FROM THE EDITOR

Dear readers

The 116th issue of MIR was prepared using the new version of the QuarkX-Press computer program, which will enable us to improve the quality of our journal. In this issue of MIR we continue to publish articles presented during the 21st IMEK. At this opportunity we are pleased to inform you that the 22nd IMEK will take place again in the city of Hódmezővásárhely (Hungary) in July 2022. It should be emphasized that co-organizer of the 22nd IMEK will be Faculty of Pharmacy of the Medical University in Szeged under the guidance of Dean prof. dr habil. István Zupkó and Vice Dean prof. dr habil. Zsolt Szakonyi. The scientific program of this important meeting was decided during a visit of an official delegation of the Faculty of Pharmacy of the Medical College of Jagiellonian University (Poland) prof. dr habil. Bożena Muszyńska, dr. Agata Kryczyk-Poprawa and prof. dr hab. Włodzimierz Opoka during a meeting in Szeged with Dean prof. dr habil. István Zupkó and Vice-Dean Zsolt Szakonyi on July 5, 2021. The proposed main themes of the 22nd IMEK are: "The new possibilities of diagnosis and treatment of civilizational diseases" and "The method of Masayuki Sajonji – history and current state. On the same day, the Polish delegation met with the mayor of Hódmezővásárhely, Dr. Péter Márki-Zay (Hungarian), who promised his help. The Organizing Committee of the 22nd IMEK will be led by a prominent Esperantist and therapist Katarina. Depending on the pandemic situation, the 22nd IMEK will take place on site in Hódmezővásárhely and Szeged or on the Internet, and the 22nd IMEK will be attended by UMEA Honorary President Dr. Imre Ferenczy and current UMEA President Dr. Christoph Klawe. Invites all participants to present their lectures in Esperanto as well as in English, Hungarian, Polish.

Professor Włodzimierz Opoka
Editor-in-Chief

Doctor Christoph Klawe
Vice-Editor-in-Chief

Professor Bożena Muszyńska
Vice-Editor-in-Chief

REDAKCIAJ VORTOJ

Karaj legantoj

La 116-a numero de MIR estis preparita uzante la novan version de la komputila programo QuarkXPress, aĉetita de Farmacia Fakultato de Medicina Kolegio de Jagelona Universitato, kio ebligos plibonigi eldonkvaliton de nia revuo. En tiu ĉi numero de MIR ni plu aperigas artikolojn prezentitajn dum la 21-a IMEK. Ĉe tiu ĉi ebleco ni kun ĝojo informas vin, ke la 22-a IMEK denove okazos en la urbo Hódmezővásárhely (Hungario) en julio 2022. Oni devas substreki, ke kunorganizanto de la 22-a IMEK estos Farmacia Fakultato de la Medicina Universitato en Szeged sub gvido de Dekano prof. dr habil. István Zupkó kaj Vic-Dekano prof. dr habil. Zsolt Szakonyi. Pri la scienca programo de tiu ĉi grava kunveno estis decidite dum vizito de oficiala delegacio de Farmacia Fakultato de Medicina Kolegio de Jagelona Universitato (Pollando) prof. dr habil. Bożena Muszyńska, dr. Agata Kryczyk-Poprawa kaj prof. dr habil. Włodzimierz Opoka dum renkontiĝo en Szeged kun Dekano prof. dr habil. István Zupkó kaj Vic-Dekano prof. dr habil. Zsolt Szakonyi la 5-an de julio 2021. La proponitaj ĉeftemojn de la 22-a IMEK estas la jenaj: "La novaj eblecoj de diagnozado kaj kuracado de civilizaciaj malsanoj" kaj „La metodo de Masayuki Sajonji – historio kaj nuna stato". En la sama tago la pola delegacio renkontiĝis kun urbestro de la urbo Hódmezővásárhely dr. Péter Márki-Zay (Hungario), kiu promesis sian helpon. La Organizan Komitaton de la 22-a IMEK gvidos elstara esperantistino Y-terapiistino Katarina Faragó. Depende de pandemia situacio la 22-a IMEK okazos surloke en Hódmezővásárhely kaj Szeged aŭ en la reto. En la 22-a IMEK partoprenos Honora Prezidanto de UMEA d-ro Imre Ferenczy kaj aktuala Prezidanto de UMEA d-ro Christoph Klawe, kiuj invitas ĉiujn partoprenontojn por prezenti siajn prelegojn en la Esperanto kaj ankaŭ en la angla, hungara, pola lingvoj.

Profesoro Włodzimierz Opoka
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Editorial Board

PROFESSOR JAN ANTONI MIKULICZ-RADECKI – ONLY THE INVENTOR OF THE PROTECTIVE MASK?

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Abstract

Professor Jan Mikulicz Radecki, a doctor of many specialties, was involved not only in many branches of surgery but also in urology, orthopaedics and gynaecology, in a broad sense of oncology, otorhinolaryngology, anaesthesiology as well as radiology.

Jan Mikulicz Radecki was a promoter and one of the pioneers of asepsis in Europe as well as in the whole world. He introduced the steam boiler for sterilization and while working in Wrocław, he developed an aseptic operating theatre. Furthermore, another innovation of Mikulicz's clinic was the use of a face mask that was made of a sterile bandage and attached to a cap.

The aim of this study was to briefly summarize the most important achievements of this outstanding doctor and inventor who, as one of the precursors of asepsis, as well as a brilliant and versatile operator, inventor and doctor, has given unequaled merit to humanity. Nowadays, when the world is struggling with the pandemic caused by the SARS-CoV-2 virus, the principles of asepsis introduced by Mikulicz-Radecki have become extremely valid and important.

Keywords: disinfection, asepsis, mask, gloves, surgery

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Jan Mikulicz-Radecki was born in the mid-19th century in Czerniowce, Bukowina [1-3] (then Austro-Hungary, now Ukraine). After completing his medical studies at the University of Vienna [4], he began his work, initially as a volunteer (operationszögling) [3-5] and then as an assistant of professor Theodor Billroth in the II Surgical Department in Vienna [3,4,5], where he was awarded the docent title [4-7]. Later he moved to Kraków where he was a professor of surgery and the director of the Jagiellonian University's Surgical Department [3-5][Fot.1]. What is more, he worked in Kalingrad (Russia) [4] and Wrocław where he also took over the surgical department [5]. Jan Mikulicz-Radecki as a doctor has always followed the principle: salus aegroti suprema lex (the well-being of the patient is the most important law) [3].

Professor Jan Mikulicz Radecki was a man of many talents. He is known as a doctor of many specialties. Mikulicz Radecki was involved not only in various branches of surgery but also in urology, orthopaedics and gynaecology as well as oncology, otorhinolaryngology, anaesthesiology and radiology [3]. Furthermore, at the time, he carried out 183 gastric resections caused by cancer [8]. He used methods of operation [3,4,5] which were hardly known and significantly innovative for his times, thoracic surgery for instance [3]. At the same time, in addition to surgical talent, he had undoubtedly a constructive mind, as he was also famous as a creator of instruments. Its thanks to him we have today the scoliosometer [4], the compessor of Mikulicz [3], the harpoon forceps [3,4], the apparatus for plastic nose surgery, or the peritoneal forceps [3]. It should be noted that he was the first in the world to construct a gastroscope [3,4] [Fot.2] and an esophagoscope [3,6,9], which is why Mikulicz-Radecki was named the father of the esophagogastroscopy [4]. Another interest of the professor was antiseptic therapy. He has become famous for using a germicidal action iodophorm. He introduced iodophorm for the treatment of surgical wounds [3,4] boil, leg ulcers, and gynaecological surgery. Mikulicz injected it also intraarticularly [3]. In the 1880s he published a work describing the effectiveness of the use of iodophorm in wound treatment [3,4,7].

Mikulicz-Radecki developed the composition of the so-called Mikulicz's ointment (containing Peruvian balsam, silver nitrate, and yellow vaseline) with a healing effect which is still used to this day (e.g. for the production of a recipe ointment), but once passed for a panacea [3]. It mainly has a healing, antibacterial and antiparasitic effect. Moreover, it is an excellent formula that supports the treatment of ulcerations or wounds which are difficult-to-treat. Mikulicz has also discovered and described a disease which has been called after him (Mikulicz's syndrome, symmetrical swelling of lacrimal and salivary glands) [1,4], as well as characteristic giant cells in the rhinoscleroma disease [3,7].

In the times of Mikulicz, a major problem for the patients and surgeons was the postoperative pain and infections, including tetanus, sepsis, phlegmon, hospital gangrene, or erysipelas. It caused postoperative mortality, which reached the level of 90% [3]. Mikulicz had to face these problems as well. During his work at the clinic in Vienna, he was interested in the antiseptic method developed by Lister and enriched his knowledge on it thanks to his scientific journey to Germany, England and France [3,4,7]. Mikulicz spread the use of Lister's method in Europe [3]. However, he soon expressed his negative opinion on the continuous spraying of carbolic acid [3,4,7] and called for this practice to be discontinued because of the harmful effects of this compound on the respiratory tract, kidneys, and the development of the carbolic gangrene of fingers by operating doctors [3].

Jan Mikulicz-Radecki was a promoter and one of the aseptic pioneers in Europe and worldwide. At the end of the 19th century, he him-

self said that he changed antiseptic into aseptic [3]. What is more, he introduced a steam boiler for sterilization [1,3] and while working in Wrocław he created a modern, aseptic operating theatre, which was then the most modern not only in Germany but across the whole Europe. At this clinic, the aseptic principles of his idea were implemented. Surgeries were no longer conducted in lecture halls, as it was generally accepted at the time, but were performed in special treatment rooms. It can be said that Jan Mikulicz-Radecki revolutionized the way and conditions in which operations were conducted. The aseptic walls of the operating theatre were coated with white tiles and the corners of the walls were rounded to improve hygiene. A chemical-bacteriological laboratory was located in the basement of the clinic. While implementing the rules of aseptic, Mikulicz used the knowledge of the bacteriologist Carl Flügge [3]. Professor Jan Mikulicz-Radecki required that only the necessary number of people be present in the operating theatre, each of whom had to be properly prepared and dressed [10]. In the late nineteenth century, he began operating in sterile gloves in his aseptic clinic [10]. He used steam-sterilized, thread gloves, but also sterile clothing, caps, and surgical gowns [3], and sterile bandages [10]. White trousers, shirts, and gowns were also introduced for daily replacement. Therefore sterile clothes, masks, and gloves were required in the operating theatre [3]. It was required not only from the surgeon but also the assistance [10]. On top of that, it should be noted that another new feature in Mikulicz's clinic was the use of a cotton mask used to cover the mouth and nose (formerly called the Mikulicz's mask), made from a sterile bandage and attached to a cap [3,11].

Before the operational procedure itself, it was necessary to wash and disinfect hands, to which Mikulicz attached great importance. The order of these measures, which he introduced, was the following: wash hands with hot soap and water, clean nails and wash them with 5% carbolic acid, wash hands with soap and hot water with a sterile brush, brush with 70 % alcohol, and wash with sublimate [10]. In addition, surgeons performing surgery had to smear the ends of their fingers in iodine [3]. If the gloves got dirty, they had to be changed each time, and in the case of digestive truct surgery, the surgeon normally changed gloves three times [10]. The operating field had to be covered with scarves and only the operated place could be exposed. Jan Mikulicz was the first to use the spiritus saponatus to wash hands with. The result of the introduced procedures was an increase in the percentage of wound healing per primam intentionem even up to 99% [3].

Summary

Jan Mikulcz-Radecki was a scholar who contributed to the development of modern surgery and medicine. His aseptic rules have become a standard over time. As one of the precursors of aseptic, an outstanding and comprehensive surgeon, inventor, and doctor, he gave unrivaled merit to humanity. Nowadays, when the world is fighting against the pandemic caused by SARS-CoV-2, the principles of aseptic introduced by Mikulicz-Radecki become even more important and relevant.

Fig.1. Photographer unknown. Prof. Jan Mikulicz together with his team of the Surgical Department Jagiellonian University in Cracow. From the left they are sitting: Czesław Górski, Rudolf Trzebicky, Jan Mikulicz, Hilary Schramm. From the left are standing: Mieczysław Dembowski, Aleksander Bossowski, NN, Roman Sondermayetr. From the collection of the Department of History of Medicine, Jagiellonian University Medical College in Cracow.

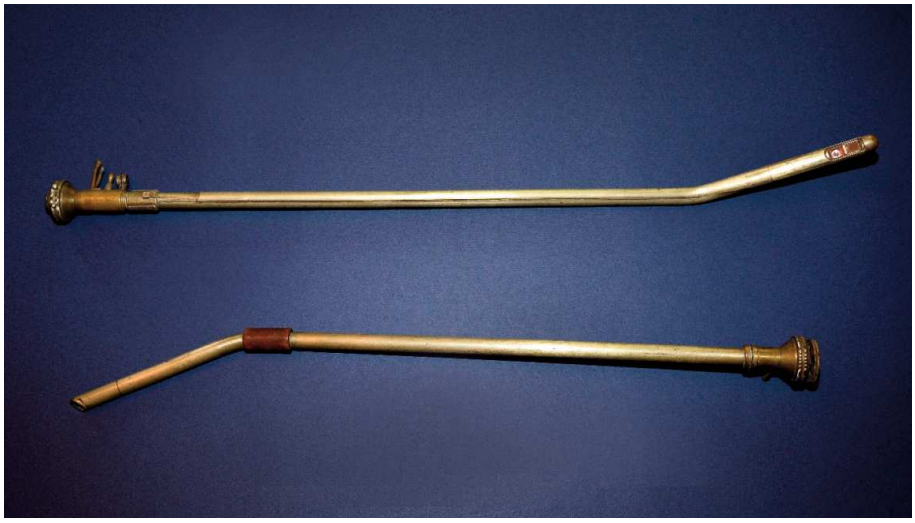

Fig.2. Mikulicz designs gastroscopes. Photography: Anna Wojnar. From the collection of the Department of History of Medicine Jagiellonian University Medical College in Cracow.

Resumo

Profesoro Jan Mikulicz Radecki, kuracisto pri multaj fakoj, okupiĝis ne nur en multaj branĉoj de kirurgio, sed ankaŭ en urologio, ortopedio kaj ginekologio, en vasta senco de onkologio, otorinolaringologio, anestesiologio kaj ankaŭ radiologio.

Jan Mikulicz Radecki estis iniciatinto kaj unu el la pioniroj de asepso en Eŭropo kaj ankaŭ en la tuta mondo. Li enkondukis la vaporkaldronon por steriligado kaj laborante en Vroclavo li disvolvis asepsan operaciejon. Krome, alia novigo de la kliniko de Mikulicz estis la uzo de vizaĝa masko farita el sterila bandaĝo kaj alkroĉita al ĉapo.

La celo de ĉi tiu studo estis koncize resumi la plej gravajn atingojn de ĉi tiu elstara kuracisto kaj inventisto, kiu, kiel unu el la antaŭuloj de asepsio, same kiel genia kaj multflanka funkciigisto, inventisto kaj kuracisto, donis grandiozan meriton al la homaro. Nuntempe, kiam la mondo luktas kun la pandemio kaŭzita de la viruso SARS-CoV-2, la principoj de asepso enkondukitaj de Mikulicz-Radecki fariĝis speciale validaj kaj gravaj.

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CORDYCEPIN AND DERIVATIVES IN PRE-CLINICAL AND CLINICAL TRIALS

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Abstract

Cordycepin (also known as 3'-deoxyadenosine) is insoluble in water, the organic compound from the group of nucleosides. This compound is produced by entomopathogenic mushrooms: Cordyceps spp. The structure of cordycepin and its potential modifications, aimed at improving pharmacokinetic and pharmacodynamic parameters as well as safety profiles, is an area for further research. It was found that cordycepin shows numerous biological activities and most of them have been demonstrated in in vitro and in vivo studies. Until now, clinical studies (or experiments involving volunteers) have confirmed, that cordycepin or its derivatives presented anti–tumor, immunostimulatory activity and symptomatic relief in multiple sclerosis.

Keywords: cordyceps spp., edible mushrooms, neuroregenerative activity, cordycepin

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1. Introduction

Cordycepin (also known as 3'-deoxyadenosine Fig.1.) is insoluble in water, the organic compound from the group of nucleosides. Structure of nucleosides are composed as nucleobases (nitrogenous bases) with an attached saccharide group. The molecule of cordycepin is created by adenine (purine) with attached a sugar unit – ribose so cordycepin is a structural analog of adenosine (nucleoside) [1; 2].

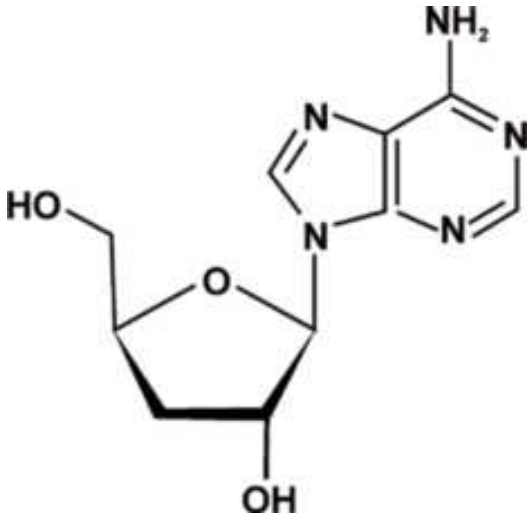

Fig.1. Cordycepin

Cordycepin is a natural biometabolite produced by entomopathogenic fungi *Cordyceps spp*., which parasitize of selected species of moths larvae. Cordycepin was isolated from *Cordyceps militaris* fruiting bodies (Photo. 1.) in 1950 for the first time [3; 4].

Fig. 1. *Cordyceps militaris* (author: Piotr Zięba)

In analytical research, cordycepin has also been confirmed in other *Cordyceps spp.* such as *Cordyceps sinensis*. So far, it has been proven that the highest concentration of cordycepin, corresponds to species *Cordyceps militaris.* Depending on the growing/breeding conditions, different values of cordycepin concentration in fruiting bodies and mycelium of *Cordyceps militaris* were obtained [5; 6].

In research of Zhou et al. 2009 content of cordycepin in fruiting bodies of *Cordyceps militaris* was estimated at 2.65 mg/g dry weight (d.w.). In mycelium *Cordyceps militaris* was obtained a lower concentration of cordycepin on the level 0.9 mg/g d.w. [5].

In 2008 analytical studies of *Cordyceps militaris* fruiting bodies and mycelium, indicate the concentration of cordycepin amounted 0.97% and 0.36%, respectively [7]. In other studies, it was found that the content of cordycepin in fruiting bodies of *Cordyceps militaris* was 1.10 mg/g d.w. , while in the mycelium of this species was estimated 1.82 mg/g d.w. [8].

Concentration of cordycepin was estimated in 2014 on the level at range 8.37 mg/g in ethanol extract, 5.28 mg/g in water extract and 1.74 mg/g in mycelial biomass of *Cordyceps militaris* [6].

In pre–clinical studies, *in vitro* and *in vivo* experiments, cordycepin has been proven to exhibit numerous biological activities, such as: immunostimulating, anti–tumor, antiviral, anti–inflammatory, ergogenic. For cordycepin was obtained also regulating activity of the endocrine system (steroidogenesis and spermatogenesis), coagulation and platelet process. For this compound was also presented its ability to inhibit adipogenesis, lipid deposition, and to improve bone structure [1].

2. Pre–clinical trials

Immunostimulating activity

An experiment conducted *in vivo* (rodents) provides evidence that cordycepin from *Cordyceps militaris* stimulated cellular and humoral immunity. The study showed an increase in the concentration of interleukins: IL-4, IL-10, IL-12. It was also confirmed the decrease in the concentration of IL-2, transforming growth factor β (TGF-β), and an effect on the level of T lymphocytes [9; 10]. An animal model was proved that *Cordyceps militaris* (enriched cordycepin) reverses immunosuppression induced by cyclophosphamide in mice [11].

Antitumor activity

In simplified terms, the main target points of cordycepin antitumor action should be mentioned: disrupting or arrest of the cell cycle, inhibiting proliferation and stimulating apoptosis of neoplastic cells. The above-mentioned effects in cell lines correlate with the biological activity of cordycepin as antimetabolite and inhibition of purine biosynthesis, disturbance in DNA or RNA biosynthesis, interaction with mTOR signal pathway, 5'AMP-activated protein kinase (AMPK), cyclin-dependent kinases (CDK), cell death such as Bcl-2 [1; 12].

Cytotoxic activity of cordycepin against neoplastic cells such as bladder, colon and stomach, has been demonstrated [13; 14].
In research from 2020, it was confirmed that cordycepin suppressed growth of human liver cancer cells. The mechanism of activity correlated with decrease in expression of CxCR4 chemokine. CxCR4 chemokine is considered as promoter invasiveness and migration of liver neoplastic cells [15].

Antiviral activity

Antiviral activity of cordycepin has been confirmed an *in vitro* experiment against various types of viruses, including influenza, Epstein-Barr (EBV) and HIV. The mechanism of action has not been fully understood, but actually research indicated that the antiviral activity of cordycepin and its derivatives is related to the inhibition of reverse transcriptase and RNA polymerase of the virus [16; 17].

In the United States in 2020 Food and Drug

Administration (FDA) approved cordycepin as a molecule with antiviral potential against coronavirus, opening new possibilities in therapy COVID-19 [18].

Anti-inflammatory activity

An *in vitro* experiment on mouse microglia proved that cordycepin acts as an inhibitor of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) enzymes and decrease concentration of inflammatory mediators: nitric oxide (NO), TNF-α, prostaglandin E2 (PGE2), IL-1β. Cordycepin has also been found to inhibit the activity of NF-κB and to inhibit the phosphorylation of mitogen-activated protein kinase (MAPK) [19].

On *in vitro* and *in vivo* experiments *Cordyceps militaris* with cordycepin reduced the ischemic area, reduced brain swelling, and limited damage of the blood-brain barrier [20]. These research results confirmed the neuroprotective activity of cordycepin and provide opportunities for further research and therapy of neurodegenerative diseases.

Ergogenic activity

Cordycepin ergogenic activity is related to its function as a precursor of ATP. It has been proven in numerous scientific evidence that precursors of ATP, such as creatine support exercise performance in human [21; 22]. Furthermore, EFSA approved health claim for creatine supplementation in daily intake of 3 g such as ergogenic aid – increases physical performance in successive bursts of short-term, high intensity exercise [23].

An animal model, confirmed the improvement of exercise capacity (grip strength test) in mice fed 12-weeks diet with the extract of *Cordyceps militaris* (concentration of cordycepin 2.33 mg/g). Intensification of ATP production, correlated with the increase concentration of markers such as AMPK, PPAR-γ, and phosphocreatine [24].

Endocrine system

In experimental rats implemented for 6 weeks a diet containing mycelium of *Cordyceps militaris* (18.92 mg/g cordycepin), demonstrated an increase motility of sperm and effect on the concentration of hormones. Confirmed increase in testosterone and estradiol levels in experimental animals. Also, an increase in concentration of cordycepin in serum of experimental rats has been noted [25].

In rats with age-related testicular dysfunction, it has been shown that the administered cordycepin, improvement of sperm quality, increased sperm motility, increased activity of antioxidant enzymes such as glutathione peroxidase (GPx4), glutathione S-transferase mu 5 (GSTm5), and peroxiredoxin (PRx4) [26].

In another scientific work was confirmed in animal models of hypogonadism, late onset (LOH) and benign prostate hyperplasia (BPH), and in vitro cell lines of the prostate, that the extract from fruiting bodies of *Cordyceps militaris* (comprising cordycepin) increased the secretion of androgens or inhibition the catabolism of androgens – testosterone and dihydrotestosterone (DHT). It was proved that the symptoms of BPH were alleviated, and hyperplasia and cells proliferation of the prostate was reduced [27].

Respiratory system

Cordycepin has been shown to reduce airway hyperresponsiveness and inflammation in murine model of asthma. Confirmed decrease in the concentration of IgE, eosinophils, as well as IL-4, IL-5 and IL-13, NF-κB [28].

In scientific work [29] in relation to cordycepin showed reduction of airway remodeling in a rat model of asthma. As in the previous cited publication, at this point has also been demonstrated decrease in IgE, eosinophil and neutrophil level, and decrease of expression TNF-α, TGF-β1, IL-5, IL-13.

Locomotor system

In vitro experiments confirmed that cordycepin inhibits osteoclast differentiation. It is associated with inhibition of the NF-κB ligand receptor activator (RANKL). In murine model of osteoporosis (induced LPS), for cordycepin has been proven to limited bone loss [30]. An

animal model of osteoarthritis in rats, cordycepin has been demonstrated to reduce pain and inflammation in the synovium. Cordycepin acts as an inhibitor of polyadenylation [31].

3. Clinical trials

On the website clinical trials.gov for the phrase "cordycepin", we can find only 23 results for this molecule. Two studies as intervention used a combination of cordycepin and pentostatin (also known as deoxycoformycin) applied in the form of intravenous (IV) infusion. Pentostatin is an inhibitor of enzyme adenosine deaminase (ADA), which is responsible for the degradation of cordycepin [32].

In the first research, phase I clinical trial, attended 14 participants with refractory acute lymphocytic or chronic myelogenous leukemia [33]. On second Interventional Open Label Clinical Trial which covered I and II phase, participate 44 patients with refractory TdT-positive leukemia [34]. Anti–tumor or cytotoxic activity of cordycepin has been confirmed in pre-clinical studies, but similar results have not been achieved on abovementioned clinical trials. As the main cause it is lability of cordycepin and susceptibility to enzymatic degradation by enzyme ADA.

Only one clinical trial (Phase I), which include 94 participants suffering from advanced solid tumours or lymphoma, given IV new phosphoramidate analogue of cordycepin signed as NUC-7738. The addition of phosphoramidate group to the cordycepin molecule is responsible for resistance and limiting the enzymatic degradation this molecule by ADA [35; 36].

Also one item study concerns of the influence, 8-weeks oral supplementation the mushroom beverage (contain mushroom extract Cordyceps militaris), on emotions regulation and explore of the antidepressant effect in humans. In this Interventional and triple-blind, randomized-controlled trial, qualified 80 participants. Volunteers received mushroom beverage with extract of *Cordyceps militaris* provides three different bioactive compounds: cordycepin, polysaccharides and mannitol. So far, not reported any research results [37].

The remaining 19 studies related to chloro derivative cordycepin – Cladribine, in combination with other drugs. In one of the studies, attended 37 participants, with secondary acute myeloid leukemia. As a chemotherapeutic intervention, it was used IV: Cladribine, Cytarabine (as antimetabolite) and Uproleselan (as glycomimetic and novel E-selectin antagonist, interferes with activation of the survival cells, enhances the response to chemotherapy and protects against the toxicity) [38]. Studies have been undertaken in patients with mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), or B cell non-Hodgkin's lymphoma. As chemotherapy interventions, a combination Vorinostat, Cladribine, and Rituximab, was used in patients [39; 40].

The European Medicines Agency (EMA) has approved active substance - cladribine as a neurological medicine, in tablet formulation, intended for oral administration at dose 10 mg, with indication for pharmacotherapy relapsing forms of multiple sclerosis [41; 42; 43].

Some study on human subjects have been conducted in Asia. Most studies have verified the consumption of *Cordyceps sinensis* or *Cordyceps militaris.* In three example studies, the active ingredient is specified as cordycepin. In some scientific works, the major active ingredient were not specified, or they were a combination (or one listed) of active ingredients, such as: adenosine, polysaccharides or mannitol (also known as cordycepic acid).

In patients with mild liver dysfunction, it was proved that 8-weeks supplementation of 1.5 g/day Cordyceps militaris (cordycepin 1.9 mg/g) contributes to support protection of the liver against lipids accumulation [44].

In group of healthy adults' males who supplemented 1.5 g/day of *Cordyceps militaris* (1.9

mg/g cordycepin) for 4 weeks, immunostimulatory activity was demonstrated, which resulted from an increase expression of: IL-2, IL-12, NK, TNF-α, IFN-γ [45].

Another study in a group of 100 patients (20–70 years) analyzed the effect of 12-weeks supplementation of *Cordyceps militaris* (cordycepin 1.9 mg/g) on upper respiratory tract infection and immune response. It was confirmed that consumption of *Cordyceps militaris* had no significant effect on the frequency and symptoms of cold. However, have been demonstrated an increase IgA concentration and stimulation NK cell activity, which are indicated immunostimulating activity [46].

4. Conclusions

It was found that cordycepin presented numerous biological activities. Most of them have been demonstrated an *in vitro* and in *vivo research.* Until now, clinical studies (or experiments involving on volunteers) have confirmed, that cordycepin or its derivatives as anti–tumor, immunostimulatory activity and symptomatic relief in multiple sclerosis. Proven immunostimulatory activity and *in vitro* antiviral activity, including against RNA viruses, made cordycepin a potential structure for the prevention and/or treatment of COVID-19 in the "era" of the coronavirus pandemic. Ergogenic activity perhaps correlates with a strengthening the physical condition of the body, which is important in the period of convalescence, recovery after traumatic injuries or in devastating diseases, in the terminal stages. Modifications of the structure cordycepin, recognition of the structure-activity relationship (SAR) and the use of additional substances that will increase the effectiveness of therapy with cordycepin or its derivatives require further research in science.

Resumo

Kordicepino (ankaŭ konata kiel 3'-deoksiadenosino) estas nesolvebla en akvo, organika komponaĵo el la grupo de nukleozidoj. Ĉi tiu kemiaĵo estas produktita de entomopatogenaj fungoj: Cordyceps spp. La strukturo de kordicepino kaj ĝiaj eblaj modifoj, celantaj plibonigi farmakokinetikajn kaj farmakodinamikajn parametrojn kaj ankaŭ sekurecajn profilojn estas areo por plua esplorado. Oni trovis, ke kordicepino montras multajn biologiajn efikojn kaj plej multaj el ili estis pruvitaj en in vitro kaj en in vivo. Ĝis nun klinikaj studoj (aŭ eksperimentoj pri volontuloj) konfirmis, ke kordicepino aŭ ĝiaj derivaĵoj prezentis kontraŭtumoran, imunostimuligan efikon kaj trankviliĝantajn simptomojn en multloka sklerozo.

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ECHOCARDIOGRAPHICALLY-GUIDED BALLOON AORTIC VALVOPLASTY

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Abstract

We report a neonate with critical aortic valvar stenosis. He was treated by bedside balloon valvuloplasty and the only imaging technique for this intervention was echocardiography. The procedure was successful. The patient has been operated twice since then, including a metallic aortic valve replacement. He is approaching his 10 years birth anniversary and fares well.

Keywords: metallic aortic valve, mini myshak, neonatal cardiac intervention

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Introduction

Balloon aortic valvoplasty (BAV) is an angiographic method for relieve of severe aortic stenosis with appreciable early and long term results (1). Bedside balloon septostomy guided exclusively by echocardiography is now an acceptable method in critical neonates needing prompt intervention while their transport to a catheterism laboratory is dangerous. However, there may be situations when other bedside interventional procedures become necessary in neonates with the same rationale. Hereby, we report a successful experience of bedside balloon valvoplasty under the guide of transthoracic echocardiography in a newborn with extremely critical aortic stenosis, with 10 years of follow up.

Case report

A 29-day-old newborn was admitted at our neonatal intensive care unit (NICU) with the diagnosis of myocarditis. He had signs of diminished cardiac output including weak pulses, cold extremities, and grayish skin color. Infusions of dopamine and dobutamine were started from the onset. Shortly after arrival at NICU, severe bradycardia and cardiac arrest developed. Successful resuscitation was performed, after which the patient needed epinephrine infusion to keep blood pressure at a level compatible with life. An emergency echocardiography revealed severe cardiomyopathy with an ejection fraction of 10%. Despite severe depression of systolic cardiac function, a 45 mmHg pressure gradient across aortic valve was detected, as well as thickness of the aortic valve leaflets. Accordingly, a diagnosis of critical aortic stenosis was made.

Due to the critical condition of the patient, an urgent valvuloplasty was considered. At that time, our catheterism laboratory was located at a nearby hospital needing car transfer of the patients. Certainly, potential dangers were anticipated in transfer of the patient. Unfortunately, an arterial switch operation was begun at our only cardiac operation room needing several hours to be completed. Therefore, we decided to perform bedside BAV under the guide of transthoracic echocardiography.

Procedure

We preferred to catheterize femoral artery due to our greater experience and less interference with the echocardiography in comparison with jugular artery catheterization. Left femoral artery was catheterized by a 5 Fr Radiofocus Introducer II (Terumo, Japan). A portable echocardiographic scanner (MicroMaxx Ultrasound System, Sonosite Inc., USA) was used to guide the procedure. Frequent changes from suprasternal to long axis parasternal view were made by the echocardiographist to guide the interventionist during the procedure. An 0.018 inch hydrophilic guide wire was passed over a 5 Fr right Judkins angiographic catheter (Cordis, USA). After several attempts, we could pass the guide wire through the stenotic valve. Then, a 5 Fr multipurpose A2 catheter was exchanged for the Judkins catheter and positioned in the left ventricle (LV). The guide wire was exchanged with a 0.014 inch moderate support guide wire, consequently. The aortic annulus was measured around 7 mm. We first used a Tyshak II balloon catheter with a diameter of 5 mm and length of 20 mm. Balloon valvoplasty reduced the echocardiographic pressure gradient (PG) across aortic valve to 25 mm Hg. Then, we used another Tyshak II balloon catheter with a diameter of 7 mm and length of 30 mm. PG was decreased to 21 mm Hg and trivial aortic regurgitation was developed. We considered the procedure successful and terminated it.

Follow up

The condition of the patient improved gradually after BAV. Epinephrine infusion was

tapered and discontinued after 3 days. The patient was extubated 7 days after the procedure. Dopamine and Dobutamine infusions were discontinued 15 days after the procedure. The patient transferred to the floor bed and discharged from the hospital. Ejection fraction increased gradually, as well as transaortic PG. Three weeks after the procedure during the first outpatient visit, EF was 80% and PG 48 mmHg. Six month after the procedure, the patient did well. There was no change in transaortic PG and EF. A complete neurologic examination showed normal neurologic condition of the baby despite prolonged underperfusion. Peripheral pulses including those of the left lower extremity were palpable.

The patient was operated twice. At the age of two years, aortoplasty was done due to high transaortic PG, and finally at the age of 8.5 years, the aortic valve was replaced by a St Jude 19 mm prosthetic valve after aortic root enlargement. The patient is now close to his 10 years birth anniversary. His cardiac and aortic valvar functions are acceptable. There is only a small paravalvar leak.

Discussion

Percutaneous balloon and surgical valvuloplasty are two treatment modalities for the neonates with critical aortic stenosis. BAV was replaced surgery due to better rsults and lower mortality (1). Normally balloon valvuloplasty is performed in the catheterism laboratory under angiographic guiding. Simultaneous use of echocardiography yielded better results, lower risk of insufficiency, and lower X-ray exposure (2, 3). Exclusive use of echocardiography during this procedure has been reported (4). However, long term follow up of these patients is unknown.

Echocardiography is used in many centers to perform bedside balloon septostomy in critically ill neonates needing this procedure (mostly transposition of the great arteries). Although the two procedures have many similarities, BAV needs more extensive wire maneuvers in the arteries and heart, and has a greater potential risk of injury when done under echocadiography. The risk of aortic wall injury after standard BAV is around 15 % (5). However, there are no data regarding this risk when echocardiography was used as the only guiding method. In the particular situation we confronted, the potential hazards of guide wire manipulation were negligible in view of the need to do a procedure without delay. To limit the potential risk of injuries, we carefully followed the tip of guide wires by changing the echocardiographic windows whenever needed. Echocardiography showed the tip of wires precisely.

Conclusion

Especially in developing countries were angiography and cardiac surgery facilities are not widely available, echocardiography guided BAV can save lifes in emergency situations.

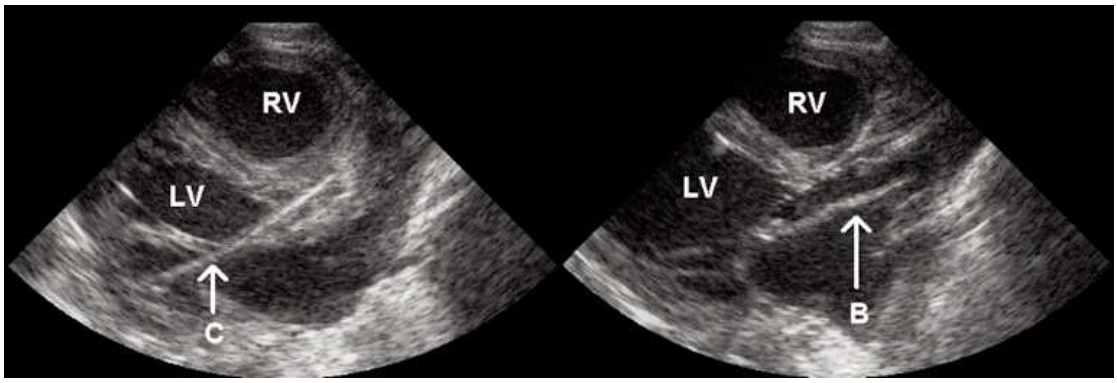

Fig. 1. Echocardiographic images when endhole catheter and Tyshak balloon were passed through the aortic valve. The balloon was inflated. LV, left ventricle; RV, right ventricle, c; catheter; b, balloon.

Resumo

Ni raportas novnaskiton kun tre serioza stenozo de aorta valvo. Li kuraciĝis per balona valvoplastio sun ununura bild-gvido de ekokardiografio. La proceduro estis sukcesa. Poste oni faris du operaciojn por li, inkluzive de la valvanstataŭigo per metala valvo. Li nun proksimiĝas al sia 10-jara naskiĝ-tagreveno kaj fartas bone.

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MICROBIOTA ON THE SURFACE OF MELANOMA MAY PROMOTE IN DERMAL INVASION OF MELANOMA

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Abstract

It is not yet known exactly what happens first: tumor invasion and the associated change in the microbiota, or, conversely, changes in the microbiota before tumor invasion.

The aim of the work was to investigate the contamination and colonization of microbes in melanoma of the skin.

In 23 primary melanoma patients the samples for microbiological examination were taken from melanoma surfaces and from non-lesioned skin before surgery. Microorganisms were cultured at optimal temperatures on elective or differential media appropriate for each taxon and were identified by bacterial analyzer. After wide local excision of melanoma the histological examination determined Breslow thickness and Clark's level of melanoma invasion; Loeffler's methylene blue staining was used to detect the colonies of microorganisms.

From intact skin 62 bacterial cultures were isolated with density of colonization in 1.2×10^3 – 6.4×10^3 CFU/cm^2. From ulcerated surface of melanoma 25 bacterial cultures were identified. The concentration of microorganisms was significantly higher on ulcerated melanomas. The colonization density of S. aureus was highest; its concentration was 5.8×10^7 comparing to 6.4×10^3 CFU/cm^2 on intact skin. Concentration of gram-negative rods was high also; e.g: E. coli and P. putida were 6.2×10^6 and 1.8×10^5 CFU/cm^2 respectively. Loeffler's staining of histological specimens revealed colonies of microorganisms at the bottom of melanoma ulcers. In case of ulcerated melanoma with Clark level IV invasion the microbial colonies were identified in the reticular dermis.

The spectrum of microorganisms on the surface of intact skin is twice as large (62 vs. 25) as on surface of ulcerative melanoma, but the concentration of microorganisms is significantly higher on ulcer tumor's (10^5–10^7) surface than intact skin (10^3–10^4).

Microbiota on the surface of the chronic ulcer may increase local pathogenicity leading to tissue degradation that may be essential for intradermal melanoma invasion.

Keywords: skin melanoma, microbiota, microbial colonies

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Introduction

Numerous studies have been published highlighting the relationship between gut microbiota and melanoma or other cancers [1–5]. They showed the host microbiome has come to the forefront as a potential modulator of cancer metabolism, and the bacterial biofilms might act as direct triggering factors contributing to cancer [6, 7].

The bacteria that reside specifically within cancer have been found to be tumor-type specific, suggesting an association with cancer development [8, 9].

Recent studies indicate the association between bacteria and the tumor microbiome is complex and remains poorly understood. How the skin microbiota affects melanoma has not yet been studied [10, 11]. However, microbiomes in inflammatory diseases of skin have been suggested to be associated with squamous skin cancer [12]. Salava et al. [13] reported minimal differences in the stratum corneum microbiome between melanoma and benign nevi, although melanoma samples seemed to have slightly less microbiome diversity. In experimental pig melanoma model was found that microbiome diversity in full thickness biopsies from nonlesion skin had a significantly different microbiome from that observed in melanoma tissue [14]. In addition, we must take into account that melanoma cells deeply interact with the tumor microenvironment and the immune system [15, 16]. Overall, further studies to unravel the potential influence of microbial exposure on tumor genesis are highly warranted and might pave a way for novel strategies for cancer therapy [17, 18].

The development of a malignant tumor is accompanied by its invasion and metastasizing, which can also affect the vector of invasion of bacteria contained in the tumor. It is not yet known exactly what happens before: tumor invasion and the associated change in the microbiota, or, conversely, changes in the microbiota induce tumor invasion.

Aim: to investigate the contamination and colonization of microbes in skin melanoma.

Material and methods

This study was approved by the Ethics Committee of the Ternopil National Medical University and conducted according to the principles of the Declaration of Helsinki. All participants were enrolled after providing written Informed Consent. Patients with primary skin melanoma T3–T4 (n=23, average age 52±9 years, 9 male and 14 female) admitted to the Ternopil Regional Clinical Oncology Hospital were included in study.

Samples for microbiological examinations were taken by a cotton-tipped swab from the melanoma surfaces (ulcers) and from the non-lesion skin (at a distance 5-6 cm of the tumor) on 3-4 days before surgery. Transport medium (AMIES) that conserves viability and prevents the growth of microorganisms was used. The skin swab sampling procedure and cultivation of microorganisms were performed in an accordance of the standard rules. Microorganisms were cultured at optimal temperatures on appropriate for each taxon elective or differential media: salt-yolk agar (for staphylococci determination), Endo medium (for enterobacteria and nonfermenting bacteria determination), Thioglycolate broth and sugar blood agar (for anaerobes cultivation), sugar meat-pepton agar (to enumerate colony forming units (CFU)), sheep blood agar (to detect hemolytic activity of microorganisms). Culture was considered positive if concentration of microbes was at least 103 CFU/cm2. Contamination level 105 CFU/cm2 was considered critical, it testified the role of bacteria in development of infectious process and probably in cancerogenesis. Microorganisms were identified by bacterial analyzer Vitek2 Compact (bioMerieux, France).

All patients underwent radical surgery – wide local excision of melanoma. During histological examination, paraffin sections were stained with hematoxylin-eosin to determine the Breslow thickness of tumor and Clark's level of

melanoma invasion; the Loeffler's methylene blue staining was used to detect the colonies of microorganisms.

Samples obtained from swabbing provide information on the superficial microbiota composition, whereas morphological specimens of removing melanomas offer the opportunity to study microorganisms that could inhabit the deepest layers of the skin.

Results and Discussion

From intact skin 62 bacterial cultures were isolated. The various species of bacteria were identified, however most of them were in low density and not included in this study. Gram-positive cocci, such as: Staphyloccocus aureus, S. epidermidis S. lentus, S. haemolyticus, Kocuria kristinae, Enterococcus faecalis, gram-positive rods Bacillus megaterium, and gram-negative rods Pseudomonas putida and Acinetobacter baumannii were presented on the intact skin in 1.2×103 – 6.4×103 CFU/cm2 (Table). They were included into 3-component-associations mostly. The frequencies of occurrence of staphylococci were highest on intact skin.

From ulcerated surface of melanoma gram-positive cocci (S. aureus, S. epidermidis) and gram-negative rods, such as: enterobacteria (E. coli, Klebsiella pneumoniae, Proteus vulgaris), and nonfermenting bacteria (A. baumannii, Pseudomonas putida) were identified (n=25). Microbial spectrum was less diverse on melanoma ulcer (Table). Similar data was shown by Mrázek et al. (2019): both bacterial composition and diversity were significantly different between the skin and melanoma microbiota. In our study the frequency of occurrence of gram-positive staphylococci on melanoma ulcer was higher in 1.8 times than same index of gram-negative rods. Similar ratio between frequency of occurrence of gram-positive and gram-negative bacteria was on intact skin. However, the concentration of microorganisms was significantly higher on melanoma ulcer. For example, the colonization density of S. aureus was the highest. Its concentration increased up to 5.8×107 CFU/cm2 compare to 6.4×103 CFU/cm2 on intact skin (Fig. 1).

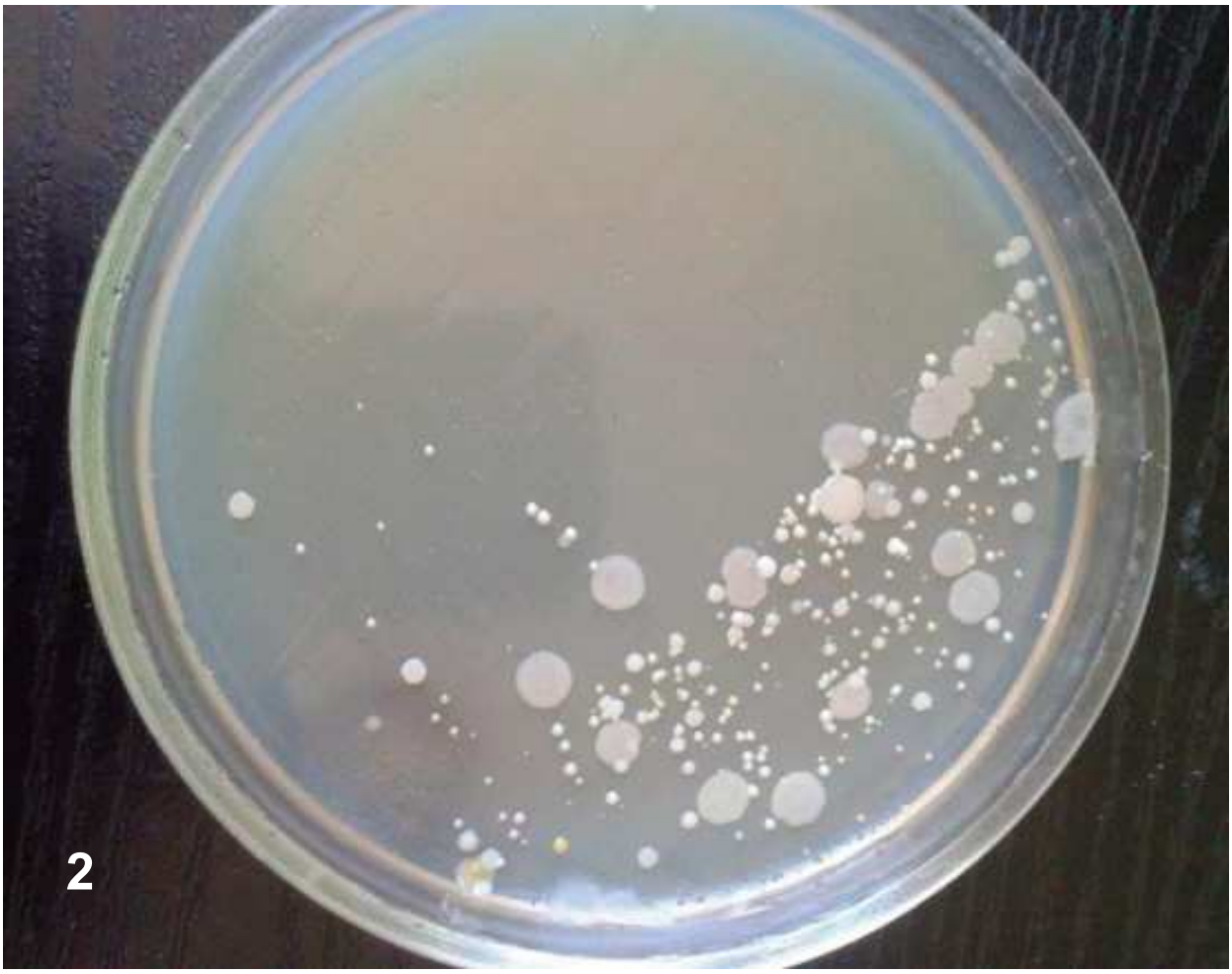

Fig. 1. The growth of bacteria, isolated from ulcerated surface of melanoma (1) and intact skin (2) by streak method, on sugar MPA.

The concentration of gram-negative rods was high also. The P. putida density of colonization was higher in 2 times (1.8×105 CFU/cm2 and 2.4×103 CFU/cm2 respectively).

It is interesting that in the study Squarzanti et al. (2020) was suggested the S. aureus may serve as marker of risk for development of squamous cell carcinoma and contribute to the cutaneous carcinogenesis through the chronic inflammatory state.

Morphological exam of removed melanomas revealed the following depth of tumor invasion: II (4 samples), III (11) and IV (8) level by Clark; 19 melanomas were ulcerated and 4 non-ulcerated. In melanoma without an ulcerative surface and the second level of Clark invasion, single colonies of microorganisms were observed at the level of the papillary dermis (Fig. 2).

In the case of ulcerative melanoma, colonies of microorganisms were determined at the bottom of the ulcer on the background of severe inflammatory infiltration by leukocytes (Fig. 3).
In samples of melanoma with ulcer on the surface and the Clark level IV invasion, microbial colonies were identified in the reticular layer of the dermis on the background of lymphocytic infiltration (Fig. 4).

Thus it is the first study to compare microbial contamination on the surface of melanoma and the presence of microbial colonies in the skin, which needs further investigation. A better understanding the role of the microbiota in skin malignancies is necessary, as it could potentially provide further insight into the different roles tissue-specific microbes in cancer progression.

Table1. Microbial diversity and density of colonization of ulcerated surface of melanoma by bacteria compare with intact skin

Microorganism		Ulcerated surface of melanoma		Non-lesion skin	
		frequency of occurrence, %	density of colonization, CFU/cm^2	frequency of occurrence, %	density of colonization, CFU/cm^2
Gram-positive cocci	*K. kristinae*	-	-	34.8	5.6×10^3
	S. aureus	43.5	5.8×10^7	47.8	6.4×10^3
	S. epidermidis	26.1	8.1×10^6	65.2	4.1×10^4
	S. haemolyticus	-		26.1	1.8×10^3
	S. lentus	-	-	17.4	1.2×10^3
	E. faecalis	-	-	8.7	1.4×10^3
Gram-positive rods	*B. megaterium*	-	-	26.1	2.6×10^3
	B. subtilis	-	-	21.7	3.1×10^3
Gram-negative rods	*E. coli*	13.1	6.2×10^6	-	-
	K. pneumoniae	30.4	-	-	-
	P. vulgaris	4.3	3.1×10^5	4.3	1.1×10^3
	P. putida	21.7	1.8×10^5	21.7	2.4×10^3
	A. baumannii	4.3	2.1×10^5	4.3	1.1×10^3

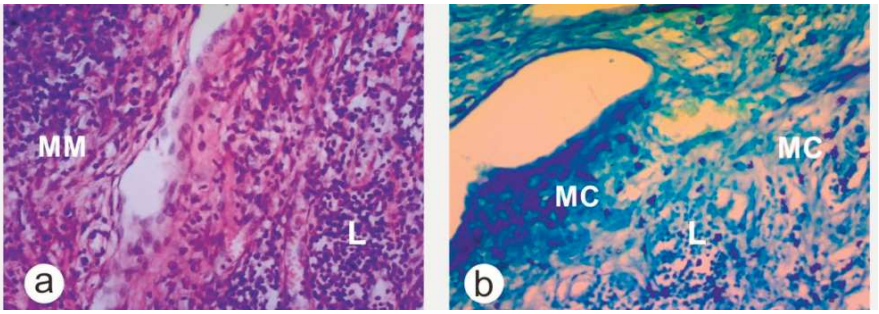

Fig. 2. Histological specimen No. 7908-9. Malignant melanoma (MM) with Clark level II invasion without ulceration: (a) hematoxylin-eosin, ×200; (b) Loeffler staining, ×400; lymphocytic infiltration (L), microbial colony (MC).

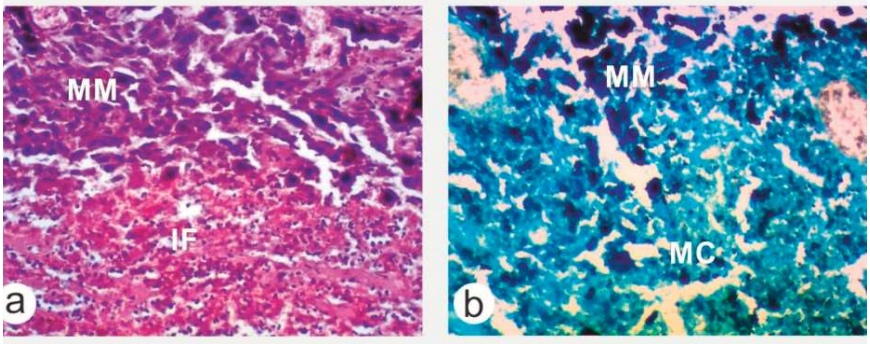

Fig. 3. Histological specimen No. 23082. Malignant melanoma: bottom of ulcer with tissue inflammation (IF), microbial colonies (MC); (a) hematoxylin-eosin, ×200; (b) Loeffler staining, ×200.

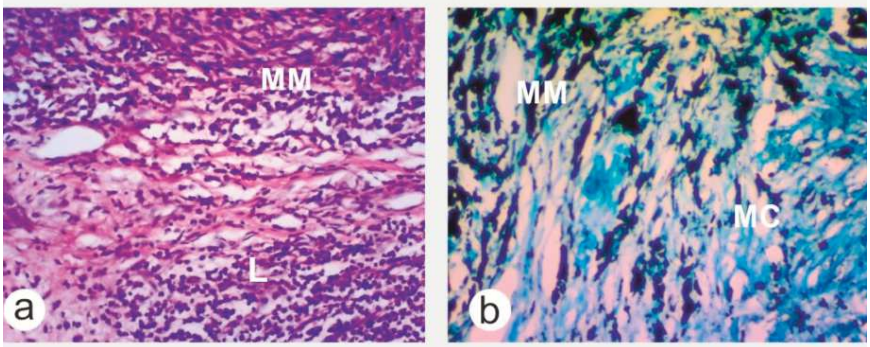

Fig. 4. Histological specimen No. 5550-1. Malignant melanoma (MM) with ulceration and Clark level IV invasion: (a) hematoxylin-eosin, ×200; (b) Loeffler staining, ×200; lymphocytic infiltration (L), microbial colonies (MC) in reticular derma.

Conclusions

Our study shows the qualitative and quantitative differences of microbiota on surfaces of ulcerated melanoma and non-lesion skin. The spectrum of microorganisms on the surface of non-lesion (intact) skin is twice as large (62 vs. 25) as on the surface of ulcerative melanoma, but the concentration of microorganisms is significantly higher on ulcer tumor's (10^5–10^7) surface than intact skin (10^3–10^4).

Microbiota on the surface of the chronic ulcer may increase local pathogenicity leading to tissue degradation that may be essential for intradermal melanoma invasion. With ulcerative melanoma and IV level of its invasion the colonies of microorganisms are located both at the bottom of the malignant ulcer and in the deep layers of the dermis (e.g., reticular).

Resumo

Nun oni ankoraŭ ne scias precize kio okazas antaŭe: tumora invado kaj la rilata ŝanĝo en la mikrobaro, aŭ, male, ŝanĝoj en la mikrobaroj estigas tumoran invadon.

La celo estis esplori la poluadon kaj koloniigon de mikroboj en haŭta melanomo.

En 23 primaraj melanomaj pacientoj la specimenoj por mikrobiologia ekzameno estis prenitaj de melanomaj surfacoj kaj de neleza haŭto antaŭ kirurgio. Mikroorganismoj estis kultivitaj ĉe optimumaj temperaturoj laŭ taŭga elektebla aŭ diferenciala medio por ĉiu taksono kaj estis identigitaj per bakteria analizilo. Post larĝa loka dekoltaĵo de melanomo la histologia ekzameno determinas Breslow-dikecon kaj la nivelon de Clark de melanoma invado; la metilen-blua kolorigo de Loeffler estis uzata por detekti la koloniojn de mikroorganismoj.

De sendifekta haŭto 62 bakteriaj kulturoj estis izolitaj kun denseco de koloniigo en 1.2 × 10^3 - 6.4 × 10^3 CFU/cm^2. De ulcerigita surfaco de melanomo identiĝis 25 bakteriaj kulturoj. La koncentriĝo de mikroorganismoj estis signife pli alta ĉe melanoma ulcero. La kolonia denseco de S. aureus estis la plej alta; ĝia koncentriĝo estis 5,8 × 10^7 kompare al 6,4 × 10^3 CFU/cm^2 sur nerompita haŭto. Koncentriĝo de gramnegativaj baciloj ankaŭ estis alta; ekz: E. coli kaj P. putida estis 6,2 × 10^6 kaj 1,8 × 10^5 CFU/cm^2 respektive. La kolorigo de Loeffler de histologiaj specimenoj malkaŝas la koloniojn de mikroorganismoj ĉe la fundo de melanoma ulcero. En kazo de ulcerigita melanomo kun Clark-nivela IV-invado la mikrobaj kolonioj estis identigitaj en la retoforma dermo.

La spektro de mikroorganismoj sur la surfaco de sendifekta haŭto estas duoble pli granda (62 kontraŭ 25) ol sur surfaco de ulcera melanomo, sed la koncentriĝo de mikroorganismoj estas signife pli alta sur la surfaco de ulcera tumoro (10^5-10^7) ol sendifekta haŭto (10^3-10^4).

Mikrobaro sur la surfaco de la kronika ulcero povas pliigi lokan patogenecon kaŭzante histan degradadon, kiu povas esti esenca por intraderma melanoma invado.

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Short article

TOBACCO AT IRISH FUNERALS: THE FINAL NAIL IN THE COFFIN

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Abstract

This article highlights the development of the traditional Irish costum to offer tobacco, snuff and beer to callers on the occasion of wakes. A change of habits in recent times as well as the support of the clergy lead to much lesser consumption of tobacco in this context

Keywords: Ireland; tobacco; funeral; wake; snuff; denormalisation

The Irish Government is committed to becoming smoke free (<5% smoking prevalence) by 2025. As part of this process it is committed to a policy of the denormalisation of tobacco [1]. The aim of tobacco denormalization in Ireland may recently have received support from an unexpected quarter that may effectively remove tobacco from Irish funerals. It is interesting to note that up until the mid-1950s, or later in more rural areas, an Irish funeral was accompanied by significant consumption of alcohol, tobacco and snuff [2]. A traditional Irish burial was often preceded by a Wake lasting up to three days (see Figure One) [2,3].

At such Wakes 'customs included the laying of clay pipes, tobacco, and snuff in the room. Every male caller was expected to take at least a puff… Usually, a pipe and tobacco were placed on a table next to the body. On some occasions, a pipe was also laid on the deceased's chest' [4]. The social standing of the deceased and their family was in-part measured through their generosity in the provision of tobacco, snuff, food and alcohol [3]. However, this tradition is fast disappearing and the increasing use of funeral homes has effectively marked the end of this practice. Funeral homes are subject to Ireland's smoke-free legislation, and as such the associated significant consumption of tobacco at such events has ceased.

One final event that may have finally removed tobacco from the Irish funeral scene relates to a recent comments about the inappropriateness of offeratory gifts that went viral [5]. It should be noted in the 2016 Census 78.3% of Ireland's population self-identified as Catholic [6]. From a strict Catholic perspective, offeratory gifts are supposed to relate purely to the bread and wine associated with the funeral Mass [7]. However, in Ireland tradition usually involves family members bringing up a few items that represent the interests or passions of the deceased.

However, controversy emerged recently in Ireland when a priest spoke out against tobacco and alcohol being used as symbolic offeratory gifts:

Bringing things such as a can of beer, a packet of cigarettes, a remote control, a mobile phone or a football jersey does not tell us anything uplifting about the person who has died… Surely items such as a flower, a family photograph, a prayer-book or rosary reveals far more about the person who has died and the loss he/she is to the family who grieve [5].

Such objections from the clergy are hardly new. There is a long history of both clerical and civil authority concerns over what was often viewed as an excuse for licentiousness [3]. However, what is perhaps different

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is that, although objections to this statements were noted in some quarters [8,9], there was significant national and international take-up on this story [5,8,9,10].

Given the attention this incident provoked it seems highly likely that the outcome will be the widespread adoption of an informal policy around the prohibition of tobacco as an offertory gift. Thus, this priest's tirade that went viral seems likely to have helped tobacco denormalization in Ireland.

Figure 1: A Stereotypical Irish Wake (note man & woman with clay pipes)

Resumo

Tiu ĉi artikolo prilumas la evoluon de la tradicia irlanda kutimo donaci tabakon, naztabakon kaj bieron al vizitantoj kadre de funebraj vigiloj. Ŝanĝo de kutimoj dum la lastaj jaroj kaj la apogo de klerikoj rezultigis konsiderindan malpliiĝon de tabakokonsumado kadre de tiu ĉi kunteksto.

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CYBERSECURITY, RANSOMWARE ATTACKS AND HEALTH: EXPLORING THE PUBLIC HEALTH IMPLICATIONS OF THE RECENT CYBERATTACK ON IRELAND'S HEALTH SERVICE

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Abstract

In May 2021, Ireland's state healthcare system, the Health Services Executive, was the subject of a devastating ransomware attack called Conti. The malware uses a number of sophisticated tools and has effectively caused the healthcare system to go off-line for an extended period of time as the state has refused to pay the $19,999,000 ransom demand. Globally, ransomware is becoming a major issue for healthcare systems, with widespread attacks, even increasing in number during the Covid-19 pandemic. Healthcare systems may be being targeted for a number of reasons including that they are necessary to a population and may be vulnerable to compromise due to a lack of cybersecurity resources. To improve the security posture of healthcare systems a rebalancing may need to occur with potential impacts on resources available for healthcare provision and consequent impact on public health. Preventative measures regarding ransomware are presented, including what to do if an attack is discovered.

Keywords: cybersecurity, ransomware attacks and health: exploring the public health implications of the recent cyberattack on Ireland's Health Service

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In mid-May of 2021 the IT systems of Ireland's state health system, the Health Services Executive (HSE), was the subject of a sophisticated ransomware attack, with the criminal gang involved allegedly demanding $19,999,000 to restore access. Whilst many details of this cyberattack are not available yet, it is being called the Conti Ransomware attack [1]. The head of the Republic of Ireland's health service has described the impact of this attack as 'catastrophic'.

It should be noted that the history of ransomware is linked to healthcare. The first recorded case of this phenomenon was the 1989 AIDS Trojan (also known as PC Cyborg), in which 20,000 infected diskettes were sent to delegates attending a WHO conference on AIDS. Subsequently a Trojan software activated to hide directories and encrypt file names. Payment to an address in Panama was required to solve the issue [2].

This attack on Ireland's HSE is only the latest in a growing number of cyber-attacks on health systems. Other recent attacks include the Brno University Hospital in Czechia [3], and attempts by the PentaGuard group to target hospitals in Romania [4]. Perhaps the most well-known health system attack was against the Hollywood Presbyterian Medical Center in Los Angeles, California, which subsequently paid $17,000 to obtain a decryption key to regain access to their files. However, other major attacks include the targeting of the US health insurance company Anthem in 2015, the Australian Red Cross Blood Service in 2016, and the global WannaCry ransomware attack in 2017, which reportedly infected approximately 200,000 systems across more than 150 countries, including 50 hospitals in the UK [5].

It must be acknowledged that the healthcare sector is particularly vulnerable to cyberattacks [6]. There are many reasons for this, the greatest of which is probably a lack of IT security resources. Expenditure on IT systems in the health industry in many European countries is approximately a quarter of that spent on IT by other sectors of the economy, with many systems out of date or unsupported. Alongside this lack of resources is the shortage of experts in the field of cybersecurity employed in healthcare. Many healthcare systems are unable to recruit or retain such professionals as they struggle to match salaries available in other sectors. Other factors that make healthcare vulnerable include governance and culture. The governance issue is particularly problematic as healthcare in many countries is routinely provided by a myriad of different organisations and clear leadership and standards on this issue are often lacking. Cultural issues are also undoubtedly a problem, with healthcare providers focussing primarily on patient health and sharing information, rather than prioritising cyber security.

The impact of the COVID-19 pandemic is also a significant factor [7]. Health systems are over-stretched with staff stressed, worn-out, and struggling to adapt to recent wholescale expansions of online systems. The volume of cyber attacks on health systems has increased dramatically since the onset of the pandemic [8].

The Public Health implications of this attack are significant. The most obvious impact of the attack was that most state health services in Ireland effectively had to go off-line for an extended period, resulting in significant loss of services to patients. It should be remembered that this de facto shutdown occurred immediately following prolonged closures and restricted operation of many health services as a result of the COVID-19 pandemic. The potential adverse health impacts caused by the threatened or actual release of sensitive health data are significant. There can be no doubt that the personal and professional lives of many individuals will be negatively impacted into the future, whether it be as a result of blackmail, identity theft, fraud, or public censure from the

publication of stolen data. Patient safety was also undoubtedly compromised as easy access to information on comorbidities, allergies, and existing prescriptions was lost.

The confidence of the public in health services is also a casualty of this attack. This widespread breach of confidentiality may have significant impacts into the future on the engagement of patients with sensitive and stigmatised health issues. This could include a range of services from testing for sexually transmitted diseases (STDs), and other reproductive health services, including fertility treatment and abortion, as well as engagement with mental health and domestic violence services.

Health providers that are subject to such cyberattacks may also be subject to legal action by patients, criminal proceedings for lack of due diligence, as well as compensation payments and regulatory fines. The reputational damage to health systems may have long term financial implications. These forms of cyberattacks may also weaken confidence in connected health, and hence significantly curtail moves towards increased telehealth, e-consultations, and mobile connected health devices [9]. Looking to the future, health services will have to re-examine the implications of information security of connected medical devices, and accessibility and utility to promote patient health and wellbeing. A final public health outcome of this attack will probably be the impact of the lost opportunity cost of this attack. Into the future, health systems will probably have to devote increasing amounts of scare resources to the issue of cybersecurity. It is highly likely that such diversion of funds will result in less resources for the provision of much needed health services elsewhere.

Although the cybercriminal group behind the attack in Ireland appear to have released the key to unlock the HSE's systems there are reports of confidential patient information appearing on the dark web, and already fraudulent attempts to obtain banking details have emerged, with criminals contacting former patients claiming that they were overcharged and offering refunds. Moving forward it is vital that all health service personnel prioritise cyber security, and understand how to prevent and respond to such attacks [10]. Basic steps in this process are outlined in Box bellow:

Action to Prevent Cyberattacks:

1. Educate end users on the prevalence of Phishing emails.
2. Update all anti-virus and computer security software.
3. Harden servers in the network and ensure local administrator passwords are unique and complex.
4. Patch and update all servers and firewalls.
5. Backup files (3-2-1 rule minimum).
6. Micro-segment the network.
7. Implement zero-trust security frameworks and technologies
8. Use multi-factor authentication (including in the air gapped backup).

In the event of an attack:

1. Do not pay the ransom.
2. Block traffic to the Internet (but do not necessarily turn off a device as anti-malware applications may need up-dates from the Internet).
3. Contact the IT department.

Resumo

En majo 2021, la ŝtata sansistemo de Irlando, la Health Services Executive, estis la celo de detrua atako per elaĉeta programaro nomita Conti. La fiprogramaro uzas kelkajn sofistikajn ilojn kaj efike kaŭzis, ke la sansistemo senretiĝis dum longa tempo, ĉar la ŝtato rifuzis pagi la rekompencan postulon de $ 19,999,000. Tutmonde elaĉeta programaro fariĝas grava afero por sanaj sistemoj, kun vastaj atakoj, eĉ pli multnombre dum la pandemio COVID-19. Kuracaj sistemoj eble estas celitaj pro multaj kialoj inkluzive de tio, ke ili estas necesaj por loĝantaro kaj povas esti facile damaĝeblaj pro manko de cibersekurecaj rimedoj. Por plibonigi la sintenon de sansistemoj rilate al sekureco, eble necesas reekvilibrigo kun eblaj efikoj al rimedoj haveblaj je la dispono de sanprizorgo kaj konsekvence efiko al publika sano. Preventaj rimedoj pri elaĉeta programaro estas prezentitaj, inkluzive kion fari se atako estas malkovrita.

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FOR AUTHORS

SUBMISSION

Manuscripts in English, Esperanto, Spanish, Italian, Polish (or any other conference language), can be submitted via electronic mail or Open Journal System (OJS). Each article has to have an abstract in English or Esperanto if only one version is submitted, the second wil be translated by the editors (free of charge).

FILE FORMAT

Manuscripts should be saved in native format, offered by text processing software (preferably .docx, .doc, .odt). All figures (apart from being embed in text, or marked) should be saved and submitted in native separate files (.jpeg, .cdr, .cpt, .svg, .tiff) with resolution suitable for printing typically 300 dpi (or greater) for color photographs and at least 400 dpi (or greater) for black-white and gray scale drawings.

TEXT

Layout of the manuscript should be left as simple as it is possible. Text has to be in single column format. Use Times New Roman 12 with 1.5 interline throughout the manuscript and avoid unnecessary formatting. Paragraphs have to be clearly separated from each other. Italic and bold face fonts can be used just as subscripts and superscripts etc. Options in word-processing software for text justification and word hyphenation must not be used.

Mathematical formula should be written in single line format (e.g. (2+2)^-2/[(2*2)-2]=2/x^-2) or written in LaTeX®Chemical formulas should be provided in a chemical drawing software format (rarely a high resolution .tif or .jpeg can be accepted but at the redaction discretion).

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All lines of text should be numbered. The maximum number of characters depends on the type of article:

Letter to the editorial board: 1000

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TITLE

Should not be exceedingly long and should state the main research goal and methodology.

ABSTRACT

The abstract should be written in English and Esperanto, left short and state briefly research goal, results, and the most important conclusions. As *Medicina Internacia Revuo* is readily willing to accept papers from authors who speak neither Eng-

lish or Esperanto, this task might be fulfilled by the editorial board on these special occasions. References in the abstract should be avoided. Abstract should be prepared in a way allowing its presentation as a stand-alone note. The maximum number of characters is 250.

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Up to six keywords can be defined, and should allow others to find the article in search forms.

REFERENCES STYLE

Vancouver, example:

Smith M, Kowalski A, Johanson MK. Medicinal activity of *Bacopa monnieri*. Med Int Rev. 2013; 1(2): 54-62.

INSTRUCTIONS FOR CITATIONS OF REFERENCES IN THE JOURNAL

In the text, sequential numbers of citations should be in order of appearence **(NOT ALPHABETICALLY)** in parentheses.

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NATURE

Mushrooms with prohealth activity for the human body (daily dosage: 300–400 g of fresh weight after processing)

Cantharellus cibarius (Chantarelle) – photo Paweł Stasiowski

Imleria badia (Bay bolete) – photo Paweł Stasiowski

Suillus luteus (Slippery Jack mushroom) – photo Paweł Stasiowski

Pleurotus ostreatus (Oyster mushroom) – photo Bożena Muszyńska

Agaricus bisporus (white bottom mushroom) – photo Bożena Muszyńska

Lentinula edodes (shiitake) – strains and fruiting bodies on solid medium in Petri dishes – photo Bożena Muszyńska