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REVISTA DE MEDICINĂ MILITARĂ

- *Diagnostic challenges in gastrointestinal infections*
- *Diagnostic value of ultrasound in comparison with the pathologic results of fine-needle aspiration of thyroid nodules for rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital*
- *Overview of bilirubin as criterion for higher military education admission*
- *Antibiotherapy: latest current affairs – state of the art 2019*
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- *Elastofibroma – an uncommon cause of upper limbs pain*
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ORIGINAL ARTICLES

Diagnostic challenges in gastrointestinal infections

Nicoleta Negrut[1], Shamim A. Khan[2], Simona Bungau[3], Dana C. Zaha[4], Corb A.R. Anca[4], Ovidiu Bratu[5], Camelia C. Diaconu[6], Florentina Ionita-Radu[7]

Abstract: *Gastrointestinal infections are among the most common infectious diseases found all over the world, varying depending on the etiological agent. Symptoms usually include diarrhea, vomiting, and abdominal pain. Water and electrolyte imbalance is the main consequence of gastrointestinal infections. Most of them are cured or self-limited in few days, but at the same time, for a specific population such as immunocompromised, elderly patients or new-borns, these infections are potentially severe. In this context, it is very important to identify the etiological agents of acute diarrhea for the appropriate treatment and infection control measures. While routine laboratory diagnosis of parasitic diarrhea still depends largely on microscopic examination of fecal samples, immunological and molecular methods are becoming increasingly commercially available and, in well-resourced settings, will ultimately displace traditional methods. The present paper presents some of the most common and well-known pathologies of this type, being a brief presentation of the variety of gastrointestinal diseases, each with characteristic clinical manifestations and diagnosis.*

Keywords: *gastrointestinal infections, Rotavirus, Salmonella, Shigella, Helicobacter pylori*

INTRODUCTION

Worldwide, acute gastrointestinal infections are the second most common infectious diseases after respiratory tract infections and a major cause of morbidity and mortality among infants and children [1]. Gastrointestinal infections can be caused by a large number of microorganisms present in the water, food, or hospital environment, that cause inflammation of different segments of the gastrointestinal tract. The prevalence of the different microorganisms causing gastroenteritis depends on several factors, such as age (children, adults), community or nosocomial acquisition, country (developing or developed), patients who have traveled abroad and immune status [2].

The common clinical picture usually looks like acute gastroenteritis, with a sudden or insidious onset, immediately after or after a while from returning to the country of origin. At the first presentation, it is important to identify the patient's journey, with detailed information on particular aspects of the trip. Failure in identification of the epidemiological link may lead to delayed diagnosis and treatment, with increased risks of complications. Empirical intervention is often important when there is a strong possibility of a certain condition and confirmatory lab tests

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are delayed or unavailable, especially for infections that are rapidly progressive or with prolonged testing duration [3].

Traveling for business and pleasure is a small proportion of the total movement of people. Modern transport ways allow more goods and people to travel around the world at a faster pace; they also open up the airways to the transcontinental movement of vectors for infectious diseases. While traveling, people carry their genetic profile, immunological sequelae from previous illnesses, cultural preferences, traditions, and patterns of behavior. They are also accompanied by microbes, animals and other biological lives [4]. The range of infectious diseases present at a certain time and in a certain region has increased, with the increasing travel possibilities from one country to another and between continents. Transiting through different geographical areas, in a short time (as with long-distance flights), may increase the risk of contamination with foreign etiological agents. Cities are becoming important centers for the transmission of infectious diseases due to international travel and migration, as shown by recent pandemics. Several factors contribute to infectious disease emergence. Others commonly listed include microbial adaptation and change, demographics and behaviour, changes in the environment, technology and economic development, deterioration of public health and surveillance programs, and international travel and trade [5].

Many tropical diseases havea polymorphic clinical feature, making the diagnosis difficult, especially for clinicians from countries outside tropical areas. In developing and developed countries, doctors in urban settings need to be aware of developments in infectious diseases. Upon travel, an ill traveler's history must analyze exposure specifics, including visited destinations, activities, ingestion of infected food or drink, contact with insects, livestock, freshwater or blood and body fluids and other possible exposures. Information on the geographic dissemination of infectious disease is significant in creating the differential diagnosis and testing [6].

The purpose of this paper is to make a brief review of the most common gastrointestinal infections, with their relevant clinical features and diagnosis.

GASTROINTESTINAL INFECTIONS

In the geographical area of Romania, southeastern Europe, Rotavirus, Salmonella species, Shigella species, Clostridium difficile, Escherichia coli and Giardia lamblia are the most common etiological agents of gastrointestinal infections [7-9]. Infections with Helicobacter pylori, Campylobacter spp., and other viruses are also frequent. Several tropical infectious diseases, such as amebiasis, malaria, leishmaniasis, dracunculiasis, schistosomiasis, brucellosis, leptospirosis, dengue fever, yellow fever and viral haemorrhagic fever, can have a digestive onset, requiring increased attention from the clinician. Experimental animal studies and clinical research have shown that the virulence of infectious microbial agents can have variable severity, sometimes being involved even in deaths because of sepsis [10, 11].

Viral gastrointestinal infections

The most important etiological viral agents of diarrhea are rotavirus, norovirus, adenovirus, astrovirus, sapovirus and enterovirus. Gastroenteritis caused by norovirus, asingle-stranded RNA (ssRNA) viruses belonging to the family of Caliciviridae, may manifest with severe nausea, vomiting and diarrhea. The genogroup I (GI), II (GII) and IV (GIV) of Rotaviruses (double stranded RNA) are the main cause of diarrhea in children under 5 yo and responsible for the death of around 200,000 children/year. Rotaviruses belong to the Reoviridae family [12]. Major symptoms are usually abdominal pain, vomiting and waterydiarrhea (AWD). Rotaviruses serogroups A, B, and C are responsible for human infections.

Adenoviruses cause respiratory diseases, bladder infections, conjunctivitis. Adenoviruses belong to Adenoviriadae family of double-stranded (dsDNA) viruses. Taking into consideration 51 serotypes of human adenoviruses (classified into six groups, from A to F), gastroenteritis is mainly caused by Adenoviruses serotype 40 and 41.

Astroviruses discovered in 1975 are single-stranded (ssDNA) viruses and belong to the family of Astroviridae; the incidence of these infections is constantly increasing [13]. Clinical signs include vomiting and severe diarrhea, with moderate dehydration. In developed countries, the Astrovirus infection incidence is between 2-9%, affecting mainly children [14]. To date, serotypes 1 - 5 are the most relevant. Serological studies indicate that most children infected with Astroviruses develop antibodies to the virus early in life, which provide protection against future infections.

Sapoviruses are part of the family of Caliciviridae and are major causative agents of gastroenteritis worldwide, causing disease in humans of all ages. The highest incidence was described for children under 5 year-old, but infections are diagnosed in adults as well, with diarrhea, vomiting, and fever (similar clinical symptoms to those of norovirus infections). However, self-limited sapovirus infections lead to less severe gastroenteritis compared to norovirus-induced gastroenteritis [15].

There is a variety of different subtypes of human enteroviruses, such as human enterovirus 70/71, poliovirus, Coxsackie virus A and B, echovirus, with different degrees of tissue tropism and virulence, although retaining a basic antigenic identity. Infections are usually asymptomatic or present with mild cold-like symptoms. Severe enterovirus infections are poliomyelitis, meningitis and myocarditis. Coxsackie viruses are present worldwide and can cause the so-called “summer diarrhoea”. Other severe infections with Coxsackie virus or human enterovirus 70/71 can lead to conjunctivitis and myocarditis, whereas echovirus infections can result in aseptic meningitis, with echovirus 30 being the most common meningitis-causing serotype in Europe, America and Asia [16, 17].

Viral hemorrhagic fever (VHF) is characterized by severe multiple organ dysfunction syndrome. Etiologically, four particular RNA viral families (Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae) lead to VHF. The viruses that cause VHFs are spread across most of the globe. Haemorrhagic viruses are considered as potential biological warfare agents. Initially, clinical symptoms include fever, cephalgia, myalgia, arthralgia and chills, which can lead to massive haemorrhages, shock or even acute multiple organ dysfunction. Paraclinical parameters show an increase in liver enzymes and a decrease in albumin levels. The coagulation panel shows an increase in prothrombin time and a decrease in fibrinogen. These results of the paraclinical tests may be similar to diseases like disseminated intravascular coagulation, malaria, haemolytic uremic syndrome. Although the confirmation of the diagnosis requires detection of viral RNA, viral antigens, or specific antibodies, the clinician must raise the suspicion quickly, before laboratory results, because these diseases require strict isolation measures and biosafety level 4 laboratory facilities [18].

Dengue fever is a global arboviral infection spread by the mosquito genus Aedes (A) (primarily A. aegypti, but also A. albopictus, and A. polynesiensis), a mosquito found in the tropical and subtropical regions of the world, also involved in spreading Zika virus disease, yellow fever, and Chikungunya. About 40% of the world population, almost 3 billion people live in regions with a dengue threat [19]. In more than 100 countries, people are affected by dengue fever resulting in up to 22,000 deaths per annum globally [20]. In 2018, Romania reported 4 cases of dengue fever, all travel-associated [21]. With incubation of 3-14 days, 50-80% of patients are asymptomatic during the period of illness [22]. Classic dengue fever (break-bone fever) is distinguished by high fever, severe cephalgia, myalgia, arthralgia, retro-orbital pain, abdominal pain or tenderness with persistent vomiting, and hepatomegaly. The diagnosis is supported by the combination of thrombocytopenia, leukopenia, modifications of the liver function tests and serological tests for dengue virus.

Yellow fever virus etiologically belongs to Flaviviridae family, which causes acute viral disease transmitted by A. aegypti, and Haemagogus mosquitoes. The name's "yellow" refers to jaundice that affects most patients. In tropical regions of Africa and Central and South America, the virus is endemic. In 2018, one case of yellow fever was reported by Romania [21]. Severe cases can have a mortality rate of up to 50% [23, 24]. With an incubation period of 3-6 days, clinical manifestations in the second phase, which generally affect liver and kidney, consist in fever, vomiting, abdominal pain, jaundice, dark urine, hemorrhage, and even shock. Yellow fever, especially during early stages, is difficult to diagnose, it can be mistaken for diseases like leptospirosis, fulminant forms of viral hepatitis, or even severe malaria. Confirmation of the diagnosis requires the detection of the virus by identifying viral ribonucleic acid (RNA), specific antigen or immunoglobulins M.

Digestive infections caused by protozoans and parasites

Parasitic infections causing diarrhea are particularly important in young children, immunocompromised patients, and those living in conditions of poor sanitation and hygiene, with unsafe food and water supplies. Travelers from developed countries are another group at risk, particularly susceptible to parasitic pathogens, because they lack protective immunity acquired from previous exposure. Several of these organisms are of zoonotic origin, and there may be occupational or recreational exposure risks [25].

Giardia lamblia, Cryptosporidium spp., Entamoeba histolytica and Dientamoeba fragilis are the most important diarrhoea-causing protozoans. Helminths are less important as agents of diarrhea, but they may produce loose or even dysenteric stools in acute or heavy infections (for example, intestinal schistosomiases). One important exception is the nematode Strongyloides stercoralis, which has the unusual capacity to maintain subclinical infections for years or decades, being discovered only when the patient is immunosuppressed for various reasons, when severe or potentially fatal disseminated infection may occur [26].

Giardia lamblia infections (synonym G. Intestinalis, G. duodenales) in developed countries occur in 6-8% of children and 2% of adults, and around 33% of the population is infected with protozoans [27, 28]. In the US for example, CDC estimated 77,000 cases/year of Giardiasis [29].

One of the several species of the genus Cryptosporidium is

Cryptosporidium parvum, which, together with C. hominis, are the most common cause's ofcryptosporidiosis in humans [29]. Similar clinical symptoms could reveal infections by other Cryptosporidium spp. such as C. felis, C. meleagridis, C. canis, and C. Muris.Around 748,000 cryptosporidiosis cases/year occurred in the US [30, 31].

The causative agent of amoebiasis is Entamoeba histolytica - the only human pathogenic species of the genus Entamoeba. Amebiasis has a worldwide distribution, prevalent in Central and South America, Africa, and Asia, being produced by trophozoites of Entamoeba histolytica.

Clinical manifestations can appear up to 4 months from the contact with contaminated food or objects. In about 90% of cases, the disease may evolve asymptomatic, self-limiting, with recurrences. The rest of the patients may have different severity clinical forms, such as acute colitis (bloody diarrhea, fever, abdominal pain), severe colitis (fulminant, abscesses), and chronic form (ameboma, and inflammatory bowel disease). The etiological diagnosis is based on the microscopic identification of cysts and trophozoites in the stool of the patients, specific antibodies and antigens detection. Around 50 million people worldwide suffer from amoebiasis each year, resulting in 100,000 deaths each year [32].

One of the most underestimated diarrhea-causing protozoansis Dientamoeba fragilis and recent studies demonstrated the direct implication as a common cause of gastrointestinal disease and, in the meantime, his pathogenic potential.The prevalence of Dientamoeba fragilis varies from 0.3% to 52% and often exceeds that of Giardia lamblia [33].

Malaria is produced by infection with Plasmodium species, by the bite of Anopheles mosquito, being recognized as the second cause of death by infections in Africa. According to the European Centre for Disease Prevention and Control (ECDC), Romania confirmed and reported 18 cases of malariain 2018 [21]. The disease is endemic in sub-Saharan Africa and South Asia and has an incubation period between 8-25 days, with a clinical picture dominated by cyclical fever. The following manifestations: insomnia, weakness, arthralgias, myalgias, diarrhoea, abdominal pain, headache, and hepatosplenomegaly can be associated with the fever in case of acute malaria. Classical clinical triad present in malaria consists of fever, splenomegaly, and anaemia. The microscopic examination of thin or thick blood smears, the detection of the parasite nucleic acid by real-time polymerase chain reaction assay, the identification of the plasmodium antigen by immunochromatography are specific tests used to confirm the diagnosis of malaria [34].

Leishmaniasis is a neglected tropical disease, protozoan vector-borne infection, whose transmission is through the bite of an infected female sand fly (Phlebotominespecies). It is present in environments ranging from tropical forests in South and Central America to desert areas in West Asia, including North of Africa with cutaneous leishmaniasis, which is the most common form, mucocutaneous, and visceral leishmaniasis, the last one being the most severe form of the disease. The period of incubation varies widely, from a few weeks to several months after the initial bite, depending on the species. The wide spectrum of clinical manifestations in visceral leishmaniasis includes fever, anorexia, abdominal pain, emesis, diarrhea, hepato-splenomegaly. Diagnostic findings are based on skin biopsy, tissue culture, and presence of antibodies [35].

Dracunculiasis, also known as Guinea worm disease, can be found in rural areas of South Sudan, Chad, Ethiopia, and Mali, which relies on contaminated water supplies. Clinical symptoms can take up to a year to appear, and may include painful blister centered on the worm, usually localized in the lower extremity, fever, nausea, vomiting, and local lymphadenopathy [36].

Schistosomiasis is a disease caused by parasitic worms (Genus Schistosoma), also known as bilharzia, which is endemic in tropical and subtropical areas and considered as one of the neglected tropical diseases. With an incubation period of about 2-8 weeks, the acute clinical picture presents a flu-like syndrome, nausea, vomiting, abdominal pain, bloody diarrhoea, hepatosplenomegaly, and lymph-adenopathy. Laboratory findings include the direct detection of eggs by the microscopic examination or of the parasite's nucleic acid in patients'stool and urine, and serological tests for identifying the specific antibodies in patients returned from endemic areas, but without eggs demonstrated in faecal or urine samples [37].

Digestive infections caused by bacteria

One of the most common bacterial causes of gastroenteritis worldwide is Campylobacter spp. frequent especially in children under 2 year-old, causing diarrhoea, abdominal cramps, vomiting, fever and even bleeding. It is usually generated by the consumption of raw or undercooked meat (frequent/especially poultry) or contaminated milk.

Up to 25% of cases of antibiotic-associated diarrhea in healthcare institutions and hospitals are generated by Clostridium difficile. Elderly and immunocompromised patients have a higher risk. The most recent emergence of highly toxigenic and resistant C. difficile strains has led to more severe outbreaks, increasing mortality and morbidity [38].

In the developing countries, especially among children, Escherichia coli is a major cause of diarrheal disease and the leading cause of travellers'diarrhoea. The infections are produced by drinking water contaminated with human or animal feces. A special strain of this genus is Escherichia coli O157:H7, a Shiga toxin producing. These bacteria generate gastrointestinal infections with symptoms including vomiting and sometimes even bloody diarrhoea. Although it is usually cured after a few days, a percentage of 5-10% of infections lead to haemolytic uremic syndrome and its consequences (destruction of blood platelets, anaemia, kidney failure) [39].

Helicobacter pyloriis the main cause of gastritis and is associated with the development of gastric and duodenal ulcers or even stomach cancers. H. Pylori is the strongest etiological factor in both of these conditions, with the World Health Organization classifying it as a class I carcinogen. According to the CDC, approximately two-thirds of the population is infected with H. pylori, but less than 20% have a risk of developing ulcers or stomach cancer [40]. On the other hand, infection with H. pylori may protect against some cancers, such as oesophageal adenocarcinoma and gastroesophageal reflux disease [41]. It can cause stomach pain or nausea, but in many cases, there are no symptoms.

Salmonella and Shigella are food-borne GI illnesses. Salmonella is common and is found in raw meats, poultry, seafood and eggs, as well as milk and dairy products. Acute symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. Shigella is frequently found in water polluted with human feces. Symptoms of shigellosis or bacillary dysentery include abdominal pain, cramps, diarrhoea, fever, vomiting, and blood, pus, or mucus in stool.

Yersinia enterocolitica is a cause of diarrhea and abdominal pain relatively frequent after eating contaminated food, especially raw or undercooked pork products, milk or ice cream. Common symptoms are fever, abdominal pain and diarrhea, which is often bloody.

The most common cause of food intoxication, characterized by violent onset, severe nausea, cramps, vomiting, and diarrhea is Staphylococcus aureus. It is also associated with a large range of food, and could be found on humans (infected cuts, skin, throats and noses).

Brucellosis is a zoonosis caused by four different species of Brucella. It is considered as a major infectious disease in the Caribbean, the Middle East, Eastern Europe, Asia, and Africa. According to ECDC, one case of brucellosis was reported by Romania, in 2018 [21].The incubation period is between 2 weeks to 5 months. General brucellosis signs are usually vague and flu-like, with gastrointestinal symptoms such as dyspepsia, diarrhoea, constipation, vomiting, abdominal pain, hepatic abscesses. However, the clinical picture is dominated by fever, with undetected aetiology through routine laboratory investigations. The diagnosis of brucellosis, although easy to confirm based on serological tests, often requires a period of up to one year, due to the presence of a polymorphic, sometimes oligo-symptomatic clinical picture.

Leptospirosis is a global zoonotic infection with a high prevalence in Caribbean and Pacific islands, Central and South America, Southeast Asia, predominantly during the rainy period, caused by pathogenic spirochetes of genus Leptospira. In 2018, 51 cases of leptospirosis were confirmed in Romania [21]. Usually the incubation period is 1-2 weeks. It presents with a wide range of clinical symptoms, which could be easily mistaken for other diseases, including flu-like syndrome, nausea, vomiting, diarrhoea, rash, dry cough, jaundice, hepatomegaly and splenomegaly, oliguria/anuria, haemorrhages, with the involvement of multiple organs. Suspicion occurs in the case of association between digestive, liver, kidney manifestations with fever, and myalgia. Confirmation requires the determination of specific antibodies.

DIAGNOSIS

In the presence of symptoms of a possible gastrointestinal infection, the diagnosis could be confirmed by different old and modern laboratory tests using stool specimens first and others such as serum for specific antibodies (serological tests). The standard culture to isolate bacteria remains the gold standard for bacterial pathogens, even if it has a relatively low sensitivity and it is time-consuming (more than 24 h), but it may be followed by antibiotic susceptibility testing. In this way, the therapeutic options may be evaluated, but also the assessment of microbial resistance to antibiotic therapy [42]. Ova or parasite examination are ordered in case of suspicion of parasites. For this purpose, a direct examination by microscopy or a concentration method is used, but it has a low sensitivity and it is, once again, time consuming and requiring specific training of the laboratory staff. In addition, there are antigen tests used to detect Clostridium difficile (GDH, toxins), Giardia lamblia, Cryptosporidium spp., Helicobacter pylori, or Entamoeba histolytica. However, antigen tests can only detect a few specific parasites and therefore cannot replace ova and parasite examination. Antigen tests are useful to rapidly detect viruses causing gastroenteritis, such as rotavirus and adenovirus; however, these tests are not available for all entero-pathogens and show a variable sensitivity.

Taking into account the wide range of pathogens that can

cause diarrhea and the similarity of the clinical manifestations produced by different pathogens; the molecular techniques seem to be the best tools for a rapid diagnosis. In the last years, some tools based on polymerase chain reaction (PCR) techniques have been developed to detect specific microorganisms or a panel of different entero-pathogens directly from stool samples [43-45]. Real-time PCR allows the simultaneous detection and differentiation of viral, bacterial and parasitic gastrointestinal pathogens. This assay also showed a high sensitivity for most pathogens included in the panel and efficient detection of coinfections. The main advantages of the molecular tools for the diagnosis of gastroenteritis are: high sensitivity, increased diagnostic spectrum, improved workflow (processing and diagnosis time is between 1 and 5 h), favorable impact on infection control and costs. Some disadvantages of molecular methods are the inability to distinguish between viable and nonviable microorganisms; they do not offer antibacterial susceptibility or epidemiological data; high cost; detection of the asymptomatic carriage could be difficult to distinguish from symptomatic/asymptomatic infections. Therefore, the application of these tools should be performed in patients selected based on clinical condition and/or the severity of gastroenteritis. Particularly in hospital settings, the rapid diagnosis provides important information for implementing infection control measures [46-48].

The clinical symptoms of all these diseases are nearly identical. Thus, it is difficult and almost impossible to determine the pathogenic cause based on clinical features alone. Rapid diagnosis, appropriate treatment, and infection control are important measures in these contexts. The early medical challenge is to include the possibility of tropical infectious diseases that are not common in the area, in the differential diagnosis. Not only the characterization of the symptoms but also the detection of infectious diseases based on a detailed history of the patient and a quick assessment can enormously help the clinicians in establishing a correct diagnosis and treatment.

Emerging evidence suggests that many gastrointestinal diseases are associated/caused by alterations in the gut microbiome. Specific microbiome signatures have been reported for irritable bowel syndrome and inflammatory bowel disease, and these have been associated with pro and anti-inflammatory effects, contributing to disruption or maintenance of gut homeostasis. Colorectal cancer is the third most common malignancy diagnosed worldwide, one of the leading causes for cancer-related mortality in both men and women. Different hereditary, environmental and inflammatory factors have been shown to increase the risk of colorectal tumorigenesis, but recently, bacteria and viruses have been described to be involved in colorectal carcinogenesis through direct modulation of epithelial cells, leading to genomic or chromosomal instability. Observational studies have found positive associations between gastric ulcers, periodontal disease, bacterial infections, and the risk of pancreatic cancer, the most rapid lethal cancer, with over 70% of patients dying within one year after diagnosis.

CONCLUSIONS

Concerning human mobility, several specific aspects must be considered, such as undiagnosed, atypical and subclinical types of diseases. These diseases are mistakenly treated as intercurrent diseases, and serious latent infections may develop. In the last few years, molecular methods have become readily available for many clinical laboratories and their utilization has resulted in a more rapid diagnosis and increased pathogens detection. As travel around the world continues, microbes will continue to evolve, adapting to new environmental conditions. The emergence of new infections will continue, and the distribution, severity and frequency of the identified infections will change, considering the new conditions. Travel or migration of people will continue to be an important factor in the occurrence and development of diseases. The analysis and monitoring of pathogens will focus the specialized actions and researches on the global movement and evolution of microbes. Integration of medical knowledge and multidisciplinary skills – social, biological and physical sciences – are therefore required.

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ORIGINAL ARTICLES

Diagnostic value of ultrasound in comparison with the pathologic results of fine-needle aspiration of thyroid nodules for rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital

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Abstract: *Background and Aim: Various studies have reported that 4-7 percent of adults with palpable thyroid nodules that 50-70 percent of it's diagnosed with high-accuracy sonography. Although most thyroid nodules are non-cancerous and do not require treatment, a small percentage of them are associated with a potential risk of malignancy, which is an important early diagnosis by ultrasound or pathology. The present study aimed to compare the ultrasound with the pathologic results of fine-needle aspiration (FNA) of thyroid nodules for the rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital.*

Methods: The study population consisted of 100 (80 female, 20 male) patients with thyroid nodules in the age range of 19-82 years referred to the radiology ward of Baqiyatallah Hospital who were studied by the cross-sectional method. All patients were first scoring by ultrasound, then fine needle aspiration with thyroid nodule ultrasound was performed, and the specimens were referred to the pathology department for further evaluation.

Results: The pathologic results from fine-needle aspiration showed that only 8 of the 100 patients studied had malignant nodules that, by ultrasound scoring, 7 patients had 4 scores and 1 patient had 3 scores. However, the other 92 patients with benign nodules 57.60% of patients had score 1, 30.43% of patients had score 2 and 11.97% of patients had score 3.

Conclusion: According to the results of this study, it seems that ultrasound scoring is useful in the rapid diagnosis of thyroid nodules and prevent unnecessary thyroid nodule FNA.

Keywords: *fine needle aspiration, sonography, thyroid nodule, malignancy*

INTRODUCTION

The thyroid gland is one of the largest endocrine glands in the butterfly-shaped transverse section of the larynx. The units that make up the thyroid gland are a sinus or follicles, which are present in the central part of colloidal proteins as a source of thyroid hormone storage [1]. The thyroid gland has several key functions, such as iodine uptake and transfer, thyroglobulin secretion, iodine binding to thyroglobulin to make thyroid hormones, facilitating thyroid hormone secretion into the bloodstream, and interfering with body metabolism [2].

Thyroid hormones

Thyroglobulin, a large iodized glycoprotein, has been shown by researchers to be the first essential step in initiating the synthesis of thyroid hormones through iodine absorption through the food. Transmission of iodine to the thyroid gland occurs by binding to serum proteins, especially albumin, and

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unbound iodine is often excreted in the urine [3]. TSH, which is secreted by the anterior pituitary cells, plays a central role in the control and evaluation of thyroid function, which is regulated by the TRH hypothalamic hormone [4]. Various studies have shown that there is an inverse relationship between thyroid hormone levels and basal TSH production, indicating the role of thyroid hormones as a major regulator of TSH production [5].

Common disorders of the thyroid gland include hypothyroidism, hyperthyroidism, goiter or enlargement of the thyroid gland, thyroid cyst, thyroiditis (Hashimoto's) and thyroid nodules. According to the latest studies, 7-8% of the world population has one of the thyroid disorders [6].

The term thyroid nodule refers to a group of thyroid cells that grow abnormally and fall into a mass, nodule, or node, which are divided into two groups of benign nodules and malignant nodules [7]. Various studies have shown that most thyroid nodules have non-cancerous and benign cells and various reports have estimated 1 in 10 patients as malignant nodules [8].

The types of thyroid nodules are divided into 3 groups of benign (colloidal), malignant (papillary) and suspicious nodules based on pathological findings. Among the types of nodules mentioned, only malignant nodules require further surgery and care.

Symptoms of patients with thyroid nodules include neck, jaw or ear pain, difficulty swallowing, itching or tingling in the throat, shortness of breath, sore throat, hoarseness, and most studies have reported that most patients Thyroid nodules are without symptoms and are often discovered during examination for other causes [9].

Researchers have identified chronic follicular thyroid nodule (Hashimoto's), congenital malformations such as failure to form a lobe, thyroid abscess, simple cyst, and non-thyroid diseases such as lymphoma and teratoma [10].

Tests for the diagnosis of thyroid nodules include measurement of thyroid hormone and thyroid-stimulating hormone, ultrasound, scanning, fine needle aspiration, and pathological examination (cellular aggregation) [12, 11].

Since a rapid diagnosis of malignancy facilitates the process of thyroid nodule healing, therefore, finding the fastest method of diagnosis is important for initiating therapeutic measures.

The present study aimed to compare the ultrasound report only with the pathologic results of fine-needle aspiration of thyroid nodules with the aim of rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital.

MATERIAL AND METHOD

Following the approval of the research plan by the University Research Council, informed written consent was obtained from patients' participation and distributed to those who referred to the thyroid nodule interventional ultrasound department and the indication of fine-needle aspiration. No entry and exit criteria were considered for the study.

Finally, 100 patients (80 females, 20 males) from thyroid nodules in the age range of 19-82 years referred to the radiology ward of Baqiyatallah Hospital and the statistical population were selected by census method and studied by the cross-sectional method. After obtaining biographies and demographic information by the student, patients were referred to a skilled sonographer for ultrasound (using Philips Affinity fog) and fine-needle aspiration (with ultrasound guidance) and the specimens were referred to the pathology department for cytological examination.

In thyroid ultrasound, tissue is examined for ultrasound by echogenicity and rate and extent of calcification. 6 modes of Hyper echo nodule (white nodule in ultrasound), Hypo echo nodule (gray and pale nodule in ultrasound), cystic nodule (absolutely Echoless), solid nodule (uniform echo), microcalcification (calcification less than 2 mm) and macro calcification (calcification greater than 2 mm) was determined by scanning ultrasound for scoring from 1-4. The four nodule states were hypo echo, solid, microcalcification, and a 1 cm-high scoring criteria of 1-4. Each of the above was given a score of 1 and the nodule with all four attributes scored 4. In fine needle aspiration, the thyroid tissue is sampled using a fine needle for the microscopic examination of existing cells.

Among the characteristics of malignant nodules can be severe porcelain, cell size and shape variation, dense and double or multilayered cell masses, etc. These features were reported by a skilled pathologist.

Data were analyzed by spss23 software.

RESULTS

The mean age of patients was 48.26±08/13 and the results of this study showed that malignant thyroid nodules were more prevalent in 40-49 years old patients and women in our study ($p<0.05$).

Of the 100 patients with thyroid nodules, 8 (8%) had malignant nodules, indicating that a thyroid nodule may be about 12 times more likely to be benign.

Ultrasound reports showed that of the 92 benign nodules, 58 were hyper echogenic, 22 were hypo echogenic, and 12

were cystic nodular, whereas of the 8 malignant nodules, 5 were hypo and 3 were iso- echogenic.

It was observed that the ratio of solid in malignant nodules was more than benign. The calcification rate in benign nodules was 17% for microcalcification and 2% for macro calcification, which was 40% and 20% for malignant nodules, respectively.

Pathologic reports showed 53 patients with nodular goiter, 28 with adenomatous goiter, 1 with a follicular lesion, 8 with papillary carcinoma, 8 with thyroiditis, and two remained unknown due to insufficient sample that both nodules were cystic.

Of the 8 patients with malignant nodules reported by the pathologist from the side of ultrasound, 7 (87.5%) cases had score 4 and one patient (12.5%) had score 3. While 57.60% of benign nodules had score 1, 30.43% had score 2 and 11.97% had score 3.

From the side of ultrasound scoring, 53 cases received score 1, 28 cases received score 2, 7 cases received score 3 and 12 cases received score 4. In terms of thyroid nodule count were 47 single nodules and 53 multinodular nodules.

From hormonal tests, 4 cases were hypothyroid, 5 were hyperthyroid and 91 cases were normal, 3 cases were hypothyroid or hyperthyroid, and 5 cases were normal. Eleven of the patients had a family history of thyroid disorders, which was found to be 5 out of 11 people and 3 in people with no history of malignancy.

DISCUSSIONS

This study aimed to compare ultrasound only report with pathologic results of fine-needle aspiration of thyroid nodules with the aim of rapid diagnosis of thyroid malignancies in patients referred to Baqiyatallah Hospital. The pathologic results of fine-needle aspiration in the present study showed that only 8 of the 100 patients studied had malignant nodules which, by ultrasound scoring, 7 cases had score 4 and 1 case had score 3. However, the other 92 patients with benign nodules 57.60% had score 1, 30.43% had score 2 and 11.97% had score 3. In other words, by taking into account the pathologic results in this study, it can be concluded that ultrasound results can be used as a quick tool in predicting thyroid nodule malignancy. The reason for the high success of sampling accuracy in this study was the use of ultrasound guidance during biopsy and slide preparation by the pathologist.

In 2015, Siyadati et al investigated the diagnostic value of fine-needle aspiration compared to pathologic results in the diagnosis of thyroid nodules in Babol Shahid Beheshti Hospital, northern Iran, for 22 years. The researchers said that fine-needle aspiration of the thyroid is a selective method of examining thyroid nodules that have recently been questioned in the management of nodules. This cross-sectional study was performed on cytology specimens of 225 patients who underwent thyroidectomy in Shahid Beheshti hospital of Babol for 22 years (1989-91). Age, sex, physician performing FNA, type, and surgical site, FNA and pathology results were recorded. Pathologic FNA results were classified into four groups of inadequate, benign, malignant and suspicious and pathologic results were also divided into two benign and malignant and compared. The results showed that in the diagnosis of malignant thyroid nodules, sensitivity was 60%, specificity 96%, positive predictive value 65%, negative predictive value 95% and diagnostic accuracy of 81.8%. Of the 225 cytological specimens, 127 were benign (56.4%), 47 suspicious (20.9%), 27 malignant (12%), and 24 inadequate (10.7%). Final pathology was also benign in 150 patients (66.7%) and malignant in 75 patients (33.3%). According to the results of this study, fine needle aspiration can be used to diagnose thyroid nodules [13].

Rosario et al., 2013 in Brazil, designed a study to predict malignancies in thyroid nodules with non-diagnostic cytology and to evaluate the value of ultrasound. The results of this study showed that in patients with thyroid nodules and repeatedly diagnosed cytology findings, ultrasound findings are an excellent parameter for choosing to continue their treatment, which would be useful for periodic ultrasound [14].

In a 2005 study in Taiwan, Lin et al., in the study of thyroid nodules by ultrasound and fine-needle aspiration, concluded that ultrasound combined with fine-needle aspiration detected 10 years earlier than other methods of malignancy [15].

In 2006, in Italy, Baratolata et al. designed a study entitled Quantitative and Qualitative Evaluation of Thyroid Nodule with Ultrasound Contrast. These researchers concluded that quantitative data corroborated the subjective findings and contrast ultrasound of the thyroid gland was a feasible procedure but found other random events during ultrasound may interfere and are one of the reasons limiting the potential of this method for the detection of thyroid nodules [16].

In 2009, Tramaloni et al. evaluated ultrasound and Doppler ultrasound and fine-needle aspiration in thyroid nodules. In this study, microcalcification, hypoechogenic nodules, and intra-nodular vascularization were introduced as criteria for malignancy [17]. In our study, as the grayscale ultrasound was used, therefore, vascularization was not used in

malignant scoring.

Rago et al. 2008 evaluated ultrasound and Doppler ultrasound and fine-needle aspiration for malignant patterns in thyroid nodules. In this study, microcalcification, hypoechogenic nodules, and intravascular nodular vasculature were introduced as criteria for malignancy [18]. In our study, as the grayscale ultrasound was used, therefore, vascularization was not used in malignant scoring.

CONCLUSIONS

According to the results of the present study, it seems that ultrasound scanning alone is useful in the rapid diagnosis of thyroid nodules and to suggest a more accurate result, the present study should be designed in a larger statistical population. More accurate and desirable results can be performed on specific types of lesions. Thyroid nodules with scoring 3 and 4 should also be subjected to the fine-needle aspiration to facilitate faster and more accurate diagnosis and to prevent the progression of malignancy. Among the properties of the nodule used in scoring, nodule size alone may not be a good criterion for nodule malignancy, whereas solidity, hypo echogenic, and microcalcification favor the malignancy of the nodule, and can exclude nodules that score 1 or 2 can be tracked by ultrasound and does not necessarily need to be sampled.

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ORIGINAL ARTICLES

Overview of bilirubin as criterion for higher military education admission

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Abstract: *This article was written because of the necessity of clarifying the recent biomedical selection criterion for the medical exam of the students who are applying for higher military education. In order to screen for or monitor liver disorders or hemolytic anemia the bilirubin is measured. The increased values of the bilirubin can be caused by multiple factors and they are presented in the following chapters.*

Keywords: *Bilirubin, liver, gall, alimentation, genetic diseases*

WHAT IS BILIRUBIN?

Bilirubin is a linear liposoluble tetrapyrrole derived from the enzymatic catabolism of the hem fraction from various hemoproteins in the body. Understanding changes in plasma levels of bilirubin under pathological conditions and differentiation of icteric syndromes involves a prior knowledge of the mechanism of formation, agglutination and elimination of this compound.

The normal values observed in the laboratory analysis are:
- Total bilirubin = 0.3 - 1.2 mg / dl, (or 0.1 - 1.2 mg / dl)
- Direct bilirubin = 0.1 - 0.35 mg / dl
- Indirect bilirubin = difference

Linear tetrapyrrole is the result of the enzymatic mechanism that catalysis the oxidative breakdown of the tetrapirolic (porphyrinic) ring of the hemoglobin heme molecule, primarily of the aged erythrocytes.

The process leads to hemoglobin formation in at least two stages. Bilirubin is mainly formed in the liver (about 80-85% up to 90% according to some authors), the rest having extrahepatic origin, in part reticuloendothelial (spleen, bone marrow).

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Involvement of the liver in bilirubin production

The main degradation product of porphyrins is bilirubin, the red-orange bile pigment, which still possesses the four pyrrole rings. In the plasma, the pigment circulates as total bilirubin, consisting of two components: bilirubin bound to unconjugated albumin, indirectly referred to as free glucuronide bilirubin (mono- and diglucuronate), related to the diazotation reaction, Hijmans Van Den Bergh. Total bilirubin level serum concentration is 0.1-1.2 mg/dL or 17 µmol/l. Under normal circumstances, bilirubin accounts for 85% of the erythrocyte hemoglobin degradation and the remaining 15% originates in the degradation of other hemproteins such as myoglobin, cytochromes and other hemic enzymes (catalases, peroxidases, cytochrom-oxidases).

Direct bilirubin (agglutinated with glucuronic acid) circulates freely in the blood. Indirect bilirubin (primary, unconjugated) is transported by binding to albumin, partially in the form of the albumin-phosphatide complex and in small amounts bound to both metals, amino acids, peptides or other small molecules. In the gall there are bilirubin esters with sulfuric

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acid and other acids. By binding the bilirubin to the elastic fibers of the skin and the conjunctiva the symptoms of jaundice appear (yellowing of the skin and conjunctiva).

Bilirubin production is 35 mg per gram of hemoglobin, yielding 250-300 mg daily bilirubin. Hemoglobin degradation in bilirubin is performed in Kupffer stellate cells in the hepatic reticulo-histocithic system (RHS), which is the primary location of the process, the spleen and bone marrow being organs involved in this activity.

The secretion of the gall

One of the most important functions of the liver is the production of its external secretion, the gall, which reaches the duodenum through the biliary tract. The gall fulfills two major functions:

• elimination of endogenous substances – end products of metabolism and foreign substances such as bromsulfonaphthalein (BSP) drugs and dyes;

•intervention in lipid digestion and reabsorption – the liver secreted by hepatocytes, the hepatic ball, differs in appearance and concentration of vesicular bile. The first one is clear, yellow-gold and almost isotonic with blood, and the second is of brownish green (oxidized bilirubin), cloudy and filthy. Vesicular vesicle is more concentrated than the liver due to water reabsorption through the bladder wall.

INCREASE IN BILIRUBIN VALUES

Without entering into anatomical and physiological details and the claim of accurately discovering the causes that cause the increase of bilirubin values, applying filters such as the elimination of neo-natal casuistry, serious diseases (cancerous disease, cirrhosis etc), which have a very low incidence at the age of the students, we can focus on two main directions that could underpin the rise of increased bilirubin at our students:

1. Physiological-behavioural and social.
2. Inherited, triggering some hereditary disease syndromes.

Prior to the two directions, two further specifications are required:

• The triggering of the studied phenomenon belongs mainly to the age of adolescent-premature.

• In specialty work, the possibility of recording normal values occurs once in 3-5 laboratory tests, at intervals of at least three weeks, repeated bilirubin tests can be associated with other investigations, to determine normal hepatic functionality.

1. On the liver tract, intrahepatic and extrahepatic, gall bladder, biliary bladder, we narrowed down the multitude of possibilities with pathological manifestation (even anicteric, cholestasis mild hepatitis, confirmed by Dr. Anton).

However, it is worth mentioning that hyperbilirubinemia has been reported in a different institution, namely the "Football Academy" (according to A. Constantinescu – Fundeni University Clinic)

We noticed an increase of bilirubin in the cases of:

• Automedication (we advise that medication is administered and supervised by the medic):
- Paracetamol, even a slightly increased dose,
- Oral contraceptives,
- Consumption of food supliments, protein for muscle mass.
- Energy drinks, sweet juices, fast food
• Alimentation (canteen, home or other sources):
- Lack of food diversity
- Lack of lipids in the diet

The most important aspects of nutrition belong to food adjuvants mainly preservatives:

- Sodium metabisulphite (E223) in biscuits, cakes, fast food, juices, pickles, medications.
- Monosodium glutamate (called "good taste") is less directly incriminating, but it induces fatigue, headaches, sometimes aggravating breathing, causing stress. It is omnipresent in soups, sausages, vegetable preserves, flavors etc. This heat-treated (E621) adjuvant gives the browning reaction resulting in very toxic compounds.
- Sodium benzoate, preservative (E211).
- Carbon tetrachloride, hepatotoxic.

An important factor is "pernicious anemia" - ineffective erythropoiesis, lack of vitamin B12 due to lack of food, or more severe, due to the lack of intrinsic Castle's factor in gastric juice, necessary for absorption. B12 or perhaps stress-related somatostatin secretion that inhibits the secretion of the Castle's factor.

Sure, the theme is vast. Surveillance over time and complementing with specific analyzes, not just laboratory (CT, eco) will explain the causes.

2. On the track from the liver, intra-, extracapsal, bile duct to gall bladder several hereditary transmission syndromes can be manifested with precise localization and with a slightly variable pathological manifestation. They are triggered at adolescent age, according to the algorithm for the interpretation of the functional - liver test results (according to Henry J.B.). The most common genetic diseases are:

- *The Gilbert syndrome*

It is characterized by chronic, benign, intermittent, familial

hyperbilirubinemia found in a routine control. It is caused by a defect in the transport and conjugation of unconjugated bilirubin. The jaundice is accentuated in feverish states, physical exertion or consumption of oral contraceptives.

- *The Crigler-Najjar syndrome*

Type I may be a homozygous of Gilbert's disease. Type II is characterized by partial glucuronide-transferase deficiency that bilirubin conjugates to the liver cell. Usually at adolescents, complications are rare and occur in the case of poor eating habits or by excluding lipids from the diet.

- *The Dubin- Johnson syndrome*

It is characterized by the inability to transport bilirubinuriuronide from hepatic cells to the tubule while the conjugation is normal. The stress periods are a factor. The jaundice is harmless and reversible and occurs after eating estrogen and contraceptives. It can be confused with mild viral hepatitis.

- *The Rotor Syndrome*

It is asymptomatic, benign; withdrawal and storage of conjugated bilirubin defect, and possibly related to transfer from liver to bile. Usually it is reported in adolescents (medication, exercise-physical stress). Other liver functions are normal.

- *Epstein-Barr virus infections (human herpesvirus 4)*

It induces bilirubin increases in about 30% of the patients, but if liver function abnormalities are not found, another diagnosis should be sought.

CONCLUSIONS

After analyzing the studied material together with the medical laboratory specialists (Chem. Stanciulescu C., Synevo, Biochem. Apircioaei O., former SMC), the two physicians mentioned above, Dr. Stanciulescu P., internal specialist and I tried to highlight some aspects of hyperbilirubinemia:

- Self-medication - forbidden.
- Nutrition - the main recommendations:
- Regular fresh fruits and vegetables consumption
- Reduced fast food and unhealthy drinks consumption.
- Reducing fast at the first deviations from the normal functioning of the body, especially during the body-growing period.
- Elimination, at least limitation of stress, in all forms of manifestation: physical, food, intellectual, psychosocial.
- Avoid fatigue of any kind (not enough sleep at night)

These landmarks must be all considered, taking care of only one recommendation cannot bring immediate positive results.

As for hereditary transmission diseases, unfortunately, the possibilities of reaction are very limited in Gilbert and Crigler - Najjar syndromes, phenobarbital brings a certain decrease (Jacques Wallach, p. 341, confirmed by Dr. Anton) only under the supervision of the doctor. The most important factor is however a varied diet, avoiding starvation and tiredness.

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ORIGINAL ARTICLES

Antibiotherapy: latest current affairs – state of the art 2019

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Abstract: *Given the continuous growth of the bacterial resistance phenomenon, the appropriate choice of antibiotherapy represents an important step in the treatment and prophylaxis of infectious diseases. Depending on the symptomatology, the dose required for the effective treatment of an infection may vary. Inadequate dosing not only reduces the effectiveness of the antibiotic, but also promotes the emergence of resistant bacterial strains. Also, too high of a dose increases the toxic side effects of antibiotics. Therefore, a personalised antibiotherapy according to: the patient's clinical status, body mass, associated chronic diseases, etc. represents a promising approach in the treatment of infectious diseases.*

The purpose of this study is to present the most important current affairs regarding the emergence of new antibiotics, the maximum doses recommended by the latest version of the "European Committee on Antimicrobial Susceptibility Testing" (EUCAST - version 9/2019) and providing an indication on whether these antibiotics are used in Romania.

Keywords: *antibiotics, antibiotherapy, maximum recommended dose, antimicrobial chemotherapics*

INTRODUCTION

Antibacterial chemotherapics (antibiotics) have become the emblematic drugs of the 20th century starting with the first antibacterial chemotherapic, Salvarsan (invented by Paul Ehrlich, of German origin, in 1909) and the beta-lactamase Penicillin (discovered by the American Alexander Fleming, in 1929). Currently, there are hundreds of antibacterial antibiotics (AB), of which 269 have been approved for antibiotherapy (ABT). It was believed that bacterial diseases could be eradicated, but the practice has shown since the middle of the last century that the phenomenon of bacterial resistance to antibiotics emerges, that much higher doses are now needed, with modified conditioning and dosage, targeted use, in phases and combined or associated, even the use of new antibiotics, as far as they can be produced [1-7].

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ANTIBIOTHERAPY

Over time, the efficacy of ABT has been reduced, despite advances in medicine in general and pharmacology in particular. Resistant, multi-resistant and all-resistant bacteria create the most serious problems in medical practice and more and more patients die from infections or over-infections that do not respond to treatment. This worrying phenomenon has been thoroughly investigated, starting with the application manner of ABT. Thus, the website www.longitudeprize.org published in 2014 an international medical statistic [6] having as results the inappropriate use and prescription of ABs:

• 90% of the physicians prescribed AB under the pressure of the patient who wanted a certain AB (namely, "Customers is King?!");

•70% of the physicians prescribed AB without being sure

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that the etiology of the disease is bacterial or viral;

• 49% of the physicians prescribed AB for a week or more, without a prolonged treatment being needed;

•44% of the physicians prescribed AB only preventively, after discharge from surgery.

Following the highlight of the multiple factors that lead to the irrational use of ABs, medical and especially non-medical, in 2017 UN/WHO launched a new conception on prophylaxis and treatment – One health – and officially formulated recommendations for the Member States, with the goal of maintaining the efficiency of ABT worldwide. Based on these recommendations, each country sets its own research directions for medical and pharmaceutical scientific research. In this respect, a prize was established worldwide, Longitude Prize (2014). It has a prize pool of £ 10m to reward a competitor who will develop a test targeted on the rapid diagnosis of the diseases that require ABT. Through the rational use of ABs, the test would lead to the conservation of efficiency and shall revolutionise healthcare worldwide. The test should be accurate, fast, inexpensive, easy-to-use and accessible to anyone, anywhere in the world. By this test, shall be identified which ABs are needed and which shall be used. But these requirements seem utopian in the current stage of development of science and technology, it being unlikely to discover something that corresponds at least partially. Therefore, the prize has not been awarded so far.

Inventing new ABs is costly and difficult. These must meet divergent conditions: high toxicity for microorganisms (thus, antimicrobial efficacy), but little or no toxicity for the treated macroorganism; also, it must not have significant side effects; it must be produced industrially with reasonable costs; it must be stored and managed conveniently etc.

For the in vitro evaluation of the antibacterial effect of the AB, the Minimum Inhibitory Concentration (MIC) testing is performed on the respective bacterium (Breakpoint by determination in microdilutions in liquid culture medium) and the Kirby-Bauer diffusimetric antibiogram (ABG) with the measurement of the diameter of the Inhibition Zone (IZ) on the solid culture medium [2, 7]. One can choose from theoretically efficient ABs [1, 3, 4, 5] those that inhibit isolated and identified bacteria from the patient, by interpreting in the SIR system (Susceptible, Intermediate and Resistant) the diameter measured in millimeters, compared to EUCAST tables [2]. Because often the result obtained in vitro was between susceptible and resistant, this is noted as intermediate and for clinicians it was equivalent to resistant, because it would not have the desired therapeutic effect. In the current conditions of spread of the bacterial resistance phenomenon towards antibiotics, it is recommended to increase the dose of ABs, where the pharmacological properties allow, so that the bacteria pass from the intermediate to the susceptible area. By this approach of bacterial susceptibility, the intermediate bacteria pass from the resistant group to the susceptible group, and the SIR system gains another significance. The paraclinical findings become a practical indicator for the guidance of the clinician:
S = susceptible to standard dosing regimen (usual dosage)
I = intermediate, susceptible to high exposure (high doses, where possible)
R = resistant (therefore, useless for use).

As a result, the most handy method of combating bacterial resistance is the justified increase of the consumption of ABs, by increasing the single dose, daily or per cure, the frequency of administration or the duration of treatment, where the pharmacological characteristics allow (for example, in order not reach the toxic dose). The maximum recommended doses can be per dose, per day or per cure, and the cure can be short (1-5 days), medium (5-10 days), long (10-15 days) or very long (15-60 days). The doses are approximated depending on the conditioning of the drug (single antibiotic package or fixed combination), as well as the body mass of the patient. Any drug is theoretically dosed for an average patient, with a net body weight of 70 kg; so for a patient over 140 kg it should be double dose and for one below 35 kg it should be half dose. Some drugs, including ABs, are given by calculating in mg/kg body weight, especially in paediatrics, where children may have very different weights. When choosing ABs and in determining the dose, one must take into account, in addition to the pharmacological criteria imposed by antibiotherapy (which are well known), also: the general and particular contraindications, the age, the general patient status, the associated diseases, possible allergies or intolerances, the need for combinations with anti-beta lactamases or combinations with ABs or other anti-infective or adjuvant drugs. Consideration should also be given to possible antagonisms, inactivation with drugs and food, unwanted adverse or secondary phenomena as well as possible Herxheimer-type reactions. All this leads to a bi-unequivocal personalized ABT: depending on the needs and characteristics of the patient as well as the susceptibility of the pathogenic bacterium [8-13].

THE CLASSIC RECOMMENDATIONS

Professor Balș, the professional owner of the hospital that bears his name formulated the basic principles of antibiotherapy (also called antibiotic therapy) regarding: indications, contraindications, errors, associations of ABs,

which are still valid today. These principles have been applied in Romanian medical practice by Prof. Angelescu and his students, with undeniable successes. But at present, although all these principles have remained valid, their effectiveness in ABT practice is progressively diminished, due to the the spread of bacterial resistance to ABs, worldwide. As a result, we must complement these principles with new observations and discoveries in order to maintain the effectiveness of ABT. The most important news for ABT are not the new ABs, but the increased doses of those already known and their grouping by type of bacterial resistance.

MAXIMUM DOSES CURRENTLY RECOMMENDED

The new maximum doses are recommended by EUCAST 2019, p.96-99, systematized on AB groups, International Common Names (DCI) and with mentions for different clinical situations (2), and in the following we shall provide a summary, with the mention, „*" If used in Romania:

Penicillins: Benzylpenicillin* 1.2 g (2MU) x 4-6 iv, or 2.4 (4 MU) x 6 iv in meningitis and pneumonia; Ampicillin* 2 g x 4 iv, or 2 g x 6 iv in meningitis; Ampicillin+Sulbactam* 3 g x 4 iv; Amoxicllin* iv 2 g x 6 iv; Amoxicllin - oral 1 g x 3; Amoxicllin+clavulanic acid* iv 2.2 g x 3 iv; Amoxicllin+ clavulanic acid - oral 1 g x 3; Piperacillin* 4 g x 4 iv; Piperacillin+tazobactam* 4.5 x 4 iv; Ticarcillin* 3 g x 6 iv; Ticarcillin+clavulanic acid* 3.1 g x 6 iv; Phenoxymethyl-penicillin 2 g x 4 oral; Oxacillin* 1 g x 6 iv; Cloxacillin 1 g x 4 oral or 2 g x 6 iv; Dicloxacillin 2 g x 4 oral or 2 g x 6 iv; Flucloxacillin* 1 g x 4 oral or 2 g x 6 iv; Mecillinam 0.4 g x 3 oral.

Cephalosporins: Cefaclor* 0.25-1 g tidpo; Cefadroxil* 0.5-1 g bidpo; Cephalexin* 0.25-1 g bid-tid po; Cefazolin* 1 g tid-qid (or 2 g tid) iv; Cefepime* 2 g tid iv; Cefixime* 0.2- 0.4 g bid po, or 0.4 g po in gonorrhea; Cefotaxime* 2 g tid iv, or 2 g qid in meningitis; Cefpodoxime* 0.1-0.2 g bid po; Ceftaroline* 0.6 g tid iv (slow infusion – 2 hrs); Ceftazidime* 2 g tid iv or 1 g x 6 iv; Ceftazidime+avibactam* 2.5 g tidpev (2 hr infusion); Ceftibuten 0.4 g po; Ceftobiprole 0.5 g tid iv (slow infusion – 2 hrs); Ceftolozane+tazobactam* 1.5 g tid iv (slow infusion – 1 hr); Ceftriaxone* 2 g bid iv, or 4 g iv in meningitis; Cefuroxime* 1.5 g tid iv; Cefuroxime 0.5 g bid po.

Carbapenems: Ertapenem* 1 g x 1 iv; Imipenem* 1 g qid iv (30 mins perfusion); Meropenem* 2 g tid iv (3 hrs perfusion), or in meningitis 2 g tid iv; Meropenem+vaborbactam 4 g tid iv.

Monobactams: Aztreonam 2 g qid iv.

Fluoroquinolones: Ciprofloxacin* 0.75 g bid po, or 0.4 g tid iv; Levofloxacin* 0.5g bid po, or 0.5g bid iv; Moxifloxacin* 0.4g x 1 po, or 0.4g x 1 iv; Norfloxacin* 0.4g bid po; Ofloxacin* 0.4 g bid po, or 0.4 g bid iv.

Aminoglycosides: Amikacin* 30 mg/kg x 1 iv; Gentamicin* 7 mg/kg x 1 iv; Netilmycin* 7 mg/kg x 1 iv; Tobramycin* 7 mg/kg x 1 iv.

Glycopeptides and glycolipopeptides: Dalbavancin* 1 g x 1 iv; Oritavancin 1.2 g x 1 iv; Teicoplanin* 0.8 g x1 iv (single dose in slow infusion – 3 hrs); Telavancin 10 mg/kg x 1 (slow infusion – 1 hr); Vancomycin* 0.5 g qid iv, or 1 g bid iv, or 2 g x 1 in perfusion.

Macrolides, lincosamides and streptogramins: Azithromycin* 0.5 g x 1 po, or 0.5 g x 1 iv, or 2 g po in single dose; Clarithromycin* 0.5 g bid po; Erythromycin* 1 g qid, or 1 g qid iv; Roxithromycin 0.15 g bid po, Telithromycin 0.8 g x 1 oral; Clindamycin* 0.3 g qid po, or 0.9 g tid iv; Quinupristin+dalfopristin 7.5 mg/kg tid iv.

Tetracyclines: Doxycycline* 0.2 g x 1 po; Eravacycline 1 mg/kg bid iv; Minocycline* 0.1 g bid po; Tetracycline* 0.5 g qid po; Tigecycline*0.1 g followed by 50 mg bid iv.

Oxazolidinone: Linezolid* 0.6 g bid po, or 0.6 g bid iv; Tedizolid* 0.2 g x 1 po.

Other antibiotics: Chloramphenicol* 2 g qid po, or 2 g qid iv; Colistin* 4.5 MU bid iv; Daptomycin* 6 mg/kg x 1 iv; Fosfomycin* 8 g tid iv or 3 g x 1 po in a single dose; Fusidic acid* 0.5 g tid iv; Metronidazole* 0.5 g tid po, or 0.5 g tid iv; Nitrofurantoin* 50-100 mg tid-qid po; Nitroxoline* 0.25 g tid po; Rifampicin* 0.6 g bid po, or 0.6 g bid iv; Spectinomycin 2 g x 1 im; Trimethoprim 0.16 g bid po; Trimethoprim+ sulfamethoxazole* 1.44 g bid po, or 1.44 g bid iv.

CLINICAL GROUPING OF ABs

Changing the posology and increasing the doses of ABs, where necessary and possible, contributes to maintaining a reasonable susceptibility of pathogenic bacteria, maintaining the efficiency of ABT. Clinicians should rationally use ABs and shall apply, where appropriate, the new recommendations, which move the category of bacteria with intermediate sensitivity, from resistant to susceptible [2]. Consideration should also be given to the recent clinical classification of ABs in the three groups: for infections with common bacteria (Access group antibiotics), with resistant bacteria (Watch group antibiotics) and the reserve (Reserve group antibiotics), as well as special antibiotics (eg anti-tuberculosis, anti-leprosy), according to the WHO Model List Essential Medicines, no 20/2017 and 21/2019 [1]. The practical advantage for medicine is immense, but if in medical practice it is found that many clinicians still do not

follow the rules of good practice of ABT, action will have to be taken. One of them could be, in extremis, that according to the recommendations of WHO 2017 and 2019, ABs from the therapeutic groups should be prescribed according to the competence of the doctor:

ABs group I, usual, by any doctor with the right to free practice,

ABs group II, for resistant or multidrug-resistant bacteria, by specialized clinicians,

ABs group III, reserved, by primary care clinicians,

Special ABs, by the clinicians, according to their specialty.

Obviously, "off-label prescriptions" could represent the exception, in justified cases; As an example, it has been found that the antiparasitic Metronidazole is also the most effective antianaerobic AB.

There are also some ABs, which, although authorized, are so little used in national medical practice that they could be considered as new ABs. It is interesting to see how many new ABs will be authorized in the coming years. It is very important to discover new anti-infective drugs, both with new molecules, and by highlighting antibacterial and/or antibiofilm properties in pharmaceuticals known for other pharmacological effects. This type of applied research involves smaller investments, and the results can be faster, going up to the patenting of new innovative anti-infective drugs, in the near future.

CONCLUSIONS

Antibiotherapy is constantly dynamic, in order to keep up with the adaptation of pathogenic bacteria to the selection of pressure exerted by ABs, We must be aware of these trends, apply new acquisitions in the field of drugs, dosage conditioning and dosage, to help maintain an effective ABT, and abandon, where appropriate, the old methods and habits. Unfortunately, it is possible that some healthcare workers may continue to prescribe ABs, not for treating the patient, but for their own peace of mind and as well as their patients' or for any other reasons.

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CLINICAL PRACTICE

Clinicopathological evaluation of Moyamoya disease. Case report and review of literature

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Abstract: *Moyamoya disease is a rare affliction, which progressively affects cerebral arteries, more frequently those in the circle of Willis and the internal carotid arteries. This condition has a genetic component, but the cause of the disease is unknown. It usually affects children but can affect adults as well, representing a health issue due to its mortality. Not in a few cases, Moyamoya disease has been difficult to recognize as a cause of ischemic or hemorrhagic strokes. In this article, we present the case of a young patient, with a medical history of cerebral hemorrhage, which was diagnosed with Moyamoya disease after clinical and cerebral angiographic corroboration in a specialized vascular center. The patient entered the Emergency Receiving Unit with a sudden comatose state. Paraclinical tests revealed massive intraparenchymal hemorrhage, with movement in the medial line and ventricular flooding.*

Keywords: *Moyamoya disease, stroke, cerebral death*

INTRODUCTION

Moyamoya disease is a rare progressive blood vessel disease characterized by progressive stenosis of cerebral arteries, more frequently those in the circle of Willis and the internal carotid arteries. A collateral blood flow is formed around the blocked arteries to compensate for the lack of blood flow, but those collateral vessels are small and fragile, thus increasing the risk of bleeding, aneurysm and thrombosis [1].

The name of Moyamoya disease comes from the Japanese language, where it means "cloud of smoke". This disease was discovered in 1957 as an internal carotid artery hypoplasia of the tunica intima [2].

This condition has a genetic component, but the cause of the disease is unknown. Patients may present chorea, headache, seizures, or cognitive impairment expression of transient ischemic attacks or ischemic stroke and a large proportion of patients, mostly adults presenting with intracerebral hemorrhage. Two age peaks are recognized: one during childhood and one in young adults [1, 3]. In most regions, women are more frequently affected than men [3].

Although Moyamoya is a vascular disease, it is associated with other medical conditions like autoimmune disease, Down syndrome, arteriosclerosis, neurofibromatosis type 1 or other multiple tumors developing in the spinal cord [4, 5, 6].

In this article we present the case of a young patient, with a medical history of cerebral hemorrhage, who was diagnosed with Moyamoya disease after clinical and cerebral angiographic corroboration in a specialized vascular center and we review the literature on this disease.

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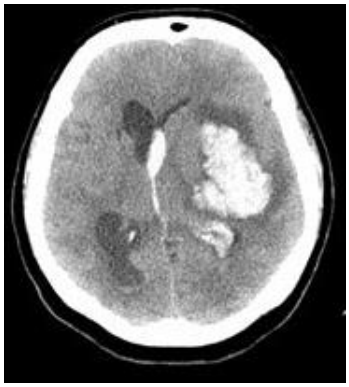
CASE PRESENTATION

We present the case of a patient aged 39, from an urban environment, known with having a medical history of cerebral hemorrhage in the previous year (twice in 6 months). The patient was investigated in a specialized vascular center in western Romania, where she was diagnosed with Moyamoya disease and was recommended a course of treatment. She is brought into the Emergency Clinical County Hospital in Oradea, Romania, with a sudden comatose state which had come on several hours before. On physical examination, neurological testing reveals asymmetric mydriasis, bilateral flexion in the plantar cutaneous reflex, symmetrical dropping of the arms and feet on the bed and a Glasgow score of 3. Other examinations (skin, extremities, abdomen, musculoskeletal system) were within normal limits.

A cranial CT scan shows massive localized hemorrhage in the left temporoparietal region, of 5.6/4/1 cm, with ventricular flooding in the third and lateral ventricles and mass effect with movement in medial line of 8 cm (Figure 1).

Figure 1: Cranial CT image shows the hemorrhagic accumulation with fresh blood; intraparenchymatous temporo-parietal densities with 5.6/4.1 cm axial diameters; slight perilesional edema, ventricular flooding in the bilateral lateral and third ventricles, and mass effect, the median line is shifted to the right by approximately 8 cm.



Laboratory results on admission showed hydro-mineral imbalance (hypokalemia and hypomagnesemia), which were corrected and remained within normal limits for the short duration of the patient's hospital visit. Complete blood cell count and examination of the peripheral smear showed normocytic anemia with normal leukocyte and platelet counts. Coagulation tests (D dimer, fibrinogen, partial thromboplastin time, prothrombin time, and thrombin time) were within normal limits.

The patient is then transferred to the Intensive Care Unit, where she receives orotracheal intubation and mechanical ventilation. Depletive and neurotrophic cerebral treatment is instituted, along with antibiotic therapy and acid-base and electrolytic balancing. The neurological state of the patient remains unchanged, with a Glasgow score of 3, fixed bilateral mydriasis and no cerebral reflexes. A control cranial CT scan is undertaken within another 48 hours, which shows the same collection of temporoparietal hemorrhage and no other modifications. The lateral ventricles have increased in size compared to the previous CT scan, with erased subarachnoid spaces (Figure 2). The suspicion of cerebral death is raised. After 72 hours, the suspicion of cerebral death was raised and the protocol in declaring cerebral death is followed, which is then confirmed by 2 EEG's, 2 apnea tests, ICU doctor, neurologist, neurosurgical assessment (Figure 3).

Figure 2: Cranial CT Image: the temporal-parietal intraparenchymal massive hemorrhagic left collection with ventricular intrusion in the right-side ventricle and ventricle 3 and relatively unchanged mass effect. The lateral ventricles have increased in dimension compared to the previous examination, bilaterally. Deleted subarachnoid spaces. Hypodensity of the cerebral parenchyma with deletion of the differentiation between the white subset and the newly developed gray substance which may suggest hypoxic ischaemia.

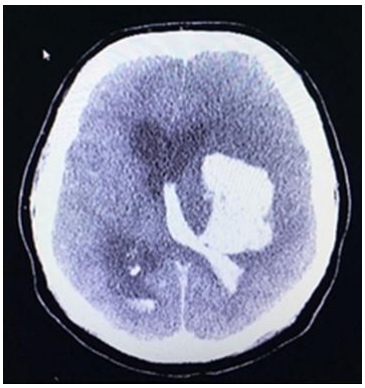
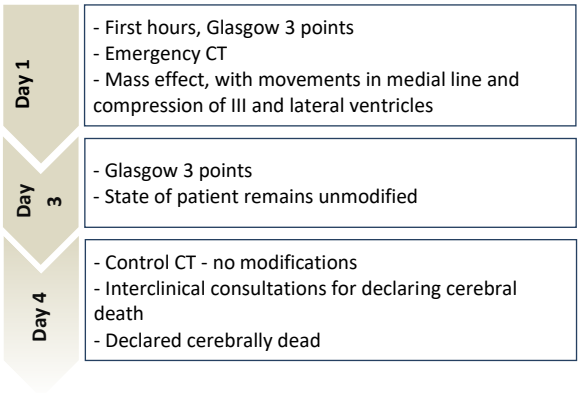


Figure 3: Evolution of patient



In the case we presented, the patient suffered a profound left cerebral vascular hemorrhagic accident, with three

ventricular flooding and mass effect, as well as movement in the medial line by 8 cm, which made her comatose. Despite proper treatment, no signs of improvement were recorded. The family grants permission for transplant and investigations begin to facilitate organ transplantation. In the subsequent necrotic examination, a massive 7/6/3 intraparenchymal hemorrhage is observed, with erased channels and flattened circumvolutions, as well as reddish cerebrospinal fluid.

DISCUSSIONS

Moyamoya disease is a rare pathology, but its seriousness needs to make us aware of the risks that can appear. This disease is a medical problem from a variety of standpoints because it can appear in both children and adults, it progressively affects cerebral arteries through stenosis and occlusion and can lead to cerebral vascular accidents or repetitive transient ischemic attacks, which can kill the patient by themselves. Usually, children present symptoms attributed to slowly progressive cerebral hypoperfusion and repetitive ischemic symptoms, while adults have hemorrhagic or ischemic events [7-8].

The cause of Moyamoya disease is unknown, but it is believed that there is a genetic component involved [9]. According to some studies, this disease could be autosomal dominant, with incomplete penetration, depending on age and genomic factors [10]. Another study shows that the RNF213 gene is the main gene associated with Moyamoya disease [11]. In some studies, arterial stenosis was found to occur in not only the intracranial arteries but also renal, pulmonary, coronary, and pancreatic arteries. Renal artery stenosis is the most frequently reported associated with Moyamoya disease and the consequence is renovascular hypertension [12].

The walls of blood vessels have three layers in their structure: tunica intima, media and adventitia. From a morpho-pathological point of view, Moyamoya disease is characterized by the thickening of the tunica intima in internal carotid arteries bilaterally, which, at this level, can contain lipid deposits. The most frequently affected arteries are the anterior, middle and posterior cerebral arteries, which form the circle of Willis and can present with different degrees of stenosis and occlusion because of thickening in the tunica intima, curling in the internal elastic lamina and thinning of the tunica media.

The transversal sectioning of a basal artery in a patient with Moyamoya disease, with hematoxylin-eosin staining shows fibrosis and thickening of the tunica intima and proliferation of the smooth muscle cells (white arrows), as well as the folding and chronic contraction of the internal elastic lamina (blue arrows) and absence of inflammatory or atherosclerotic changes [13].

The incidence of the disease is greater in children around the age of five and adults around the age of 40. The condition is more frequent in women than in men, with a higher incidence in Asian population and a lower one in the European population. Women can present with transient ischemic attacks or cerebral hemorrhages in their medical history, or they can be asymptomatic. The recurrence in cerebral vascular accidents can be higher in these patients [14]. Clinically, patients with Moyamoya disease may present seizures, transient ischemic attacks, ischemic and hemorrhagic strokes. Headache, speech disturbances, visual deficits, intellectual deterioration, cranial nerve paralysis can also be seen in both children and adults. Mental retardation can be seen in children having Moyamoya disease.

Moyamoya disease is an important diagnosis to consider in children or young adults presenting stroke. Cerebrospinal fluid results of a lumbar puncture or electro-encephalography can be abnormal but are usually nonspecific. This pathology can be most frequently diagnosed in a quick fashion by using modern cerebral imaging methods such as an angio-CT, angio-MRI or 3D-DSA (rotational digital subtraction angiography), in a disease that is sometimes asymptomatic. Knowing all the radiological signs have a major influence on placing early and correct diagnosis, which can lead to prompt treatment and a possible increase in life expectancy in these patients.

Unfortunately, the therapeutic options are limited or inappropriate. Treatment in the acute phase is symptomatic for maintaining cerebro-vascular perfusion. Anticoagulant and antiplatelet agents, corticosteroids have shown no remarkable benefit. Surgical treatment procedures have been used to manage the hemorrhagic and ischemic consequences of Moyamoya disease to restore and maintain adequate cerebral perfusion, but there are serious adverse effects including cerebrovascular infarct or hemorrhage, motor or sensory dysfunction, cognitive decline, and death [15].

CONCLUSION

This pathology needs to be taken seriously because it presents a significant risk factor in creating cerebral vascular accidents, both ischemic and hemorrhagic, in young adults. Surgery can be beneficial, particularly if the diagnosis of moyamoya disease is made early. In patients with a history of cerebral accidents, angio-CT or angio-MRI and/or obtaining a protein RNF213 dosage would be

recommendedto confirm or exclude Moyamoya disease in its early stages and institute proper specialized treatment.

Conflict of interests

None.

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CLINICAL PRACTICE

Elastofibroma – an uncommon cause of upper limbs pain

Natalia Dumitrescu[1], Bianca Sandu[2], Silviu V. Dumitrescu[2]

A female patient age 65 came to our clinic forcontinuous pain of the upper limbs (left more than right), increased when moving the scapulae. Patient declared that in the past few months the dorsal decubit became gradually painful and she cannot achive a good sleep. One year ago patient was diagnosed with bilateral suprascapular lipoma and a surgical removal was proposed.

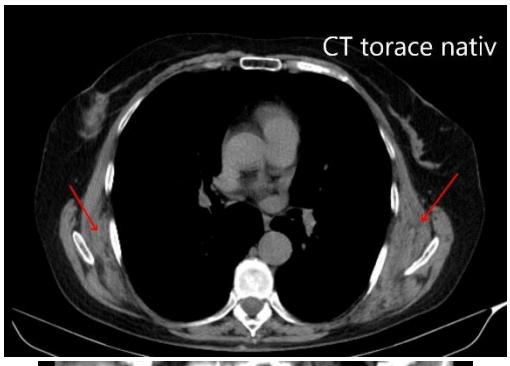
At the visual examination, we found two well-circumscribed suprascapular tumor mobile with arms movements. At palpation, we noticed that each scapula was pushed from below from an underlying tumor with a very firm consistency.

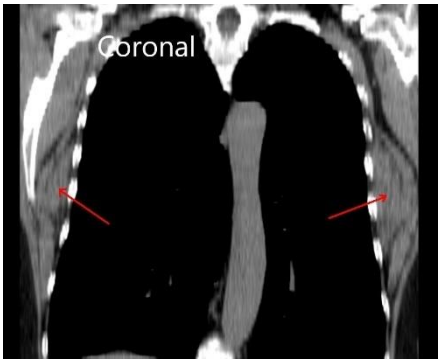
Figure 1: Clinical aspects

Chest radiography was normal.

A CT scan of torax was performed and revealed two subscapular unencapsulated, soft tissue masses with skeletal muscle attenuation interspersed with strands of fat attenuation [1, 2].

Figure 2: CT aspects





The patient was directed to thoracic surgery and the tumors were removed. The histological findings were consistent with the diagnosis of elastofibroma [3]. The recovery period was long, allmost two months passed before complete healing.

The long period needed for healing, usually burdened with local complications and the fact that the tumor is benign makes the surgery not recommendable for the patients with

no discomfort or impared arms movement [4].

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CLINICAL PRACTICE

Crossed renal ectopia

P. Bordei[1], I. Bulbuc[1], A.C. Rusali[1], C.R. Jecan[2,3], V. Ardeleanu[1,4,5,6]

Abstract: *The case of 73 years - old male patient with inferior crossed renal ectopia diagnosed upon imaging workup using a GE LightSpeed 16 Slice CT, with urothelial carcinoma, at the level of the left kidney, nephrolithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery. The upper lobe of the right kidney fused with the lower lobe of the left kidney, on the left side of the midline. There were 6 renal arteries (4 on the left and 2 on the right) originating from the left side of the abdominal aorta, at the level of the termination of the abdominal aorta, the left common iliac artery and the right internal iliac artery. The patient presented with 6 renal veins (3 on the left and 3 on the right) that drained anteriorly on the left side of the inferior vena cava into the left common iliac vein and into the right internal iliac vein.*

Keywords: *crossed renal ectopia; arteries – veins*

INTRODUCTION

Crossed ectopic kidney (CFE) is an anomaly within the fused kidneys (FKs) group of malformations, consisting of the fusing of the upper pole of one kidney with the opposite lower pole of the other kidney traversing the midline, with a reversed L-shaped view. [1] describes 6 types of fused kidneys (FKs). [2], quoting Sousa Escandon and Aguilar Tubet, mentions that the first case of crossed renal ectopia was described by Pamarolus in 1654 and the classification of fused kidneys was done in 1957 by McDonalds and McClellan. According to [3], most frequently, the left kidney is the one fusing on the right part, aspect met in the case described by [1]. In our case, as well as in the first two cases described by [4] and in the case described by [5], it was the right kidney that traversed the midline in order to fuse with the left kidney. Numerical variation are frequently met in crossed fused ectopia of the renal veins, comporting 1 to 6 arteries originating from the aorta or the iliac veins (common and internal), and one or more veins that terminate into the inferior vena cava or in the iliac veins (common or internal). The ureters usually do not present variants or anomalies, being just one for each kidney. The renal hilum is anterior or anteromedial. Frequently, crossed fused ectopia is asymptomatic [2, 3, 5, 6] and incidentally discovered during a consult for different other medical reasons, although cases of crossed fused ectopia may present a pathology associated with congenital malformations. As associated pathologies, we have : hematuria, dysuria, urinary tract infections, renal failure, nephrolithiasis, obstruction of the ureteropyelic junction, hydronephrosis, urinary stasis, ectopic ureterocele, urothelial carcinoma [2, 3, 5, 6, 7]. As for congenital malformations, we may encounter the following: cardiac malformation, skeletal-muscle malformation, genital malformations, gastrointestinal malformation [3, 6, 7, 8, 9]. The case presented in our review was diagnosed with urothelial carcinoma of the left kidney, nephrolithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery.

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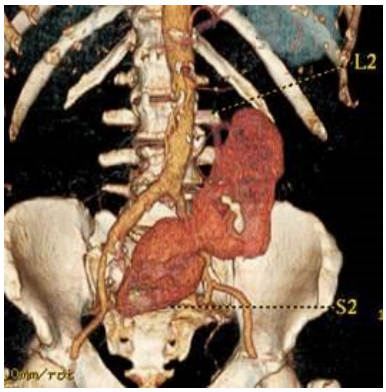
CASE STUDY

73-year old male patient, admitted at the Emergency Clinical County Hospital "Sf. Andrei" in Constanta, diagnosed with urothelial carcinoma of the left kidney, renal lithiasis and thrombosed aneurysm at the level of the origin of right common iliac artery. After the CT scan that was performed using GE LightSpeed 16 Slice CT, the patient was diagnosed with crossed renal ectopia. The right kidney crossed the midline – its upper pole fused with the lower pole of the left kidney, forming a 129,4^{o} angle, showing a reversed L-shape aspect. The diagnosis of inferior crossed renal ectopia – type D was made, according to the classification of [1].

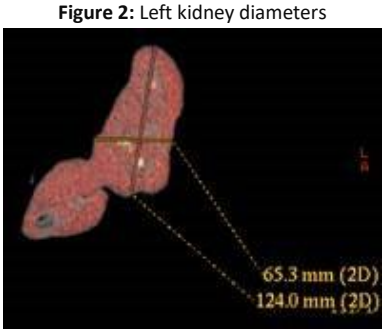
The left kidney was located vertically in the upper part and presented with the upper pole laterally to the costal processes of the L2 vertebra, covering the inferior top of the 12th rib. Its inferior pole reached the pelvis, at the level of inferior half of the S1 vertebra, at the level of sacral ala and the upper medial of the left iliac fossa. The renal isthmus, slightly oblique-inferior to the right, was located above the superior margin of the L5 vertebra and at the level of the L5 – S1 intervertebral disk and at the level of the left lateral part of the S1 vertebral body, at the level of the sacral ala. The inferior pole of the left kidney was positioned anteriorly to the sacral ala and also anteriorly to the upper medial part of the left iliac fossa. The left renal hilum was positioned anteriorly. The right kidney was horizontal (slightly oblique-lateroinferior to the right) with its superior pole corresponding to the inferolateral part of the L5 vertebral body, to the right lateral part of the L5 – S1 intervertebral disk and to the left sacral media ala, and also corresponding to the anterolateral sides of the sacral S1 – S2 vertebras. Its inferior pole overpassed the midline of the sacrum corresponding to the right lateral side of the S3 vertebra, above the anterior third sacral hole (Figure 1). The right hilum was positioned anteroinferiorly.

Figure 1: Our case of CFE in relation with vertebral column



The complex of the 2 kidneys had a longitudinal diameter of 190.6mm, a transverse diameter of 113.8mm and with a volume of 295,788 cm^3. The left kidney (Figure 2) had a bipolar diameter of 122.9mm, a transverse diameter of 65.3mm and an anteroposterior diameter of 36.5mm, with a parenchymal thickness of 20mm and a volume of 160 cm^3.

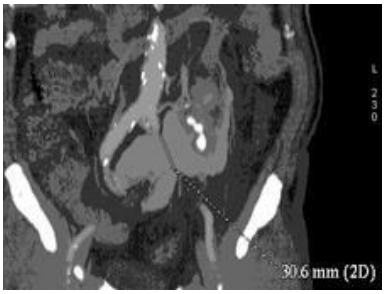
Figure 2: Left kidney diameters



The renal isthmus (Figures 3, 4) had an anteroposterior diameter of 18.1mm, a length of 31 mm, located next to the L4 – L5 intervertebral disk, 30 mm laterally to the midline.

Figure 3: Isthmus antero-posterior diameter (18.1 mm)

Figure 4: Isthmus lenght (30.6 mm)



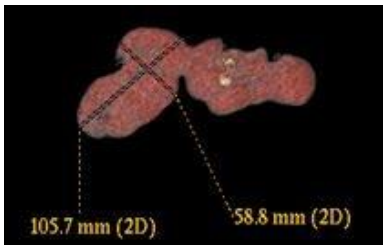
The right kidney (Figure 5) had a bipolar diameter of 105.7 mm, a transverse diameter of 58.8 mm, an anteroposterior diameter of 35.8 mm, with a parenchymal thickness of 20.3 mm and a volume of 136,295 cm^3.

The right ureter had a diameter of 6.0 mm and the left ureter presented with a diameter of 5.0 mm.

The 6 renal arteries of the cross fused ectopia (CFE) were

positioned one under the other, originating at the level of the abdominal aorta and at the level of the internal and common iliac arteries, each artery with its own vascularization territory.

Figure 5: Right kidney diameters



The first renal artery originated on the left side of the aorta, approximately 19mm inferiorly to the origin of the superior mesenteric artery, at the inferior 1/3 of the L1 vertebral body, with a diameter of 1.7 mm that penetrated the upper pole of the left kidney giving birth to a branch the descended anteriorly to the bassinet.

The second renal artery also originated from the left side of the aorta, approximately 55mm inferiorly to the first renal artery, next to the right upper part of the L3 vertebra, with a diameter of 4.3 mm penetrating the renal sinus from behind the bassinet of the left kidney.

The third renal artery originated at the level of the aortic termination, next to the L4 vertebral body, approximately 40 mm inferiorly to the second renal artery, with a diameter of 4.6 mm. It bifurcated into an anterior branch penetrating the renal sinus behind the bassinet and into a posterior branch penetrating the renal parenchyma of the inferior pole issuing a branch that penetrated the renal isthmus and branches for both renal poles.

The fourth artery originated in the left common iliac artery, approximately 60 mm from the bifurcation of the aorta, with a diameter of 2.6 mm. The artery bypasses the lower left pole of the kidney, penetrating the renal sinus anterior to the bassinet flowing into the inferior mesorenal.

The fifth renal artery originated in the right internal iliac artery, at the level of its origin, approximately 62mm from the bifurcation of the aorta, with a diameter of 6.8mm; it penetrated in the right renal sinus where it bifurcated into a prepyelic and retropyelic branches.

The sixth renal artery originated in the posterior trunk of the right internal iliac artery, approximately 62 mm inferiorly to the fifth renal artery, with a diameter of 2.2 mm, perforating the renal parenchyma next to the left inferior kidney.

The left testicular artery originated from the left side of the abdominal aorta, 28 mm under the first renal artery, next to the middle of the L2 vertebral body, with a diameter of 1.7mm and in its ascending route passing behind the left bassinet, issuing a branch for the tumor formation.

There were 6 renal veins (Figures 6, 7, 8), 3 veins for each kidney – for the left kidney we had V1, V2 and V4 and for the right kidney, we had V3, V5 and V6.

Figure 6: CFE 6 renal arteries (R1, R2, R3, R4, R5, R6) and vascular territories

Figure 7: CFE 6 renal veins (V1, V2, V3, V4, V5, V6)

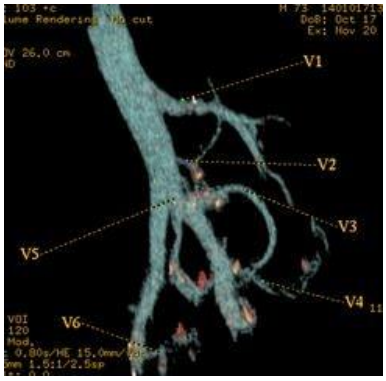


Figure 8: CFE veins and renal drainage territories

The first renal vein ended into the left side of the inferior vena cava, next to the L2 vertebra, it drained the left kidney,

with a retropyelic and retroaortic trajectory and with a diameter of 9.3 mm.

The second renal vein formed by the fusing of an anastomotic branch with the first renal vein with a left lumbar branch, also ending into the left side of the inferior vena cava, next to the L3 vertebra, with a retroaortic trajectory, draining the left kidney and having a diameter of 4.8 mm.

The third renal vein originated from the right renal sinus anterior to the bassinet (also draining the upper pole), ending into the left common iliac vein, 19 mm from the formation of the inferior vena cava, with a diameter of 8.7 mm, draining the right kidney.

The fourth renal vein originated from the left renal sinus next to the inferior pole, accompaning the fourth renal artery, ending into the left common iliac vein, approximately 68 mm from the formation of the inferior vena cava, with a diameter of 5.4 mm.

The fifth renal vein originated from the renal sinus on the posterior side of the right bassinet along with the fifth renal artery was bypassing anteriorly the right common iliac artery, ending on the anterior side of the inferior vena cava, approximately 10 mm above the IVC origin and approximately 22 mm inferiorly to the second renal vein, with a diameter of 8.3 mm.

The sixth renal vein originated from the right inferior pole along with the sixth renal artery, ending in the right internal iliac vein at 33 mm inferiorly to the formation of the right common iliac vein, with a diameter of 5.2 mm.

DISCUSSION

Within the fused kidneys (FKs) group of malformations, the most frequently met is the horseshoe kidney (HK), the crossed fused ectopia (CFE) being the second anomaly in terms of frequency. The horseshoe kidney has a bigger rate of incidence: 1/400 cases according to Lawsley, Eisendrath, Bauer (quoted by 10), 1/600 according to Moore, or 1/1,200 according to Rubin (both quoted by [4]). According to [7], CFE is a rare anomaly of the kidneys, with a prevalence rate of 1/1000 – 1/7500 of cases (Wilmer, Abeshouse, quoted by 10), although a renal anomaly, according to [3] and Bauer (quoted by [7]) incidence rates of 1/300 – 1/7500 of cases. [10] considers the ratio between the frequency of CFE/HK is of 1/7.7. CFE is most frequent in male cases, i.e. double the cases in relation to female cases [9], Bauer (quoted by [7]), 3/1 Boyan (quoted by [5]) or 2.3/1 according to Muttarak (quoted by [5]). [2] describes 3 cases of CFE, 2 of which are female cases and 1 male case.

[4] describes 2 cases of CFE: in one case he finds 6 right renal arteries (2 arteries originating from the lateral side of the aorta and were destined to the left kidney; 1 artery originating from the posterior side of the aorta, vascularizing the isthmus and the left inferior kidney; 1 artery originating at the level of the bifurcation of the aorta that penetrated the left kidney on the posterior side and issued a voluminous branch for the right kidney; the right kidney was vascularized by 2 arteries – one originating on the anterolateral side of the aorta and the other originating from the right common iliac artery). In this case, he finds only 3 renal veins (2 right renal veins and 1 left renal vein). In the second case, he also finds 6 renal arteries: 2 left renal arteries originating on the lateral side of the aorta and 4 right renal arteries - 3 originating on the lateral side of the aorta and one artery originating at the level of the terminal bifurcation of the aorta. In this case, he finds only 2 renal veins – right and left.

[5] describes the case of a CFE that presented 4 renal arteries – 1 artery for the superior renal parenchyma (left kidney) and 3 renal arteries for the inferior renal parenchyma (right kidney). De venous drain was composed of 2 renal veins.

According to Papin, quoted by [7], fused renal anomalies may present with 2 renal arteries (in 20% of the cases), 3-5 renal arteries (in 66% of the cases) and more than 5 renal arteries (in 14% of the cases).

[3] quoting Bauer and bailey, considers that the total number of the arteries for a crossed fused ectopic kidney varies from 1 to most frequently met cases ranging from 2 to 4 arteries; he discovered 3 renal arteries for the left kidney and 1 renal artery for the right kidney.

[6] describes a case of CFE that presented 2 renal arteries originating in the common iliac arteries.

[9] describes one single case presented with only 2 renal arteries (originating on the sides of the aorta), the right renal artery having a precaval trajectory and 2 renal veins – the left renal vein with a retroaortic trajectory.

The origin of the CFE arteries varies, usually the main arteries or in the case where there are only 2 renal arteries originating in the abdominal aorta. [6] finds the origin of the 2 renal arteries in the common iliac arteries.

We found that out of the 6 renal arteries, 2 renal arteries (R1 and R2) originated on the left side of the abdominal aorta, one renal artery originating at the level of the terminal ramification of the aorta (R3) and into the left internal iliac artery (R4), the last 2 arteries originating in the right internal iliac artery – 1 at the level of the unique trunk of the artery (R5) and the other at the level of the posterior trunk of the right internal iliac artery (R5).

[34] finds that most frequently the origin of the CFE arteries is at the level of the abdominal aorta (lateral right side or left side, posterior side and aortic bifurcation), considering the origin of the artery at the level of the right common iliac artery.

In the case of [5], he finds the origin of the 4 renal arteries only at the level of the lateral sides of the aorta – on the right side, 1 artery and on the left side, 3 arteries.

According to [3] the origin of the fused ectopic kidney is at the level of the superior abdominal aorta (in 25% of the cases), whereas for the rest of the cases, he considers the origin at the level of the inferior aorta and the iliac arteries.

The 6 arteries penetrated the kidney at its superior pole (R1), at the level of the renal hilum (R2, R4 and R5), or at the level of the inferior pole of the kidney (R6). R3 terminated into a bifurcation that issued a hilar branch and another branch that inserted in the kidney through its inferior pole. R2 penetrated the hilum posteriorly to the bassinet whereas R4 anteriorly to the bassinet and R5 issued one prepyelic branch and another retropyelic branch.

In two of his cases, [4] finds the CFE arteries penetrating the kidneys about the same levels as ours and he also describes a renal artery for the left kidney that bifurcated into one hilar branch and into an isthmic branch, whereas for the right kidney he finds 2 renal arteries that penetrated the kidney through its anterior side, respectively at the level of its posterior side.

The most voluminous of the arteries was the fifth renal artery that vascularized the right kidney and the less voluminous artery was the sixth renal artery also destined to the right kidney.

There was no renal artery for the renal isthmus. It receives a branch through an artery destined to the inferior pole of the left kidney or through an artery destined to the right kidney.

Out of the 6 renal veins of the CFE, 3 of them terminated in the inferior vena cava, 2 in the left common iliac vein and 1 in the right internal iliac vein. The veins of the left kidney left the kidney through the upper pole (V1) and through the renal hilum (V2 and V4) whereas the veins of the right kidney left the kidney through the renal hilum (V3 and V5) and through the lower pole of the kidney (V6). Out of the veins of the right kidney, the first 2 renal veins had a posterior trajectory to the bassinet, presenting also with a retroaortic trajectory, and out of the veins of the right kidney, the fifth renal vein had a posterior trajectory to the bassinet. The veins of the left kidney terminated on the left side of the inferior vena cava (V1 and V2) and into the left common iliac vein, and the veins of the right kidney terminated into the anterior side of the inferior vena cava (V5) into the left common iliac vein (V4) and in the right internal iliac vein (V6). The first renal vein (V1) was the most voluminous one and belonged to the left kidney, and the less voluminous one was the second vein and originated also from the left kidney. There was no vein to serve exclusively the renal isthmus. Out of the 3 veins of the left kidney only the renal vein had a retroaortic trajectory.

In one of the cases [4] described, he met 3 renal veins: 2 right renal veins – one collecting the blood at the level of the left pole of the left kidney and from the level of the isthmus and flew into the left internal iliac vein, and the other collecting the blood of the upper part of the isthmus ending in the inferior vena cava, above its termination into the cava of the left renal vein. The left renal vein was normally positioned but it received an anastomosis from the left common iliac vein. The other case of CFE of [4] presented a normal left renal vein and 2 right renal veins: one originated at the level of the left pole of the kidney and terminated into the inferior vena cava, 2 cm under the termination of the left renal vein and the left pole of the right kidney with the isthmus presented a vena that terminated in the left iliac vein.

In the case described by [5] there were only 2 renal veins: the upper renal parenchyma was vascularized by a vein that terminated in the left side of the inferior vena cava and the lower renal parenchyma was drained by the second renal vein that terminated also in the left side of the inferior vena cava.

CONCLUSIONS

The case we presented is special due to its uniqueness, as we have not found a similar case of crossed renal ectopia presented with 6 renal arteries (4 left and 2 right) and 6 renal veins (3 left and 3 right) in the specialist literature.

There was no artery – vein correspondence in all 6 cases, such a correspondence was met only at the level of the pole vessels and there was only one case at the level of the hilum vessels. Regarding arteries, the most voluminous was a hilum artery, whereas the veins; the most voluminous, was a superior polar one.

We consider MDCT examination to be the best method of describing vascular and uretheral abnormalities in case of crossed fused ectopia, being able to indentify also, other associated malformation, aspect noted also by [3, 5, 7].

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VARIA

The synthesis and in vitro testing of symmetric bisquaternary salts with pyridin-isonitrosoacetanilide structure, active in the exposure to neurotoxic chemical agents

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Abstract: *Neurotoxic chemical agents induce complex toxicological effects with major adverse effects for those who are exposed to them. Medical countermeasures include administering atropine, a cholinesterase reactivating oxime and an anticonvulsant. The purpose of this reseach is the optimization of an antidote-based treatment in the intoxication with organophosphates through the synthesis and toxicological testing of new compounds with a pyridin-isonitrosoacetanilide structure and their comparison with obidoxime as reference.*

The objective of this study was the in vitro/in vivo assessment of the antidote properties and acute toxicity of synthethic oximes active in the exposure to neuroparalytic organophosphates.

The CT50 value for obidoxime was estimated, through calculus, to be 3.24 mg/ml and 9.33 ×10 -6 M respectively. The CT50 value for Bis1,3[2-hydroxyimino-N-(pyridyl)acetamide]oxapropandichloride (HIN 2) was estimated to be 2.72 mg/ml and 6.29 ×10 -6 M respectively.

The studied compounds showed values close to cellular viability at low and moderate equimolecular concentrations, administered in vitro to a cellular culture of fibroblasts. Bis1,3[2-hydroxyimino-N-(piridyl)acetamide]oxapropandichloride (HIN 2) showed an in vitro cellular viability value smalled than obidoxime at higher equimolecular concentrations, proving itself to be more toxic than obidoxime at high concentrations. Taking into account the close values of CT50, the newly synthethized compound, having this particular characterization from a toxicological standpoint, qualifies for further studies as an antidote.

Keywords: *paraoxon, acetylcholinesterase, obidoxime, N-acetyl-2-hydroxyiminomethylpyridinbromide (PYC8); 1,3(2-hydroxyimino-N-pyridin-3-propan bromide (HIN1); 1,3(2-hydroxyimino-N-pyridin-3-oxapropanchloride (HIN2)*

INTRODUCTION

High risk organophosphates (sarin, soman, tabun) are extremely toxic substances with a phosphoric acid esther structure. Due to their extremely high toxicity, they were registered on the list of substances that require destruction according to international regulations (Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction – Geneva). Their production, stockpiling and use are monitored by international organizations (Organization for prohibition of chemical weapons – OPCW). [4, 7, 23].

Neuroparalytic chemical agents have the property of irreversibly inhibiting erythrocyte acetylcholinesterase (AChE). The coupling reaction of the organophosphate at the level of the active site of the enzyme is shown in Figures 1, 2. [23]

The inhibition of AChE leads to the acummulation of acetylcholine (ACh) at the level of the synaptic cleft and the hyperstimulation of muscarinic and nicotinic receptors. The

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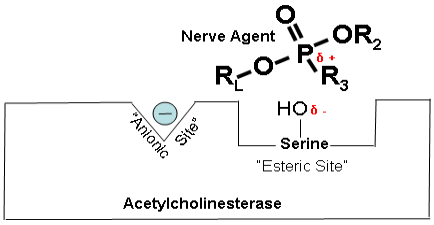
clinical signs of mild intoxication are pupil constriction, sweating, muscular fasciculation, and the decrease of cardiac frequency and of arterial tension.

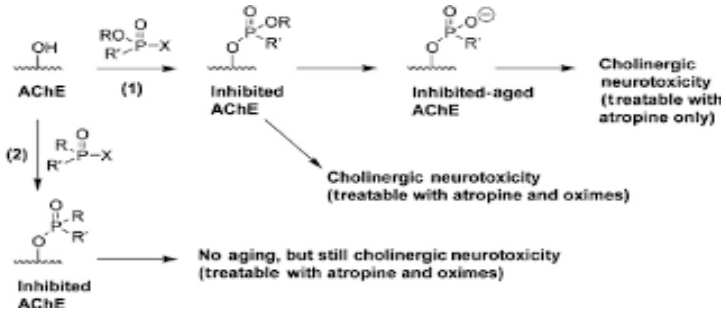
Figure 1: The coupling mechanism of the organophosphate at the level of the active site of AchE



In high concentrations of the toxic compounds, convulsions occur and death takes place through respiratory insufficiency caused by the paralysis of respiratory centers. Cholinesterase-inhibiting neurotoxic organophospates possess, in their molecule, two organic groups, identical or different, and an active group that is inorganic or organic, bound to a pentavalent phosphorus atom. This atom can be bound to an oxygen or sulphur atom (phosphates, phosphonates, tiophosphates, tiophosphonates). [1, 4, 9, 23] In scientific literature, they were presented as potential simulants for each class or warfare chemical agents [4, 16, 22].

The laboratory use of neurotoxic chemical agents is restricted, as they are extremely toxic substances. Thus, antidote studies and those refering to their pharmacotoxicological properties are performed utilizing simulants of the paraoxon type. A simulant is defined as a compound possessing all relevant physical and chemical properties of the corresponding neurotoxic chemical agent, but with lower intrinsical toxicity; The utilization of simlants in research and testing activities contributes to the reduction of the stress and risk the personnel involved in the testing procedures is subjected to by handling chemical agents with high toxicity. [1, 23]

Figure 2: The interaction between AchE and the organophosphate



The general emergency measures necessary to stop the exposure are masks, decontamination and the evacuation of the individual from a contaminated environment. Medical countermeasures are the administration of atropine, of a cholinesterase reactivating oxime and of an anticonvulsant. The increase of antidote treatment efficiency in the intoxication with organophosphates is one of the main objectives of research activity at national and international level, taking into account both aspects of clinical toxicology of organophosphates used as pesticides and of experimental toxicological research of neuroparalytic war chemical agents. [4, 8, 12, 16, 19] Cholinesterase-reactivating compounds used in the UN member states (pralidoxime, obidoxime, HI6) possess therapeutic efficiency for different neuroparalytic chemical agents [1, 2, 3, 5, 11, 15, 17, 22]. Having quaternary and bisquaternary salts chemical structures with pyridinic rings, this substances are known and studied as efficient antidotes in the processes of activation-reactivation of acetylcholinesterase in the intoxication with organophosphates [6, 10, 22].

Bisquaternary compounds such as Hagedorn, HI-6, Obidoxime, are obtained through the quaternization of pyridine bases substituted (oximes, carboxamides) with reactive dihalogenated derivatives such as symmetric dichloromethylether (DCDMS) or 1,3-dibromopropane [6, 14, 22]. Symmetric quaternary salts obtained through the quaternization of pyridine substituted with oxime or amide groups in a 2:1 molar ratio to the dihalogenated derivate are intensely studied. The typical example is represented by obidoxime, the product of 4-pyridinaldoxime bisquaternization with exces symmetric dichlorodimethylether. This compound is easily obtained and has good stability. [6, 7, 10, 12, 14, 22].

Through the monoquaternization of a pyridine base or of another cyclic tertiary amine with DCDMS or 1,3-dibromopropane in excess, the monoquaternary salt is obtained, usually unisolable that, in the next step, can be quaternized with another different base, obtaining mixed products with an antidote efficiency that is higher and a

reduced stability. In (bis)quaternization, tertiary bases that can be utilized are pyridines which are monosubstituted with aldoxime or carboxamide groups in all three positions, 1,3-N-methyl-2-imidazolilaldoxime or quinuclidinic bases: 3-quinuclidinonoxime, 3-quinuclidinyl-N,N-dimethylaminocarbamate etc, which, theoretically, through combination with dihalogenate derivatives cand lead to a large variety of antidotes [6, 14, 19, 20].

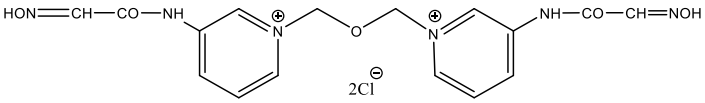
The present paper proposes the synthesis and characterization of new symmetric bisquaternization compounds with 3-pyridinylisonitroso-acetanilide as a precursor, the quaternization agents being symmetric dichlorodimethylether and 1,3-dibromopropane. These compounds are PAM-2 analoges, N-Methyl-2-hydroximinomethyl pyridine chloride being utilized for the first time as an antidote in the intoxications with neurotoxic organophosphates. [14, 19, 21]

MATERIAL AND METHOD

The reactivating potential of two oxime compounds with pyridin-isonitrosoacetanilide structure was tested in vitro: 1,3(2-hydroxyimino-N-pyridin-3-propane bromide (HIN1); 1,3(2-hydroxyimino-N-pyridin-3-oxapropanchloride (HIN2). The HIN 1 compound did not disolve in the culture medium. The acute toxicity of obidoxime and of HIN 2 was assessed through the determination of cellular viability the two produced on cell cultures from 3T3 fibroblast lines. The cells were incubated for 24 hours at concentrations of 1 mg/ml, 3 mg/ml, and 5 mg/ml and optical density was read at 490 nm.

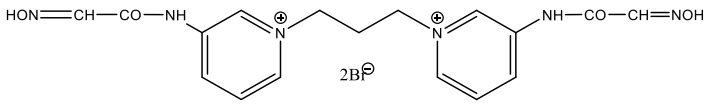
For this purpose, two compounds with pyridinisonitroso-acetanilide structure were tested, compounds considered active in the intoxication with neuroparalytic chemical agents due to the presence in their molecule of the oxime group R=N-OH, as well as of the pyridine structure resembling obidoxime. (Figure 3)

Figure 3: Chemical structures of newly synthethized compounds



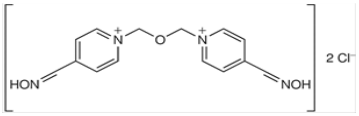
$C_{16}H_{18}Cl_2N_6O_5$
Masa Moleculara; 445,26

bis 1,3[2-hidroxiimino-N-(piridin-3-il)] oxapropandiclorura



$C_{17}H_{20}Br_2N_6O_4$
Masa Moleculara: 532,19

bis 1,3[2-hidroximino-N(piridin-3-il)]propandiona



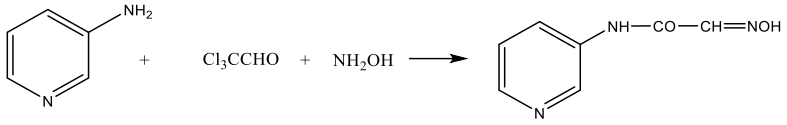
$C_{14}H_{16}N_4O_3Cl_2$
1,1-bis-metilen-bis-hidroxiiminometilpiridinium
Molecular mass 358,75

The synthesis of new symmetric pyridin-isonitroso-acetanilide bisquaternary salts with cholinesterase reactivation properties

The sythesis of the isonitrosoacetanilide of 3-aminopyridine named 2-(hydroxyimino)-N-(pyridin-3-yl) acetamide.

The isonitrosoacetanilide of 3-aminopyridine is obtained from 3-aminopyridine, chloral hydrate and hydroxylamine chlorohydrate in a saturated solution of sodium sulphate according to the following reaction in Figure4.

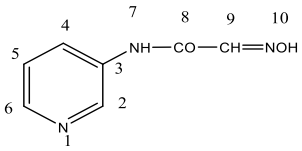
Figure 4: The synthesis of 2-(hydroxyimino)-N-(pyridin-3-yl) acetamide.



In a 2L balloon with efficient mechanical agitation, around 400ml of water are introduced. Under agitation, the sodium sulphate is brought in portions, and the chloral hydrate is added to the saturated solution within 2-5m. Seperately, the amine chlorhydrate is prepared in an aqueous solution of amine, 10ml HCl 35 and 20ml water. It is added under agitation to the previously prepared solution within 5m. Then, it is heated at 30-40^{0}C, and the hydroxylamine chlorhydrate is added in small portions after being dissolved in 50ml of water. The temperature is steadily increased to 80-90^{0}C within 1h, followed by boiling for another 15m. Then it is left to cool down at room temperature overnight and the sodium sulphate is filtered. The sodium sulphate is then washed twice with 50ml of water each time, and the filtered solution is added to the previous one. The reunited filtered solutions are neutralized with saturated ammonia solutions and are cooled down to 0-5 degrees. The precipitate is filtered, and the resulted solution undergoes extraction three times with 150ml methylen chloride each time. Through distillation, the solvent is removed, and the residue is recrystallized from methanol (through boiling with animal charcoal). The isonitrosoderivative is obtained with a yield of 65-70 %.

The compound was characterized through spectral RMN analyses, hydrogen and carbon spectrums which confirm the structure(Figure 5).

Figure 5



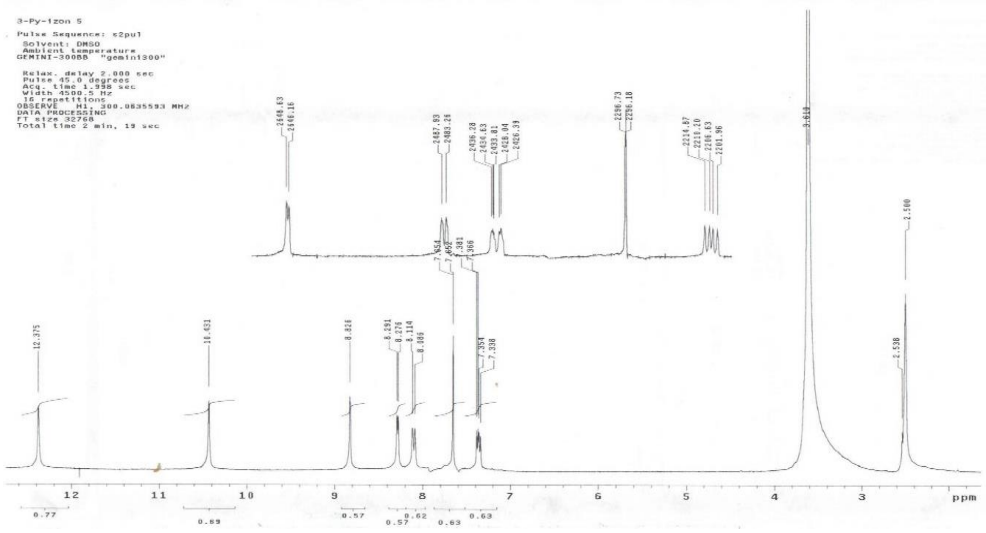
RMN characterization

1H-RMN – 1H-RMN (DMSO-d6, δ ppm, J, Hz) spectrum: 10.32(s, 1H, H-10); 8.85(dl, 2.6, 1H, H2); 8.29(dd, 4.4, 1.3, 1H, H-6); 8.12(dl, 4.3, 1H, H-4) (Figure 13).

Quaternization with symmetric dichlorodimethylether (DCMS) to bis1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamidî oxapropandichloride

The symmetric quaternization reaction of the isonitrosoacetanilide of aminopyridine with dichloroether in a 2:1 ratio is performed in anhydrous dimethylformamide at temperatures of 30-40^{0}C. The quaternization agent, symmetric dichlorodimethylether, is obtained from paraformaldehide and thionyl chloride (Figure 6).

Figure 6: Isonitrosoacetanilide 3-aminopyridine –H-RMN spectrum



13C-RMN – 13C-RMN (DMSO-d6, δ ppm) spectrum: 160.91(C-8); 144.77(C-9); 143.80(C-6); 141.60(C-2); 135.22_C-3); 127.04(C-4); 123.69(C-5).

In an Erlenmayer, dimethylformamide and isonitroso-acetanilide are introduced. They are magnetically agitated to complete dissolution, and then dichlorodimethylether that was previously dissolved in 5ml of anhydrous

dimethylformamide is added. The recipient is then sealed and agitated for 10h at room temperature and, finally, another 5h at 30-40^{0}C. It is cooled at 0-5^{0}C for 3h and is then filtered through vacuum filtering. The precipitate is repeteadly washed with anhydrous acetone and then dried in a vacuum, in the desiccator. 2.3 g of white product are obtained with m.p=145-150^{0}C, which corresponds to a yield of 52%. The synthethized compound, the bisquaternary salt, was characterized through spectral RMN and proton and carbon spectrum analyses which confirm its structure.

Figure 7: Isonitrosoacetanilide 3-aminopyridine - C- RMN spectrum

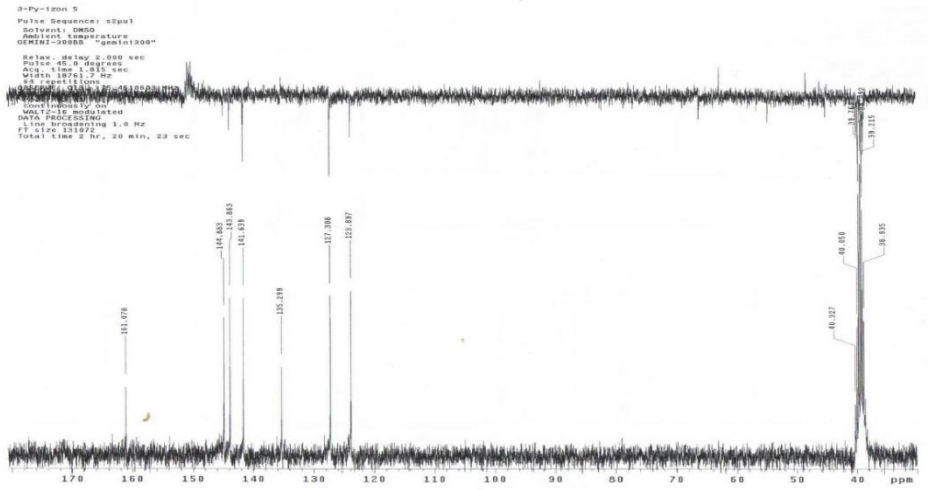
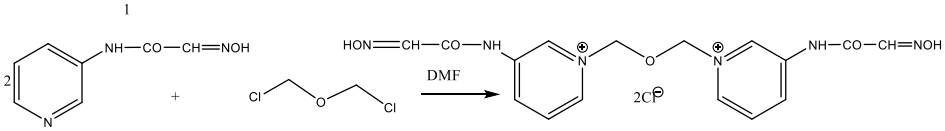
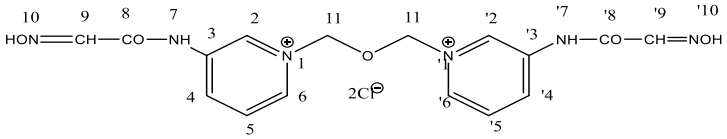


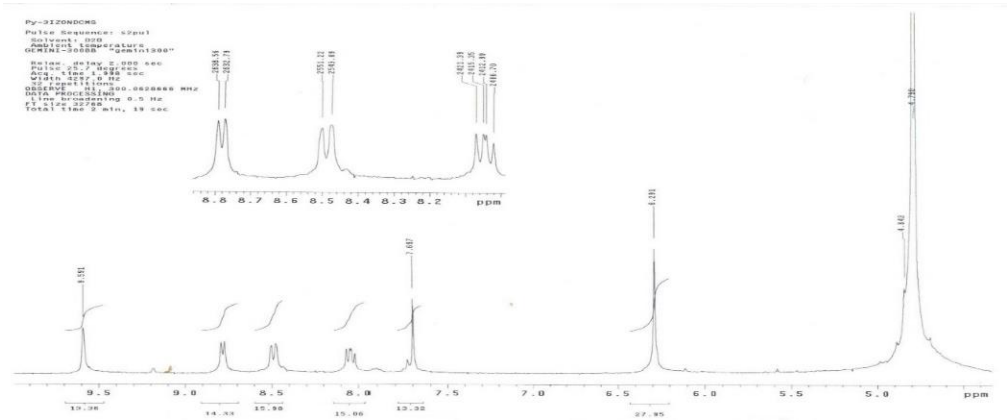
Figure 8: The synthesis of bis1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride



RMN characterization of the 1H-RMN hydrogen spectrum is shown in Figure 9 and the13C-RMN carbon spectrum is shown in Figure 10.

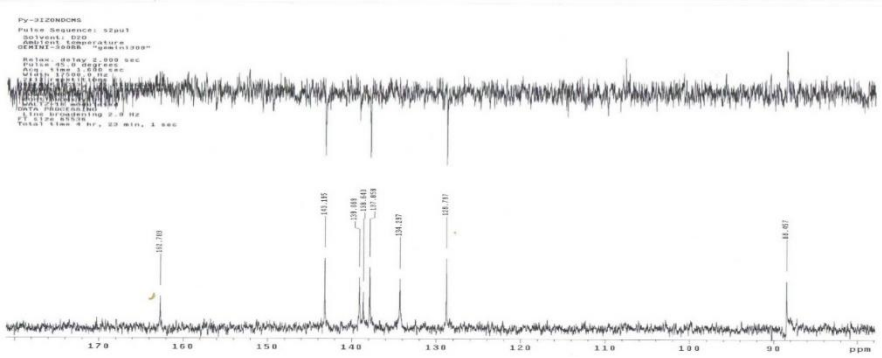
Figure 9: Bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride compounds - 1H-RMN spectrum





1H-RMN (D2O, δ ppm, J, Hz) hydrogen spectrum: 9.58(sl, 2H, H-2, H-2'), 8.77(dl, 6.0, 2H, H-6, H-6'), 8.48(dl, 9.7); 8.04(dd, 8.7, 6.3, 2H, H-6, H-6'), 6.28 (s, 4H, H-11, H-11')

Figure 10: Bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]oxapropandichloride compound - C-RMN spectrum

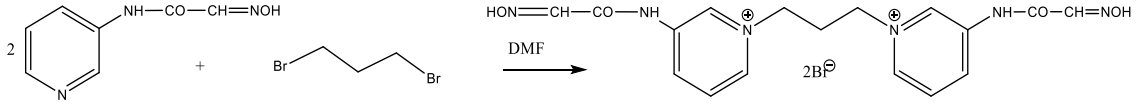


3C-RMN (D2O, δ ppm) carbon spectrum: 162.69(C-8, C-8'), 143.18(C-9, C-9'), 139.08(C-6, C-6'), 138.69(C-2, C-2'), 137.84 (C-4, C-4'); 134.30(C-3, C-3'); 128.79(C-5, C-5'); 88.48(C-11, C-11')

Quaternization with 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide.

The symmetric quaternization reaction of 3-pyridyliso-nitrosoacetanilide with 1,3-dibromopropane in a 2:1 ratio goes as described below and is also performed in anhydrous DMF DMF, at a temperature of 40-55^0 C (Figure 11).

Figure 11: The synthesis of bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propane dibromide



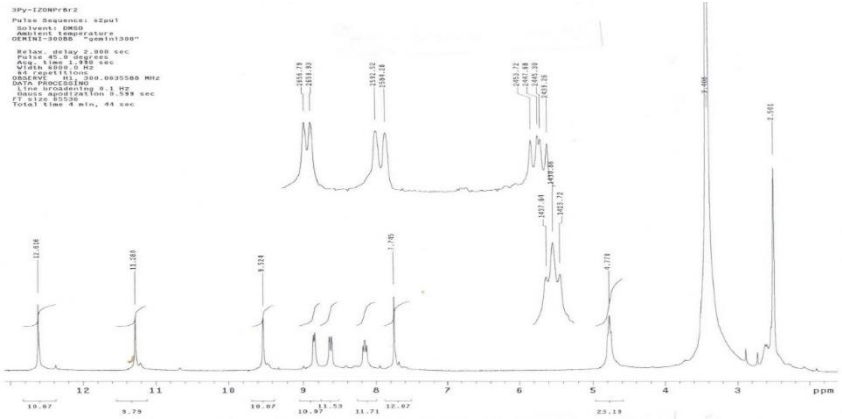
In an Erlenmayer glass, dimethylformamide and isonitrosoacetanilide are introduced. They are magnetically agitated to complete dissolution, then 1,3-dibromopropane that was previously dissolved in 5ml of anhydrous dimethylformamide is added. The recipient is sealed and agitated for 4h at room temperature and for another 10h at 40-60^{0}C. It is cooled down to 0-5^{0}C, and then vacuum filtered. The precipitate is repeteadly washed on the filter, with anhydrous acetone. It is dried in a desiccator in a vacuum overnight. 3.1 g of white crystal product are obtained with mp.180-182^{0}C and a yield of 59.0%.

The synthethized compound, the bisquaternary salt, was characterized through spectral RMN analyses, which confirm its chemical structure. (Figures 12, 13)

RMN characterization

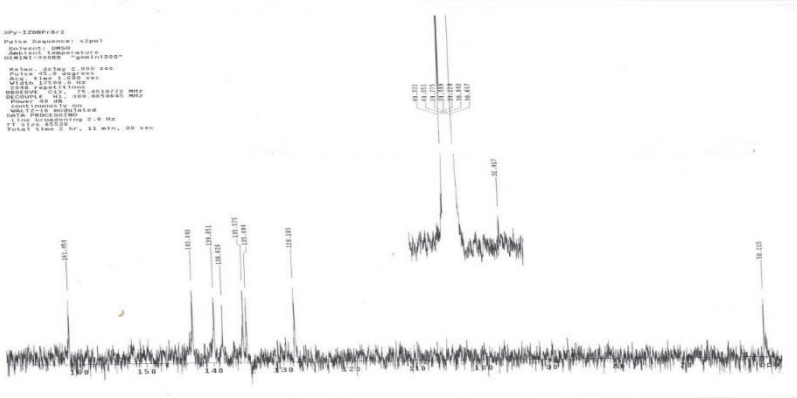
The 1H-RMN hydrogen spectrum is shownin Figure 12and the 13C-RMN carbon spectrum is shownin Figure 18.

Figure 12: 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide - H-RMN spectrum



1H-RMN (DMSO-d6, δ ppm, J Hz) spectrum: 10.52 (s, 2H, H-7, H-7); 8.8 (dl, 2.4, 2H, H'-2, H-2); 8.3(dd, 5.0; 1.6, 2H, H-6, H-6); 8.1 (dl, 8.4, 2H, H-4,H-4)', 7.68(s, 2H, H-9, H-9'); 7.43(dd, 8.4, 4.7, 2H, H-5, H-5'), 4.71(t, 7.1, 4H, H-11, H-11'); 2.48(t, 7.1, 2H, H-12)

Figure 13: 1,3 dibromopropane to bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propan dibromide - C-RMN spectrum



13C-RMN (DMSO-d6, δ ppm) carbon spectrum: 160.84 (C-8, C-8'); 143.97 (C-6, C-6'); 143.64 (C-2, C-2'); 140.70(C-9, C-9'), 135.36 (C-3, C-3'); 127.69(C-4, C-4'); 123.95(C-5, C-5'), 59.11(C-11, C-11'); 33.13(C12)

In vitro testing and toxicological characterization of symmetric bisquaternar compounds with a pyridin-isonitrosoacetanilide structure

The experiment was performed in sterile conditions in a microbiological niche.

In vitro testing incldued the two synthethized compounds: bis 1,3[2-hydroxyimino-N-(pyridin-3-yl)acetamid]propane dibromide (coded under the name of HIN 1) and bis 1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2), utilizing obidoxime as reference. Each of these compounds was dissolved in a culture medium, and 3 solutions were obtained for each, with the following concentration: 5mg/ml; 3 mg/ml and 1 mg/ml. HIN 1 was insoluble in the culture medium at the above mentioned concentration.

On a 3T3 cellular line (fibroblasts) from Lonza from which a cellular subculture with a concentration of 1×10^5 cells /ml was obtained as follows:

- the cells were counted initially with the Burger Turck hemacytometer to establish the initial concentration of cells per ml;

- the dilution was performed so that a final concentration of 105 cells/ml would be obtained;

- the cells were cultivated with a proper medium (supplemented DMEM, Lonza reactive) for 24 hours at a temperature of 37 degree Celsius in a CO_2 atmosphere;

- 2 ml of tripsine solution are added (Sigma reactive);

- 5 minutes of centrifugation at 500 G at a temperature of 5-8 degrees Celsius;

- the supernatant is removed;

- resuspension in 5 ml of culture medium (DMEM);

- a volume of 200 microliters is placed in a plate with 96 wells;

- 6 h of incubation in CO_2 atmosphere for adherence;

- after 6 h of incubation for adherence, obidoxime and HIN2 are added after dissolution in the culture medium, equimolecularly, in 3 concentrations: 5 mg/ml, 3 mg/ml and 1 mg/ml;

- 24 h of incubation;

- after 24 h of incubation, 20 microliters of MTS reactive (Lonza) are added to each well;

- 4 h of incubation at 37 degrees Celsius in a CO_2 atmosphere.

The samples were read at 490 nm with a Biorad microplate reader and compared to the control sample represented by the culture medium.

RESULTS

The in vitro toxicity of HIN 2 and obidoxime was assessed through the values of their optical densities corresponding to different molar concentrations. The results are shown in Table 1 and in Figures 14 and 15.

The molar concentrations of studied compounds can be correlated with the cellular viability percentage.

The results are shown in Table 2 and in Figures 16 and 17.

In lower concentrations of 1 mg/ml, values that were close to the in vitro cellular viability were recorded (73.43% for obidoxime, and 82.42% for HIN 2).

For medium molar concentrations (3 mg/ml), cellular

viability values were 67.75% for HIN 2 and 67.36% for obidoxime.

Table 1: The variations of optical density values of obidoxime and HIN 2

	Well1	Well2	Well3	Mean	Substraction blank
Untreated CTRL	1.068	1.112	1.67	1.283333±0.174	1.16083
Obi 5mg/ml	0.362	0.353	0.449	0.388±0.02	0.2655
Obi 1mg/ml	0.875	1.075		0.975±0.025	0.8525
Obi 3mg/ml	0.837	0.972		0.904 ±0.033	0.782
HIN 2 5mg/ml	0.141	0.149	0.152	0.147±0.012	0.0248
HIN2 1mg/ml	0.895	1.078	1.265	1.079±0.030	0.9568
HIN2 3mg/ml	0.849	0.969		0.9090±021	0.7865
CTRL BLANK	0.122	0.123		0.1225	0

Figure 14: Variation of optical density (DO) correlated with concentrations of obidoxime

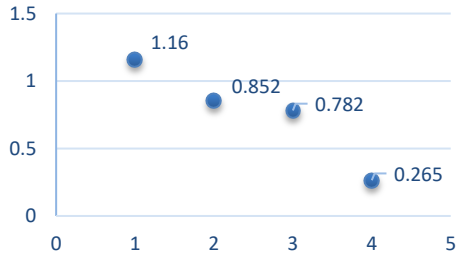


Figure 15: Variation of optical density (DO) correlated with concentrations of HIN 2

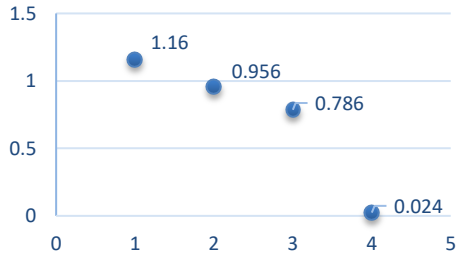


Table 2: Percetange variation of cellular viability for obidoxime and HIN 2

Dose mg/ml	DO Obidox	Cellular viability vs control (%)	DO HIN2	Cellular viability percentage vs control (%)
0	1.160833	100	1.160833	100
5	0.2655	22.8715	0.024833	2.139268
1	0.8525	73.43862	0.956833	82.42642
3	0.782	67.3654	0.7865	67.75305

For high doses, (5 mg/ml) HIN 2 showed a cellular viability value of 2.13%, much lower than that of obidoxime, and 22.87% respectively. Thus, HIN2 is more toxic than obidoxime.

Figure 16: Cellular viability percentage corresponding to different concentrations of obidoxime (y = -13.873 + 97.379)

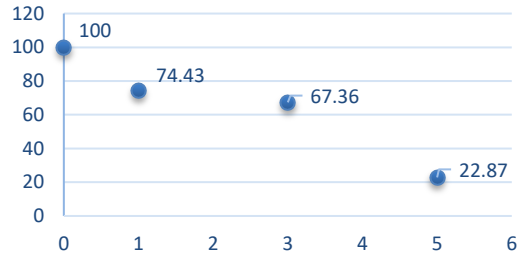
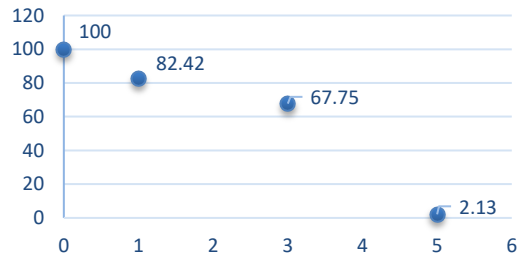


Figure 17: Cellular viability percentage corresponding to different concentrations of HIN 2 (y = -18.397 + 104.47)



The assessment of CT50 was performed according to the working protocol described in the Romanian Pharmacopoeia.

In the regression line ecuations shown in Figures 14 and 15, the y value was replaced with the probit corresponding to the ideal mortality of 50 %. The probit is a statistical measure resulted from the transformation of the percentage of positive answer into a numeric value and is linearly dependent on the logarithm of the concentration. Thus, the x value in the ecuation of the regression line represents the logarithm of CT50. Through calculus, we could estimate CT50 for obidoxime at a value of 3.24 ± 0.005 mg/ml. The CT50 value for HIN 2 was estimated at 2.72 ± 0.009 mg/ml. The CT50 value being inversely proportional to the toxicity of the

substance, it can be understood that the CT50 values are comparable (around the value of 3 mg/ml), HIN 2 being more toxic than obidoxime.

The statistical analysis of experimental results obtained showed statistically significant variations between the determined cellular viabilities of the two compounds in equimolecular concentrations only for high doses. The experimental results are represented in Tables 3-5.

Table 3: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 1 mg/ml (2.29×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	2.29×10^{-6} M;	1.160833333	0.276632	P ≥0,05 – mean values do not significantly differ
2	HIN 2	2.29×10^{-6} M;	0.956833333		

Table 4: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 3 mg/ml (6.94×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	6.94×10^{-6} M;	0.7820	0.4820	P ≥0,05 - mean values do not significantly differ
2	HIN 2	6.94×10^{-6} M;	0.7865		

Table 5: The representation of probability P associated with mean values of optical density (OD) correlated with cellular viability corresponding to a concentration of 5 mg/ml (11.57×10^{-6} M)

No.	Compound	Adm. Molar Conc.	Mean value of OD	Probability (P)	Observations
1	Obidoxime	11.57×10^{-6} M;	0.2655	0.000723	P≤0,05 – mean values significantly differ
2	HIN 2	11.57×10^{-6} M;	0.0248		

CONCLUSIONS

The syntheses took into consideration the principle of obtaining a cholinesterase reactivating chemical compound that is stable, with a possible action at central nervous level, which could prove to be an alternative therapy to obidoxime, being usable in antidote studies.

Following solubility tests, only one compound, bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2) qualified for in vitro testing, the other being insoluble.

The CT50 value for obidoxime was estimated through calculus to be 3.24 ± 0.005 mg/ml, or 9.33×10^{-6} M;

The CT50 value for bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride (HIN 2) was estimated to be 2.72± 0.009 mg/ml or 6.29×10^{-6} M;

The in vitro tested compounds showed values close to cellular viability in low and moderate equimolecular concentrations in the fibroblast cellular culture (73.43% for obidoxime, and 82.42% for HIN 2).

The bis1,3[2-hydroxyimino-N-(pyridyl)acetamid]oxapropandichloride compounds (HIN 2) showed a value of cellular viability in vitro lower than obidoxime at high equimolecular concentrations, proving to be more toxic. (2.13%, for HIN2, and 22.87% for obidoxime)

Taking into account the close CT50 values, the newly synthethized compound that is as such characterized from a toxicological standpoint can qualify for further studies as an antidote.

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VARIA

Orthodontic treatment and periodontal disease – between advantages and disadvantages

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Abstract: *Orthodontic treatment and periodontal disease can have an inter-conditional relationship, particularly when the accumulation of bacterial plaque, favoured by orthodontic appliances and poor oral hygiene can cause periodontal inflammation. The objective of the study is to highlight the influence of the risk factors' polymorphism on the therapeutic response in periodontal disease. 64 patients with mixed or definitive dentition and poor oral hygiene were included in the study, of whom 22 patients followed treatment with fixed orthodontic dispensers, 22 patients were treated with mobile orthodontic braces and 20 were non-attendants. From all patients gingival crevicular fluid was collected and assessed, before and after applying a chlorhexidine-based gel antiseptic agent.*

Keywords: *periodontal disease, orthodontic treatment, chlorhexidine gel, gingival fluid*

INTRODUCTION

Periodontal disease inflicts a wide range of oral changes, ranging from gingival tissue inflammation to tooth loss. During some orthodontic treatment protocols patients may not to compliant to keeping a good oral hygiene, possibly due to the difficulties that certain orthodontic appliances may impose on efficient teeth brushing [1]. Microbiological patterns involved in the genesis of periodontal disease are more common in orthodontic treatment patients, so the bacterial plaque is left undisturbed for a long period of time around the orthodontic appliances and can grow in bacteria number and pathogenic potential. Microbial periodontal pathogens, especially Gram-negative bacteria, are more frequently found around fixed/mobile orthodontic appliances [2]. Periodontal disease may have different clinical forms, with a wide range of manifestations, from gum tissue damage to periodontitis, which can even lead to tooth loss in some cases [3].

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The clinical manifestations and evolution of periodontal disease may vary in accordance with the intensity of the bacterial challenge. In orthodontic treatment patients, the periodontal tissues are also challenged by the orthodontic forces which generate teeth movement. During teeth movement, all periodontal structures, including the gingival, the periodontal ligament and the alveolar bone are under constant mechanical stress [4]. This mechanical stress can cause a decrease of the tissue's defensive capabilities. Combined with the increased bacterial challenge, caused by poor oral hygiene, this creates very favourable conditions for the onset of periodontal disease [5]. Consequently, a good understanding of the periodontal disease's existing aetiology and risk factors is required for a correct diagnosis and

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treatment plan for a patient undergoing orthodontic therapy.

The objective of this study was to highlight the influence that certain risk factors have on the therapeutic response in periodontal disease in orthodontic patients and to assess the local impact that the use of an anti-plaque agent has in these patients.

MATERIAL AND METHOD

The study included a group of patients who were clinically examined and expressed similar symptoms during the first visit to the dental office. In order to collect the data obtained from the anamnesis, observation charts were prepared for each patient. The inclusion criteria used for the study were the following: (1) patients who were treated or not with orthodontic braces/mobile dispensers; (2) age 12-20 years; (3) the presence of mixed or definitive dentition; (4) the presence of gingivitis subjective and objective clinical signs (gingival bleeding, gingival oedema, halitosis). Following the clinical examination, a bacterial plaque index was calculated for the participating patients, its values being directly proportionate with the degree of gingival inflammation.

A total of 64 patients with mixed or definitive dentition and poor oral hygiene were selected, being divided into 3 study groups: (1) 22 patients carrying a mobile orthodontic device; (2) 22 patients with fixed orthodontic appliances and (3) 20 patients who did not require or have refused orthodontic treatment.

Before initial periodontal therapy (airflow cleaning and professional brushing) gingival crevicular fluid was sampled from the gingival sulcus of the participating patients. For sampling sterile paper, cones of 20 mm were kept for 30 seconds in the periodontal pocket/gingival sulcus, after the pre-removal of the supragingival bacterial plaque, in order to avoid contamination of the sample with supragingival bacteria. The collection and transport of the biological products and the stages of microbiological diagnosis were performed by the clinician and their assistant.

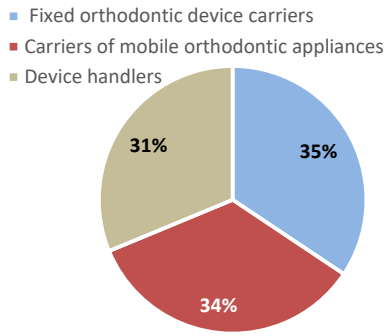
The results of microbiological investigations depend to a large extent on the quality of the sampling procedure, which needs to follow specific rules:
(a) the patient was informed about the aimed analysis and about the correct way of sampling procedure;
(b) the sampling instruments were sterile, disposable and adapted to the investigation;
(c) the pathological product was harvested prior to administration of any topical or general topical treatment; it is harvested from areas where it is most likely to find the bacterium that produced the infection;
(d) the outside of the container was disinfected if it has been contaminated during sampling procedure;
(e) the name of the patient and his provenance, the type of biological product, the date and time of collection was written on the container;
(f) each pathological product was accompanied by a bulletin containing information about the sample and the patient;
(g) transport was as quick as possible to the laboratory to prevent the degradation of the pathological product;
(h) transport media used ensured good survival of bacteria;
(i) for the oral cavity, the sampling procedure consisted in carefully cleaning the edge of the gum and tooth surface to remove saliva and food debris, afterwards a periodontal scalpel was used and the lesion secretion was removed;
(j) transport was carried out using an anaerobic transport system in less than 2 hours from sampling.

Microbiological tests were performed to detect aerobic and anaerobic gram-positive and gram-negative bacteria. After the initial periodontal treatment, the patients were randomly re-divided into two groups, as follows: (1) 12 patients with orthodontic devices (six fixed devices, six mobile devices) and (2) 12 appliance donors, who received applications of a chlorhexidine-based gel (Elugel 0.02%, Pierre-Fabre, France). The other 24 patients were only instructed for good oral hygiene and monitored. The ethical standards of medical research and GDPR (Global Data Protection) and the approval of the Ethics Committee of the Faculty of Dentistry of the "Titu Maiorescu" University in Bucharest were followed by the present study.

RESULTS

The distribution of the participating patients regarding orthodontic treatment type was similar between the patients with mobile and fixed orthodontic appliances (Figure 1).

Figure 1: Selection criteria: fixed/mobile orthodontic handlers/ carriers



The microbiological assessment revealed bacteria of the violet periodontal complex: (Veillonella parvula,

Actinomyces odontolyticus), yellow complex (Streptococcus spp), green complex (Eikenella corrodens, Capnocytophaga spp.) red complex (Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola) and of the orange complex (Prevotella intermedia/nigrescens, Micromonas micros, Campylobacter rectus, Fusobacterium nucleatum) (Figure 2). Compared to the microbial flora normally found in gingival tissue of healthy individuals with no periodontal problems, microbiological tests revealed the exaggerated growth of germs in the case of fixed carriers and a significant increase in the case of mobile carriers, as shown in Table 1.

Streptococcus spp, P. gingivalis, and F. nucleatum predominantly occured in fixed-bearer carriers, while Treponemma is constantly present whether or not the patient is treated with fixed or mobile devices. The Actinomyces spp were present within the saprophytic flora and does not develop during orthodontic treatment.

Figure 2: Bacteria type frequency in periodontal heath vs. gingival inflammation (%)

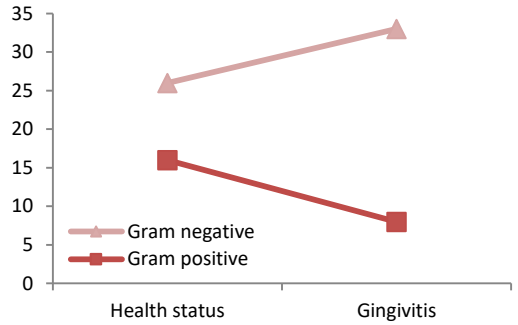
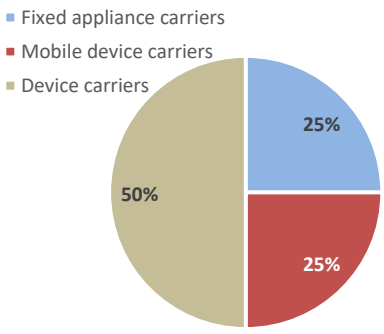


Table 1: Distribution of bacterial pathogens according to study groups

Pathogen	Health	Not carriers of orthodontic braces	Carriers of orthodontic braces	Carriers of mobile devices
Streptococcus	+++	+	+ ++	+ +
Actinomyces	+++	+	+	+
Veillonella	+	+	+	+
Aggregatibacter actinomycetemcomitans	-	++	+	?
Capnocytophaga	+/-	+/-	+/-	+/-
Treponeme orale	+/-	+++	+++	+++
Porphyromonas gingivalis	-	+	+++	++
Prevotella intermedia	+/-	+	+++	++
Tannerella forsythia	-	++	++	?
Fusobacterium nucleatum	+	++	+++	++

Figure 3: Patients treated with chlorhexidine-based gel antiseptic substance



Following the application of the chlorhexidine-based gel antiseptic substance to orthodontic patients, it was found that after two weeks the results of the microbiological analysis of the gingival sulcus exudate revealed a reduction of the number of pathogenic bacteria in the orthodontic treated subjects and their disappearance in the non-attendant devices (Figure 3).

For the patients who did not benefit from the application of chlorhexidine-based gel substance, it was found that for the orthodontic handlers the microbial flora was still highly present. After 30 days, when the patients were reassessed, it was discovered that the pathogenic flora disappeared in all patients treated with chlorhexidine-based gel antiseptic substance, both with orthodontic treatment or not.

DISCUSSION

According to the literature, a study by Al-Bazi et al. from 2016 demonstrated that the formation of dental biofilm is a result of the increase in the number of microorganisms on the tooth surface, which was found to be 2-3 times higher in patients under orthodontic treatment than in young people without orthodontic treatment [6]. Previous studies have shown that regular dental brushing and the use of dental floss alone do not decrease levels of S. mutans in patients

treated with fixed orthodontic appliances [7]. In our study, subjects treated with mobile orthodontic devices, compared to healthy individuals without periodontal problems, revealed an exacerbated growth of germs in the case of fixed-bearer carriers and a significant increase in the case of the mobile-bearer carriers. Some bacterial species (Streptococcus, Porphyromonas, Prevotella and Fusobacterium) are found mainly in fixed-bearer carriers, while Treponemma is constantly present whether or not the patient is a carrier of fixed or mobile devices and Tannerella forsytia is absent in patients' exudates.

Different protocols have shown the ability of CHX (Chlorhexidine) to inhibit the formation of dental biofilm and to reduce plaque development [8]. Randomized, controlled clinical trials demonstrated the efficacy of 0.50% and 0.75% CHX to control gingivitis and bleeding in orthodontic patients, concluding that lower concentrations of chlorhexidine may reduce the risk of tooth colouring, without compromising its effectiveness level against pathogenic bacteria [9]. In our study, applying the chlorhexidine-based gel antiseptic substance significantly reduced the number of bacterial colonies in all patients of the study. However, antiseptic treatment should not replace dental mechanical treatment, but be applied in a complementary manner [10]. Combining mechanical treatment with local application of antiseptics gives statistically better results than each of them taken separately does.

CONCLUSIONS

Given the results of the study, we can conclude that for the patients treated with orthodontic appliances, either fixed or mobile, it is impossible to completely clean all oral surfaces and to decrease the number of microbial colonisation without the application of local antiseptics during the treatment. The results of the study can be used to prevent periodontal disease in patients with orthodontic treatment.

Conflict of interest:
Nothing to declare.

Contribution Note:
All the authors equally contributed to the drawing up of the present paper.

Patient participation at the study respects ethical standards in medical research and GDPR.

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VARIA

Acute kidney injury incidence and models for mortality prediction in acute coronary syndromes

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Abstract: *Background and aim. The presence of acute kidney injury (AKI) in subjects diagnosed with acute coronary syndromes (ACS) represents a major predictor of a negative outcome.The purpose of this study is to reveal the incidence of AKI in ACS subjects and to illustrate the prognosis of these subjects, using special models for mortality prediction, based on the creatinine values and the presence of acute heart failure (AHF).*

Methods. Using multiple linear regression, several models were designed to predict the mortality including: comorbidities of the subjects, initial and highest value of the serum creatinine (sCr) during hospitalization, presence of theAHF and Killip class compared with sCr values, the last model illustrating the influence of all the previous variables.

Results. AKI had a significant incidence in the chronic kidney disease (CKD) group (53.11%). The mortality rate and the hospital readmission were higher in the CKD subjects (41.14%) versus the patients with normal kidney function (21.23%, p<0.01). The association of AKI and AHF, known as cardio-renal syndrome, was related to a negative outcome in the CKD group. The models for prediction of mortality emphasize that the age, dyslipidaemia, worst sCr, Killip class and AHF presence can be used to assess the mortality risk of patients with AKI and ACS. Previous pathological conditions such as diabetes, peripheral artery disease, coronary artery disease (CAD) and obesity, failed to achieve statistically significance for prediction of death.

Conclusions. AKI represents a serious pathological condition with a high prevalence in subjects with CKD; the association with AHF increases the risk of hospital readmission and fatal outcome.

Keywords: *acute coronary syndromes, acute kidney injury,acute heart failure, serum creatinine, predictors, mortality*

INTRODUCTION

AKI represents a pathological condition that resumes a complex interaction between various factors like subsequent renal failure, utilization of iodinated contrast substance during percutaneous coronary intervention (PCI), renal hypoperfusion caused by decreasing of the cardiac output during an ischemic event [1]. Manifestations of AKI may consist in a simple increase in sCr or severe renal failure with anuria requiring kidney replacement therapy [1]. There is a strong relationship between the renal function impairment and the CAD development, regardless the other common risk factor involvement. The prospective study realized by Jurkovitz et al. including 13,329 participants,

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without previous CAD, reflected that increased values of baseline sCr (defined as ≥1.2 mg/dL in women and ≥1.5 mg/dL in men) were associated with a high incidence of acute myocardial infarction (AMI) and death due to ischemic events, compared to normal values of the sCr [2]. The renal impairment has an important influence on the patient's outcome during cardiovascular events and a small decrease in glomerular filtration rate worsens the prognosis of these patients [3].

The complexity of the atherosclerosis process in CKD subjects is a result of the interaction between common and uncommon risk factors. Diabetes mellitus, hypertension, dyslipidaemia and smoking represent essential factors in atherosclerosis development by promoting chronic inflammation, oxidation of the low-density lipoproteins and reactive oxygen species formation [4]. Even secondary hypertension in young persons has an important role in atherosclerosis occurrence and the level of plasma renin in these subjects represents a clear indication [5, 6]. Systolic and diastolic values of the blood pressure are strongly related to alimentation in young persons and there are several actions to change the unhealthy eating habits, beginning with education regarding healthy diet from early age [7].

Various terms were attributed to AKI and that is the reason why the comparison of studies is difficult. There are more than 30 definitions of AKI and the most used are: the Risk, Injury, Failure, Loss and End-stage renal disease (RIFLE), criteria revised by the Acute Kidney Injury Network (AKIN) and the Kidney Disease: Improving Global Outcomes (KDIGO) AKI Work Group classification.

AKI definition (according to KDIGO) expresses an increase in sCr by 0.3 mg/dL (≥ 26.5 µmol/L) within 48 hours or increase in sCr 1.5 to 1.9 times baseline value, with known or presumed abrupt onset within the prior 7 days. There are 3 stages according to the sCr values and the urine output. The first stage is consistent with sCr variations mentioned in the definition and a urine output of less than 0.5 mL/kg/h for 6-12 h, whereas the second stage involves increase in sCr 2.0 to 2.9 times baseline and a urine output of 0.5 mL/kg/h for 12 h. The last stage represents an association between increase in sCr to ≥ 4.0 mg/dL (≥ 353.6 µmol/L) or ≥ 3.0 times baseline or usage of renal replacement therapy and a urine output of less than 0.3 mL/kg/h for ≥ 24 h or anuria for ≥12 hours [8, 9, 10].

Usually, AKI remains undetected due to the fact that the onset is earlier in ACS patients and the sCr from admission represents the reference value, without actually knowing the evolution of the creatinine before hospitalization. If the sCr does not have a remarkable increase after admission, there may be an error of AKI diagnosis. New biomarkers able to early detect the AKI are required in order to initiate the preventive treatment and minimalize the renal insult [11]. The beginning of AKI is usually in the early phase of the ACS, fact that determines the late detection of this condition.

ACS represents a challenging diagnosis in CKD subjects because most of them remain asymptomatic. The explanation is suggested by the fact that uremic milieu and associated diabetic neuropathy influence the perception of pain in these patients [12, 13].Inflammation and oxidative stress represent common elements for both CKD and ACS, and the association of these diseases increases the risk to develop AKI. High levels of inflammatory markers (such as C reactive protein (CRP), fibrinogen and procalcitonin (PCT)) were found in subjects who developed AKI after PCI. Kurtul et al. revealed that PCT represents an independent factor for the development of AKI in ACS patients undergoing PCI [14]. Subjects without AKI emphasize PCT values less than 0.1 µg/L15. Gao et al. analysed 7000 PCI and illustrated that CRP levels were higher in subjects with AKI16. Gu et al. illustrated that determination of both markers, CRP and PCT, has higher predictive value in identifying subjects at high risk of AKI development. Also, the AMI is an independent risk factor involved in AKI occurrence [17].The role of antioxidants in the cardiovascular field is well known and vegetables/fruits consumption has demonstrated its effect for people at risk of cardiovascular diseases, on immunologic, clinical and antioxidant markers [18].

The main purpose of the present research is to highlight the incidence of AKI in ACS patients, and to present prediction for these patients based on particular models for mortality prognosis taking into account the presence of AHF and creatinine values.

METHOD

This study designed a retrospective single-centre analysis, which enrolled 435 patients admitted with ACS diagnosis in the Cardiology department of the Clinical Emergency County Hospital of Oradea, January 2016 - January 2018. The study lot involved 209 patients with ACS and CKD; the control lot comprised 226 patients with ACS and normal kidney function. The exclusion criteria included allergy to contrast media, exposure to the contrast media 2 days before the coronary angiography procedure, malignant conditions.The AKI patients were detected using the KDIGO classification because it contains lower values of sCr than the AKIN criteria, so the underdiagnosis of the condition was avoided.

ACS was revealed according to the Fourth Definition of the

Myocardial Infarction using the association of thoracic pain, elevated cardiac necrosis enzymes, electrocardiographic changes and imaging evidence of new regional wall motion disturbances.ST-segment elevation myocardial infarction (STEMI) was defined due to the presence of persistent ST-segment elevation more than 1 mm in two contiguous leads with the exception of precordial leads that require an elevation of 1.5 mm in women, more than 2.5 mm in men under 40 years-old and less than 2 mm in men older than 40 years. Myocardial infarction in subjects with previous left bundle branch was revealed due to Sgarbossa's criteria. Non-ST-segment elevation myocardial infarction (N-STEMI) illustrates either a ST-segment depression ≥0.5 mm in two contiguous leads or deep inverted T wave more than 1 mm, along with elevated myocardial necrosis enzymes. Unstable angina (UA) expresses cardiac necrosis enzymes in normal limits associated with T negative wave, ST-segment depression or transitory ST-segment elevation [19]. Left ventricle ejection fraction (LVEF) was assessed using the Simpson's method. The normal value was considered between 50-72% in men and 52-74% in women [20].

Cardiac necrosis enzymes, cTnI (cardiac troponin I) and hscTnI (high-sensitive cardiac troponin I), were tested and abnormal values were defined as elevated when the result showed at least one value above the upper limit of the 99th percentile. Normal ranges were considered whenthe values were lower than 0.04 ng/mL for cTnI and 29 ng/L for hscTnI. Serial measurements were required to exclude thediagnosis of myocardial injury [21].

Killip Kimball classification was assessed in each patient with STEMI and the four classes. The first class illustrated subjects without signs of cardiac decompensation, the second class included patients with rales, third heart sound and increased jugular venous pressure. The third class was consistent with acute pulmonary oedema and the last stage revealed subjects with cardiogenic shock [22].

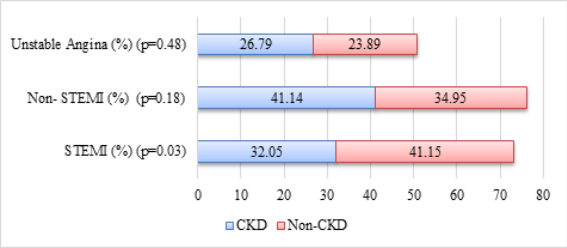
The statistical analysis was performed using Biostat software. Chi-square test was used when comparing prevalence in the two-groups, while Anova test was used to compare the mean values of certain parameters in the two groups. Univariate and multivariate regressions were done to assess the factors that correlate with the risk of death. A value of p<0.0 was considered statistically significant.

This research was performed respecting the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects), and it was approved by the Ethics Committee of the Clinical County Emergency Hospital of Oradea, Bihor County, Romania (decision no. 30088/December 10th, 2019); each patient included in the study signed the informed consent form.

RESULTS

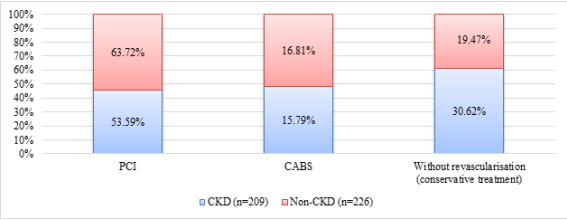
The prevalence of the common risk factors and associated comorbidities was higher in the CKD lot. Conditions like hypertension, diabetes mellitus, dyslipidaemia and peripheral artery disease were present in an increased proportion in CKD subjects in comparison with patients without CKD (84.21% vs 63.27%, p<0.01; 58.85% vs 33.62%,p<0.01; 60.09% vs 49.95%, p=0.02; 20% vs 4.9%, p<0.01).The distribution of the ACS types was different between the two lots. STEMI was characteristic in subjects without CKD whereas the CKD lot revealed mostly N-STEMI cases, UA had a similar incidence in both CKD and non-CKD lots (Figure 1).

Figure 1: Categories of coronary syndrome according to the presence/absence of CKD



Myocardial interventional revascularization through PCI and stent implantation represented the therapeutic option for 53.59% of CKD subjects and 63.72% non-CKD patients (p=0.03). Coronary artery bypass surgery (CABS) was performed in 15.79% of CKD subjects, whereas the non-CKD lot had a proportion of surgical myocardial revascu1arization of 16.81% (p=0.77). The use of conservative treatment instead of PCI, known as renal nihilism, was objectified mostly in the CKD lot (p<0.01) (Figure 2).

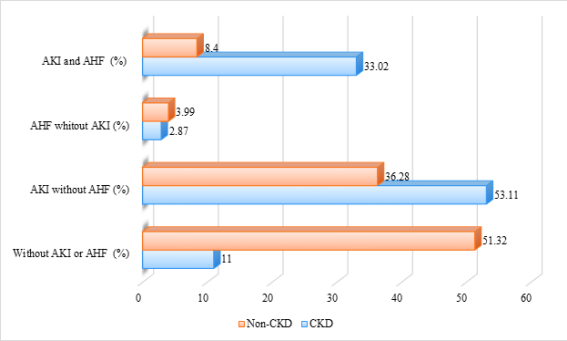
Figure 2: Treatment of ACS according to the presence/absence of CKD



AKI has occurred in the CKD lot, revealing statistically significance (p<0.01). The association of two pathological conditions, such as AKI and AHF, had a major impact in subjects' outcome and was revealed in the CKD group (p<0.01). The uncomplicated evolution of the ACS cases was

noticed in patients with normal kidney function (p<0.01) (Figure 3).

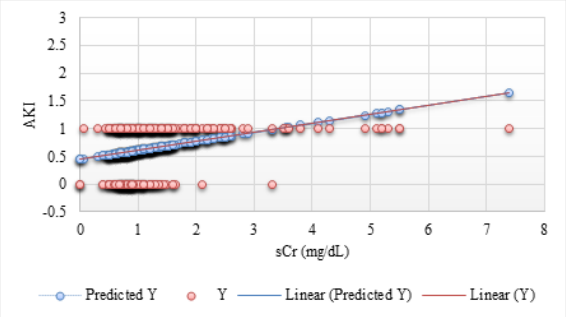
Figure 3: Presence of AKI or AHF according to the presence/ absence of CKD



Deteriorated LVEF was noticed in a higher proportion in CKD lot, compared to patients without CKD (23.5% vs 20.17%, p=0.4). The average value of sCr was higher in the CKD lot (1.66 mg/dL vs 0.8, p<0.01), the standard deviation (Q1=1.01, Q2=0.20). Another important parameter was the worst value of the sCr which revealed a high level in the CKD lot with statistical significance (2.71 mg/dL vs 1.1 mg/dL, p<0.01), standard deviation (Q1=2.11, Q2=0.36).The analysis of the average sCr variation illustrated an increased value in the CKD group (1.03 mg/dL vs 0.3 mg/dL, p<0.01).The mortality rate was statistically significant in CKD subjects compared to patients with normal kidney function (13.39% vs 6.1%, p<0.05, SD CKD 8.39-17.61, SD non-CKD 4.39-7.69). Killip class assessment revealed that most of the subjects without CKD had first Killip class at admission (p<0.01), whereas the CKD patients were included in the third and fourth Killip classes (p<0.01).

The initial value of sCr illustrated importance in univariate analysis and predicted the development of AHF and AKI (p<0.01) (Figure 4, Figure 5, Table 1).

Figure 4: Correlation between worst value of sCr (mg/dL) and AKI development



Models for mortality prediction were designed using multivariate analysis and including different factors to demonstrate the impact of AKI and other comorbidities or biomarkers regarding the outcome of patients with ACS (Table 2). The first model, based on the medical history and the associated cardiovascular risk factors of the subjects, illustrated that only the age and dyslipidaemia reached statistical importance to predict the mortality risk in ACS subjects with and without CKD (p=0.0404, p=0.0332).

Figure 5: Correlation between worst value of sCr (mg/dL) levels and AHF development

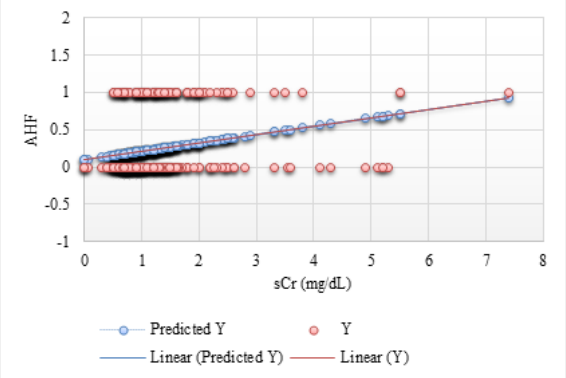


Table 1: Parameters of linear regression

	Coefficient	Standard error	LCL	UCL	t Stat	p value
Between worst value of sCr (mg/dL) and AKI						
Intercept	0.4489	0.0389	0.3724	0.5253	11.5427	*0.0000
Worst value of sCr (mg/dL)	0.1619	0.0263	0.1102	0.2136	6.1518	1.7451E-9
Between sCr (mg/dL) and AHF						
Intercept	0.1005	0.0352	0.0314	0.1696	2.8576	*0.0045
Worst value of sCr (mg/dL)	0.1120	0.0238	0.0652	0.1587	4.7053	3.4162E-6

**p<0.05, respectively p<0.001; LCL –low confidence limit; UCL–upper confidence limit; sCr – serum creatinine*

Diabetes, hypertension, smoking condition, history of peripheral artery disease, stroke did not predict the risk of death in the presented model. The second model included an analysis regarding the mortality risk of the study groups based on the presence of complications and biomarkers values. The presence of AHF and Killip class were statistically significant in anticipating the fatal outcome (p=0.0002, p<0.01). Other parameters like sCr value, age, troponin, LVEF

failed to demonstrate their role in predicting the mortality. The third model evaluated the difference between baseline sCr and the worst value of sCr in identifying the patient's outcome. The worst value of sCr was the most important factor in mortality prediction in this model (p=0.0117). The fourth model represented an evaluation of all the variables in detecting the mortality risk. The Killip class and the AHF demonstrated statistical importance (p=0.0132, p= 0.0000).

Table 2: Prediction of mortality risk using multivariate analysis, based on 3 models

Parameter	Coefficient	Standard error	p value
Model 1. Associated cardiovascular risk factors and comorbidities			
Intercept	-0.0037	0.1521	0.9805
Age	0.0027	0.0013	*0.0404
Gender	-0.0004	0.0342	0.9918
BMI (kg/m^2)	-0.0030	0.0034	0.3700
Smoking	0.0137	0.0187	0.4656
Hypertension	-0.0342	0.0348	0.3270
Diabetes mellitus	-0.0111	0.0162	0.4945
CKD	0.0498	0.0327	0.1288
Anaemia	0.0496	0.0328	0.1318
Peripheral artery disease	0.0866	0.0469	0.0661
Chronic obstructive pulmonary disease	0.0131	0.0583	0.8229
Stroke	-0.0728	0.0491	0.1389
CAD	0.0020	0.0117	0.8651
Model 2. Complications and paraclinical results			
Intercept	-0.2572	0.0972	*0.0085
Age	0.0009	0.0010	0.3625
Initial value of sCr (mg/dL)	-0.0066	0.0328	0.8410
Worst value of sCr (mg/dL)	0.0144	0.0176	0.4138
AKI	-0.0175	0.0271	0.5200
AHF	-0.2284	0.0598	*0.0002
cTnI (ng/mL)	0.0057	0.0286	0.8426
Killip class	0.2300	0.0303	*<0.001
Decreased LVEF	0.0431	0.0322	0.1818
Model 3. Initial sCr and worst value of sCr			
Intercept	0.0176	0.0249	0.4791
Initial sCr (mg/dL)	-0.0027	0.0350	0.9375
Worst value of sCr (mg/dL)	0.0439	0.0173	*0.0117

**p<0.05, respectively p<0.001; BMI – body mass index; CKD – chronic kidney disease; CAD – coronary artery disease; AKI – acute kidney injury; AHF – acute heart failure; ctnI –cardiac troponin I; LVEF – left ventricular ejection fraction; sCr – serum creatinine.*

DISCUSSIONS

The present study outlines two significant ideas: (1) The serial determination of sCr is essential in identifying subjects with AKI and special attention should be paid to subjects with CKD undergoing the coronary procedure; (2) The presence of AKI in patients with ACS and CKD can be a fatal prognosis, especially when associated with AHF. Despite following accurate indications regarding coronary imaging, like the use of a low-osmolarity contrast agent (600-800 mOsm/kg) and the corresponding post-procedural hydration associated with diuresis stimulation, the incidence of AKI varies from 5% to 55% [1, 23, 24]. The variable incidence resides in variations of the nomenclature of this term [25].

As there is no safe and highly efficient prevention method for AKI, it is considered necessary to develop prediction scores to identify high-risk patients [26]. A first element that should be included is the Killip class, evaluated upon admission to the hospital, along with the presence of AHF. These elements should be identified particularly for CKD subjects, as multivariate analysis demonstrates the role of independent risk factors for the risk of death in these patients. The reduction of the number of functional nephrons, evidenced by the initial sCr analysis and the worst value of the sCr, together with an increased rate of reduced

LVEF are major predictors of AKI and AHF occurrence, an association known as cardio-renal syndrome [27].

Common cardiovascular risk factors have failed to outline the risk of mortality in multivariate analysis, except for dyslipidaemia, since in the case of CKD there are also factors specific to the uremic environment that significantly influence the prognosis of these patients. Early myocardial revascularization reduces the risk of AHF. A recent study confirms the importance of this therapeutic method, especially in subjects with N-STEMI and CKD, that associate increased values of cardiac biomarkers, such as the N-terminal fragment of the brain natriuretic peptide (N-terminal pro b-type natriuretic peptide, NT-proBNP, BNPT) or hscTnI, or a Killip class three or four [28]. For patients with ACS undergoing PCI, an independent predictor of developing AKI is the level of NT-proBNP at admission, being connected with high costs, extended hospitalization, high rates of terminal-stage renal failure, repeat revascularization, myocardial infarction and early and late mortality [29]. LVEF shows low values in subjects with ACS and CKD due to the increased incidence of multivariate CAD and especially to the impairment of left coronary artery trunk. Choosing the method of myocardial, interventional or surgical revascularization should consider the possibility of resolving all significant coronary lesions, the complexity of CAD and the surgical risk for the subjects [30].

The increased mortality evidenced in the group of subjects with CKD is explained by the increased incidence of AKI in these subjects. The mean hospitalization period was longer for patients with ACS and CKD who developed AKI, highlighting the severity of this complication (15.71 days vs. 13.15 days, $p < 0.01$). For patients with STEMI, accentuated oxidative stress, sympathetic activation and pronounced inflammatory response were found to synergistically accelerate AKI [31]. The most powerful independent predictor of mortality within hospital in case of dangerous settings like for STEMI associated with cardiogenic shock was identified to be AKI [32].

A recent and further argument of the way AKI influences the long-term effect for patients suffering from STEMI subjected to primary PCI is presented by Narula et al. The 3-year follow-up revealed that the mortality rate for patients suffering from AKI was 16%, while for those without AKI it was 4%. As for the association of severe ischemic cardiac side effects and serious bleeding, the intensification of the side effects was more accentuated after three years, being of 40% for subjects with AKI and about 25% for patients without AKI. Corroborated, these data show that AKI evolution influences the complete prognosis of ACS patients [33]. Throughout primary PCI for STEMI, increased contrast volume is connected with increased levels of AKI and mortality, the contrast ratio and contrast volume determining AKI evolution [34]. A general complication in the case of patients suffering from shock and myocardial infarction is nephropathy, as recently revealed by a CULPRIT-SHOCK study that showed a high percentage of subjects (11.6-16.4%) who needed renal replacement therapy [35].

The decrease of renal mass is thought to be the main pathophysiology that is associated with CKD, AKI and the end stage renal disease. Considering these interesting findings and the fact that the common therapies do not influence the short-term prediction in AKI, supplementary preventive actions meant to ameliorate the health status for AKI survivors are extremely important. There is general evidence that most of the patients suffering from AKI are not regularly surveyed by nephrologists and the current clinical practice does rarely include follow up evaluation for AKI, dealing with this issue representing a considerable option for important change and better caring for this category of population [36]. Even patients with mild and moderate AKI, which in most cases is short lived and clinically reversible renal failure, had an elevated risk of death after hospitalization that did not dissipate over time. These conclusions arenovel and do not support the conventional teaching that AKI in its mild-form is inconsequential [37].

CONCLUSIONS

AKI negatively influences the short- and long-term prognosis of subjects diagnosed with ACS. CKD and AHF are other negative predictors of ACS evolution and their association with AKI has shown the highest mortality rate. Although it is not an accurate marker, the initial sCr and the worst value of sCr can be used in AKI detection to establish appropriate therapeutic measures. New predictive scores able to identify AKI in a timely manner are necessary, considering the fatal prognosis for patients with ACS and CKD.Early and complete myocardial revascularization in ACS is an optimal method, which decreases the risk of developing AHF and AKI, known as cardio-renal syndrome.

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VARIA

Bucharest mobile military hospital – response to the COVID-19 pandemic

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Abstract: *The idea of using mobile hospitals - a fully functional hospital is a system with a high degree of complexity - in case of a pandemic crisis, is not new, but shows how much they are needed.*

Solutions belonging to the category of mobile architecture are increasingly common, technical and logistic difficulties are solved in more perfect way. This applies mostly to objects with simple layouts and functional relationships, with relatively little technical equipment. In the case of healthcare facilities there are several factors increasing the difficulty: multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure.

The Romanian military and civilian specialists from Bucharest Central Military Emergency University Hospital (SUUMC) showed that is possible to build a hospital as a modular and mobile structure in order to fight COVID 19, but the strategic actions and the effort needed to organize and run mobile hospital far exceeds the expenditure, protocols and specialized M.D.s necessary to run the stationary hospital.

Keywords: *mobile hospitals, COVID-19, strategic actions*

INTRODUCTION

SARS-CoV-2, the causative agent of COVID-19, is a newly discovered coronavirus. It is transmitted through respiratory droplets and direct contact; other modes of transmission (i.e. airborne and digestive) have been proposed, but they are still under investigation. The average incubation period is estimated at 5 to 6 days, ranging from 1 to 14 days [1]. There is currently no approved specific treatment or vaccine against COVID-19.

Business-as-usual service delivery approaches are not sufficient to respond in the fight against COVID 19, once cluster of cases or widespread community transmission is registered and superior capacity will be needed.

While World Health Organization (WHO) advises that aggressive measures to find, isolate, test, treat and trace are the best ways to stop the spread of this virus, public health interventions to "flatten the curve" may slow transmission and mitigate peak capacity needs. Modelling studies point to the impact of nonpharmaceutical interventions (NPIs), such as physical distancing, school and university closures, banning of mass gatherings, and remaining indoors, on spreading the number of cases over a longer period to give health systems the opportunities they need to cope with caseloads [1, 2, 3, 4]

These models have helped to reinforce the message from WHO about the implementation of such measures, alongside widespread testing, to detect cases and break chains of transmission. In addition to these measures, phased plans for creating surge capacity in acute and intensive care will help respond to COVID-19 cases while maintaining essential health services.

Surge capacity is the ability of a health system to manage a sudden and unexpected influx of patients in a disaster or

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emergency situation.

Surge capacity can be created from intrinsic and extrinsic resources. The former covers all local resources that can be used for the response, while the latter involves leveraging resources from outside the affected area (across geography or across specialty).

As the COVID-19 pandemic necessitates both in tandem in all countries, this note covers both these aspects together. Creating surge capacity involves a comprehensive approach linking the 4 S's of surge capacity: space, staff, supplies and systems. [5]

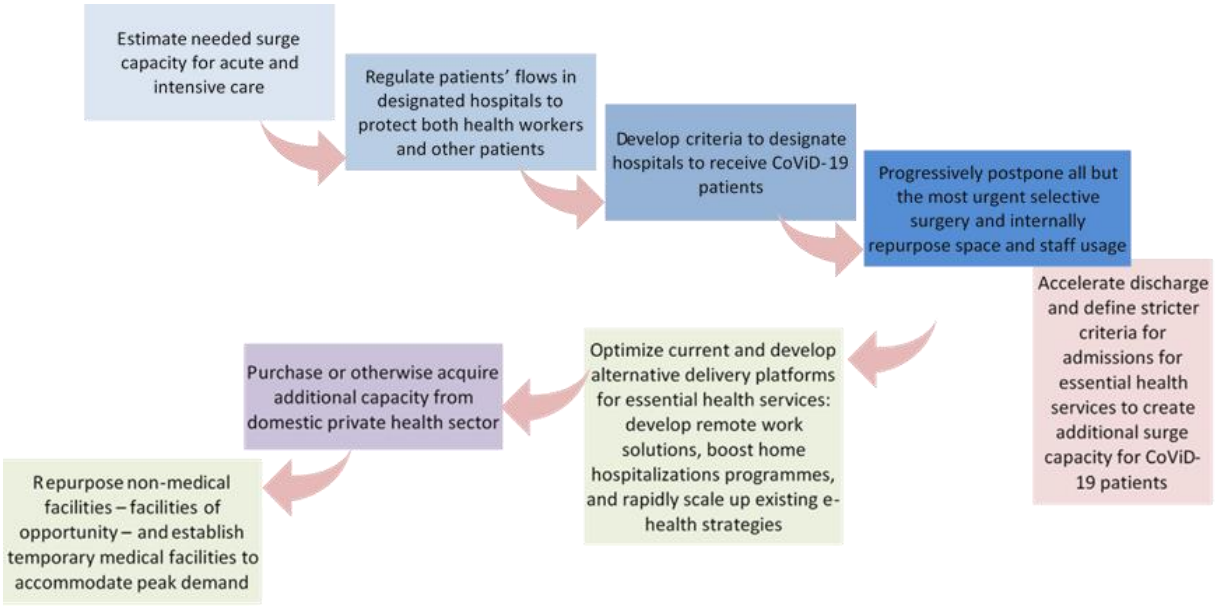
RECOMMENDATIONS AND STRATEGIC ACTIONS

In the following, are presented the recommendations and strategic actions to activate surge capacity in line with the 4 S's drawing on past experiences in emergencies and on the emergent experiences of world countries in the COVID-19 response.[6]

Strategic action 1. **Create and activate a stepwise plan to expand physical space to care for COVID-19 patients respecting infection prevention and control protocols**.

This action requires the following steps, and is schematically presented in the Figure 1.

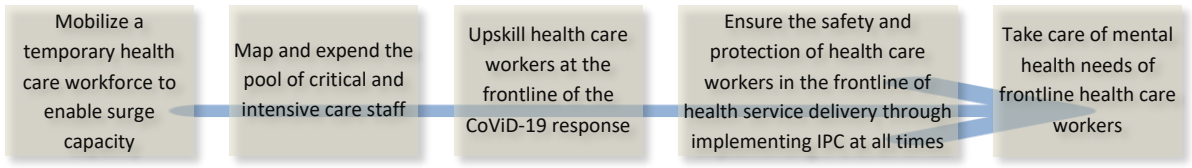
Figure 1: Necessary steps for strategic action 1



Strategic action 2. Identify the health workforce available for surge capacity demands, and repurpose and upskill for rapid deployment to meet surge capacity needs

The steps necessary for this strategic action are depicted in Figure 2.

Figure 2. Necessary steps for strategic action 2



Strategic action 3. Ensure adequate supplies to support surge in demand

The necessary steps for strategic action 3 are presented in Figure 3.

Strategic action 4. Establish systems to manage and align policies to meet surge in demand

The steps that are necessary for strategic action 4 are mentioned in Figure 4.

Figure 3: Necessary steps for strategic action 3

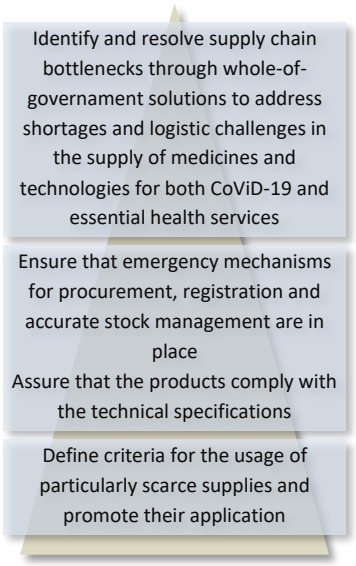
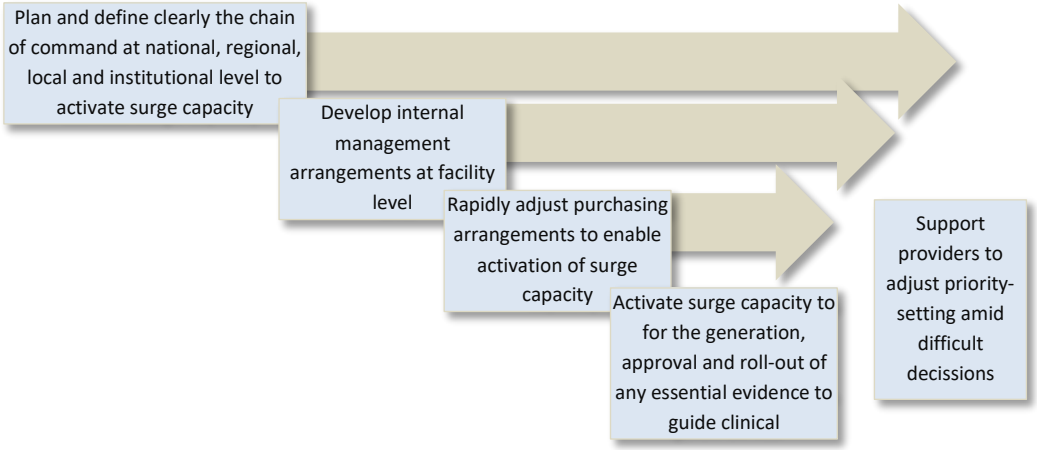


Figure 4: Necessary steps for strategic action 4



THE ROL 2 MILITARY HOSPITAL AT THE ANA ASLAN INSTITUTE IN OTOPENI, NEAR BUCHAREST – A CASE STUDY

The ROL 2 military hospital was installed at the Ana Aslan Institute in Otopeni city, near Bucharest, to support the Romanian government as a response to the Covid-19 pandemic. The ROL 2 was declared operational on Saturday, March 28, in the presence of President Klaus Iohannis and Prime Minister Ludovic Orban, the Ministry of Defense announced.

Once accredited by the Ministry of Health, the ROL 2 hospital will operate as an external facility of the Central Military Emergency University Hospital (SUUMC). The mild and medium cases of COVID-19 will be admitted and treated in this medical facility (Figure 5).

Figure 5: The ROL 2 first medical team

The ROL 2 mobile military hospital operate as a stand-alone entity (as in the American solutions), or in conjunction with the whole hospital – Bucharest Central Military Emergency University Hospital (but still autonomously, as in European solutions). These two variants are taking into account

organisation of space or technological line flow. The difference between the two models comes down to one important aspect: in the European pattern patients' flow is unidirectional, almost linear, from the entrance of the patient to the segregation area (TRIAGE), through diagnostic and treatment facilities ending at the hospital bed wards). This is due to the principles of Emergency Department (DE) operation. ED is an integral part of the hospital and at the same time it is an emergency path of hospitalization. The American ED is more autonomous unit, operating often as an outpatient clinic compared to European standards: the TRIAGE and central nurse station become the most important areas of the ED. Other functions of the ED are grouped around the focal point of the nurse station. The flow of patients through the ED is less structured, without clearly separate movement paths, always through the nurse point.

In the case of building a mobile military hospital there are several factors increasing the difficulty: multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure, which includes both the military and civilian objectives.

The mobile military hospital is built as a reaction to disasters, both natural and those resulting from human factor, for "emergency" (acute and crisis situations) activities. An emergency medicine, no matter of the form of its organisation, is a set of medical actions, serving a life rescue.

The technical solutions are related to 2 terms: modularity and mobility. Modularity should provide a construction of possibly most numerous and diverse structures of various purposes. Mobility is to ensure a solution which enables the module (typical commodity container) to be loaded and transported by standard ways: truck or a ship, or even air-borne.

For this achievement, the management of the Bucharest Central Military Emergency University Hospital (SUUMC) conceived a procedure which has been developed to support hospital preparedness for the management of COVID-19 patients, based on European Centre for Disease Prevention and Control recommendations [7]. The procedure started with the procurement and stock management. A procurement procedure to acquire the necessary materials and supplies was activated on short notice and alternative suppliers have been identified. The acquisition team took into account that if main suppliers should run out of stock (especially for personal protective equipment (PPE)) a buffer stock of key supplies has been acquired (e.g. for hand and respiratory hygiene, PPE, isolation, ICU supplies, mechanical respirators). A procedure to monitor and regularly update the inventory and a stock inventory have been conducted. In order to avoid misuse or overuse, a plan was developed to keep track and custody of key supplies (e.g. PPE, ventilators, cleaning and disinfection material, alcohol solution etc.).

The maximum facility capacity, including the maximal number of ICU beds, mechanical ventilators, bins for infectious waste, and the required human resource capacities (M.D.s, healthcare workers and cleaning personnel) and supply capacity have been calculated.

Storage facilities which meet all demands with respect to temperature, humidity, cold-chain, logistics etc., for additional stock have been identified, also a proper amount of cleaning and disinfection products shown to be effective against coronaviruses is available. A procedure to ensure that equipment is in perfect working order and can be quickly replaced whenever necessary was developed.

All staff, healthcare and non-healthcare workers, have been informed and trained on the following topics: Case definitions, Hand and respiratory hygiene, How to use PPE's and know the documents outlining the procedures, Triage procedures, Placement and movement of patients in isolation, Internal and external communication lines and rules, Sick-leave policy and what to do if staff members show symptoms and Security plan.

Procedures for patient prioritization (e.g. triage, discharge criteria, triggers to postpone elective hospitalizations or interventions) are in place A patient' system before they arrive at the hospital is in place: phone/email/telemedicine services are in place for possible cases.

A procedure for the cleaning of the rooms on a regular basis and after a patient's discharge has been established.

Figure 6: The ROL 2 mobile military hospital – general view

The ROL2 mobile military hospital, made up of medical tents and containers (Figure 6) is intended for the triage, diagnosis and hospitalization of patients confirmed with COVID-19. The structure of the hospital is composed of two parts: the medical and the administrative part. The medical part is structured in the following sections/laboratories/

compartments:
- Emergency Room Unit (ERU) with 20 beds
- Infectious Diseases Department (IDD) with 120 beds
- Intensive Care Unit (ICU) with 24 beds
- Imagistic Laboratory
- Medical Analysis Laboratory
- Pharmaceutical point

The hospital was equipped with medical equipment necessary for the diagnosis and treatment of patients with COVID-19, as follows: necessary RT-PCR for the identification of COVID-19, biochemistry analyzer, mobile radiology apparatus, ultrasound device, oxygen concentrators, pulse oximeters, laryngoscopes, intermittent mechanical compression devices, patient heating system, trolleys for the storage of sanitary materials and medicines, , air purification devices, wheel sprayers for car-trucks disinfection.

The Intensive Care Unit (ICU) was equipped with specific beds, secretion aspirators, oxygen pumps, automatic syringes with related stations, mechanical fans, and monitors for monitoring vital functions. Very important is the fact that telemedicine system has been installed with video cameras for the monitoring of patients, so the medical staff can keep them under observation, with minimal contact.

The Emergency Room Unit (ERU), adjacent to the patient triage area, is equipped with 8 beds and a stationary area featured with 6 compartments of 2 beds for patients waiting for lab results and for carrying out complementary investigations (interdisciplinary evaluations, imaging investigations). If the number of patients presented is greater than the capacity to take in the ERU, there are two waiting areas for patients featured with chairs located at a distance of at least 1 meter, in an adjacent tent (Figure 7).

The Infectious Diseases Department (IDD) is designated for the admission of patients confirmed with COVID-19. The section is provided with 120 beds equally distributed in 30 individual reserves with own sanitary group. Each reserve is provided with medical equipment and equipment for the permanent monitoring of patients, with the possibility of ensuring the oxygen requirement to patients who do not meet the criteria for admission to the intensive care unit. All IDD rooms are sterile environments; all personnel wear protective clothing (scrubs), shoe covers, masks, caps, eye shields and other coverings.

The inventory for 1 IDD reserve (Figure 8) includes: 4 beds, disposable linen, 1 TV set, 1 telephone and equipment for the medical supervision of patients (nurse-call system), 1 refrigerator, 4 stands of infusion, 1 acute care physiologic monitoring system (comprehensive patient monitoring systems that can be configured to continuously measure and display various parameters via electrodes and sensors connected to the patient), 4 pulse oximeters, 1 treatment table, 4 bedside tables. Lingerie is changed daily and whenever necessary.

Figure 7: The Emergency Reception Unit (ERU)

Upon admission to the IDD, each patient will receive a disposable kit containing: 1 disposable pajamas, 1 pair of disposable slippers, 1 toothbrush and toothpaste, 2 absorbent fields (towels) size 90/140 cm^2. These materials will be later distributed, whenever appropriate.

Figure 8: IDD reserve

The medical part also comprises:

• **The Intensive Care Unit** (ICU) is undergoing an arrangement with 24 beds and it is located in the annex building of the ROL 2 hospital. Patients with severe respiratory disorders cannot be properly treated in tents, as

they must benefit, for a certain period of time, of special care and connection to mechanical fans.

• **Two own laboratories** (Figure 9) where diagnostic tests (RT-PCR) and respectively hematological and biochemical investigations (complete blood count, CBC (hemoglobin), Erythrocyte Sedimentation Rate (ESR), coagulation test, myoglobin, D-Dimer, blood chemistry tests including Ferritin, troponin test, procalcitonin (PCT), presepsin (P-SEP), HBV, HCV, HIV Screening Tests, detection of SARS CoV 2 with RT-PCR) will be performed.

Figure 9: Laboratory for diagnostic tests (RT-PCR) and hematological - biochemical investigations

• **Imaging laboratory** provided with a mobile radiology device and Computer Tomography machine.

• **Pharmaceutical point** (Figure 10) where antiviral drugs necessary for the treatment of patients confirmed according to the national protocol adopted at the level of the Ministry of Health and taken over by this structure will be available.

Figure 10: Pharmaceutical point

At the ROL 2 hospital level, the functional circuits with the delimitation of the red and green areas, are ensured by providing separate flows for patients and medical staff, with rooms for equipping the medical and auxiliary staff and separate rooms with their own sanitary for undressing.

The sections and compartments of the ROL 2 hospital are computer interconnected between them and the command point, as well as with the computer network of S.U.U.M.C "Carol Davila".

The payroll comprises a total of approximately 270 positions distributed over each structure. For the operationalization of the ROL 2 Hospital, the personnel will be provided from the human resources of the S.U.U.M.C. and partly from the Medical Directorate.

The work program of the staff is structured on each separate compartment, in 12-hour shifts, over a 7-day cycle and is provided by working medical corps, made up of doctors, nurses, stretches. After this period of 7 days, the work team enters the period of self-isolation for a period of 7-14 days, a necessary measure for both the rest and the physical and mental recovery of the personnel and for the epidemiological supervision. The activity will be continued by other working medical corps, following the same structure.

At the entry and exit of the 7-day work cycle, the entire workforce will be clinically evaluated and the diagnostic test using RT-PCR for COVID-19 will be performed. Also, the staff will be tested as needed if it becomes symptomatic during the activity.

The work program is structured as follows:

- 7 consecutive days with 7 days rest for doctors and 14 days for nurses, nurses, stretches and medical administrators;
- Daily, there will be two shifts (day and evening) at 12 hours intervals; the ranges are from 08:00 – 20:00, respectively 20:00 – 08:00;
- Each 12-hour work program is provided by separate working medical corps.
- The structure and work schedule for a 7-day cycle of the IDD
- 2 specialist/consultant doctors (infectious diseases and pneumology or internal medicine);
- 6 residents (infectious diseases – 2 and internal medicine – 4);
- 12 nurses (also includes the head nurse);
- 4 orderlies;
- 2 stretches.

The procedure described may not be applicable to all military or civilian hospitals and may need to be adapted to the specific characteristics of the hospital, the individual national health system, legislation and community where the hospital is located.

"Today I visited the ROL 2 Military Hospital, a medical facility made in record time by the Army and the military medical personnel. Romania is prepared to cope as well as possible with this epidemic and I assure you that we will continue to

take all necessary measures to be even better prepared," Klaus Iohannis (Figure 11) said after visiting the hospital. [9]

Figure 11: The Romanian President Klaus Iohannis and Major General Assoc. Prof. Florentina Ioniță-Radu MD, PhD at the inauguration of the ROL 2 military hospital installed at the Ana Aslan Institute in Otopeni (Photo: Presidency.ro)

The same ROL 2 military hospital was deployed in 2015 when it was supposed to treat potential Ebola infection cases. It was also used in NATO military exercises in 2019, including Vigorous Warrior – the biggest medical military exercise ever organized by NATO, which was hosted by Romania.

"*I have entrusted the supreme ally commander of Europe with the task of coordinating all the means of supporting, coordinating and directing all the excesses at national level to the allies who need support at this particular moment, a first step taken by NATO, at this moment, is to provide the logistics of allies who need support in the fight against the coronavirus pandemic*." said Mircea Geoana, NATO Deputy Secretary General. [10]

CONCLUSIONS

The set up of the mobile military hospital installed at the Ana Aslan Institute in Otopeni, near Bucharest, to support the Romanian government as a response to the Covid-19 pandemic complies all the health requirements regulations defined by the National Authority of Quality Management in Health (Autoritatea Naţională de Management al Calităţii în Sănătate – ANMCS) throughout specific accreditation standards for medical units.

Further studies are necessary in order to determine the Covid-19 pandemic amplitude actions to end it, and future research projects destined to cover the need and development of these mobile hospitals, which involve a multitude of functional connections, sanitary requirements, technical equipment, and internal infrastructure is necessary.

Note

The ICU is arranged in the annex building of the ROL 2 hospital. Patients with severe respiratory disorders cannot be properly treated in tents, as they must benefit, for a certain period of time, of special care and connection to mechanical fans.

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Guidelines for authors

Thank you for your interest in *Romanian Journal of Military Medicine.* Please read the complete Author Guidelines carefully prior to submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review.

Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at rjmilmed@yahoo.com.

We look forward to your submission.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians and Pharmacists. The Journal publishes peer-reviewed original papers, reviews, meta-analyses and systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine.

Papers cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and historical aspects of the subject areas.

Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor will not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer reviewed under the direction of an Editor. The Editor reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials) are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

(1) Accessible to the public at no charge;

(2) Searchable by standard, electronic (Internet-based) methods;

(3) Open to all prospective registrants free of charge or at minimal cost;

(4) Validates registered information;

(5) Identifies trials with a unique number; and

(6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow-up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

Plagiarism Detection

The journal employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

Committee on Publication Ethics

The journal subscribes to the principles of the Committee on Publication Ethics (COPE).

MANUSCRIPT CATEGORIES AND SPECIFICATIONS

All articles, with the exception of Editorials, must contain an abstract of no more than 250 words. Abstracts for original articles should be formatted into subheadings, as detailed below. Titles must not be longer than 120 characters (including spaces).

Editorials

These are invited by the Editor-in-Chief or their delegated editor, and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure and cite up to 15 references, including the source article [this should be cited as Military Med. Today (year); (vol): [this issue].

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RJMM welcomes reviews of important topics across the scientific basis of medicine, and advances in clinical practice. Most published

reviews are in response to editorial invitation, including thematically related "mini-series" of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are limited to 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

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RJMM particularly welcomes submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for preparation of Meta-Analysis and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-Analyses and Systematic Reviews have a word limit of 3500–5000 words, with an abstract of up to 250 words and up to 75 references and 3–7 figures or tables.

Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical and/or historical aspects of the subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with a clear relevance to disease mechanisms and new therapies. Original articles are limited to 3000 words, with an abstract of up to 250 words and up to 50 references and 3–7 figures and tables.

Education and Imaging

The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or diagnosis of unusual cases. Contributions will be reviewed by the Education and Imaging Coordinating Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In part 1, a case will be described briefly, including a summary of the presentation, clinical features and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to diagnosis or which are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, presentation of analogous cases or related images from a similar case might be appropriate. Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background to and description of the disorder of interest together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

MANUSCRIPT PREPARATION

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain (i) a short informative title that contains the major key words. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgements. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer-review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented in the following order: (i) abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: *Background and Aim, Methods, Results, Conclusions*. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser—(http://www.nlm.nih.gov/mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: *Introduction, Methods, Results, Discussion Acknowledgments and References*.

References

The Vancouver system of referencing should be used. In the text, references should be cited using superscript Arabic numerals in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text. In the reference list, the references should be numbered and listed in order of appearance in the text. Cite the names of all authors when there are six or less; when seven or more list the first three followed by et al. Names of journals should be

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Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for *P*-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure legends

Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

Figures

All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, publication requires high quality images to prevent the final product being blurred or fuzzy.

SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at rjmilmed@yahoo.com
A cover letter containing an authorship statement should be included.
The cover letter should include a statement covering each of the following areas:
1. Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author.
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3. A statement outlining how ethical clearance has been obtained for the research, particularly in relation to studies involving human subjects, and animal experimentation. The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki) and this must be stated.
4. For research involving pharmacological agents, devices or medical technology, a clear Conflict of Interest statement in relation to any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.
5. For clinical trials, that these have been registered in a publically accessible database.
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Two Word-files need to be included upon submission: A title page file and a main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures which should be supplied separately.
The main text file should be prepared using Microsoft Word, doubled-spaced. The top, bottom and side margins should be 30 mm. All pages should be numbered consecutively in the top right-hand corner, beginning with the first page of the main text file.
Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, low-resolution figures saved as .jpg or .bmp files should be uploaded, for ease of transmission during the review process. Upon acceptance of the article, high-resolution figures (at least 300 d.p.i.) saved as .eps or .tif files will be required.

PUBLICATION PROCESS AFTER ACCEPTANCE

Accepted papers will be passed to production team for publication. The author identified as the formal corresponding author for the paper will receive an email, being asked to complete an electronic license agreement on behalf of all authors on the paper.

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ROMÂNIA
MINISTERUL APĂRĂRII NAȚIONALE

Atestat

Ministrul apărării naţionale

dorind a răsplăti meritele

redacției pubilcației „Revista de Medicină Militară",

în semn de apreciere a contribuţiei avute la creşterea prestigiului structurilor medicale din Armata României, cu ocazia aniversării a 120 de ani de la înfiinţare,

conferă ***Emblema de Onoare a Armatei României***
-cu însemn de pace-

Acordat în Bucureşti, 15 Noiembrie 2017

Ministrul apărării naţionale

Mihai-Viorel Fifor

Ordinul nr.M.P.3 din 15.11.2017
Atestat E.O.A.R./nr.1

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