous mycobacteria against adverse environmental conditions [48]. This particular location allows both the protection of the mycobacterium from adverse environmental conditions and their rapid release from the wall of the ameobian cyst, when the environmental conditions are favorable.

In **conclusion**, the morphological and functional peculiarities of opportunistic mycobacteria are those that ensure their resistance and spread in the environment. Therefore, understanding the mycobacteria ecology is an essential condition in preventing and treating these infections.

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RĂSPÂNDIREA ȘI ECOLOGIA FIZIOLOGICĂ A MICOBACTERIILOR NONTUBERCULOASE (NTM)

REZUMAT

Micobacteriile nontuberculoase (MNT) sunt microorganisme omniprezente, care se gasesc nu numai în mediul natural, apa, sol și praf, dar și în mediul uman. Datorită proprietăților lor morfofuncționale, ele pot supraviețui în toate mediile naturale și artificiale. Apa din sistemele de distribuție și instalațiile sanitare reprezintă locul ideal de dezvoltare și multiplicare pentru MNT, precum și principala cale de transmisie a MNT din natură în sistemul artificial. MNT formează biofilme pe suprafața obiectelor din gospodării, iar prin aerosolubilizare și inhalarea ulterioară de către oameni reprezintă principala cale de producere a bolii pulmonare MNT. MNT includ microorganisme saprofite si oportuniste. Majoritatea dintre ele au o patogenitatea scăzută, motiv pentru care necesită o gazdă afectată pentru a produce boală.

Cuvinte cheie: MNT, microorganisme oportuniste, ecologie fiziologica

AN UNUSUAL CASE OF MORBUS LEDDERHOSE IN A CAUCASIAN, YOUNG MALE PATIENT. CASE REPORT AND LITERATURE REVIEW

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ABSTRACT

Introduction: Plantar fibromatosis also known as Morbus Ledderhose, first described in 1897 by Georg Ledderhose is characterized by slow-growing benign extra-abdominal desmoid nodules on the plantar aponeurosis. The etiology of this disease is unknown but is often associated with diabetes mellitus, liver disease, alcoholism, and repeated traumatic episodes of the soles. New possible causative agents reported in the literature are: drugs from the class of beta-blockers, anticonvulsants, nutritional supplements high in glucosamine or chondroitin, and high doses of vitamin C. **Case presentation:** In this report, we present the case of a 33-year-old caucasian male with a 5-year history of palpable nodule within the medial plantar region of the left sole, who denied any personal or family history that could be related to this disease, medications or alcohol consumption. Instead, he did report daily intake of nutritional supplements high in vitamin C, which corresponds to the onset of disease. **Conclusion:** Because our patient did not have any other predisposing factors that could lead to this pathology, we intend to highlight the fact that prolonged consumption of nutritional supplements high in vitamin C can influence the occurrence of Morbus Ledderhose. Further investigation between the long-term use of these nutritional supplements and the development of this pathology is necessary.
Key-words: plantar fibromatosis, vitamin C, Morbus Ledderhose, nutritional supplements

INTRODUCTION

Plantar fibromatosis also known as Morbus Ledderhose, was firstly described in 1897 by Georg Ledderhose and is characterized by slow-growing benign extra-abdominal desmoid nodules on the plantar aponeurosis [1,2].This condition is characterized by three developmental phases: the first phase with high proliferative cellular activity, a subsequent acquisition of myofibroblastic characteristics with tissue contraction, and a final phase characterized by few mature fibroblasts embedded in a dense matrix [3]. The etiology of this disease is unknown, but it is often associated with diabetes mellitus, liver disease, alcoholism, and repeated traumatic episodes of the soles. Recent studies report in the literature possible new causative agents: beta-blockers, anticonvulsants, and nutritional supplements high in glucosamine or chondroitin and high doses of vitamin C, thus promoting the production of excess collagen [4]. The pathophysiological perspective states that growth factors play a role in increasing fibroblastic activity causing the formation of the fibromas. The identified growth factors are the platelet-derived growth factor, the transforming growth factor-beta, free oxidized radicals, interleukin-1 alpha, and interleukin-1 beta[1,5,6].]. As a benign disease, treatment has involved, in the last decades, symptomatic management, as it follows: steroid injections, verapamil, radiation therapy, extracorporeal shock wave therapy, and not least tamoxifen. When conservative measures fail, surgical removal of the fibromas and adjacent plantar fascia is often conducted, although recurrence is common [7].

Case report

We present the case of a 33-year-old caucasian male with a 5-year history of palpable nodule within the medial plantar region of the left sole, who presented to the plastic surgery department of our institution. The patient reported increasing size of the nodule and local tenderness over the past 10 months, particularly after standing for a long period. The patient has denied any personal or family history that could be related to this disease, medications, or alcohol consumption. He did report daily intake of nutritional supplements high in vitamin C, which corresponds to the onset of disease.

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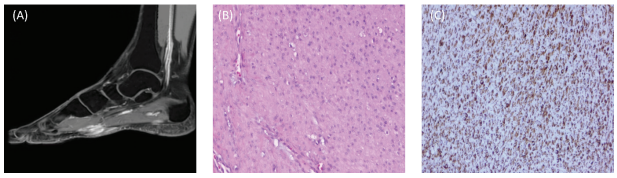
Clinical examination revealed a 6 x 3 cm lesion of the plantar surface, hyperalgesic and erythematous. At the time of the referral, an MRI showed a large deep plantar mass located on the middle segment of the left sole (Figure 1A), imprecisely contoured with contrast, highlighting the flexor muscles of the toes. The subcutaneous adipose tissue has also been infiltrated on the medial contour. Due to worsening pain and limitations in daily living, the patient elected for surgical intervention. Following the surgery, the tumor was sent to the pathology department of our institution for a definite diagnosis to be established.

The examined fragments were represented by connective-adipose tissue in which multinodular, fusiform fibroblastic cells were observed (Figure 1B). No cytonuclear atypia was identified. For a more accurate diagnosis, we performed the positive-expressed Vimentin immunohistochemical reaction, which revealed the activity of fibroblasts (Figure 1C).

Regarding follow-up, the patient returned to our clinic 2 weeks post-surgery, and the incision was noted to be healing well with no evidence of infection.



DISCUSSION

As described in the previous lines, the diagnosis of Ledderhose disease is usually clinical and does not require additional testing. In our case, the clinical findings did not raise awareness in the direction of this diagnosis. The tumor was present unilaterally. This aspect led us to think of a malignant tumor, in contrast to data from the literature that supports the presence of plantar fibromas on both soles, simultaneously. Therefore, we included as differential diagnosis the following: plantar fasciitis, leiomyoma, rhabdomyosarcoma, and liposarcoma, as mentioned by other specialty studies [9-11]. Last but not least, another aspect that has raised concern was the size of our patient's tumor. The National Institute for Health and Care Excellence recommends the prompt referral to a sarcoma treatment center for patients presenting with soft tissue swellings that are greater than 5 cm in diameter, painful, deep to the fascia, increasing in size, or recurring after a previous excision [12]. Histological features have highlighted the benign nature of this disease.

Imaging Assessment

Magnetic Resonance Imaging proved to be the preferred option for diagnosis and it has the added advantage of examining the anatomy of the structures involved, the adjacent soft tissue planes, and identification of other pathologies. If imaging characteristics are ambiguous or typical imaging features of a plantar fibromatosis are absent, tissue histology should be performed to exclude other pathologies [10]. MRI is influential in surgical planning and can help define the margins of the lesion allowing for better surgical resection [1].

The most important aspect, like in any disease, is to relieve the pain, to get patients' comfort, and to use therapeutic strategies, which do not activate or aggravate underlying chronic pathologies [13-15].Conservative therapies can be applied in the early stages of the disease, without pain, walking discomfort, or balance problems. Unfortunately, this Regarding shock therapy produces direct trauma of the lesion unleashing an intensive healing response and lesion lysing, by increasing its vascularity [16]. Furthermore, radiotherapy is effective in the first stages, targeting the disease progression and preservation of the feet function, but few studies support this hypothesis.

Ionizing radiation reduces the proliferative activity of the fibroblasts and myofibroblasts by interacting with TGF-β production [1]. This therapy presents acute side effects like lethargy, local edema, pain, or local skin reaction. Late side effects are represented by fibrosis and local skin modifications. It can produce a decrease in patients' immunity status leading to general complications. In the case of young patients, as in our case, we do not recommend the use of this therapy due to the risk of promoting secondary malignancy like soft tissue sarcoma or skin cancer.

Intralesional steroid injections, decrease the expression of vascular cell adhesion molecule-1, thus dropping the production of TGF-β, basic fibroblast growth factor, IL-1α, and IL-1β. As a result, the proliferation of fibroblasts and collagen production is decreased [16]. Another conservative therapy is represented by collagenase Clostridium histolyticum injections, containing two types of collagenase: AUX-1 and AUX-2. Both degrade collagen and appear to be a safe and effective alternative treatment to surgery [18].

Surgical excision is usually used as a last resort, in cases with inefficient conservative therapies, progressive lesions, advanced stages of the disease, unsupportable pain, walking or balance problems, as our patient also reported. It can be performed classically or endoscopic, with partial or complete fascia resection. Some surgeons prefer the local excision, removing only the nodules, but this method has a recurrence rate from 57–100% [19]. The endoscopic plantar fasciectomy has the benefit of reducing the wound size with better cosmetics, decreasing the incidence of painful hypertrophic scars, necrosis, or infection. In case of lesions invading the muscle, plantar skin, or neurovascular bundles, this technique is contraindicated. Another impediment of this approach is, after all, technically demanding and should be performed only by experienced arthroscopists [20]. A personalized therapeutic plan depending on the individual characteristics, presence, and intensity of the symptoms and the stage of pathological modifications is, also, strongly recommended.

CONCLUSION

Because our patient did not have any other predisposing factors that could lead to this pathology, we intend to highlight the fact that prolonged consumption of nutritional supplements high in vitamin C can influence the occurrence of Morbus Ledderhose. However, a further investigation between the long-term use of these nutritional supplements and the development of this pathology is necessary.

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UN CAZ NEOBIȘNUIT DE MORBUS LEDDERHOSE LA UN TÂNĂR PACIENT CAUCAZIAN. PREZENTARE DE CAZ ȘI ANALIZA LITERATURII DE SPECIALITATE

REZUMAT

Introducere: Fibromatoza plantară, cunoscută și sub numele de Morbus Ledderhose, descrisă pentru prima dată în 1897 de Georg Ledderhose, se caracterizează prin apariția unor noduli desmoizi extra-abdominali cu caracter benign și creștere lentă, la nivelul aponevrozei plantare. Etiologia bolii este necunoscută, însă este adesea asociată cu diabetul zaharat, bolile hepatice, consumul de alcool și episoadele traumatice repetate la nivelul tălpilor. În literatura de specialitate au fost raportați noi posibili agenți cauzali, precum: medicamentele din clasa beta-blocantelor și anticonvulsivantelor, precum și suplimentele nutriționale bogate în glucozamină, condroitină sau cu concentrații crescute de vitamina C. **Prezentarea cazului:** În acest raport dorim să prezentăm cazul unui bărbat caucazian în vârstă de 33 de ani, cu un istoric de 5 ani a unui nodul palpabil în regiunea plantară mediană a tălpii stângi, și care a negat orice antecedente personale sau familiale, consumul de medicamente sau alcool, care ar fi putut determina apariția bolii. În schimb, pacientul a raportat consumul zilnic de suplimente nutritive bogate în vitamina C, fapt ce corespunde cu momentul declanșării bolii. **Concluzie:** Având în vedere că pacientul nostru nu prezenta alți factori predispozanți, intenționăm să subliniem faptul că un consum prelungit de suplimente nutritive bogate în vitamina C, poate influența apariția bolii Ledderhose. Pentru viitor, sugerăm efectuarea unor investigații suplimentare în ceea ce privește legătura dintre utilizarea pe termen lung a acestor suplimente nutritive și dezvoltarea patologiei Ledderhose.

Cuvinte-cheie: fibromatoză plantară, vitamina C, Morbus Ledderhose, suplimente nutritive

POST-STROKE DEPRESSION – INSIGHTS ON THE PATHOPHYSIOLOGICAL MECHANISMS

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ABSTRACT

Post-stroke depression (PSD) is one of the most common disorders encountered in stroke patients, having an unfavorable impact on the person's recovery and, implicitly, on his/her quality of life. Numerous epidemiological studies have highlighted the significant impact of this phenomenon, both at the individual level (approximately 1 in 3 patients with stroke will suffer from depression) and at the socio-economic level. Although intensively studied, the occurrence of depression in only a part of people who have had a history of stroke is not fully explained, currently existing several incomplete and possibly interdependent theories. In this context, the aim of this paper is to bring a new perspective on the phenomenon of depression associated with stroke, the authors presenting in the first part the latest results from the literature regarding PSD's pathophysiological mechanisms, while in the second part, current and future therapeutic approaches are detailed.

Key words: stroke, depression, pathophysiology, neuroinflammation, oxidative stress

INTRODUCTION

Depression is common in neurology, with a significant percentage of neurological patients also suffering from major or minor depression (1)(2). Stroke, the third cause of death and having a great negative impact at individual and socio-economic level, is frequently associated with post-stroke depression (PSD), this condition affecting approximately one third of survivors (3). Because of the important burden generated, PSD has increasingly become a concern to researchers and clinicians, with numerous studies and meta-analyzes having focused on the epidemiology, risk factors and treatment possibilities of this psychiatric pathology. Nowadays, it is known that the predisposing factors for PSD can be divided into several categories, including personal unchangeable (female gender, personal history of pre-stroke depression), modifiable (cognitive and physical impairment, level of independence), and independent risk factors such as lack of family and social support (4).

Regarding the pathophysiological mechanism of PSD, there are still many unknowns, as the currently accepted theories are not enough to completely explain the phenomenon. It is clear that depression diagnosed after stroke is a complex and multifactorial process that has at least two generating elements: ischemia-induced neurobiological dysfunctions induced by the acute vascular event which also have chronic effects and, additionally, the psychosocial distress. Recent research considers that neurobiological changes are more important than the mental damage resulting from the sudden onset of disability or due to an unsatisfactory outcome following recovery therapy (5). In addition, other studies have shown a positive correlation between certain genetic factors such as polymorphisms of serotonin (5-hydroxytryptamine, 5-HT) transporter 5-HTTLPR, 5-HT2A receptor and of the Brain-Derived Neurotrophic Factor (BDNF) genotypes and the occurrence post stroke depression (6). In this context, the aim of this article is to offer a detailed view of the most important pathophysiological theories that try to explain the appearance of PSD syndrome, remembering also possible therapies that are born from them, but also the missing elements that require future research.

Most relevant pathophysiological mechanisms of post-stroke depression

Nowadays, numerous pathophysiological mechanisms are being discussed that partially explain the occurrence of PSD in stroke patients, some of them common with the mechanisms of primary depression. Among the theories regarding the neurotransmitter imbalance as a cause of depression, we mention first of all the monoaminergic hypothesis. Although one of the first hypotheses raised by the medical world, it is no longer accepted as the only pathophysiological explanation for the depressive mood disorder. Considering this theory applicable in the case of PSD, following the acute cerebral infarction, it is thought that there are destructions of the aminergic neurons that connect the brainstem and the upper levels of the cerebral nervous system (CNS), especially the left cerebral cortex. Consequently, a low production of serotonin and norepinephrine results in the temporal and frontal lobes and in the basal ganglia. This serotonin depletion is

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supported by studies that have demonstrated low concentrations of 5-hydroxyindolacetic acid in CSF (7) and up-regulated PET measured 5-HT2A receptors in patients with PSD compared with non-depressive stroke patients (8).

Another neurotransmitter which CNS concentration is altered in the case of PSD and could play an important role in the pathophysiology of this disorder is glutamate. The glutamate-induced toxicity hypothesis is based on the hyperactivity of the glutamatergic system manifested during the first moments to months after stroke. Paraclinical evidence from studies shows a correlation between the appearance of early-onset PSD and a low plasma glutamate level following acute ischemic stroke (9).

Along with the involvement of the neurotransmitters imbalance at CNS level, there are also systemic theories that emphasize a more complex imbalance, with possible implications for the whole human body, thus considering PSD more as a "system disfunction". Among these theories, we mention neuroinflammation, a frequently encountered theory used to explain many neurological pathologies. This hypothesis is based on both cellular elements such as microglia activation and disturbance of the microenvironment of the neurovascular unit, and non-cellular elements, the heterogeneous group of proinflammatory cytokines (IL-1β, IL-6, IL-18 and TNF-α) playing an essential role in generating and sustaining inflamation. It is known that upregulated activity of proinflammatory cytokines is found both in response to ischemic stroke and in major depression, thus becoming a common element that explains the coexistence of the two conditions. Accordingly, IL-1β has a clearly defined pathologic effect in stroke, different trials assessing that this cytokine increases infarct size, while blocking its activity through administration of recombinant human receptor antagonist has protective effects (10).

Regarding IL-6, there are only a few reports on its modulating functions in experimental stroke conducted mainly on rodent models. IL-6 seems to have neuroprotective functions, by reducing infarct volume (11). A recent meta-analysis was conducted in order to clarify the role of another important cytokine, IL-18, after stroke, author's findings revealing that increased IL-18 level contributed to the development and severity of ischemic stroke, suggesting also the potential of IL-18 as an early biomarker (12).

Regarding depression, a recent meta-analysis reveals the positive association of this disorder with IL-1 and IL-6, both in clinical trials and in community samples. Moreover, time tracking of samples suggests a dose-response relationship between the depressive phenomenon and inflammatory markers, body mass index being a mediating factor in this association (13).

TNF-α, one of the most extensively studied cytokines in human and animal stroke models, seems to have both neurotoxic and neuroprotective impact, demonstrating that the survival of ischemic neurons is modulated by the TNF (14). In support of this theory come studies that confirm the positive association between elevated levels of TNF-α and IL-1 β and the early appearance of PSD (at 2 weeks). Moreover, this association also depends on the genetic polymorphism, Alleles associated with reduced anti-inflammatory cytokine function were associated with PSD (15).

In case of neuroinflammation, the release of cytokines subsequently means the activation of the hypothalamic-pituitary-adrenal (HPA) connection, with the upregulation of corticosteroids production. Elevated cortisol levels have been associated with a more unfavorable prognosis in terms of post-stroke recovery, higher mortality, but also a higher risk of PSD. Glucocorticoids found in high amounts for long periods of time have many side effects on many organs and systems, including the CNS, which cause decreased neurogenesis, neuronal survival and dysfunction of neural circuits, especially in the hippocampus (16). These harmful effects have been highlighted in animal models that simulate pre or post stroke (17). Older studies have already shown an association between HPA axis deficiencies and the serotonergic system that has been shown to be affected in PSD (18). Specifically, it is a bidirectional relationship between the two seemingly unrelated systems, with serotonin binding and transport in the CNS being influenced by HPA, while serotonin transporter polymorphism involves a chronic state of stress, i.e. chronic hyperproduction of cortisol.

Another theory that is gaining more and more popularity is based on brain damage associated with decreased performance of compensatory mechanisms, ultimately translating into decreased neuroplasticity. Multiple evidence has shown a connection between chronic stress in disorders such as PSD and localized brain degeneration, especially in the hippocampus, where at the neuronal level is translated in axonal damage, downregulated dendritogenesis and decreased synaptogenesis (6). At the core of this theory lays the brain-derived neurotrophic factor (BDNF). Also known as abrineurin, BDNF exerts its action to support the viability of existing neurons, and the growth and differentiation of new neurons and synapses in certain specific regions of the brain, such as the hippocampus, the cortex or the basal ganglia. Shortly after stroke, low levels of BDNF (detected in serum, for example) were shown to correlate directly with early-onset PSD, approximately 3 months' post-cerebrovascular event, and indirectly with stroke severity (19). The explanations are incomplete. On the one hand, it is possible that in some patients, the BDNF level before stroke is already low, thus creating a predisposition for a depressive syndrome, and on the other hand, the hypoxic neuronal microenvironment found in cerebral ischemia could cause downregulation of BDNF expression.

Finally, two theories directly related to the ischemic episode and tilting the balance towards cerebrovascular pathology are worth mentioning. We must not forget the vascular factors such as the lesions of the white matter which, by destroying some anatomical tracts or structures with important neurological implications, could support, at least from a structural point of view, the validity of the other hypotheses. Regarding other risk factors such as hypertension, obesity or dyslipidemia, by promoting an initially local chronic inflammatory environment and by partially known and explained mechanisms, they may be able to represent an additional condition for the occurrence of PSD. In the same context, researchers take into account the metabolic-energy

theory, being already known that the CNS is a large energy consumer, with an impressive number of mitochondria, and the sudden decrease in blood flow in ischemia means altered aerobic metabolism and switch to anaerobic, with the detrimental impact of oxidative stress, which according to the latest research, could support the occurrence of depression (20).

Current and future therapeutic approaches based on today's knowledge

Although incomplete, each of the aforementioned hypothesis is a possible therapeutic direction which is worth to be followed when thinking of novel treatments. Approaches have already been attempted in preclinical and clinical studies based on the majority of the stated theories, and the use of drugs and other non-pharmacological therapies with encouraging results in other cases of depression has provided at least a starting point for future studies.

Thus, similar to primary depression and taking into account hypotheses based on the lack of certain neurotransmitters, a first natural step is to replace the missing substance from exogenous sources. This is the explanation for the successful use of selective serotonin reuptake inhibitors (SSRIs) which are the first-line treatment in PSD, although there are no definite data to show better efficacy compared to other drugs used in depression (tricyclic antidepressants). In addition, SSRIs were linked with a tendency of increased hemorrhagic risks, including intracerebral hemorrhage (21). Of course, based on the same clinical-pharmacological replacement principle, other antidepressants have been tried in clinical practice, such as serotonin-norepinephrine reuptake inhibitors (SNRIs) which, theoretically, by improving the level of two different neurotransmitters (serotonin and norepinephrine) should be more effective. However, meta-analyzes show similar results between SNRIs and SSRIs, both in terms of beneficial effects and in relation to their side effects, especially bleeding (22). Another drug, mirtazapine, which modulates the noradrenergic and serotonergic systems, has been effective in preventing and treating PSD after a cure of at least 8 weeks (23), but with the adverse effect of sedation by antagonizing histaminergic H1 receptors.

Among the hypotheses mentioned above and which so far have not been exploited at all in clinical trials, we mention the theory of neuroinflammation, including the cascade of pro-inflammatory cytokines and oxidative stress. Even simple drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), with clinically proven effectiveness in case of various local and general inflammations and depression, have not yet been explored in PSD. From the heterogeneous group of NSAIDs, selective COX-2 NSAIDs should be tried, given that the effects of Cox-2 at CNS level are known. Regarding cytokines, novel therapies such as monoclonal antibodies and cytokine inhibitors could focus selectively on one cytokine, or, with reduced selectivity, inhibit more than one cytokine at a time. Anti-cytokine treatment, especially anti-TNF-alpha drugs, when used as adjunctive therapy combined with SSRIs, is also effective in cases of treatment-resistant depression (24). However, to be effective, anti-cytokine monoclonal antibodies must cross the blood-brain barrier and properly modulate the entire cytokine pathway in order to minimize possible adverse effects, thus, improved pharmaco-engineering is required in order to obtain novel potent drug particles.

When thinking of the HPA axis, although strong preclinical and clinical evidence already exists, no drug has been approved until today that targets specifically this neuroanatomical structure. Moreover, comparative to other hypotheses such as the neuroinflammatory one, where at least in clinical studies, cytokine detection is more routinely used, with cytokines becoming increasingly valuable biomarkers, regarding the HPA alteration, no biological or imagistic test are yet available. One simple explanation could be that not all depressed patients display alterations of the HPA axis, as this fact raises another future therapeutic concern, mainly personalized medicine.

In addition to drugs, non-pharmacological therapies could be used additionally to treat PSD. Among these, the cognitive behavioral therapy (CBT) has been tried in several clinical trials, a recent meta-analysis of 23 studies with 1,972 participants supporting the effectiveness of this procedure, with variations depending on the mean age and gender of the patient, baseline depression score, and elements related to duration, manner and number of CBT administration sessions (25). Alternative and complementary treatment methods should not be neglected, for example acupuncture, which can be an additional way of treatment, along with drug therapy, currently several trials are underway whose results will be later systematized in order to confirm or not the benefit of these therapeutic methods (26).

Finally, control of vascular factors remains important and, although often neglected by both the patient and the physician, relatively simple non-pharmacological methods and drugs exist, both having an acceptable safety and efficacy profile. Observing the lack of international accepted consent on PSD therapies, fundamental and clinical studies are urgently needed in order to explain as completely as possible the other factors related to PSD, such as the roles of mitochondria in the pathophysiology of this disorder.

CONCLUSION

PSD, due to the increased prevalence and the significant impact on the individual's quality of life, remains a topic of great interest for clinicians dealing with rehabilitation after stroke. It is clear that we cannot focus only on motor or language parameters when considering a good recovery of stroke patients, ensuring a life without depression being a goal at least as important. Currently, it is necessary to continue studying in this field, with an emphasis on the most complete explanation of the pathophysiology of PSD. Once the biological mechanisms are understood, it will be possible to create highly effective treatments with much lower side effects compared to those related to currently used medication. Checking the effectiveness of alternative therapies and other non-pharmacological means of treatment can only bring an extra benefit to the patient, increasing the person's quality of life after stroke.

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DEPRESIA DUPA ACCIDENTUL VASCULAR CEREBRAL – PERSPECTIVE ASUPRA MECANISMELOR FIZIOPATOLOGICE

REZUMAT

Depresia dupa accident vascular cerebral este una din cele mai frecvente tulburari aparute la pacientul cu accident vascular cerebral, avand un impact nefavorabil asupra recuperarii si, implicit, asupra calitatii vietii acestuia. Numeroase studii epidemiologice au evidentiat impactul semnificativ al acestui fenomen atat la nivel de individ (aproximativ 1 din 3 pacienti cu accident vascular cerebral va suferi de depresie), cat si la nivel socio-economic. Desi intens studiata, aparitia depresiei doar la o parte dintre persoanele care au avut un accident vascular cerebral in antecedente nu este pe deplin explicata, existand la acest moment mai multe teorii incomplete si posibil interdependente. In acest context, scopul acestei lucrari este de a aduce o noua perspectiva asupra fenomenului depresiei associate accidentului vascular cerebral, autorii prezentand in prima parte cele mai recente rezultate din literature in ce priveste mecanisme fiziopatologice ale depresiei post accident vascular cerebral, iar in partea a doua, mijloacele terapeutice actuale si viitoare sunt detaliate.

Cuvinte cheie: accident vascular cerebral, depresie, fiziopatologie, neuroinflamatie, stres oxidativ

SOLUBILITY, STABILITY AND DEGRADATION KINETICS OF NICOTINAMIDE RIBOSIDE BORATE, A VITAMIN B_3 DERIVATIVE, IN PHYSIOLOGICAL SOLUTIONS

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ABSTRACT
Nicotinamide riboside (NR), a natural nicotinamide adenine dinucleotide (NAD^+) precursor, is a nutrient that has been shown to have significant therapeutic potential due to its activity on sirtuins' signaling pathways. Moreover, esterification of borate with nicotinamide nucleosides could be of physiological significance since the ribose-borate complex released after their hydrolysis could be an important physiological boron reservoir. These are reasons to undergo investigations in order to evaluate the effect of nicotinamide riboside borate (NRB) supplementation in experimental models of various diseases and becomes critical to determine the solubility and stability of the complex NRB in different physiological pH conditions that are found in the human body. Solubility and degradation kinetics of nicotinamide riboside borate (NRB) in the following physiological media, 0.1 N hydrochloric acid (pH 1.5), sodium acetate buffer (pH 5.0), water (pH 7.0) and phosphate-buffered saline (pH 7.4), were investigated using high-performance thin-layer chromatography. Stability studies of NRB were also performed to determine the effect of different pH media and temperature on the degradation kinetics. Solubility and stability of NRB varied significantly in tested biological solutions. Solubility of NRB was pH-dependent and augmented with the increase in pH.
Keywords: Nicotinamide riboside borate, solubility, stability, degradation kinetics.

INTRODUCTION

Vitamins, especially those included in B group, have gained considerable attention due to several benefits over drugs, including a wide range of therapeutic activities, fewer side effects and structural complexity [1]. More than a hundred clinical trials are underway to evaluate the effectiveness of natural drugs. After oral administration, the level of vitamins in plasma depends on the absorption through the gastrointestinal tract (GIT). The stomach and intestines are the main sites for the absorption of a vitamin molecule. Therefore, it becomes critical to determine the solubility and stability of a drug at the different physiological pH conditions that are found in the GIT. Many researches have highlighted the therapeutic effects of a nicotinamide nucleoside, nicotinamide riboside chloride (NRCl), but only a systematic and adequate study evaluates solubility and stability under different pH conditions in environments simulating the physiological conditions of the GIT and blood plasma [2]. Comparatively to this study, we investigate the effect of pH and storage temperature on nicotinamide riboside borate (NRB) stability.

A simple and fast high-performance thin-layer chromatography (HPTLC) method confirms that both NRB and nicotinamide riboside (NR) have an alkaline hydrolytic degradation to nicotinamide (NAM) and ribose–borate complex in simulated GIT solutions. Any formulation should promote the stability of NRB and NR and protect them from hostile environments to prevent the accumulation of a potentially antagonistic degradation product. With the current study, we hope to enrich the NR literature with the data obtained useful for developing and processing a future formulation of the new NR compound stabi-

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lized with borate and not with hydrochloric acid [3–6]. The aim of this study was to characterize NR stabilized with borate *versus* NR regarding the physicochemical properties, including p*Ka*, log*P*, solubility, degradation kinetics, its stability and behavior under thermally and physiologically relevant conditions.

MATERIALS AND METHODS

Chemicals and reagents

NAM was purchased from Sigma-Aldrich (Taufkirchen, Germany), while NR stabilized with dextrin (21.23% content of 93.4% purity) was obtained from BOC Sciences (Shirley, NY, USA) and the boric acid was bought from Merck (Darmstadt, Germany). Hydrochloric acid, formic acid, acetic acid, phosphate-buffered saline (PBS), sodium acetate and ammonium acetate were purchased from Merck. The solvents, namely, ethanol, methanol and water were also bought from Merck.

Analytical method

NR was analyzed in different solutions using HPTLC (CAMAG, Muttenz, Switzerland) and ultra-high performance liquid chromatography (UHPLC, ACQUITY Arc, Waters Corporation, Milford, MA, USA) coupled with tandem quadrupole mass spectrometer (MS, ACQUITY TQD, Waters) [7]. The CAMAG system consists of an application device, Linomat V, capable of applying the samples in bands and a TLC Scanner 3 which was used to obtain the densitograms, both devices being controlled using the visionCATS software package version 3.0.

The samples were applied with the CAMAG Linomat V in 8 mm length bands on HPTLC silica gel 60 F_{254} glass plates (Merck) at 8 mm from the bottom edge. The mobile phase used was ethanol–1M ammonium acetate–formic acid (7:1:0.1, *v/v/v*). The elution was conducted in 20×10 cm glass twin-trough chamber on a 60 mm distance. After elution, the plate was dried with the help of an air drier at room temperature for about 5 minutes. After drying, the plates were documented at 270 nm (the maximum absorbance peak of NR). The densitograms were obtained with the CAMAG TLC Scanner 3 in absorbance mode optimized for resolution, with a scanning speed of 20 mm/s, a slit of 5×0.2 mm and a data resolution of 100 µm/step. The lamps used for detection were deuterium and tungsten. The evaluation of the resulted densitograms were processed using the visionCATS software package version 3.0.

The UHPLC method employed a CORTECS C18 column (4.6×50 mm, 2.7 µm) and the mobile phase consisted of an isocratic elution of methanol–10 mM ammonium acetate (40:60, *v/v*). The flow rate was kept at 1 mL/min, at a column temperature of 60°C [8, 9]. The MS/MS parameters used for NR and NRB were positive mode, collision energy 12 eV and cone voltage 14 eV. The NR sample for the stability testing was obtained by solubilizing in methanol an amount of the dextrin stabilized product to obtain a concentration of 2 mg/mL NR. To compare the stability of NR with the NRB, an additional sample was prepared by adding to the previous NR stock solution an amount of boric acid to obtain a 1 mg/mL concentration. The concentration of the samples for MS/MS analysis was 10 mM.

NRB determination

The coupling of NR with boric acid was investigated by the means of the UHPLC–MS/MS assay (see "Analytical method" subsection).

pH stability testing

Stability studies were performed in 0.1 N hydrochloric acid (pH 1.5), sodium acetate buffer (pH 5.0), water (pH 7.0) and PBS (pH 7.4) containing NR and NRB with the concentration as mentioned in the "Analytical method" subsection (see above). All the solutions were incubated at 37°C for 24 hours after which the HPTLC method was employed to determine the stability of the compounds.

Temperature stability testing

The sample preparation used for the temperature stability testing was described in the "Analytical method" subsection (see above). The temperatures at which the samples were kept for 15 minutes were 30°C, 40°C, 50°C and 60°C. The reference temperature was 20°C. Aliquots were

removed and prepared for the HPTLC analysis.

Statistical analysis

All experiments were performed in triplicate and results are reported as a mean ± standard deviation (SD). The *p*-values less than 0.05 were considered as statistically significant.

RESULTS

NRB synthesis

The synthesis of NRB was performed according to the US Patent [6] and verified *via* the method described in the "Materials and Methods" section. The obtained spectra for both NR and NRB are shown in Figures 1 and 2.

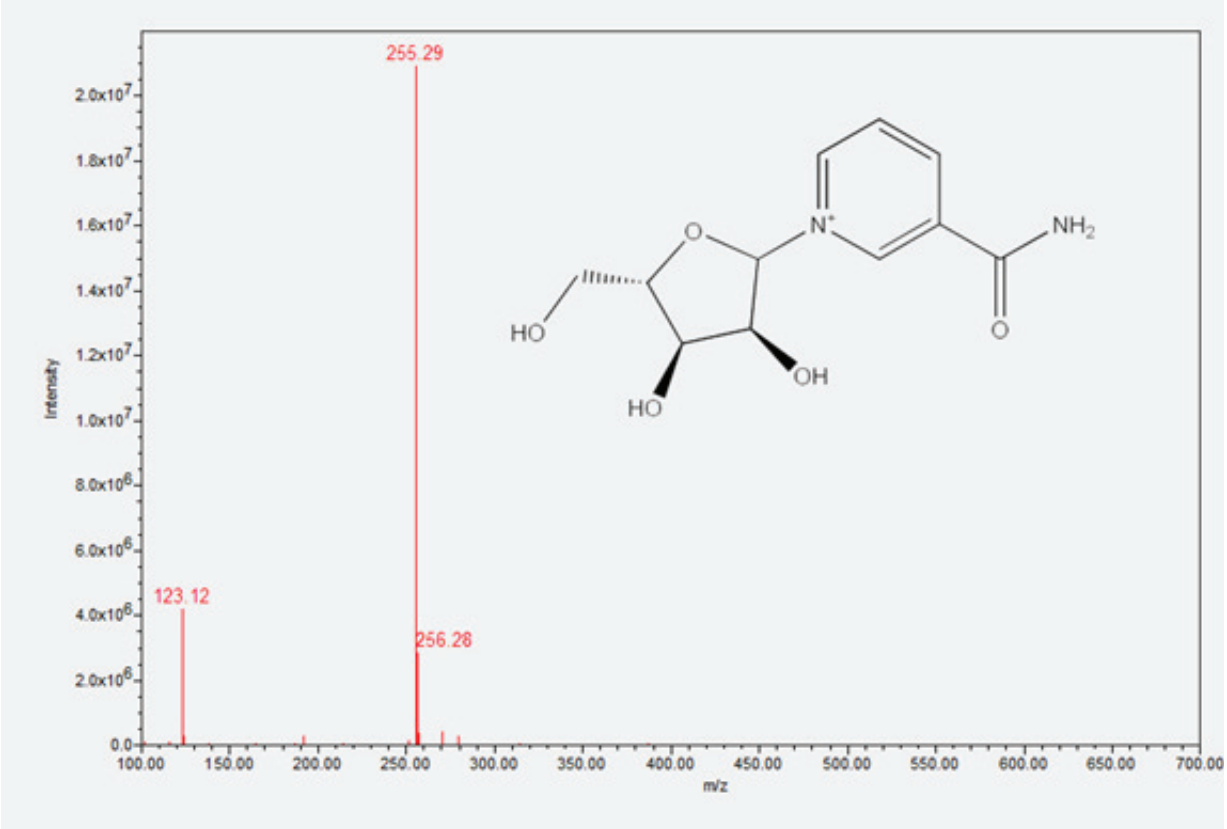

Fig.1. NR mass spectrometry spectrum (NR – *m/z* 255; NAM – *m/z* 123). NAM: Nicotinamide; NR: Nicotinamide riboside

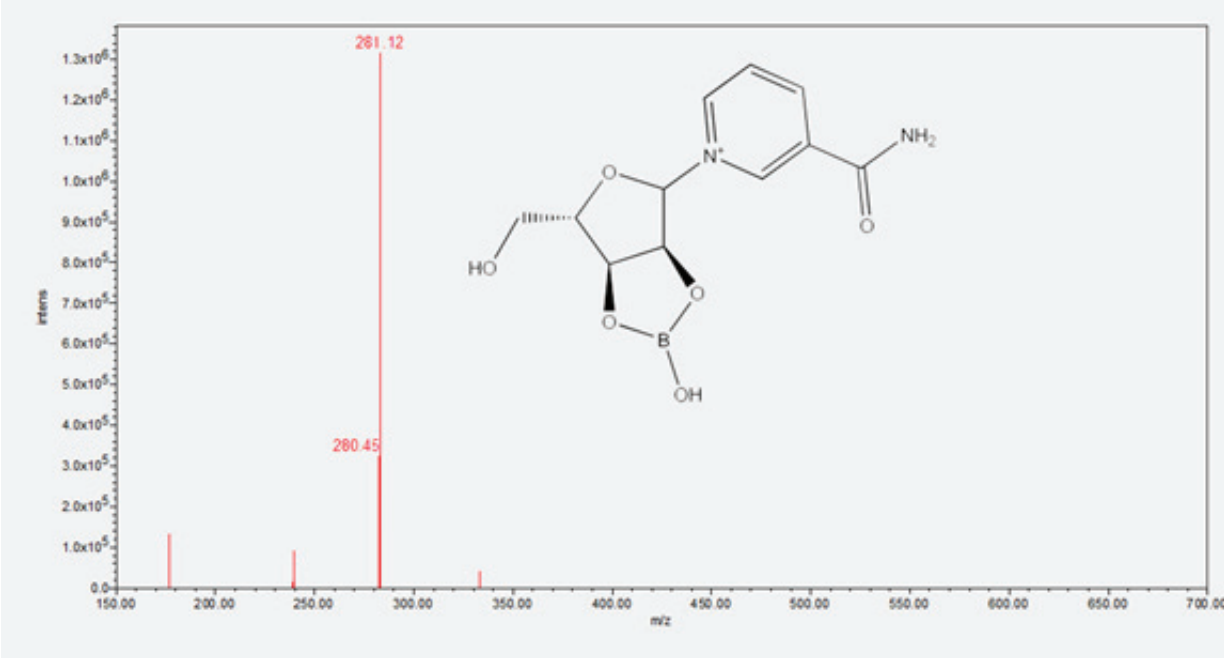

Fig. 2. NRB mass spectrometry spectrum (NRB: ^{11}B – *m/z* 281; ^{10}B – *m/z* 280). NRB: Nicotinamide riboside borate.

pH stability testing

The results regarding the pH stability testing are accurately presented in the graphs (Figures 3 and 4) and Table I from below. The R_F obtained for NAM and NR/NRB during the pH stability test were 0.62±0.01 and 0.255±0.01, respectively. The areas of each peak were integrated using the visionCATS software package version 3.0 and shown in Table I. The graph was computed using the GraphPad Prism 8 software and it shows that the NRB begins to degrade at pH 7.0 (Figure 4).

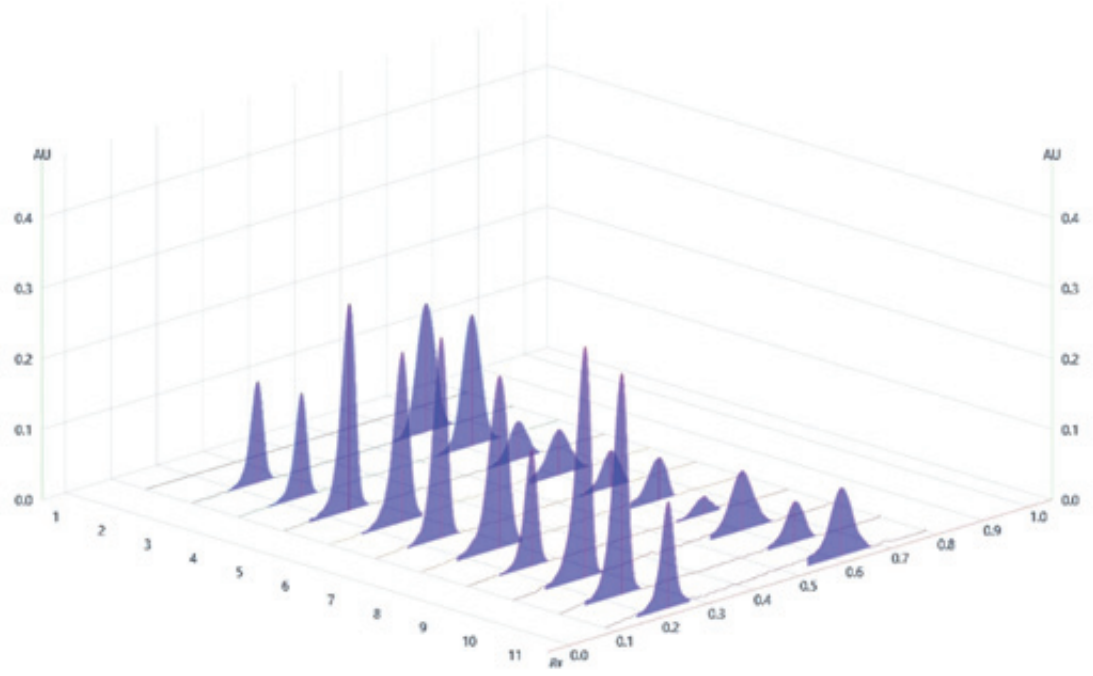

Fig. 3. Overview of pH stability test densitograms..

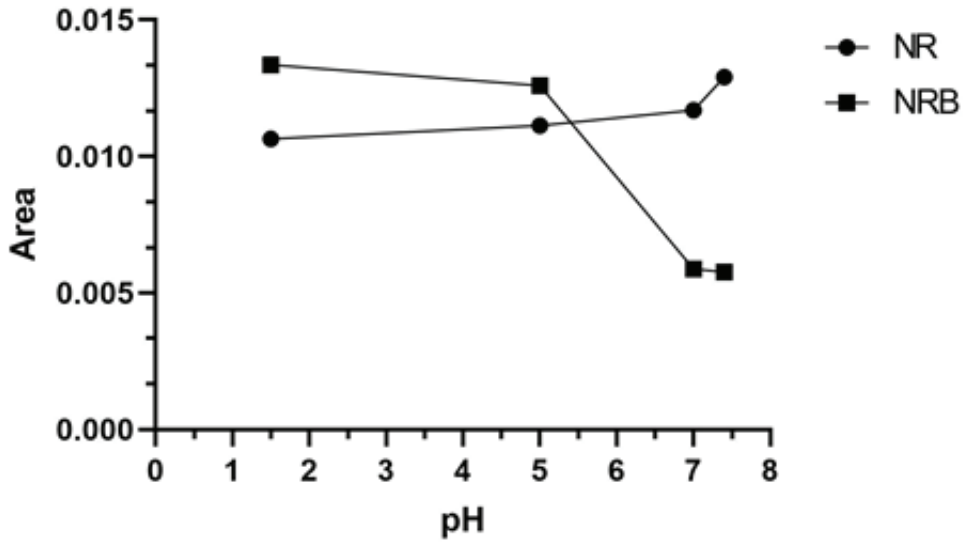

Fig. 4. Graph showing pH stability for both NR and NRB.NR: Nicotinamide riboside; NRB: Nicotinamide riboside borate.

Table 1. Sample areas for the pH stability testing analysis extracted from densitograms.

pH	NR	NRB
1.5	0.01064	0.01335
5.0	0.01113	0.01259
7.0	0.01169	0.00588
7.4	0.01290	0.00577

NR: Nicotinamide riboside; NRB: Nicotinamide riboside borate.

Temperature stability testing

The R_F obtained for NAM and NR/NRB during the temperature test were just slightly different (mainly due to the difference in the laboratory environment temperature and humidity), 0.615±0.01 and 0.250±0.01, respectively (Figure 5).

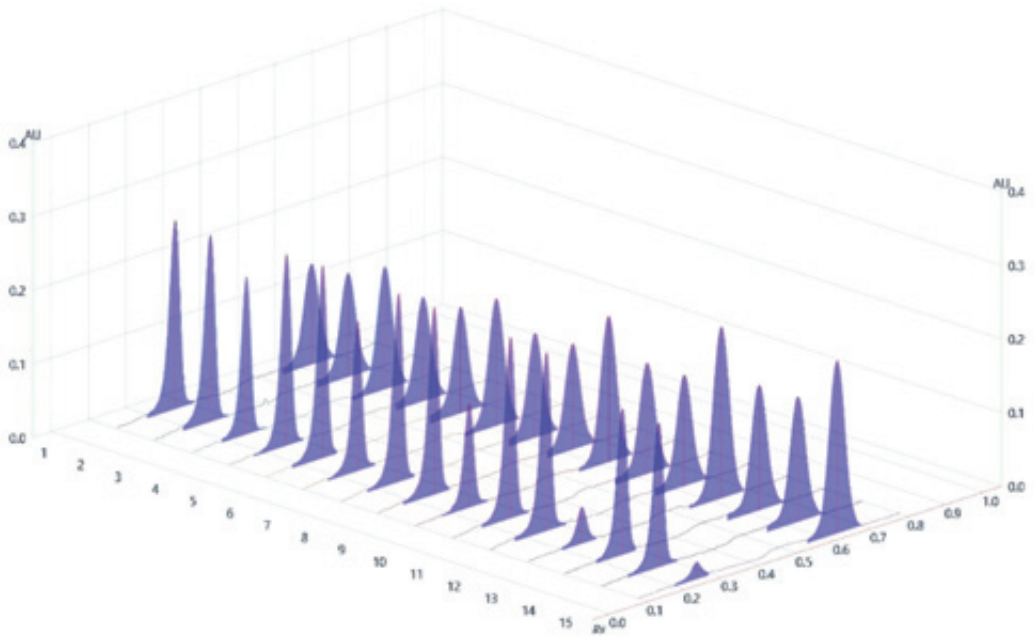

Fig. 5. Overview of temperature stability test densitograms.

Same as before, the areas of each peak were integrated using the visionCATS software package version 3.0 and shown in Table II.

Table 2. Sample areas for the temperature stability testing analysis extracted from densitograms.

Temperature [°C]	NR	NRB
20	0.009475	0.009410
30	0.009501	0.009695
40	0.009504	0.009482
50	0.009112	0.009208
60	0.007205	0.007322

NR: Nicotinamide riboside; NRB: Nicotinamide riboside borate.

The computed graph shows that both NR and NRB begin to degrade starting 50°C (Figure 6).

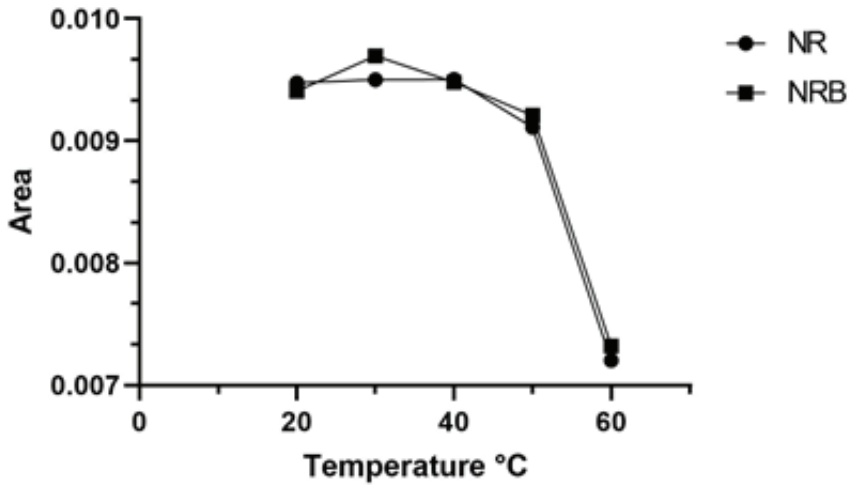

Fig. 6. Graph showing the temperature stability of both NR and NRB. NR: Nicotinamide riboside; NRB: Nicotinamide riboside borate.

Aqueous solubility of NRB

The solubility of NRB was determined by the shake-flask method and Table III summarizes the solubility in different solvents, at room temperature [10]. The solubility of NRB at pH 1.5, 5.0 and 7.4 was measured to be 1972.7±15.4 mg/mL, 1060.5±31.0 mg/mL and 926.0±34.4 mg/mL, respectively. NRB was highly soluble in all three solutions with different pH, taking into account the recommendations of the United States Pharmacopeia [11].

Table 3. Nicotinamide riboside borate solubility in different solutions

pH	Solubility [mg/mL]
1.5	1972.7±15.4
5.0	1060.5±31.0
7.4	926.0±34.4

pKa determination and logP prediction

An isosbestic point is observed at the λ_{max} 270 nm for all tested pH solutions. A predicted log*P* of –6.25 was obtained by MarvinSketch (ChemAxon Ltd., Hungary) inputting the chemical structure of NR, value which suggest that NR is a very hydrophilic compound. The p*Ka* of NR was predicted to be 11.25, using the same software package. The predicted log*P* for NRB was -4.17 and the p*Ka* was 13.509.

Degradation kinetics of NRB

Gibbs free energy (*ΔG*) for comparative degradation was determined to study the spontaneity of NR and NRB degradation in different pH solutions. *ΔG* was calculated using the following equation:

$$\Delta G = -2.303RT(A/A_0),$$

where A and A_0 are the absorbance values of the sample at 0 h and 24 h, respectively.

Table IV and Figure 7 show the values for the pH-dependent NR and NRB solutions under different storage conditions, to deduce spontaneous NRB degradation. The *ΔG* values were -96.46 kcal/mol and 2.43 kcal/mol for NR and NRB, respectively.

Table 4. ODs for storage temperatures

Temperature [°C]	NR	NRB
20	0.961	0.998
30	0.999	0.998
40	1	0.999
50	1	0.997
60	0.989	0.985

NR: Nicotinamide riboside; NRB: Nicotinamide riboside borate; ODs: Optical densities.

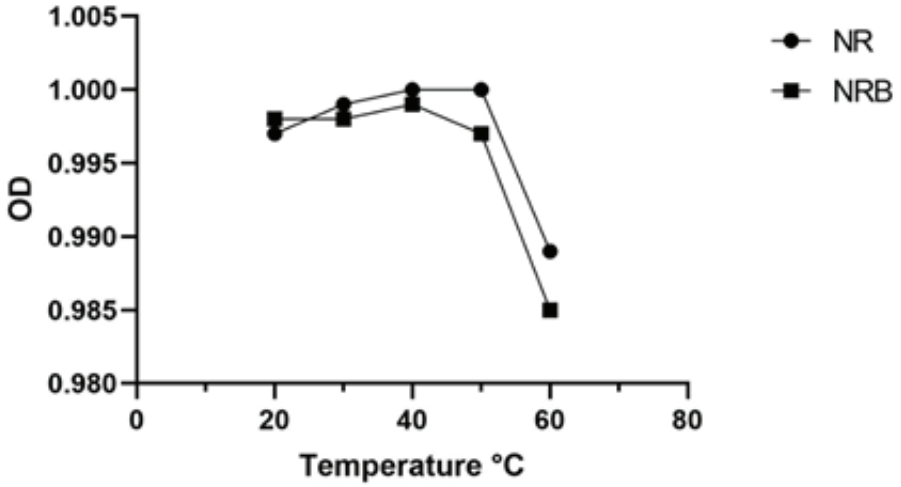

Fig. 7. Graph compiled from the ODs. NR: Nicotinamide riboside; NRB: Nicotinamide riboside borate; OD: Optical density.

DISCUSSIONS

NR is a vitamin B_3 derivative endowed with a significant therapeutic potential and has undergone investigations to assess its benefits as a nutritional supplement. NR enhances NAD^+ biosynthesis and have beneficial effects in multiple disorders [12–15]. Accumulating data has validated its efficiency across numerous animal and human studies for the treatment of cardiovascular, neurodegenerative, metabolic disorders, and retina degeneration [16,17]. NR, like any other nutrient administered to the body, can be exposed to a wide range of pH conditions in various compartments. An intravenous injection of NR would be exposed to pH 7.4 in the blood, not to mention the more complex pH environment along the GIT [2]. When NRB is tested as a nutritional supplement, the production of NAM (an important vitamin involved in the same biochemical processes as NR) and of ribose–borate complex (a sugar–borate that is a physiological boron reservoir) as degradation products it is a significant advantage [6]. However, the release of NAM is not desirable, since NAM may counteract some positive effects attributed to NR and NRB supplementation. NAM and NR are opposite in the regulatory mechanism of sirtuin action *in vitro* and *in vivo* [18]. It is known that NAM acts as a sirtuin inhibitor while NR increases NAD^+ supply for sirtuins actions [18-20]. That's why is of the outmost importance to understand the physicochemical conditions under which NR and NRB are stable. It is known that the presence of the anionic borate can inhibit NR dissolution to NAM and stimulates SIRT.

There are few scientific data that show a minor degradation of NR in simulated gastric fluids but it seems that there is a significant degradation in the intestinal environment due to its high pH [2]. Neutral conditions, such as pH 7.4 used in this study, have a significant effect on NR degradation. In the Arrhenius model of NRB degradation, the rate of NR degradation is higher at pH 7.4 compared to pH 1.5 and pH 5.0 and supports the hypothesis that NRB degradation will be related to the proportion of hydroxyl ions in the solution. Given the fact that it undergoes hydrolysis, care must be taken when one uses an aqueous solution of NRB, especially at high pH values with excessive hydroxyl ions promoting NRB degradation. Based on our results, we presume that there is a slightly different mechanism of NR and NRB degradation in acidic conditions compared to their degradation in neutral/alkaline conditions. So, this model can be used as a general guide for understanding the stability of NRB in various buffered solutions. Temperature also has an effect on NRB content in solution, a 10°C increase approximately doubling its degradation in any pH condition.

logP estimation is important to predict NRB permeability. It is known that the compounds with estimated logP values less than 1.72 have a low permeability [21]. According to the biopharmaceutical classification system (BCS), NRB should be class III due to its high solubility but expected low permeability [22] related to a logP of -4.17. Physiologically, this could be related to the proportion of degradation products released. UHPLC spectra suggested that the degradation products of NRB in aqueous solutions are NAM and ribose-borate complex. When degrades, each molecule of NRB forms a molecule of NAM and one of ribose-borate. For this reason, NAM concentration can be used as a marker for NRB degradation as well as for NRCl

[2], the commercial form existing on the market. HPTLC method developed can separate and quantify NR and NAM peaks with a high separation resolution (R = 1.8) that far exceeds the acceptable resolution (R>1) recommended by the Food and Drug Administration [23]. This method provides rapid detection of NR and its degradation product, NAM, as well as ribose-borate complex.

Our research data provide, for the first time, an explicit upper temperature limit for the processing of NRB and its products, which may have an impact on the production of supplements in several stages. Optimizing the administration of NRB will have a significant impact on its effectiveness. Through this study, we tried to reveal a mechanism by which NRB degradation forms a physiological product when exposed to simulated physiological fluids *in vitro*. Development of the industrial pilot manufacturer NRB will allow further research with a potential high impact on its perspective therapeutic use. Therefore, under this condition, NRB solutions and formulations could be very stable. The results of this study will be helpful for pharmaceutical scientists in handling NRB working solutions and designing appropriate formulations for NRB delivery. Clear pH-dependent NRB solubilization was observed. NRB was found to be extremely unstable at pH greater than 8.0. However, it was metastable in an acidic and neutral environment.

CONCLUSIONS

NRB could be better as a nutritional supplement when compared to NRCl due to the blockade of the glycosidic bond between the pyrimidine base and ribose by boric acid residue. The study revealed the main physicochemical properties to be considered when NRB is used for *in vitro* and *in vivo* experiments. The HPTLC method described could be useful to discriminate between NRB and its degradation products in media with pH conditions similar to those from the GIT. The data obtained are also important for processing, production, and storage of this supplement.

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CINETICA DE SOLUBILITATE, STABILITATE ȘI DEGRADARE A BORATULUI DE NICOTINAMIDĂ RIBOSIDĂ, O DERIVATĂ A VITAMINEI B3, ÎN SOLUȚII FIZIOLOGICE

REZUMAT

Nicotinamid ribozida (NR), precursor natural al nicotinamid adenin dinucleotidei (NAD^+), este un nutrient ce a dovedit un potențial terapeutic important datorită acțiunii sale asupra căilor de semnalizare a sirtuinelor. În plus, esterificarea nicotinamid nucleozidelor cu acid boric poate avea semnificație fiziologică deoarece complexul riboză-borat eliberat după hidroliza acestora ar putea fi un important rezervor fiziologic de bor. Acestea sunt câteva motive pentru studii pe modele experimentale ale unor afecțiuni variate pentru evaluarea efectelor suplimentării cu nicotinamid ribozid borat fiind importantă cunoașterea solubilității și stabilității complexului NRB în condițiile mediilor cu pH fiziologic variat din organismul uman. Au fost investigate solubilitatea și cinetica de degradare a NRB în medii fiziologice diferite, precum acid clorhidric 0.1 N (pH 1.2), tampon acetat (pH 5.0), apă (pH 7.0) și tampon fosfat salin (pH 7.4), prin HPTLC (high-performance thin-layer chromatography-cromatografie în strat subțire de înaltă performanță). Studiile de stabilitate au fost realizate pentru a determina efectul mediilor cu pH diferit și al temperaturii asupra cineticii de degradare a NRB. Solubilitatea și stabilitatea NRB au variat semnificativ în soluțiile biologice testate. Solubilitatea NRB a crescut odată cu creșterea valorilor pH-ului.

Cuvinte cheie: nicotinamid ribozid borat, solubilitate, stabilitate, cinetică de degradare.

CURRENT TRENDS IN THE ASSESSMENT OF SUICIDE ATTEMPTS

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ABSTRACT

Scientific acquisitions in recent decades generate new perspectives in deciphering the suicidal phenomenon, defining it as a multi- and interdisciplinary problem still under the sign of scientific interrogation, despite its efforts and its age.

This research, with a theoretical justification, starts from the hypothesis that suicidal behavior goes beyond pathological conditioning, extending into the individual system of moral values and the personal way of responding to psychostressing factors.

The risk for suicide in the context of somatic diseases is significantly correlated with the presence of a mental disorder, particularly depression, but also involves the personality traits of the patient, his ability to adapt, availability for social support, the presence of psychosocial stressors and history personal suicidal behavior.

From these results it can be concluded that the frequency of suicidal act could be influenced in the sense of reducing it, in the conditions of primary, secondary and tertiary prophylaxis, emphasizing the therapeutic adequacy and socio-familial reintegration measures.

Keywords: suicidal phenomenon, pathological conditioning, moral values, psychostressing factors.

INTRODUCTION

Both the criteria for defining mental illness and the criteria for considering a suicide or attempt differ and show instability of the approach. It is recognized that a fairly small number of suicidal acts is related to clear, obvious mental illness. Otherwise, it is up to the person carrying out the research whether to study a transient reaction, classified it as a disease or as a normal response to special situations. Secondly, difficulties may also arise in the way in which the presence of a mental illness is assessed at the time of committing the suicidal act. On the other hand, data from subjects with suicide attempts cannot be correlated with information obtained in the case of completed suicides, as they represent two different groups.

This research, with a theoretical justification, starts from the assumption that suicidal behavior goes beyond pathological conditioning, extending into the individual system of moral values and the personal way of responding to stressing factors. At the same time, the approach of the ethiopathic study of suicide in depression was motivated by the reality that depressive disorder is the most common psychiatric syndrome found in practice, with heterogeneous and multifactorial etiology and a multitude of clinical types.

Scientific acquisitions in recent decades generate new perspectives in deciphering the suicidal phenomenon, defining it as a multi- and interdisciplinary problem still under the sign of scientific interrogation, despite its efforts and its age.

ETIOPATHOGENIC IPOTESIS IN SUICIDE

There are currently numerous arguments for involvement in the ethiopathogeny of depression and schizophrenia, conditions associated with increased suicide rates, neuroanatomical circuits and neurotransmitter systems.[14]

Neuroimaging studies CT and MRI have shown ventriculomegaly associated with suicides with low L-tryptophan levels and increased cortisolemy (presynaptic serotonin depression). [25]

PET studies in patients with recurrent suicide attempts showed decreased cerebral blood flow and metabolism in the left frontal cortex, in contrast to significant increase in right temporal lobe activity. [29]

SPECT studies have shown in suicide patients a significant increase in the activity of 5-HT2 receptors in the right hemisphere, frontal, parietal and temporal, with a decrease in the number of GABA receptors. [16]

Current trends in the assessment of suicide attempts are based on different neurobiological models, with two variants: high level and low level of risk of death. These studies (Oquendo, 2003) are of particular clinical importance by identifying differentiated neurobiological bases. [21]

In subjects with high-risk suicide attempts, serotonin levels and indirect biochemical indicators for serotonin function show sharp decreases compared to depression levels and low-risk lethal risk attempt.

The differences between the two groups relate to

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the relative hypo functionality of the prefrontal cortex and decreased activity in the anterior cingulate gyrus, significantly correlated with frontal dysfunction, either in the bilateral frontal median gyrus or in the right upper frontal gyrus.

These data allow the assessment of suicidal behavior and its lethal risk independently of impulsiveness. In this context assessment of a suicide attempt will take into account a set of criteria:

- Neuroimaging changes;
- the clinical aspect of the depressive disorder;
- the presence of impulsivity and the planning of the suicidal act;
- hypercortisolemy;
- cognitive deficit.

The association of low dopamine levels and markers in CRL, as well as the history of extrapyramidal phenomena, are additional risk factors for the severity of suicidal act.

From a psychopharmacological perspective the occurrence of extrapyramidal symptomatology in a patient treated with serotonin antidepressants (SSRIs) may be a marker for predicting suicidal risk. The same mechanism can be considered in the case of patients with major psychotic disorders, antipsychotic treatment and extrapyramidal side effects.

Biological-biochemical vulnerability is the background on which autolytic behavior can be carried out, the psychological, cognitive and social components being favorable factors, genuine triggers of suicidal drama.

In the ethiopaty of suicidal behavior we can speak of a socio-genetic vulnerability in which neurobiochemical vulnerability is distinguished. Rice has shown an increased risk for monopolistic affective disease in subjects from families with depression, alcoholism and suicidal or aggressive behavior since 1987.

Extensive studies have evaluated the presynaptic release of monoamines, intrasynaptic or intracellular degradation, and the phenomenon of receptor "binding", the place and role of receptors in the mechanism of action of psychotropic substances.

Serotonin deficiency

Post-mortem studies have objectified the deficiency of the serotonin system by:

- decrease in 5-HT levels and 5-hydroxyindolacetic acid (5-HIAA), predominately in the frontal cortex;
- significant decrease in binding sites for tricyclic and paroxetine antidepressants, marked with radioactive isotopes (binding studies);
- increase in the number of 5-HT2 receptors in the frontal cortex, as an adaptive factor of the post synaptic floor compared to the decrease in presynaptic 5-HT activity;
- modification of pre and postsynaptic 5-HT receptors in the hippocampus and basal ganglia.

These changes may or may not be associated with diagnostic psychopathological disorders at the time of suicide. Postmortem studies take on a special dimension, as argumentative elements, objectives that support or disprove suicide, in unclear cases.

"In vivo" studies show a marked decrease in 5-HIAA concentration in CRL, significantly associated with impulsive-violent suicidal behavior and its recurrence. Dynamic tracking of 5-HIAA levels in the LCR reveals two types of activity:

- Consistently low levels of 5-HIAA – translate a primary presynaptic serotonin deficiency correlated with low L-tryptophan levels and low platelet MAO activity (strong genetic brand);
- Variable levels of 5-HIAA whose decrease anticipates suicidal behavior, being determined by the decrease in the effectiveness of the system of second messengers in the serotonin neurotransmission chain, with inositol-phosphate at the center (Shimon, 1998) [26]

Altering the functioning of the system of second messengers raises the problem of "collapse" of serotonin transmission at the postsynaptic level, by the inability of the receptor system to decode the presynaptic signal.

The decrease in presynaptic serotonin transmission appears to be significantly correlated with violent suicide and antisocial behavior, with a strong genetic determinism:

- positive family history for antisocial behavior, correlated with low levels of 5-HIAA in CRL (Constantino, 1997) [7], faithful witness of aggression and hostility;
- Cloninger noted in alcoholism type I, an impulsive antisocial behavior and a tendency to violent suicide, that the deficit of 5-HT is associated with the decrease in the level of MAO platelet activity and 5-HIAA in the CRL.
- Serotonin central activity is also related to the weak hormonal response to the administration of a serotonin agent - d-phenfluramine (Coccaro,1996) [6]; prolactin secretion does not increase significantly in people with violent self- or heteroagressive violent behavior.

Recurrent aggressive and impulsive behavior is correlated with low values of 5-HIAA in CRL (Asberg, 1986; Virkkonen, 1994) and low platelet levels of paroxetine binding (Coccaro, 1996), and the seasonality of depression correlates with the decrease in tryptophan (Neumeister A,, 1998) [20], with presynaptic serotonin deficiency (Carroll BJ, 2007) [5]

Dopaminergic dysfunction

The objective of reversing the ratio between prolactinic stimulation with d-phenfluramine and 5-HIAA in the LCR brings into question the intrigue of 5-HT and DA activity in a genuine functional balance of the two neurotransmission systems.

This view is supported by research, which has shown in people with behavior/ excitable (impulsively the existence of D4 "variant" receptors D4.7 – compared to the normal D4 receptor D4.4 (present in 66% of subjects).

The D4.7 receptor is much more common in people with ADHD (Attention Deficit Hipereactivity Disorder) syndrome and excitable – impulsive behavior.

The interference of serotonin and dopaminergic systems in suicidal behavior seems all the more evident since, as early as 1988, Lopez Ibor jr. correlated serotonin deficiency with

attention deficit, hyperkinetic syndrome and aggressive and/or suicidal behavior.

Consistent with those supported, depression by DA deficiency (depressive dopaminedependence – Mouret, 1988) [19] is considered to have the most important suicide risk.

Noradrenergic dysfunction:

In severe forms of depression accompanied by suicidal behavior/risk, there is an increased plasma level of NA significantly influenced by electroconvulsive therapy.

It can be assumed that there is a NA/5HT junction, since in major depression, the NA system behaves bimodally: NA deficiency – inhibited depression, NA hyperactivity – depression with aggression, hostility, anxiety and suicidal risk.

The involvement of the noradrenargic system by the NA/5-HT junction in hyperkinetic syndrome and ADHD explains the obvious therapeutic results with amphetamine derivatives in the specific treatment of these syndromes.

Tiihonen – 1997 [27], argues that the seasonality of pathological crime is superposable over that of violent suicide, both manifestations being related to a primary "presynaptic" defect; Bandecchi – 1994 [2], considers that the seasonal peak of pathological crime can also be influenced by the level of DA transmission and testosterone secretion, which significantly raises the rate of the aggressive behavior.

EPIDEMIOLOGICAL DATA

Unlike other medical clinical conditions, estimating the suicide mortality rate is difficult to achieve due to epidemiological entropy factors, among which it is a masked way, suicide is often recorded as an accident that remains etiologically undetermined. In other cases, it cannot be determined whether death is caused by suicide or murder (Cooper, J, , 2006) [10]. Among people whose deaths are recorded as accidental, some had previously suffered from depression, drug or alcohol dependence, thus presenting risk conditions for suicide (Haris, Barraclough,1997) [13]. In England and Wales, official figures depend on verdicts in the courts, with a strict rule that suicide must be proven by evidence, and if there is doubt about the verdict, investigations are resumed. In this situation, the source of error practically depends on the legal criteria. In other countries, less stringent criteria are used.

Any national or international research on the suicide rate raises the issue of the validity of data collected from descriptions, analyzes or even national mortality statistics. The methods of accomplishment and the criteria for declaring suicide vary greatly in different peoples of the globe, with differences in the calculation of mortality, cultural attitudes towards suicide affecting official statistics to such an extent that they become inoperative (Cosman, 1999) [11].

Sex

A number of studies show that men commit suicide 3 times more often than women, and the latter commit about 14 times more suicide attempts. Regarding the comparative incidence by sex of suicide attempts, most authors noted that this ratio is not constant, but variable with age: thus under the age of 20, the ratio is 10: 1, after which it shows a decrease continues, so that in the 41-50s, this ratio reaches 3: 1 (Haw, 2005).

In countries with high suicide rates such as Lithuania, Estonia, Russia, the male / female suicide rate is much higher than average. An exception is China, for which studies have suggested a suicide rate in women 25% higher than in men (O.M.S., 2006). The maximum value in men is after 45 years and in women after 55 years.

In the United States, the suicide rate has risen, especially among men aged 15-24, the third leading cause of death after accidents and homicides, and suicide attempts in this age group range from 1 to 2 million annually. Also, in the last 10 years, suicide has increased by 30% in men between 25 and 34 years old (Sadock and Kaplan, 2007).

There is a common opinion that the increased suicide rate in men is also due to the highly lethal methods used by them: hanging, firearms, precipitation or defenestration from high altitudes, as opposed to overdose intake of psychoactive substances or poisons used by women. (Hawton, 2000). In some European countries and in the USA, the most common method of suicide remains hanging, a trend common to industrialized countries.

In principle, suicide methods in the elderly are much more violent in men than in women. The pattern of increasing suicide rates with aging is similar in both sexes, although the rate is higher in men probably in relation to the frequency of problems related to alcohol or substance abuse, without depressive symptoms.

One can discuss the existence of protective factors present in women, such as pregnancy and the presence of children in the family, but it must also be taken into account that women commit more unsuccessful suicide attempts compared to men (Kutcher and Checil, 2009).

Age

There is unanimous recognition that the suicide rate increases with age. In North America, Western Europe, including the United Kingdom, and in most countries that report validated data, the suicide rate increases with age, but there is a tendency for two peaks: adolescents and young adults.

Mortality declared by suicide before the age of 15 is little recognized. Suicide is the second leading cause of death among young people between the ages of 15 and 19, and is one of the most serious problems facing society today. A number of specific risk factors with a favorable role were identified: family factors (conflicting relationships with the family, family abuse, divided families, parental divorce, death of a close person); school performance (children and adolescents feel pressured by the family to get better results); social stressors (lack of communication, emotional expression and relationship skills); individual factors (emotional, cognitive, behavioral, health); family history of suicidal behavior, mental disorders or alcohol

dependence [1].

The risk of suicide, which accounts for 12% of adolescent mortality, is significantly associated after Sadock and Kaplan with depressive disorders. However, the same authors state that in the last 15 years, both the rate of attempted and completed suicide has decreased in adolescents, apparently in relation to the introduction of antidepressant treatment in the group of selective serotonin reuptake inhibitors, prescribed to adolescents with mood and behavior disorders.

Regarding the differences in the suicide rate according to age, Sadock and Kaplan – 2002 [24], state that the highest rate of 40 per 100,000 inhabitants is in men over 65 years. Older people commit fewer suicide attempts, but have a higher incidence of completed autolytic acts. Although in the USA it represents only 10% of the population, the elderly commit 25% of the total suicides, the rate over 75 years being three times higher than among young people.

The consideration that most suicides in the elderly are "rational, comprehensible" in response to irreversible life situations is not supported by clinical trials, depression being the most important predictor of suicide in old age.

Conwell – 1996 [8], studying the relationship between age and Axis 1 on a sample of 141 complete suicides in people aged 21-92 years, found affective disorders in 71% and 84% of cases in the cohort 75-92 years and 55 respectively -74 years, compared to 30% of the age group 21-34 years.

Major late-onset depression was diagnosed in approximately 60% of suicides in old age, with non-affective psychosis and addiction disorders being rare.

Barraclough – 1971 [3], outlined the symptomatic profile of elderly people with suicidal depression, revealing: insomnia in 90% of cases, weight loss 75%, guilt 50% and hypochondria 50% of cases. Isolated depressive symptoms are risk factors for major depression and should not be considered as normal in the aging process or as a natural consequence of the social and economic difficulties faced by this category of patients.

Many countries have reported a decline in the suicide rate among the elderly in recent years, with the exception of men over the age of 85. The gender ratio in the elderly is 3: 1 for men.

Marital and professional status

The status of unmarried, divorced or widowed is a factor that doubles the risk of suicide, compared to marriage with children.

Professional status is another factor that can influence the risk of suicide, being recognized by many authors the fact that a high professional and social position increases the risk of suicide but also its loss. Unemployed people and the unemployed have a higher suicide rate. Work is generally recognized as a protection factor against suicide. The suicide rate increases during periods of economic recession and decreases during times of war.

Depending on the occupational category, doctors are traditionally considered to have a higher risk of suicide. The conclusion of several studies is that in the US there is an increased suicide rate in doctors of both sexes. Women doctors have a higher risk of suicide than other women, 410/0000 compared to 120/0000 over 25 years.

Recent data from England and Scandinavia have shown that the suicide rate in male physicians is 2 to 3 times higher than in men of the same age in the general population.

Both women and men doctors commit suicide more frequently by overdose and less by firearms than the general population, this being determined by access to drugs and knowledge about their toxicity.

Among doctors, psychiatrists are considered to be at the highest risk, followed by ophthalmologists and anesthetists, but all specialties are vulnerable (Sadock, 2007) [18].

Somatic diseases

Somatic diseases may increase the risk of suicide especially if they are associated with functional and / or cognitive impairment, chronic pain, disfigurement, increased dependence on others, or sensory impairment of sight or hearing; Neurological diseases such as epilepsy, multiple sclerosis, Huntington's chorea or brain or spinal injuries are associated with an increased risk of suicide. Other somatic conditions recognized as being associated with an increased risk of suicide include: AIDS / HIV, neoplasms, peptic ulcer, disseminated lupus erythematous, heart disease, chronic obstructive pulmonary disease (Kutcher and Chehil, 2009) [15].

The risk for suicide in the context of somatic diseases is significantly correlated with the presence of a mental disorder, particularly depression, but also involves the personality traits of the patient, his ability to adapt, availability for social support, the presence of psychosocial stressors and history personal suicidal behavior. In patients with chronic somatic disorders, special importance should be given to depression especially that associated with suicidal ideation; there is a tendency to underdiagnosed depression.

Ways to commit suicide

Hanging is one of the most common forms of suicide worldwide with an increasing incidence over the past 30 years. Studies conducted in England and the United States have shown an increase in this suicidal modality in parallel with the decrease in the number of suicides with firearms.

Only a small proportion, about 10% of hanging suicides occur in controlled areas such as hospitals, prisons or police custody, most of them in the community.

In England, hanging deaths account for about 70% of attempts in this way, and most patients, 80-90%, who arrive at the hospital alive, survive this trial.

Materials used, rope, cables, etc. they are easy to procure, for this reason prevention cannot be focused at this level as in the case of limiting access to firearms or the sale of toxic substances, but on general measures of prophylaxis.

However, in the United States, gun suicide accounts for more than half of all suicides. In 2005, an average of 46

Americans committed suicide with firearms per day (Miller and Hemenway, 2008) [18].

SUICIDAL BEHAVIOR IN PSYCHOLOGICAL DISEASES

Suicide research has been conducted in a variety of ways, the most important methods including studies on the general or clinical population. An example would be the method of psychological autopsy, in which survivors or other people can give information about victims, and medical records are studied in order to establish the mental state.

Of the general population studies conducted in the last 30 years, six provided similar information on Robins suicide - 1960, Dorpat and Ripley - 1960, Rich - 1986, in the United States, Baraclough - 1974 in the United Kingdom, Besskow - 1970 in Sweden, Chynoweth - 1980 in Australia, despite differences in methodology, diagnostic criteria, location of studies and year of completion reached several common conclusions:

- 90% of suicides suffer from a major mental disorder at the time of suicide, and about half had clinically manifest depression and a third were chronic alcohol users.
- Mental disorders such as schizophrenia, organic psychiatric disorders, anxiety disorders and those caused by substance abuse have been rare among suicides.
- Few suicides have been committed by people considered without a mental disorder, suggesting that "rational" suicide is rare.
- Men over 40, divorced make up 2/3 of the number of suicides.
- Psychiatric diagnoses tend to differ depending on age groups, so Dorpat and Ripley - 1960, found schizophrenia as the most common diagnosis under 40 years, alcoholism between 40-60 years and depression over 60 years; Rich - 1986 found substance abuse and antisocial personality disorders predominant under the age of 30, and affective disorders over the age of 30.

The six studies have shown the importance of major mental disorders, but Murphy - 1983, noted that the intervention of other factors is "necessary", the psychiatric diagnosis not being "sufficient" to determine suicide.

Studies on the clinical population performed on groups of inpatients or outpatients have led to different conclusions:

- Black - 1985, Kraft and Babigian - 1976, Martin - 1985, Pokorny - 1964, showed that most suicides among patients with mental illness were committed by those with emotional disorders, schizophrenia or alcoholism.
- Schizophrenia tends to be responsible for most suicides in patient groups, unlike the general population.
- Suicide in the clinical population has a closer distribution by sex, although men predominate.
- The risk of suicide tends to be higher in younger patients compared to the general population.
- After 69 years there are only significant differences in the suicide rate between the two populations studied (Black, 1985).

In a study of 5,412 patients hospitalized Black - 1985, found that 68 people committed suicide immediately after discharge, 38% in the first 6 months, 75% in the first year and 79% within 2 years.

Almost 95% of all people who commit suicide or attempt have a diagnosis of mental disorder. Depressive disorders account for 80% of these, schizophrenia 10%, dementia or delirium 5%. Of all people with psychiatric disorders, 25% are also addicted to alcohol and have 2 diagnoses. People with psychotic depression have the highest risk of suicide. The presence in the personal history of impulsive behavior or acts of violence increases the risk of suicide (Mousavi et al, 2004). Also, previous hospitalizations in psychiatric wards for any psychiatric disorder (Qin et al., 2003)[23].

The risk of suicide in psychiatric patients is estimated by most authors to be 3-12 times higher than in the general population.

The degree of risk is variable depending on age, sex, diagnosis and whether or not they were admitted to a psychiatric hospital unit. The risk for those who were hospitalized, regardless of sex, is estimated in the US as 5 to 10 times higher than in the general population, and for outpatients who have not had any hospitalization in the personal history is only 3- 4 times higher. The highest risk for both sexes is mood disorders, and within them major depression.

A study of 5,000 patients discharged from a psychiatric hospital in Iowa showed that in the first 3 months after discharge, the suicide rate in women was 275 times higher than in the female population in Iowa, and in men 70 times bigger. Multiple studies have shown that one-third or even more of patients with depression commit suicide in the first 6 months after discharge, probably due to a relapse. The most important risk groups for suicide are patients with depressive disorders, schizophrenia and substance abuse. Patients with panic disorder who call the emergency department are also at increased risk (Sadock, 2007).

Suicide in depressive disorders

Depressive disorders are most commonly associated with suicide, although advances in psychopharmacology should have reduced the risk of suicide in depressives. Patients with depression commit suicide more frequently during the onset of the disease, and the higher the risk, the greater the risk factors for suicidal behavior. It is known that the risk of suicide does not decrease with the relief of depression, but on the contrary manifests itself more intensely after the patient has gone through the depressive episode. The patient often requests discharge and conceals his symptoms in order to implement his suicidal plan. Patients with major depression may have different types of self-aggressive behavior: refusal to eat, self-mutilation "symbolic suicide", elaborate suicide, suicidal frenzy (in depressions with marked anxiety), "altruistic" suicide (in which heteroagressive behavior has delusional motivation).

The data presented are arguments for the importance of early diagnosis of depressive disorders and the establishment of antidepressant treatment according to the principle of therapeutic adequacy. To this end, we consider it useful to present information on the epidemiology of depressive disorder, diagnostic criteria and clinical and evolutionary features of clinical forms of depression.

The division of depression into the two categories seems to be dependent on factors of genetic and biological vulnerability. The figures of epidemiological studies have important variations, being also influenced by ethnic, cultural, social components. Psychopharmacogenetic studies have confirmed the risk of dispositional turn when administering psychotropic drugs. This traditional nosographic dichotomy is all the more difficult as the influence of the biological brain factor is dependent on some comorbidity that may be associated with the initial depressive disorder: vascular, metabolic, traumatic and toxic. Depression is estimated in current practice in primary care in 20% of all patients with psychiatric disorders. The latest data suggest that 30% of depressed patients make a dispositional turn at some point in their evolution, becoming bipolar depression.

Ethnic variations can be significantly correlated with genetic differences in different populations, explaining different epidemiological values and indirectly confirming the neurobiological background. Thus, studies performed on the Chinese population estimated prevalence values of 0.4% for bipolar disorder and 1.4% for major unipolar depression. For incidence, rates of 2.3% per year were suggested for unipolar depression, compared to annual incidence rates of 2.5% for manic episode and 10.3% for major depression [9].

Psychotic depression. It occupies an intermediate position in clinical nosography between affective disorders and schizophrenia, the presence and recognition of this entity calling into question the unique psychosis hypothesis. The prevalence of depression with psychotic symptoms is 12.5% - 16% in contrast to the prevalence of schizoaffective disorder, estimated at 0.2% - 0.5%. Epidemiological data show a significant difference between unipolar and bipolar depressive disorder associated with psychotic symptoms and schizoaffective disorder, anticipating different neurobiological patterns for psychotic depression in the spectrum of bipolar or unipolar disorder and depression in the spectrum of schizophrenia.

Approximately 20% of patients with major depressive disorder with psychotic symptoms, 2 years after the initiation of drug treatment with antidepressants show poor quality with persistent depressive symptoms and worsening cognitive dysfunction. After multiple drug interventions, 10% of these patients do not show significant improvements and an unfavorable evolution, with a significant increase in suicide. Incomplete remission of depressive disorders with psychotic symptoms increases the risk of somatic manifestations, obscuring the prognosis (Marinescu D., 2009).

Suicide in bipolar disorder

Bipolar affective disorder is a chronic condition characterized by extreme and severe mood swings. Prospective studies on the natural evolution of bipolar disorder have shown that, most of the time, patients are in the depressive phase (30% - 50%), compared to the manic phase (1% - 10%) or the mixed phase (2% - 6%). .

Patients with bipolar disorder have an increased risk of suicide. it is estimated that 20% - 25% had attempted suicide during their lifetime. The risk for suicide is higher in bipolar II than in bipolar I, in light of the predominance of recurrent severe depression, although the rate of attempts is similar.

In a meta-analysis of English studies of mortality in mental disorders, the average suicide rate in bipolar patients was estimated at 0.40% per year, versus the average of 0.017% in the international population. (Vieta, 2007). This leads to a standardized mortality rate of 22, compared to about 20 in unipolar depression and 8.4 in schizophrenia.

Suicide attempts in patients with bipolar disorder have a high risk of lethality. One in five attempts are complete in bipolar versus one in 10-20 in the general population. Bipolar patients may attempt earlier in a depressive episode than other psychic patients, and those who develop a rapid cycling pattern also have marked depressive symptoms and a very high risk of suicide attempts. Among the phases of bipolar disorder, depressive episodes are burdened with the highest suicidal risk, followed by mixed states and the presence of psychotic states, with manic episodes having the lowest risk.

Suicide in schizophrenia

Bleuler - 1950 considers suicide to be "the worst symptom of schizophrenia"[22]. In contrast to therapeutic advances, suicide remains the leading cause of premature death in patients with schizophrenia. The rate of suicidal behavior remains high with an obvious upward trend. Suicide initially estimated at 7% (Johnston, 1986) then increases to 13% (Caldwell, 1990) and 18% (Baldwin; Sinclair, 2004), while (Meltzer, 1995) reports a 20% attempt rate - 40%.

Harris - 1998, estimates suicide as a mortality rate at 23%, and the main modalities are evaluated by Foster – 1999 [17], differentiated by sex: hanging predominates in men, and intoxication in women.

Also Foster identifies a number of risk factors for patients with schizophrenia: male gender, white race, lack of service, chronic relapses, pre-existing depression, a history of treatment for depression, depression during the disease episode, and recent discharge.

Most patients with suicidal schizophrenia are young. In a many studies, there were no cases of suicide among chronic patients over 40 years of age. The increased suicide rate requires a reassessment of depressive symptoms in schizophrenia. Wiersma, 2000, considering that depression in schizophrenia is of secondary type and describes 4 psychopathological sub models:

a) depression as a reactive symptom to the amelioration of positive psychotic symptoms (awareness of the disease and

implicitly of its severity);

b) depression secondary to loss of communication skills directly proportional to autistic retraction and loss of self-independence;

c) depression as an active and independent psychotic symptom, evolving even after the acute episode;

d) prodromal symptoms such as atypical depression [28].

Elements of neurobiology may partially explain suicidal behavior based on levels of primary vulnerability (biochemical and genetic abnormalities), secondary (related to disease and treatment), and cognitive vulnerability. At the first hospitalization, Haw's meta-analysis - 2005, estimates the suicide risk at 5.6%; moreover, the completed suicide rate increases in direct proportion to the evolution of the condition and the number of readmissions. Negative life events are associated with suicidal behavior in schizophrenia in a proportion of 52% in outpatients and 22% in hospitalized patients. The catamnestic study performed by Siris - 1995, on 30 studies estimates the rate of depression as variable between 7% and 65%, with an average of 25% [4].

The psychopathological inspiration model does not provide sufficient explanations for suicidal behavior. Based on the premise that in about 2/3 of patients with schizophrenia, the prodromal period contains depression, the therapeutic objectives of the acute episode of schizophrenia were reconsidered, improving cognition and preserving affectivity occupying an important place, along with positive symptoms.

Suicide in substance use disorders

The relationship between alcoholism and suicide-depression has been revealed in the literature since 1896 by many authors.

Chronic alcoholism is a predisposing risk factor for suicidal behavior, but must be viewed in relation to a number of other demographic or psychopathological risk factors that modulate its predictive value:

- alteration of family and social relations;
- diminishing work capacity;
- job loss;
- compromising psychophysical health;
- progressive isolation.

According to Sadock and Kaplan, alcohol use and alcohol-related disorders are associated in about 50% of homicide cases and 25% of all suicides (Sadock B.J., Kaplan 2007) [24].

Alcohol-related disorders are found in people of all socio-economic backgrounds. Frequently, alcoholism overlaps with some pre-existing mental or organic disorders (personality disorders, mood disorders, schizophrenia, organic brain disorders).

Their association with somatic and psychopathological disorders induced by alcohol abuse and the coexistence of stressful socio-cultural and situational factors, determines a cumul of risk factors for suicide. Suicidal behaviors are quite common in subjects with excessive alcohol and / or drug use, regardless of gender. Often, however, suicidal acts are performed against the background of psychotic or depressive decompensations (Grecu Gaboş M, 2000) [12].

Suicide in personality disorders

The suicide rate in Borderline Disorder has been compared in multiple studies to that of bipolar disorder or schizophrenia, despite the fact that most patients with borderline disorder live longer than those with psychosis. Many patients after the age of 30 significantly improve their condition and no longer meet the criteria for Borderline Disorder, so 8% or 10% of adolescents or young adults with Borderline Disorder will develop various forms of affective disorder (bipolar II or unipolar depression), almost no one schizophrenia. Factors associated with a poor prognosis, including suicide, include incest, parental cruelty, comorbidity with antisocial disorder, and marked impulsivity (Grecu Gaboş M, 2000 [12]). Those who continue to abuse alcohol have a suicide rate of 37%. The average suicide rate was 10% and was usually accompanied by substance abuse. Long-term studies have had encouraging results compared to short-term studies suggesting that there are no major differences between patients with borderline disorder and schizophrenics.

We used the method of retrospective statistical study, analyzing cases of suicide attempt with lethal end, admitted to the Craiova Psychiatric Clinic between 2019-2020 and then recorded as completed suicide at IFM Craiova.

A lot B = 58 cases was selected from all admissions, the inclusion criteria being:

- The presence of a suicide attempt as the main reason for the admission recorded in the observation sheet;
- Completeness of the data in the observation sheets on the items studied.

It should be noted that the suicide attempt is not codified in the ICD-10 classification system used in psychiatry as a diagnosis in its own right, which has made our research more laborious, since the attempt as such was mentioned only in the referral diagnosis.

B Group Distribution of group B by sex

Table I. Distribution of group B by sex

Men		Women		Total	
Abs.	%	Abs.	%	Abs.	%
20	34.5	38	65.5	58	(100)

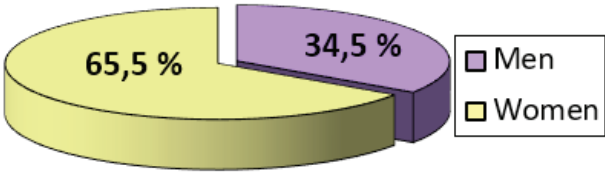

Fig. 1. Distribution of group B by sex. In accordance with the data from the specialized literature, there is an increased percentage (almost double) for females, which can be correlated with the existential situation, as well as the status of "inferiority" attributed to this sex. Also, the suicide rate in women corresponds to the number of suicide attempts - much higher than the completed suicide (Table I, Fig. 1).

Sex and age at the time of the suicide

Table II. Distribution of group B by sex, by age at the time of suicide. Relative values were calculated for the whole batch

Sex / Age	Men		Women		Total	
	Abs.	%	Abs.	%	Abs.	%
Under 30	4	6,9	25	43,1	**29**	**50,0**
30-45	6	10,3	6	10,3	**12**	**20,6**
Over 45	10	17,3	7	12,1	**17**	**29,4**
Total	**20**	**34,5**	**38**	**65,5**	**58**	**(100)**

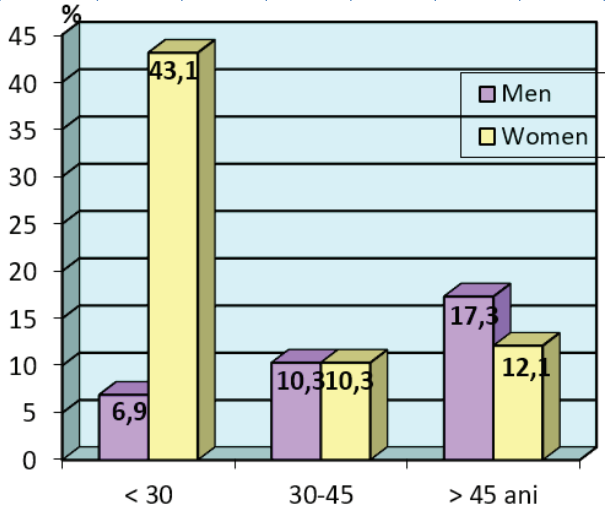

Fig. 2. Distribution of group B by sex, by age at the time of suicide The results obtained reveal - worryingly, the fact that half of the suicides belong to the age group under 30, with a net predominance of females (Table II, Fig. 2). In males, a progressive increase is observed with age, which could be correlated both with the increase of family / social responsibilities and with alcohol consumption.

Sex and profession

Table III. Distribution of group B by sex, by profession
Relative values were calculated for the whole batch.

Sex / Profession	Men		Women		Total	
	Abs.	%	Abs.	%	Abs.	%
Workers	2	3,4	11	19,0	**13**	**22,4**
Technicians, students, sub-engineers	4	6,9	7	12,1	**11**	**19,0**
Intellectual	2	3,4	2	3,4	**4**	**6,8**
Without	8	13,9	15	25,8	**23**	**39,7**
Pensioners	4	6,9	3	5,2	**7**	**12,1**
Total	**20**	**34,5**	**38**	**65,5**	**58**	**(100)**

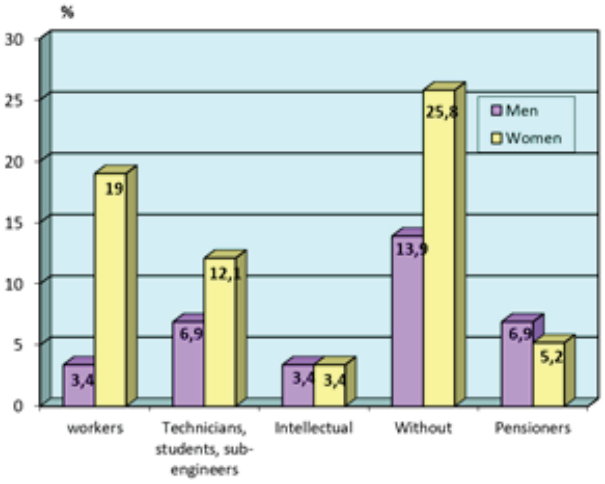

Fig. 3. Distribution of group B by sex, by profession The fact that suicide is much more common in the "disadvantaged" categories (workers, farmers or unemployed) is consistent with the socio-cultural level, but not with the moral-religious. Intellectuals have a low rate of suicide in terms of depressive disorder, the distribution by sex being equal, which cannot be said in the other categories where females have "supremacy" (Table III, Fig. 3).

Sex and marital status

Table IV. Distribution of group B by sex, by marital status.
Relative values were calculated for the whole batch

Sex Marital status	Men		Women		Total	
	Abs.	%	Abs.	%	Abs.	%
Married	6	10,4	11	19,0	17	29,4
Unmarried	3	5,2	2	3,4	5	8,6
Divorced	9	15,5	20	34,5	29	50,0
Widower	2	3,4	5	8,6	7	12,0
Total	20	34,5	38	65,5	58	(100)

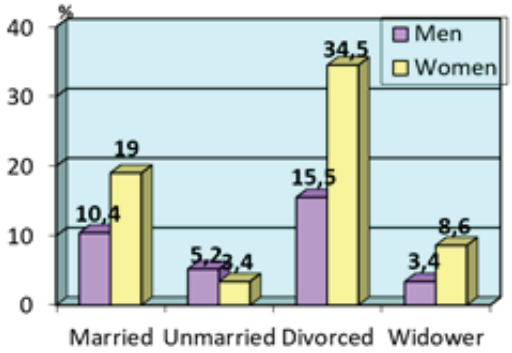

Fig. 4. Distribution of group B by sex, by marital status. The status of divorced obviously predominates, being explicable by sudden changes from a socio-economic point of view - which justifies the much higher percentage of females. Interesting, and even contrary to the above, is the proportion of married people, marital incidents seem to play a decisive role. In these two categories, the percentage distribution by sex is almost unequal, which cannot be stated in the case of widowhood status where the ratio in favor of the female sex is reversed (Table IV, Fig. 4).

Sex and hereditary history of mental illness or alcoholism

Table V. Distribution of group B by sex, according to heredocolateral antecedents for mental illness or alcoholism. Relative values were calculated for the whole batch

Sex AHC	Men		Women		Total	
	Abs.	%	Abs.	%	Abs.	%
Positive	14	24,1	24	41,4	38	65,5
Negative	6	10,4	14	24,1	20	34,5
Total	20	34,5	38	65,5	58	(100)

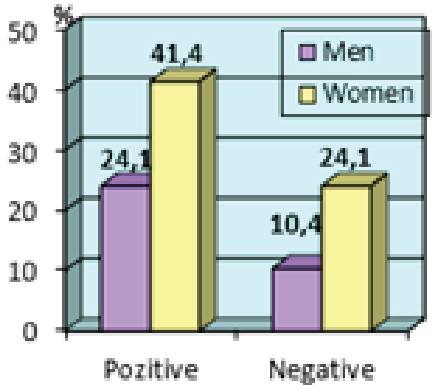

Fig. 5. Distribution of group B by sex, according to heredocolateral antecedents for mental illness or alcoholism. Although the predominance of heredocolateral antecedents for mental illness and alcoholism is obvious - 2/3 of the cases, the correlation of the data obtained by one's own study may not be relevant due to the difficulties in obtaining anamnestic data. However, the results obtained are consistent with those in the literature (Table V, Fig. 5).

Sex and suicide

Table VI. Distribution of group B by sex, by suicide. Relative values were calculated separately by sex.

Sex Method	Men		Women		Total	
	Abs.	%	Abs.	%	Abs.	%
Hanging	14	70,0	12	31,6	26	44,8
Defenestration	4	20,0	9	23,7	13	22,4
Drug ingestion	1	5,0	14	36,8	15	25,9
Other methods	1	5,0	3	7,9	4	6,9
Total	20	34,5	38	65,5	58	(100)

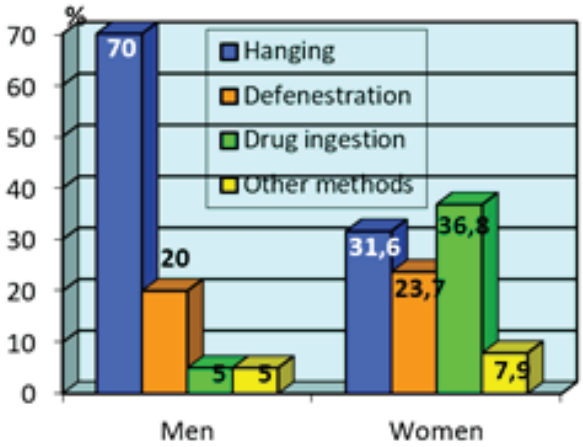

Fig. 6. Distribution by sex, by suicide. There is an obvious discrepancy between suicidal methods regarding the sex of the suicide bomber. Thus, hanging is the method of choice for males, while in women drug ingestion predominates (compared to males) - Table VI, Fig. 6. With the exception of drug ingestion and other methods - which may be the result of "unexpected" accidents by the patient, it should be noted that unlike suicide attempts, completed suicide occurs through brutal methods that do not give a chance of survival. This reflects the intensity of the desire for death and, implicitly, of depression.

Sex and onset-suicide interval

Table VII. Distribution of group B by sex, after the onset-suicide interval. Relative values were calculated for the whole batch

Sex Suicide-Debut	Men		Women		Total	
	Abs.	%	Abs.	%	Abs.	%
Under 1 year	1	1,7	---	---	**1**	**1,7**
1-3 years	2	3,4	3	5,2	**5**	**8,6**
4-10 years	12	20,7	13	22,4	**25**	**43,1**
Over 10 years	5	8,7	22	37,9	**27**	**36,6**
Total	**20**	**34,5**	**38**	**65,5**	**58**	**(100)**

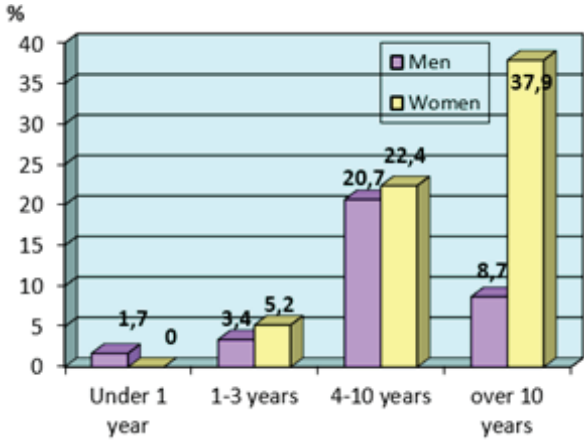

Fig. 7. Distribution by sex, after the onset-suicide interval. The research shows the increased frequency of suicide between 4-10 years and over, as well as the impressive share of suicide in women after 10 years from the onset of the disease (Table VII, Fig. 7). The fact that some men commit suicide less than 1 year after onset can be explained by alcohol consumption or impulsive behavior. It should also be noted that between 4-10 years the distribution of suicide by sex is almost equal, which may raise the issue of inadequacy or therapeutic compliance.

CONCLUSIONS

1. Following the study, the following risk factors (prediction) for suicide in people with depressive syndromes were highlighted:
• Female sex;
• Age under 45 years;
• Acute onset of the disease;
• Low level of training;
• Divorced or married people with marital problems;
• Positive hereditary collateral history of mental illness and alcoholism.

2. Regarding the suicidal modality, the share of suicide by hanging in men and drug ingestion in women was noted.

3. The interval between the onset of the mental illness and the commission of the suicidal act clearly reveals the increase of the suicide frequency after at least 4 years of evolution.

From these results it can be concluded that the frequency of suicidal act could be influenced in the sense of reducing it, in the conditions of primary, secondary and tertiary prophylaxis, emphasizing the therapeutic adequacy and socio-familial reintegration measures.

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TENDINŢE ACTUALE ÎN EVALUAREA TENTATIVELOR DE SUICID

REZUMAT

Achiziţiile ştiinţifice din ultimele decenii generează noi perspective în descifrarea fenomenului suicidar, definindu-l ca o problemă multi- şi interdisciplinară rămasă încă sub semnul interogaţiei ştiinţifice, în ciuda eforturilor depuse şi a vechimii sale.

Cercetarea de faţă, cu o bază teoretică justificatoare, porneşte de la ipoteza că un comportament suicidar depăşeşte condiţionarea patologică, extinzându-se în sistemul individual de valori morale şi la modalitatea personală de răspuns la factori psihostresanţi.

Vulnerabilitatea biologic-biochimică constituie fundalul pe care se poate derula comportamentul autolitic, componentele psihologică, cognitivă şi socială fiind factori favorizanţi, veritabili triggeri ai dramei suicidare.

Riscul pentru suicid în contextul bolilor somatice este corelat în mod semnificativ cu prezenţa unei tulburări psihice, în mod particular a depresiei, dar sunt implicate şi trăsăturile de personalitate ale bolnavului, capacitatea sa de adaptare, disponibilitatea pentru suport social, prezenţa stresorilor psihosociali ca şi antecedentele personale de comportament suicidar.

Din aceste rezultate se poate conchide că frecvenţa actului suicidar ar putea fi influenţată în sensul reducerii acestuia, în condiţiile unei profilaxii primare, secundare şi terţiare, punând accentul pe adecvanţa terapeutică şi măsurile de reintegrare socio-familială..

Cuvinte cheie: fenomen suicidar, condiţionarea patologică, valori morale, factori psihostresanţi

PROGNOSTIC FACTORS IN ISCHEMIC STROKE

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ABSTRACT
Stroke represents one of the most important public health problems nowadays as it is a major cause of both mortality and morbidity worldwide, but especially in highly developed countries[1-2, 6-12]. The study is a retrospective one, carried out on a group of patients with ischemic stroke admitted to the Neurology Clinic of the Clinical Neuropsychiatry Hospital in Craiova from January 1, 2020 to June 30, 2020.Prognostic factors for patients with ischemic stroke are the age of the patient, the severity of the infarction, the mechanism of infarction production, related comorbidities, complications of ischemic stroke, which currently contribute to the morbidity and mortality of these patients.[1-4]
Keywords: ischemic stroke, prognostic factors, hypertension, atrial fibrillation, diabetes mellitus

Introduction

Stroke represents one of the most important public health problems nowadays as it is a major cause of both mortality and morbidity worldwide, but especially in highly developed countries [1-4].All over the world it is expected that by 2030 the number of patients with stroke will reach 23 million.[1-6] According to both the European Commission and the Organization for Economic Development and Cooperation (OCED), Romania is on the 10th place in the world in terms of stroke incidence and on the second position in what mortality caused by stroke is concerned [1-6,37,48].Studies on ischemic stroke, performed on animal models, highlight the importance of the proinflammatory cascade, the free radicals in generating ischemic stroke [1-6].Improving the management of risk factors is essential in decreasing mortality in patients presenting stroke [2]. The presence of extracranial cerebral atherosclerosis, emphasized on Doppler ultrasonography, affects the functional outcome and represents a risk factor for ischemic stroke [3]. The risk factors for stroke also include higher blood glucose levels whichis linked to a worse clinical outcome and early brain edema, however, pathophysiological pathways are not yet fully understood [4]. Moreover, the presence of cardiac comorbidities influences the incidence and the prevalence of stroke. Compared with patients that do not present cardiac comorbidities, patients with hypertension, coronary heart disease and atrial fibrillation associate a higher stroke risk (hypertension alone doubles the risk for stroke). Comorbidities such as coronary heart disease, cardiac failure, atrial fibrillation doubled the stroke risk in men and trebled the risk in women. The elderly represent a vulnerable category to stroke when atrial fibrillation is also present [5]. Stroke is one of the major health problems our society is facing today.

1. Experimental part

The purpose of this work is to analyse the clinical, biological and imaging parameters of a group of 200 patients diagnosed with ischemic stroke and furthermore to identify the prognostic factors associated with this pathology.

Materials and methods

The study is a retrospective one, carried out on a group of patients with ischemic stroke admitted to the Neurology Clinic of the Clinical Neuropsychiatry Hospital in Craiova from January 1, 2020 to June 30, 2020.

Relevant data have been considered for the diagnosis resulting from the anamnesis, clinical exam, neurological examination, biological characteristics, but also imaging features. The parameters resulting from the anamnesis refer to: age, gender, main diagnosis, secondary diagnoses, reasons for hospitalization, personal pathological history, associated risk factors.

During the clinical examination the following parameters

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were noted: particular attitudes observed through inspection, involuntary movements taken into consideration in terms of rhythmicity, recurrence, frequency, speed and amplitude, the presence or absence of coordination and balance disorders. There has been an assessment of: the expressive, spontaneous, repetitive, automatic speech, the name of the colours of the objects, the shapes and the images, but also the expressive speech was evaluated. Speech disorders have been observed, such as dysarthria, aphasia.

From a paraclinic point of view, there has been monitoring of the values of creatinine (mg/dl), blood glucose (mg/dl) and cholesterol (mg/dl). The computed tomography scan of the brain highlighted: the presence or absence of tomodensitometric changes in favour of certain recent or sequellar vascular lesions, craniocerebral traumas, intracranial haemorrhages, aneurysms, hematomas, intracranial tumours, the affected vascular territory, the presence of sequelae.

2. Results

In a first stage we followed the distribution of patients with ischemic stroke according to age and we noted that the highest frequency of ischemic stroke occurs in the age group between 70 and 95 years (119 patients representing 59.5% of the total number of patients under study), followed by the age group of 60-69 years (59 patients namely 29.5% of the total number of patients).

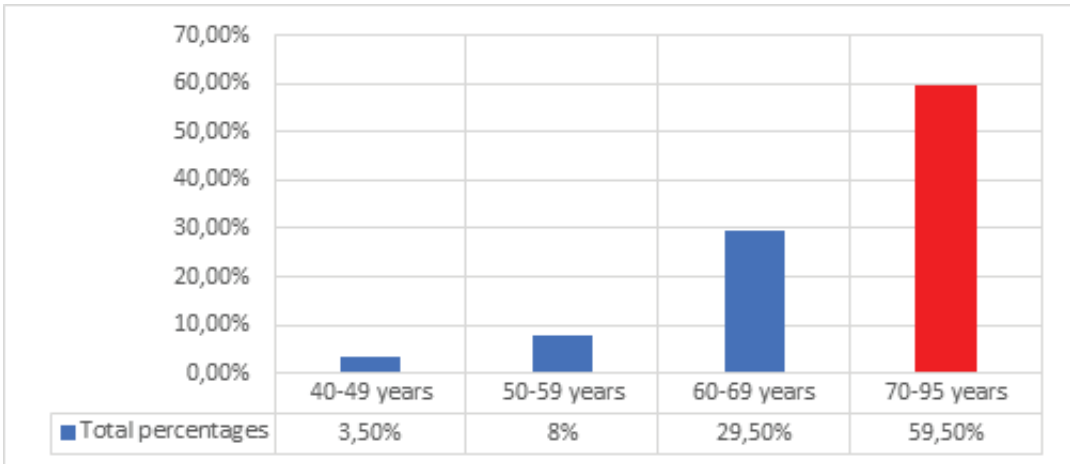

Fig 1. The percentage and graphical distribution of patients according to age

With regard to the distribution of patients by gender, there has been identified an approximately equal prevalence of ischemic stroke for both genders, respectively 50%.

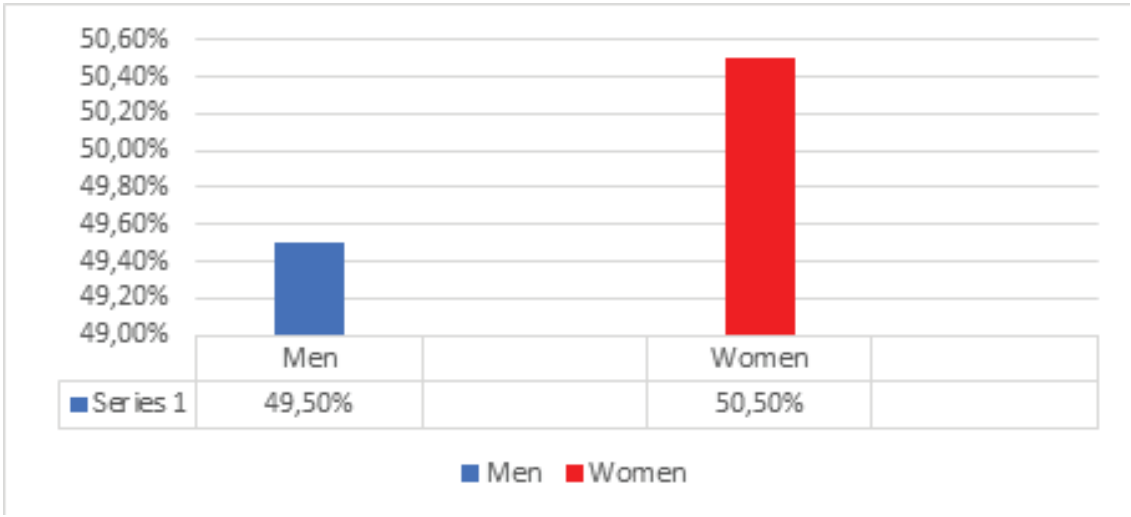

Fig 2. Graphical and percentage distribution of patients according to gender

A.The distribution of the group of patients according to the reasons for hospitalization

A.I. The distribution of patients according to the presence of hemiparesis / monoparesis

The highest incidence corresponds to left-sided hemiparesis, respectively 24.5% (49 patients), followed by right-sided hemiparesis with a percentage of 22.5% (45 patients). All the other types of hemiparesis had similar percentages: right-sidedpredominantly brachial hemiparesis 5.5% - 11 patients, right-sidedpredominantly crural hemiparesis 1.5% -3 patients, left-sidedpredominantly brachial hemiparesis 3.5% -7 patients, left-sided predominantly crural hemiparesis 1% - 2 patients, left-sided predominantly brachial monoparesis 1.5% - 3 patients.

A.II. The distribution of patients according to the presence of hemiplegia / monoplegia

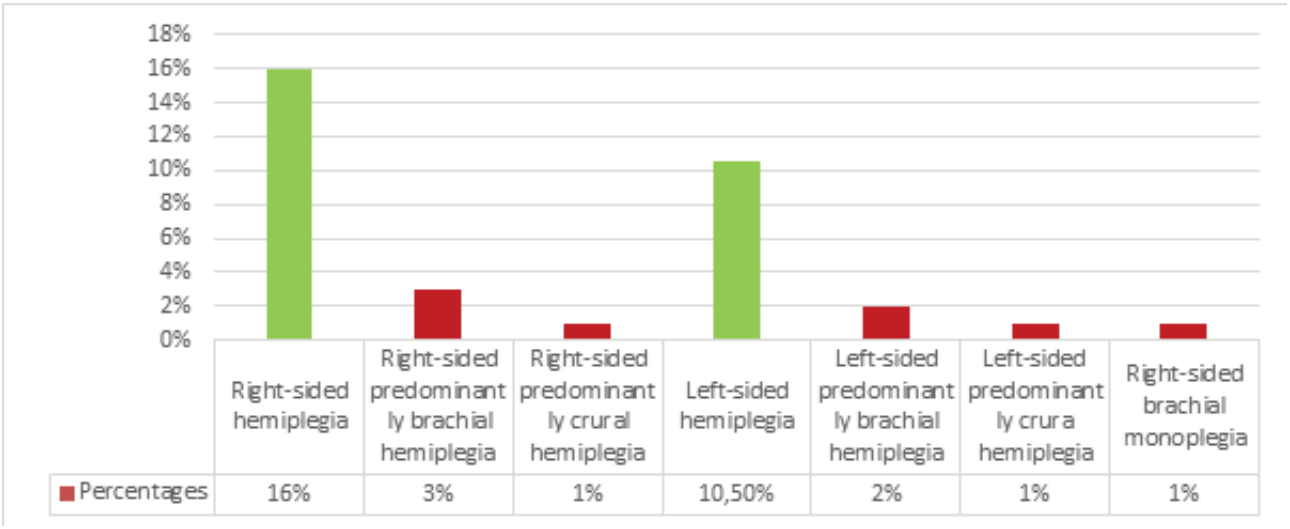

Fig 3. The graphical and percentage representation of patients with hemiplegia / monoplegia

The incidence of right-sided / left-sided hemiplegia is quite high as shown in the figure 3. The right-sided hemiplegia was detected in a number of 32 patients and the left-sided hemiplegia in 21 patients. The rest of plegia deficits were found in approximately equal percentages.

B.The distribution of the group of patients considering the presence of speech disorder

Of the 200 patients assessed, almost half of them, more precisely 42% of the patients presented dysarthria. A percentage of 31% (61 patients) presented mixed aphasia, 8% (15 patients) presentedmixed predominantly motor aphasia and 2% mixed predominantly sensitive aphasia.

C. The distribution of patients with ischemic stroke considering the results of the neurological examination.

The prevailing elements of the clinical picture in the studied group were the pyramidal syndrome with a percentage of 60% of entire group and speech disorders with a percentage of 79.5%.

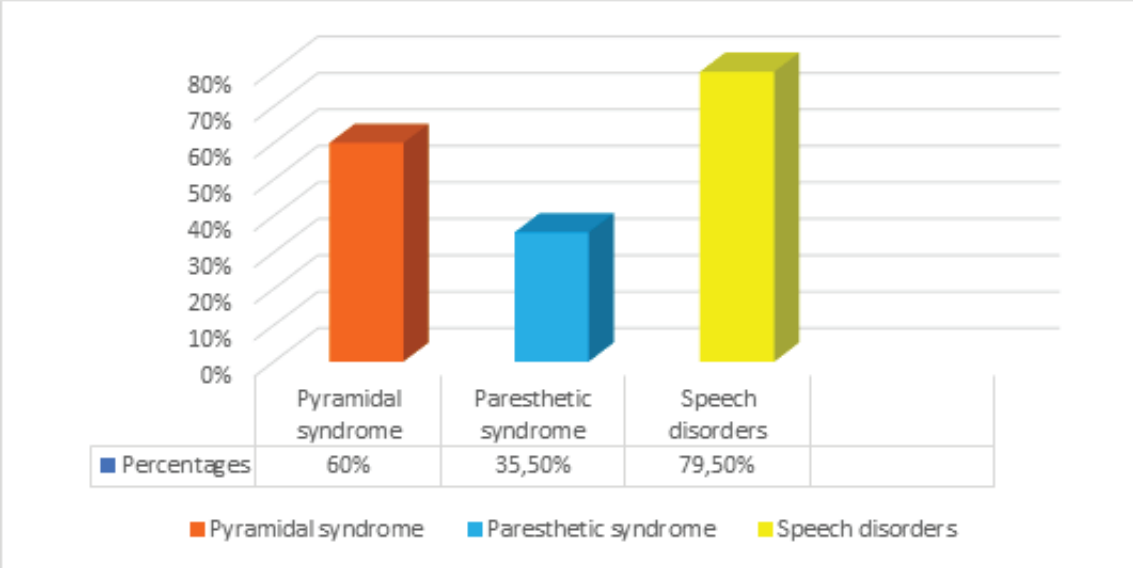

Fig 4. The graphical and percentage representation of the symptoms evaluated following the neurological examination.

D. The distribution of patients with HBPconsideringthe degree of hypertension
Of the total number of patients diagnosed with high blood pressure (170 patients), most of them presentedgrade IIhypertension, respectively 69.5%. A percentage of 11% presented grade I hypertension and 4.5% grade III hypertension.

E. The distribution of patients with stroke depending on the presence of atrial fibrillation in their history was also discussed, atrial fibrillation being a high risk factor, but at the same time a prognostic factor in ischemic stroke. Randomized studies have shown that antiplatelet medication reduced the incidence of stroke in patients with nonvalvular AF. In the study group, we encountered a significant percentage of 29.5% of patients presenting atrial fibrillation, which certifies once more the implications of this pathology and of cardiovascular pathology in stroke ethiopathogeny.

F. The distribution of patients with stroke considering the presence of diabetes mellitus in their history
Of the group of 200 patients with ischemic stroke, a percentage of 18%, respectively 35 patients had type II diabetes mellitus in their medical history and 34%, respectively, 68 patients had a blood glucose level> 126 mg/dl. Patients with diabetes mellitus have a higher susceptibility to arteriosclerosis, prospective epidemiological studies confirming the increased risk of ischemic stroke in patients with diabetes.

G.The distribution of patients with ischemic stroke considering the blood glucose levels
A significant percentage of 33.5% was represented by patients registering a blood glucose level above 126 mg/dl (67 patients). Values of blood glucose ranging between 110.1-126 mg/dl were found in 21.5% of patients, the remaining 42.5% being represented by patients with normal blood glucose levels: 70-110mg/dl (85 patients).

H. The distribution of patients with diabetes mellitus considering the average value of glycosylated haemoglobin
Of 35 patients with type II diabetes, we analysed the glycosylated haemoglobin in 17 of them, resulting in an average value of 8.7%. This increased value is associated with an unfavourable prognosis for the evolution of a patient with ischemic stroke.

I.The distribution of the group of patients considering the cholesterol values Given the fact that the values of serum cholesterol have a significant impact on the pathogenesis of cerebrovascular diseases, we proceeded to measure it in the studied group. Therefore, we identified119 patients (59.5%) with cholesterol values below 200mg/dl, 23.5% of patients with cholesterol values ranging between 201-250 mg/dl and 15% of patients with the value of cholesterol above 250 mg/dl.

J. The distribution of patients with chronic kidney disease
We have also discussed the presence of chronic kidney disease in the group of patients included in the study. Of the 200 patients diagnosed with ischemic stroke, 18 patients (9%) also suffered from chronic kidney disease. Chronic kidney disease is a risk factor for the onset of ischemic stroke, but also a negative prognostic factor for the evolution of a patient who suffered an ischemic stroke. By affecting the kidney function, the glomerular filtration rate decreases, the decrease of excretion being accompanied by the accumulation in the body of toxic or metabolic compounds and thus the appearance of complications: hypertension, anaemia, sodium and water retention, nitrogen retention, hyperlipoproteinemia, acidosis.

K.The distribution of the group of patients consideringthe presence of pneumonia as a prognostic factor
Of the group of patients included in the study, 8.5% (17 patients) suffered from bacterial pneumonia. Bacterial pneumonia is one of the most significant complications in patients undergoing a stroke and it is mainly caused by aspiration. Aspiration occurs most frequently in patients with swallowing disorders and those with impaired consciousness.

L. Other associated pathologies that represent a negative prognostic factor in the patient with ischemic stroke: myocardial infarction in his/her medical history, vascular dementia, COPD, asthma, acute chronic bronchitis, acute respiratory failure, chronic renal failure, right latero-basal pachypleuritis, congestive heart failure, decubitus ulcers, epileptic seizures, gangrene in the lower limbs, chronic obliterative arteriopathy, neoplasm of the colon operated on, prostate neoplasm, cervical neoplasm.

M. The distribution of patients according to CT examination results

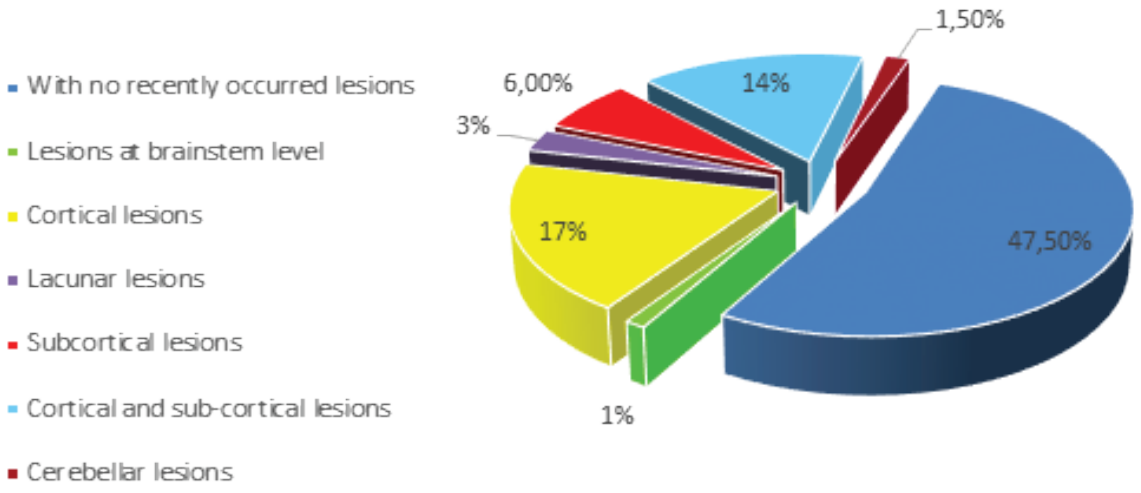

Fig 5. Graphical and percentage representation of the CT examination results of the studied group of patients

Most of the patients in the group did not present anyrecently established lesions, more precisely in a number of 95 patients corresponding to a percentage of 47.5%.
Most patients suffered from ischemic cortical stroke, respectively 17%, followed by a percentage of 14% corresponding to patients with cortical-subcortical infarctions and 6% with subcortical infarctions. Large infarctions were noted in a total of 74 patients. By affecting a large area of brain tissue, the patient suffers from a variety of disorders: motor, sensory, mixed, speech, cognitive, behaviour, memory, awareness disorders etc.

The increased size and the location of the infarction are associated with an unfavourable prognosis on the evolution of the patient suffering from ischemic stroke. It is also worth mentioning the percentage of 1% infarctions in the brainstem and 1.5% in the cerebellum, highly severe infarctions, with potentially severe evolution given the fact that at these levels are found a lot of structures playing a vital role for the body.

N.The distribution of patients with ischemic stroke depending on the affected area

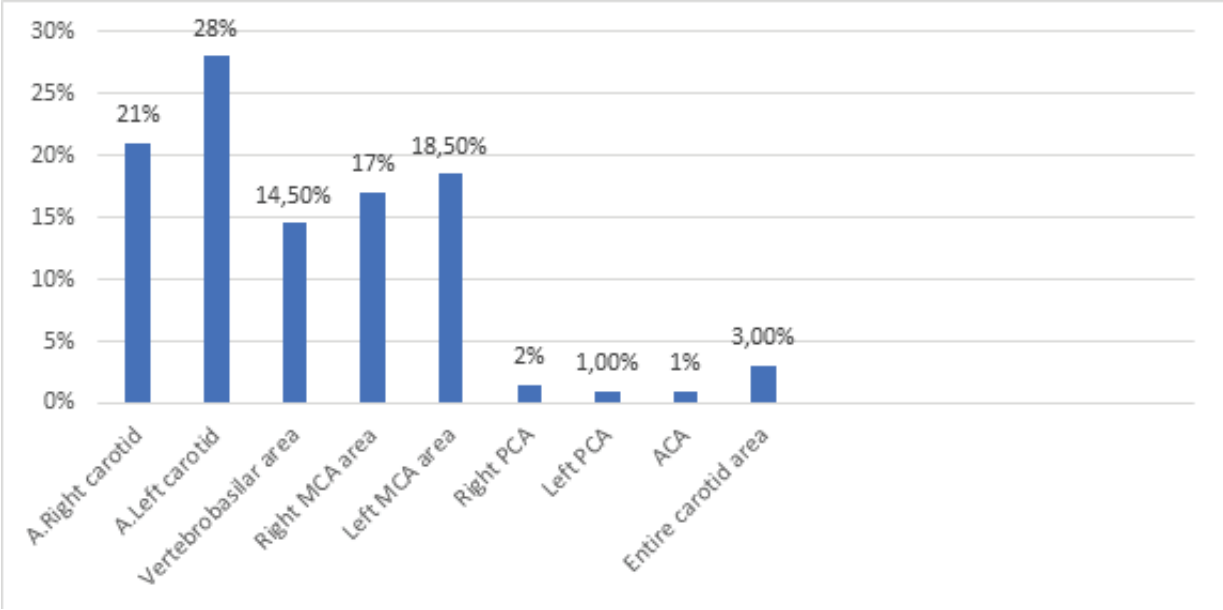

Fig 6. Graphic and percentage representation of patients depending on the area involved in ischemic stroke

The distribution of patients depending on the affected cerebral area is particularly important, because the affected area has an influence on the evolution and the prognosis of ischemic stroke. From this point of view most patients suffered an ischemic stroke in the area of the left carotid artery, namely 28%. On the 2nd place in terms of frequency was identified the area of the right carotid artery (18.5%), the following being left MCA (18.5%) and respectively right MCA (17%).

3. DISCUSSIONS

Prognostic factors for patients with ischemic stroke are the age of the patient, the severity of the infarction, the mechanism of infarction production, related comorbidities, complications of ischemic stroke, which currently contribute to the morbidity and mortality of these patients[1-4,7-12,47,49]

The clinical study was carried out with a view to analyse a number of 200 patients diagnosed with ischemic stroke in the Neurology Clinic II over a 6-month period. By analysing the group of patients, it can be noted that most patients with ischemic stroke were found in the 70-95 age group, there is a relatively equal distribution between men and women, and most patients were from the rural area. These data are in line with data found in the specialty literature.[1-6,13-17,35,48]

The current study helped to identify a series of clinical signs or symptoms suggesting ischemic stroke, of the type: right-sided hemiparesis, left-sided hemiparesis, right-sided hemiplegia, left-sided hemiplegia.

With regard to the presence of speech disorders, in this study we encountered: dysarthria, mixed aphasia, mixed motor aphasia, mixed sensory aphasia.

Following the analysis of comorbidities associated with ischemic stroke[2-4, 23-29,32,41-47], the main aggravating factor is high blood pressure. In the group of patients included in the study 85% were hypertensive, and of these, most presented grade II hypertension. The next aggravating factor is diabetes found in a percentage of 18% of patients, then atrial fibrillation which was found in a number of 29.5% of patients and chronic ischemic cardiomyopathy encountered in more than half of the patients.

One of the parameters individualizing the group of patients in the study is the severity of the ischemic stroke, which is assessed after the neurological examination and represents one of the most important prognostic factors affecting the symptoms in the short or long term[2-4, 23-29]. Most patients presented a pyramidal syndrome (120 patients), paresthetic syndrome (71 patients), speech disorders (159 patients).

Another significant parameter that helps to evaluate the prognosis is brain imaging, which provides information on the volume and location of the infarction[1-6,32-36]. In the group of patients included in the study the volume of the infarction was correlated as follows: cortical infarctions encountered in 17%, subcortical infarctions 6%, cortical-subcortical infarctions 14%, lacunar infarctions 3%, infarctions at the brainstem level 1%, infarctions at the cerebellum level 1%.

The location of the infarction is another prognostic factor with influence on the evolution of ischemic stroke[10,15,48]. Obstruction of the basilar, vertebral artery or any of the large intracranial vessels is associated with a very high risk of unfavourable outcomes[1,35,41-43]. In the study performed we encountered the obstruction of the right carotid artery in 21%, the left carotid artery obstruction 28%, the vertebrobasilar area 14.5%.

The complications of ischemic stroke represent prognostic factors for post-stroke evolution [8-10,43-49]. In the group of patients included in the study we encountered bacterial pneumonia in 8.5% of patients, urinary tract infection in 9.5% of patients.

4. CONCLUSIONS

Following the analysis of the results obtained by studying the group of patients diagnosed with ischemic strokes, we concluded:

1. Ischemic strokes were common in both genders, with similar percentages: men 49.5% and women 50.5%.

2. The most affected age group was between 70-95 years, followed by the age group of 60-69 years. The other age groups represented a smaller percentage.

3. The main reasons for hospitalization were: right-sided hemiparesis (45 patients), left-sided hemiparesis (49 patients), right-sided hemiplegia (32 patients) and left-sided hemiplegia (21 patients).

4. By analysing speech disorders we noted that most frequently the patients suffered from dysarthria 42% and mixed aphasia 31%.

5. 85% of patients with ischemic stroke had high blood pressure in their personal pathological history, and most of them presented stage II hypertension 69.5%.

6. 29.5% of the group patients presented atrial fibrillation.

7. Following the study carried out 18% of the patients had type II diabetes and 25.5% had dyslipidaemia.

8. Following the analysis of the laboratory medical analyses, it was found that most patients had normal value of creatinine in 72% of patients and only 26% had increased values, the cholesterol value was normal in 59.5% of patients and increased at a percentage of 38% patients. The blood glucose value was between 70-110mg/dl at 42.5% and over 110 mg/dl at 55%.

9. The vascular areas were affected in various percentages: left carotid artery (28%), right carotid artery (21%), vertebrobasilar territory (14.5%), left middle cerebral artery (18.5%), right middle cerebral artery (17%), ACA (1%), right PCA 2%, left PCA (1%) and entire carotid area (3%).

10. Based on the CT exam, it was found that most patients suffered from cortical infarctions (17%), followed by 14% of patients whose ischemic strokes were located in the cortical-subcortical area, while only 6% had suffered from subcortical infarctions, then 1.5% patients had cerebellar infarctions, 1% patients underwent infarctions at the brainstem level and 3% of patients underwent lacunar infarctions.

11. As prognostic factors, along with age, the severity of neurological impairment and the size of the infarction, shall be added comorbidities that accentuate the negative prognosis of ischemic stroke: urinary tract infection 9.5% of patients, pneumonia 8.5%, chronic kidney disease 9%, myocardial infarction 2.5%, dementia 3.5%, COPD 1.5%, tumours 2% of patients, acute chronic bronchitis 2.5% of patients, acute respiratory failure 1% of patients, chronic renal failure 1% of patients, latero-basal pachypleuritis 3% patients, decubitus sores 2.5% of patients, epileptic seizures 1% of patients, congestive heart failure 1% of patients.

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FACTORII DE PROGNOSTIC ÎN ACCIDENTUL VASCULAR CEREBRAL ISCHEMIC

REZUMAT

Accidentul vascular cerebral reprezintă una dintre cele mai importante probleme de sănătate publică în zilele noastre, deoarece, la nivel mondial, dar mai ales în țările foarte dezvoltate este o cauză majoră de mortalitate cât și de morbiditate.[1-2, 6-12] Studiul este unul retrospectiv, efectuat pe un lot de pacienti cu AVC ischemic internati in Clinica de Neurologie a Spitalului Clinic de Neuropsihiatrie din Craiova in perioada 1 ianuarie 2020- 30 iunie 2020. Factorii de prognostic pentru pacienții cu accident vascular cerebral ischemic sunt vârsta pacientului, severitatea infarctului, mecanismul de producere al infarctului,comorbiditățile asociate, complicațiile accidentului cerebral ischemic, care contribuie în momentul actual la morbiditatea și mortalitatea acestor pacienți.[1-4]

Cuvinte cheie: accidentul vascular cerebral ischemic, factori de prognostic, hipertensiunea, fibrilatia atriala, diabetul zaharat