LEKARZ WOJSKOWY
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- Hospitalization time after laparotomy, laparoscopy and robotic procedures in patients with endometrial cancer
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Informacje dla autorów

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Letter from the Editor-in-Chief

We are pleased to present the 2nd issue of the 100th volume of “Military Physician”. It is more extensive and includes more papers than the first issue. We owe this to our colleagues who have decided to publish their works in our journal. Being aware of the difficult publishing market, I would like to thank the authors on behalf of the Editorial Board for their efforts related to paper preparation. At the same time, I would like to assure you that the editorial team will do their best to make the publication process smooth, fast and timely, and to ensure that the graphic layout of the journal meets contemporary standards.

In the current issue, we would like to draw your attention to the papers on robotics in medicine from the Military Institute of Medicine, which are richly and interestingly illustrated. An extremely interesting case report is devoted to treatment involving the consequences of trauma, using a neuroprotective agent. There are also papers on the current problems associated with the SARS-CoV-2 virus infection.

As we announced, we publish papers from a wide range of topics and from all branches of medicine. We would like this time to encourage you to read the news from paediatrics and medical biology, as well as a report on the 22nd Congress of the European Society of Gynaecological Oncology. The finishing touch is an article describing the history of the Epidemiological Diagnostic Center in Puławy.

I hope you have an enjoyable read, and please remember to send in your articles. Let's work together on improving the profile of our journal!

Prof. Bolesław Kalicki, MD, PhD

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CONTENTS

THE THREATS CONNECTED WITH CRANBERRY USE IN URINARY TRACT INFECTIONS DURING PREGNANCY

Zagrożenia związane ze stosowaniem żurawiny w uroinfekcjach w ciąży

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Abstract: Urinary tract infections can be a severe problem in pregnant and lactating women. They are usually treated with antibiotics, which have certain side effects and can negatively affect the developing foetus. Cranberry and its extracts are often recommended for pregnant and lactating women as an alternative or complementary therapy to antibiotic treatment therapy. It is commonly believed that the use of cranberry as an antibacterial treatment is safe for both the mother and the developing foetus. The article describes potentially undesirable mechanisms contained in cranberry compounds on the development of foetus and newborns, emphasizing its potential anti-angiogenic effect on the foetus.

Streszczenie: U kobiet w ciąży i karmiących poważnym problemem są infekcje dróg moczowych. Zazwyczaj leczy się je antybiotykami, które mają pewne skutki uboczne i mogą negatywnie wpływać na rozwijający się płód. Żurawina i jej ekstrakty są obecnie często polecane kobietom w okresie ciąży i laktacji jako terapia alternatywna bądź uzupełniająca do leczenia antybiotykami. Powszechnie uważa się, że stosowanie żurawiny w terapii przeciwbakteryjnej jest bezpieczne zarówno dla matki, jak i rozwijającego się płodu. W niniejszym artykule opisano potencjalnie niepożądane mechanizmy działania związków zawartych w żurawinie na rozwój płodu i noworodków, ze szczególnym uwzględnieniem jej potencjalnego antyangiogennego wpływu na płód.

Key words: pregnancy, angiogenesis, cranberry, breastfeeding, supplementation.

Słowa kluczowe: ciąża, angiogeneza, suplementacja, żurawina, okres karmienia piersią.

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Introduction

Antibiotic therapy in pregnancy

Urinary tract infections pose a serious problem for pregnant patients. They are usually treated with antibiotics, which have certain side effects and can negatively affect the developing foetus. The FDA (Food and Drug Administration) divides common antibiotics used in humans into five categories, defining the risk of their application in pregnant women (A, B, C, D, X), where only group A is considered to be safe for use in this group of patients [1]. The studies we carried out for many years on a pregnant mouse model confirmed the validity of this classification. Animals born by mothers who received penicillin and cephalosporins (group B antibiotics) during pregnancy had abnormal reactivity of the immune system, manifested by a reduced cellular immunity and increased humoral response, which in the future may lead to autoimmune reactions. Therefore, physicians try to use natural plant products, preparations or extracts for the treatment of infections in pregnant women.

Cranberry

Cranberry and its extracts are often recommended for pregnant and lactating women as an alternative or complementary treatment to antibiotic therapy. It is commonly believed that using cranberry as part of an antibacterial therapy is safe. Studies conducted in elderly

patients with recurrent urinary tract infections did not demonstrate any adverse reactions associated with the use of cranberry extract [2]. It is also often assumed that the use of cranberry preparations is safe both for the mother and the developing foetus. This assumption is not supported by strong scientific evidence.

Cranberries are a group of evergreen dwarf shrubs or trailing vines in the subgenus *Oxycoccus* of the genus *Vaccinium*. Traditionally, cranberry was used by native Americans as a source of food and medicine, especially in the treatment of wounds, urinary disorders, diarrhoea and diabetes. The unique combination of compounds found in cranberry fruits may provide synergistic health effects. Cranberry is an excellent source of vitamins A, B and C as well as folic acid, fibre and many minerals, including iron, calcium, phosphorus and potassium. Moreover, it contains numerous active substances, such as organic acids, plant polyphenols (compounds demonstrating immunotropic, antioxidant and anti-inflammatory properties), terpenes and many other substances that contribute to the extraordinary qualities of these fruits [3-14]. Cranberry contains 3 classes of flavonoids (flavonols, anthocyanins and proanthocyanidins), catechins, hydroxycinnamic acids, other phenolic acids and triterpenoids. The characteristics of the active subfraction of proanthocyanidins include the presence of dimers and catechin/epicatechin oligomers, monomeric catechins and quercetin glycosides [15]. The main anthocyanins found in cranberry are galactosides and arabinosides of cyanidin and peonidin [16].

Antioxidant and antineoplastic activities

Fruits and fruit products (juices, extracts) demonstrate significant antioxidant properties due to the high content of flavonoids and phenolic acids [17]. It has been shown that cranberry inhibits oxidative and inflammatory vascular endothelium damage, oxidative processes including oxidation of low-density lipoproteins and oxidative neuron damage during simulated ischaemia in rats [18, 19].

Compounds found in cranberry demonstrated a significant level of antineoplastic activity involving various mechanisms of action. Total polyphenol extract inhibited the proliferation of the colon (HT-29, HCT-116, SW480, SW620), oral (CAL27, KB) and prostate (RWPE-1, RWPE-2, 22Rv1) cancer cell lines [6]. Pentacyclic triterpenoid ursolic acid found in the skin of cranberry fruit inhibited the growth of several neoplastic cell lines [7, 8]. It has also been demonstrated that ursolic acid inhibited the growth of HT-29 cancer cell lines more effectively than the proanthocyanidin fraction from cranberry. Additionally, triterpene hydroxycinnamates (identified by HPLC and NMR as cis- (1) and trans- (2) isomers of 3-O-p-hydroxycinnamoyl ursolic acid), isolated from whole cranberry fruits, revealed a greater antiproliferative activity in the breast cancer MCF-7 cell line, the cervical cancer ME180 cell line and the prostate cancer PC3 cell line compared to quercetin or cyanidin-3-galactoside [8]. Resveratrol, epigallocatechin gallate and quercetin (the main flavonoid in cranberry fruits) have the potential to induce apoptosis in cancer cells [9, 10]. It is believed that cranberry partially inhibits carcinogenesis, by affecting the expression of matrix metalloproteinases (MMP). MMPs participate in the proteolysis of extracellular matrix, which may lead to tumour progression [11]. Proanthocyanidins, quercetin and ursolic acid reduce both the expression and activity of MMPs [12].

Antiangiogenic effects of cranberry

A large group of antineoplastic properties of cranberry is associated with their antiangiogenic effects. Researchers have proved repeatedly that anthocyanins, proanthocyanidins, ursolic acid, quercetin and triterpene acids demonstrate antiangiogenic effects, inhibiting tumour progression and metastasis [13, 20-23]. Cranberry extract in human keranocytes inhibited VEGF expression induced by hydrogen peroxide or TNF-alpha. The antiangiogenic effect of cranberry extract was also observed in studies on tumour endothelial cell lines (EOMA) derived from haemangiomas developing in children. Cranberry extract inhibited the transcription of the angiogenesis-stimulating MCP-1 factor produced by macrophages. In mice with melanoma, ursolic acid reduced the levels of VEGF, nitric oxide and pro-inflammatory cytokines, as well as increased the level of tissue inhibitor of metalloproteinases (TIMP1) and interleukin 2 (IL-2) in blood serum [12].

Angiogenesis and polyphenols

Pregnant women consume various food products with a high content of different polyphenols (fruit, vegetables, cocoa, tea, chocolate, coffee, fruit teas and herbal infusions, and dietary supplements commonly available on the market). Simultaneous consumption of numerous plant products containing antiangiogenic substances may adversely affect the foetus and cause various disorders in adult offspring. Angiogenesis is an important component of many physiological processes and, together with vasculogenesis, it is a driving force behind the development of tissues and organs in the embryo and foetus. Our previous studies demonstrated that both pure compounds: polyphenols (catechins, phenolic acids and glycosides, resveratrol), triterpenoids (ursolic acid) and the plants that contain them-(*Echinacea*, *Rhodiola*, *Cocoa*) and their extracts have immunotropic and antiangiogenic properties [24-26]. Using the pregnant mice model that we developed, we demonstrated that caffeic acid, chocolate and theobromine and the catechins that it contains (catechin, epicatechin and epigallocatechin) significantly impair angiogenesis in the foetus, as well as the production of angiogenic cytokines (VEGF and bFGF). These changes resulted in the development of organ dysfunctions, expressed in adult offspring as impaired limb growth and changes in the kidneys (abnormal glomerular number and size, increased levels of serum creatinine and urea). Moreover, preliminary studies demonstrated abnormal immune response to antigens in the adult offspring of mice receiving caffeic acid, chocolate (with a high catechin content) or theobromine in pregnancy [27, 28]. In the offspring of these mothers, we also observed impaired bone mineralisation and growth, resulting in upper and lower limb shortening, as well as effects on the immune response, both cellular and humoral. In addition, the adult offspring of mice fed with chocolate demonstrated significant morphometric abnormalities in

the renal structure, and pathologically high serum creatinine concentrations [29-32].

Cranberry during pregnancy

Cranberry contains strong antiangiogenic compounds: polyphenols (flavonoids and phenolic acids) and triterpenoids (ursolic acid and its derivatives) which inhibit neoplastic angiogenesis. It is possible that the increased intake of these substances, especially high concentration extracts, during pregnancy and lactation may affect embryonic, foetal and postnatal angiogenesis, and later, during the postnatal period, affect the maturation and function of organs in the progeny. Sohn et al. [22] observed that ursolic acid and oleanolic acid inhibit angiogenesis in the chick embryo chorioallantoic membrane model, which clearly indicated that cranberry active compounds may affect embryonic angiogenesis, at least in birds. These outcomes provided a premise for further studies on pregnant and lactating mice that received a commercial product containing concentrated American cranberry extract (*Vaccinium macrocarpon*) and many antiangiogenic compounds, in order to assess the effect on the development and function of certain organs (kidneys and spleen) in their progeny [33]. The study evaluated the effect of feeding pregnant and lactating mice with American cranberry extract (daily dose of 0.88 mg) on the morphology and selected parameters of the spleen and kidney function in their adult offspring. Six weeks post-partum, a morphometric assessment was made of the spleen and kidney, cytometric analysis of spleen lymphocytes and an assessment of the humoral response to sheep red blood cells (SRBC) and the concentration of serum creatinine/urea was determined in the offspring. The spleens of the progeny from the experimental group (E) differed from those of the offspring of the control mice, as they contained fewer germinal centres and their diameter was larger. A cytometry of the spleen cells from the progeny of group E mothers demonstrated higher levels of CD19+ and CD8+ lymphocytes than the control group. No differences were observed in the humoral response to immunisation with sheep red blood cells (SRBC) between the control offspring and the E offspring. An increase of the glomerular diameter in the kidneys of the experimental group was observed, compared to the control group. No abnormalities were found in serum creatinine and urea concentrations. Although the observed differences in the above parameters between the control group and the experimental groups were not large, cranberry and its extracts should be used with caution in pregnant women, until further studies on this problem are conducted.

Apart from the antiangiogenic effect of cranberry, recent reports of the potential anticoagulatory effect of cranberry supplements could raise concerns. In one study it was shown that cranberry supplementation led to massive intraoperative haemorrhages due to aspirin-like platelet inhibition [34].

Conclusions

Both the content and properties of cranberry components, as well as the absence of toxic compounds, make this fruit a “perfect natural supplement” that can support treatment or replace medications. There is a common belief that cranberry therapy is beneficial to the mother and the developing foetus. Dugoua et al. [35] suggest that cranberry consumption during pregnancy is safe, while they indicate that there is no direct evidence to support this safety. However, potential delayed effects of the therapy have never been studied (especially in adult offspring), and the belief in its safety is not supported by any experimental evidence. Potential adverse effects of using cranberry during lactation have not been studied either. It is also believed that cranberry has a prophylactic effect and protects women from recurring urinary tract infections [36, 37], which can additionally extend the period of cranberry use in pregnant women. However, some authors did not demonstrate the effectiveness of this therapy in the prevention and treatment of URI [38, 39].

A failure to continue studies on the safety of using cranberry in pregnant and lactating women may result in kidney failure in the adult children of mothers using cranberry extracts during pregnancy, and the causes of this phenomenon might not be understood (due to a long time interval between the treatment and its consequences). Moreover, as suggested in our previous studies, the immune response may shift towards the humoral response (Th2), which can increase the incidence of autoimmunisation and autoaggression. Pregnant and breastfeeding women who use cranberry long-term as a preventive or supporting treatment in therapy of urinary tract infections should be informed about the lack of robust safety studies regarding the use of large quantities of cranberry in this period and the associated adverse effects.

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REVIEW ARTICLE

NEW LOCAL INFLAMMATORY MARKERS IN THE DIAGNOSIS OF URINARY TRACT INFECTIONS AMONG INFANTS

Nowe lokalne markery stanu zapalnego w diagnostyce różnicowej zakażeń układu moczowego u najmłodszych dzieci

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Abstract: Urinary tract infections (UTI) are among the most common childhood diseases. Proper urine collection in the youngest children is technically difficult and can therefore lead to false-positive results from bacteriological tests obtained after several days of waiting. In case of inconclusive urinalysis results, antibiotic administration is delayed until obtaining the result of the bacteriological test, which may aggravate the infection and increase the risk of complications. At the same time, an antibiotic is often administered in cases where leukocyturia is due to inadequate urine sampling, and in cases when pathogens are cultured in a bacteriological test as a result of contamination. This exposes children to unnecessary destruction of normal intestinal bacterial flora and a gradual build-up of bacterial resistance to antibiotics. Therefore, new inflammation biomarkers are being sought to differentiate urinary tract infections from febrile illnesses of other causes. The paper presents new local inflammatory markers that may be helpful in the diagnosis of UTI. These include: YKL40 protein, neutrophil gelatinase-associated lipocalin, interleukin 8 and 6, Kidney Injury Molecule-1 and calprotectin.

Streszczenie: Zakażenia układu moczowego są jedną z najczęstszych infekcji wieku dziecięcego. Prawidłowe pobranie moczu w grupie dzieci najmłodszych stwarza trudności techniczne, a w konsekwencji prowadzi do uzyskiwania, po kilkudniowym czasie oczekiwania, fałszywie dodatnich wyników badań bakteriologicznych. W przypadku niejednoznacznego wyniku badania ogólnego moczu włączenie antybiotyku bywa opóźnione do czasu otrzymania wyniku badania bakteriologicznego, co może przyczynić się do nasilenia zakażenia oraz zwiększenia ryzyka powikłań. Jednocześnie często dochodzi do sytuacji włączenia antybiotykoterapii w przypadkach, gdy leukocyturia wynika z niepoprawnego pobrania próbki moczu, a wyhodowane w badaniu bakteriologicznym patogeny są wynikiem zanieczyszczenia. Naraża to dzieci na niepotrzebne wyjaławianie naturalnej fory jelitowej oraz stopniowe narastanie oporności bakterii na stosowane antybiotyki. Z tego względu poszukuje się nowych biomarkerów stanu zapalnego różnicujących zakażenia układu moczowego od zakażeń gorączkowych o innej przyczynie. W artykule przedstawiono badane nowe lokalne markery stanu zapalnego, które mogą być pomocne w diagnostyce ZUM. Są to: białko YKL40, lipokalina związana z żelatynazą neutrofili, interleukina 8 i 6, Kidney Injury Molecule-1 oraz kalprotektyna.

Key words: urinary tract infections, infants, calprotectin, YKL40.

Słowa kluczowe: zakażenia układu moczowego, dzieci, kalprotektyna, YKL40.

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Introduction

Urinary tract infections (UTI) are among the most common childhood diseases. Depending on their location, they may be classified as upper or lower urinary tract infections. Those involving renal parenchyma are typically characterised by a sudden symptom onset, high fever and abdominal or lumbar pain. Lower urinary tract infections are accompanied by dysuric symptoms (painful micturition, pollakiuria), changed odour of urine and – in children – loss of appetite. In the group of youngest children (up to 2-3 years of age) the course of the disease is typically oligosymptomatic, with fever frequently being the only symptom. This poses significant diagnostic problems in differentiating UTI from other causes of fever; hence general urinalysis (UA) is frequently ordered in this age group.

Urinalysis, together with bacteriological tests, are a gold standard in the diagnostics of UTI. A midstream urine sample for UA and bacteriological tests or, alternatively, catheter collection or suprapubic aspiration from the urinary bladder is recommended. According to the Polish Society for Paediatric Nephrology (PSPN) guidelines, significant leukocyturia is defined as the presence of over 5 leukocytes per high-power field (HPF) in uncentrifuged

urine, or the presence of over 10 leukocytes per HPF in centrifuged urine. Bacteriuria is considered to be significant if bacterial growth in a culture is over 10^5 CFU/ml in a mid-stream urine sample, 10^4 CFU/ml in urine from catheter collection, or any growth of pathogenic bacteria in a urine sample obtained by suprapubic aspiration [1]. In the most recent PSPN guidelines from 2021, presently being published, the authors recommend that in the case of suprapubic aspiration, the criterion for significant bacteriuria should be bacterial growth of at least 10^3 CFU/ml.

In the youngest children, proper mid-stream urine collection is technically challenging. The sample is often contaminated, resulting in non-diagnostic results. The test also does not allow for quick exclusion of an infection exclusion, as culture results are available after a few days. Therefore, new biomarkers of inflammation are sought to differentiate between UTIs and febrile infections of other origin. Earlier studies placed hope in such parameters as YKL40 protein, neutrophil gelatinase-associated lipocalin, interleukins 8 and 6, Kidney Injury Molecule-1 (KIM-1) and calprotectin.

Human cartilage glycoprotein 39 (YKL40)

Urine YKL40 is also known as human cartilage glycoprotein 39 (HC gp-39) or chitinase-3-like protein 1 (CHI3L1). YKL40 participates in acute and chronic inflammatory processes, angiogenesis and fibrosis. It is secreted by a number of immune cells, including neutrophils and macrophages accumulating at the infection site, which causes a local increase of urine YKL40 (uYKL40) concentrations in UTI [2].

Hyun Hee Kim et al. assessed the usefulness of YKL40 in the diagnostics of febrile urinary tract infection in children. They determined uYKL40 concentration in febrile children with UTI and compared them with a control group of children with fever of different origin. The diagnosis of urinary tract infection was based on a positive result of a urine culture, with significant bacteriuria correlating to the clinical symptoms. The obtained values of the evaluated uYKL40/creatinine ratio were significantly higher in children diagnosed with UTI, compared to the control group. In children in the control group, whose urinalysis demonstrated leukocyturia with negative urine culture results, this ratio was lower than in the UTI group. Based on the results, a cut-off point for uYKL40/creatinine was established at over 125 pg/mg as a value that supports the UTI diagnosis. With this value, the assessment of the ratio was characterised by high sensitivity and specificity [3]. El-Saeed Mashaly et al. made similar conclusions in their study. They measured uYKL40 and uNGAL concentrations in a group of febrile children with UTI confirmed in a bacteriological test, compared to children with fever and negative urine culture results and to a control group of healthy children. Each group comprised 50 children. Both uYKL40 concentrations and uYKL40/creatinine ratios were significantly higher in children with confirmed urinary tract infection, compared to the other two groups. The differences in the mean values of these parameters between the healthy children and those with fever due to causes other than UTI were not, however, statistically significant. Based on data analysis, the authors established a cut-off point in differential diagnostics for uYKL40 concentration at 171.5 pg/mg, and for the uYKL40/creatinine ratio at 159.2 pg/mg, which provided sensitivity of 84% and specificity of 82%, and sensitivity of 72% and specificity of 71%, respectively. Moreover, uYKL40 demonstrated a higher sensitivity and specificity in diagnosing urinary tract infections than uNGAL. Despite the promising results, the researchers indicated the limitations of the study, including small study group size, inability to exclude the correlation between increased urine YKL40 concentrations and serum YKL40 concentrations in subjects and a lack of control value of YKL40 concentrations after treatment and the dynamics of its variability during the therapy [4]. In the light of previous reports the determination of YKL40 as another marker in UTI diagnostics appears to be useful; however, further research on this parameter is required.

Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein in the lipocalin family. Its promising function as a biomarker of early renal damage and urinary tract infections is associated with a significant increase of the protein's concentration in blood and urine in response to infection [5].

Valdimarsson et al. attempted to assess the utility of NGAL as another inflammation marker in the diagnostics of UTI in young children. Their study results indicate that NGAL concentrations were significantly higher in children with UTI compared to those with fever due to other causes. Urine NGAL concentration and the ratio of urine NGAL concentration to urine creatinine concentration had a sensitivity of 93% and 96% and a specificity of 95% and 100%, respectively, for diagnosing urinary tract infection, with a cut-off concentration of 38 ng/ml and 233 ng/mg. According to the authors, in febrile children with high uNGAL concentration, urinary tract infection is highly probable and provides an opportunity to introduce early treatment. In the case of fever and low uNGAL concentration, other causes of symptoms should be considered [5].

Krzemień et al. analysed the utility of blood serum NGAL concentration and its correlation with CRP and PCT in the differential diagnostics of acute infectious tubulointerstitial nephritis (AITN) compared to lower urinary tract infection in youngest children. Median sNGAL concentration, as well as PCT and CPR concentrations, were significantly higher in children with AITN compared to those with lower urinary tract infection. Simultaneously, children with AITN more often had fever, while its duration until the start of treatment, was definitely longer [6].

Forester et al. observed that the simultaneous increase of NGAL in plasma and urine may be indicative of a generalised infection in the course of acute pyelonephritis, whereas if the urine NGAL concentration is increased, while the plasma NGAL concentration is normal, lower urinary tract infection without a generalised inflammatory reaction is likely [7].

Interleukins 6 and 8

Interleukin 6 is a proinflammatory cytokine, considered to be one of the principal factors regulating the defence mechanism, including induction of fever and increased production of acute phase proteins. Interleukin 8 is a chemotactic cytokine responsible for migration of granulocytes to the infections site and for the development of pyuria. Interleukins 6 and 8 are present only in trace amounts in the urine of healthy individuals, whereas in children and adults with various forms of urinary tract infection the concentration of these cytokines in the urine is significantly increased. In urinary tract infections, IL-6 and IL-8 are produced by the urothelium in response to bacterial infection [8]. Therefore, determination of IL-6 and IL-8 concentrations in the urine may be useful for the assessment of the location and severity of the urinary tract inflammation. Krzemień et al. observed that the increase in the concentrations of both cytokines is significantly higher in children with febrile urinary tract infection, compared to those with non-febrile UTI. The intensity of IL-8 response may thus correlate with the severity of UTI and the associated renal damage in children [8]. IL-8 concentration decreases quickly after treatment [9]. Increased IL-8 levels were also observed in other pathologies: vesicoureteral reflux, congenital defects of the urinary tract [5], as well as in the haemolytic-uraemic syndrome, Kawasaki disease or chronic glomerulopathies [9]. Therefore, it is a sensitive, but not particularly specific marker of urinary tract infections [5]. In the presented cases, an increase of the Il-8/creatinine ratio correlates with disease exacerbation. Determination of IL-8 level in infants without urinary tract infection appears to be a promising marker in the assessment of patients at high risk of congenital defects, including infants diagnosed with renal pelvis dilatation [9].

Kidney Injury Molecule-1 (KIM-1)

Kidney Injury Molecule-1 (KIM-1) is a type 1 transmembrane protein, with an immunoglobulin and mucin domain, whose expression is markedly increased in the proximal tubule in acute renal failure. Similar results were observed in chronic kidney diseases of various aetiology: primary inflammatory renal disease, such as glomerulonephritis and vasculitis, in diabetes, allograft nephropathy and renal cell carcinoma. The activity of KIM-1 increases earlier than that of conventional biomarkers of acute renal injury (creatinine and BUN). This protein is not a marker of inflammation, but it may be used as a urine marker of acute renal tubular injury. Children in the youngest age group with urinary tract infection may develop prerenal acute kidney injury due to dehydration and kidney hypoperfusion due to fever, reduced appetite and vomiting. Therefore, a change in KIM-1 concentration in urinary tract infections in children can be observed [10].

In their study, Krzemień et al. demonstrated that median serum KIM-1 concentration (sKIM-1) was significantly higher in the group of infants with febrile urinary tract infection as well as those with non-febrile UTI, compared to the control group. No significant difference was found in sKIM-1 concentrations between the group of children with febrile UTI and non-febrile UTI, whereas mean urine KIM-1 concentrations (uKIM-1) were markedly lower in the group of infants with febrile urinary tract infection compared to the group of non-febrile UTI and the control group. However, no significant difference was observed in uKIM-1 concentrations between the control group and infants with non-febrile urinary tract infection.

In summing up, the study noted that serum KIM-1 concentration increases in both febrile and non-febrile UTI, while urine KIM-1 concentration decreases in febrile UTI. Krzemień et al. demonstrated that sKIM-1 has a high specificity, but low sensitivity in the diagnosis of febrile UTI; however, both sKIM-1 and uKIM-1 are not useful for the diagnosis of non-febrile UTI [10]. Yim et al. and Lee et al. demonstrated in their studies that urine KIM-1 concentration may be used in the diagnostics of urinary tract infections in children. They found that the ratio of urine KIM-1 to urine creatinine in children with febrile UTI increases. However, the sensitivity of this marker was relatively low (45%-76%) [11, 12]. Inconsistent outcomes of the presented studies may result from an increase of urine KIM-1 concentration in children with UTI and undiagnosed subclinical AKI [10].

Calprotectin

Another biomarker studied for its utility in the diagnostics of UTI is urine calprotectin concentration. Calprotectin in a heterodimer of two proteins: calgranulin A (S100A8) and calgranulin B (S100A9). It is secreted in the organism, primarily by granulocytes and monocytes, as acute phase protein during an inflammatory reaction. In low concentrations it is found in all body fluids and the stools, including in healthy individuals [13]. Determination of elevated calprotectin concentration is widely used in the diagnostics of such diseases as rheumatoid arthritis, Crohn's disease and ulcerative colitis. Researchers have so far assessed the utility of urine calprotectin in paediatric patients primarily as a parameter that allows us to differentiate between acute kidney injury (AKI) due to prerenal and renal causes. In their study, Basiratnia et al. compared urine calprotectin concentrations in children with acute renal parenchymal disease (45 children), prerenal AKI (30 children) and the control group (20 children). Mean urine calprotectin concentrations in children with renal AKI were 36 times higher than in the group with prerenal AKI, and 44 times higher than in healthy children. Moreover, calprotectin concentrations were found to increase with the severity of renal injury. Also the calprotectin/creatinine ratio in urine was 140 times higher in children with renal AKI, compared to those with prerenal AKI. Urine calprotectin concentration of 230 ng/ml enabled to differentiate, with 95% sensitivity and 100% specificity, between prerenal and renal AKI. One of the study exclusion criterions was a diagnosis of urinary tract infection, because a local increase in calprotectin concentration might affect the results. In a group of the youngest children, UTI is often associated with renal parenchymal involvement. Therefore, for future studies, it is worth considering the assessment of the utility of urine calprotectin in the diagnosis of UTI in this age group [14].

Conclusions

Considering the commonly observed difficulties in diagnosing UTI based on urinalysis results, including leukocyturia being a consequence of incorrect sample collection, finding a marker that would enable differentiation between false-positive results of urinalysis and an actual infection would be of considerable diagnostic value. Perhaps, based on future studies, one of the proteins discussed in this article will become a gold standard in the diagnostics of urinary tract infections in the youngest children.

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REVIEW ARTICLE

RETURN TO PHYSICAL ACTIVITY AFTER SARS-COV-2 INFECTION - IN THE EYE OF A CARDIOLOGIST

Powrót do aktywności fizycznej po przebytym zakażeniu wirusem SARS-CoV-2 - okiem kardiologa

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Abstract: Regular physical activity is the cornerstone of cardiovascular disease prevention. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has significantly reduced physical activity in the healthy population. In addition, SARS-CoV-2 infection is associated with a higher risk of cardiovascular complications, than other viral infections. A return to physical activity after coronavirus disease depends primarily on the severity of the illness, cardiovascular abnormalities found during the infection and intensity of the planned effort. Patients with confirmed or probable diagnosis of recent myocarditis should be advised to abstain from all forms of sport until the active inflammation is resolved, and return to physical activity should be considered 3 to 6 months after resolution of symptoms, following a comprehensive cardiac evaluation. In the remaining group of patients (without diagnosed myocarditis), because of the limited follow-up time and the lack of randomised trials concerning the return to physical activity after recovery, the recommendations published so far are expert opinions. These opinions emphasise that it is important to stratify the risk, and return to sport after a SARS-CoV-2 infection in a gradual, symptom-limited and individualised way.

Streszczenie: Regularna aktywność fizyczna to podstawa profilaktyki chorób sercowo-naczyniowych. Pandemia koronawirusa SARS-CoV-2 (*severe acute respiratory syndrome coronavirus 2*) w istotny sposób wpłynęła na ograniczenie aktywności fizycznej w populacji osób zdrowych. Ponadto infekcja SARS-CoV-2 związana jest ze zwiększonym, w porównaniu z innymi infekcjami wirusowymi, ryzykiem powikłań sercowo-naczyniowych. Powrót do aktywności fizycznej po chorobie koronawirusowej zależy przede wszystkim od stopnia ciężkości choroby, stwierdzanych w czasie infekcji nieprawidłowości w badaniach układu sercowo-naczyniowego oraz intensywności planowanego wysiłku.
Pacjentom z pewnym lub prawdopodobnym rozpoznaniem świeżego zapalenia mięśnia sercowego należy zalecić powstrzymanie się od uprawiania jakichkolwiek sportów do czasu ustąpienia aktywnego zapalenia, a powrót do aktywności fizycznej należy rozważyć po 3-6 miesiącach od ustąpienia objawów, po przeprowadzeniu kompleksowej oceny kardiologicznej. W grupie pozostałych pacjentów (bez rozpoznanego zapalenia mięśnia sercowego) ze względu na ograniczony czas obserwacji i brak badań randomizowanych na temat powrotu do aktywności fizycznej po przechorowaniu, dotychczas opublikowane zalecenia mają charakter opinii ekspertów. W opiniach tych podkreśla się znaczenie stratyfikacji ryzyka i stopniowy, ograniczony objawami, indywidualnie dopasowany powrót do uprawiania sportu po zakażeniu wirusem SARS-CoV-2.

Key words: rehabilitation, cardiovascular complications, COVID-19, SARS-CoV-2, return to physical activity.

Słowa kluczowe: rehabilitacja, SARS-CoV-2, COVID-19, powrót do wysiłku, powikłania sercowo-naczyniowe.

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Introduction

Regular physical activity is the basis of cardiovascular prevention, reducing all-cause mortality and cardiovascular mortality by 20-30% [1]. The SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) pandemic and the associated restrictions of the movement of people, as well as the recommended social distancing, significantly reduced physical activity in the general population. A varied course of SARS-CoV-2 infection may result in the phenomenon of "prolonged recovery", described in the literature. Symptoms reported by patients, such as fatigue, weakness, dyspnoea, atypical chest pains persisting after COVID-19 infection, suggest a cardiac background, which delays the decision to return to regular physical activity [2]. The aim of this study is, therefore, to present the current recommendations regarding a return to physical activity of patients after SARS-CoV-2 infection, considering the time, type and intensity of the planned activity in individuals who

were healthy before, and practised sports recreationally or for cardiovascular prophylaxis.

Current guidelines of the European Society of Cardiology regarding physical activity in cardiovascular prevention

According to the most recent guidelines of the European Society of Cardiology from 2021 [1], the following is recommended (class of recommendation - I, level of evidence - A):

- for adults of all ages, at least 150 - 300 min per week aerobic exercise of moderate intensity or 75 - 150 min per week aerobic exercise of vigorous intensity, or an equivalent combination of both forms, to reduce all-cause mortality, cardiovascular mortality and morbidity;
- adults who cannot perform 150 min of moderate-intensity physical activity per week should stay as active as their abilities and health condition allow;
- limitation of a sedentary lifestyle including a commitment to engage in at least one light activity throughout the day to reduce all-cause and cardiovascular mortality and morbidity;
- performing resistance exercise, in addition to aerobic activity, on 2 or more days per week to reduce all-cause mortality.

Classification of physical activity intensity comprises three levels, as presented in Table 1:

Table 1. Levels of physical activity intensity, based on the ESC guidelines [1]

Intensity	Description
Light exercise	1.1 to 2.9 MET*, which corresponds to light household work, walking <4.7 km/h, 10-11 on the Borg scale**
Moderate exercise	3 to 5.9 MET, walking 4.1 to 6.5 km/h, cycling up to 15 km/h, ballroom dancing; exercise that results in faster breathing, but one can talk easily, 12-13 on the Borg scale
Vigorous exercise	>=6 MET, running, cycling >15 km/h, tennis; exercise during which talking is impossible, 14-16 on the Borg scale

** MET- metabolic equivalent, 1 MET is an equivalent of using 3.5 ml of oxygen/ kg/min. ** Borg scale - a subjective scale of exertion; during an activity, patient rates the experienced degree of fatigue by indicating the level of exertion on a scale from 6 to 20, where 6 means lack of exertion and 20 mean maximum exertion.*

Cardiovascular complications in the course of SARS-CoV-2 infection and their effect on physical activity

Coronavirus disease 2019 (COVID-19) is associated with a risk of significant cardiovascular complications. These include: myocarditis, myocardial infarction, heart failure, arrhythmias and thromboembolic complications. Previous studies demonstrate that acute myocardial injury (defined as an increase of cardiac troponin concentration above the 99th percentile of the upper reference limit, associated with ECG and/or echocardiographic abnormalities) is found in 7 to 17% of all patients hospitalised due to COVID-19, and the rate is significantly higher compared to other viral infections [3]. The exact mechanism of myocardial and vascular damage by the SARS-CoV-2 virus is the subject of research. The present knowledge is mainly based on observations from the time of SARS (severe acute respiratory syndrome) pandemic and assumed pathophysiological analogy to the SARS-CoV virus of a similar structure. Myocardial and vascular damage in the course of SARS-CoV-2 infection is associated with a membrane receptor, angiotensin-converting enzyme 2 (ACE2), and the renin-angiotensin-aldosterone (RAA) system. ACE2 receptor expression was demonstrated in many organs, also in pneumocytes that are the main entry gates for SARS-CoV-2 virus. Moreover, ACE2 membrane receptors are present in vascular endothelium, myocytes and other organs, including the kidneys, which elevates the risk of multiorgan injury. ACE2 expression decreases after virus attachment. It leads to a local increase in concentration of angiotensin II (Ang II), the main ACE2 substrate, thus promoting stimulation of the RAA system and proinflammatory response. The virus may damage the heart not only directly, but also by its effect on the circulatory system in the mechanism of generalised inflammatory response ("cytokine storm") or as a consequence of ARDS (acute respiratory distress syndrome) [4].

Myocarditis as a complication in the course of SARS-CoV-2 infection

The most serious complication of SARS-CoV-2 infection is myocarditis. Using animal models, it was demonstrated that exercise could cause an accelerated and progressive inflammatory response, thus increasing the risk of death. Therefore, patients with an established or probable diagnosis of fresh myocarditis should be advised to avoid sports, including recreational exercise, until resolution of active inflammation. Following the guidelines of the European Society of Cardiology, after recovery from the acute phase of the inflammation, a comprehensive cardiac assessment should be performed (including imaging tests, exercise test and Holter ECG monitoring) to assess the risk of sudden cardiac death due to physical exercise (class of recommendation - I, level of evidence - B). Return to physical activity, including performance sports, should be considered after 3-6 months in asymptomatic patients with normal concentrations of troponin and inflammatory markers, normal left ventricular systolic function in echocardiography and MRI, without signs of active inflammation or muscle fibrosis in MRI, with good functional capacity and without frequent or complex ventricular arrhythmia in Holter ECG or during the exercise test (class of recommendation - IIa, level of evidence - C). In patients with residual myocardial injury and persisting left ventricular dysfunction, intensive engagement in recreational or performance sports is not recommended (class of recommendation - III, level of evidence - C) [5].

Stages of returning to physical activity according to type and intensity of exercise

All the previously published recommendations on returning to sports after COVID-19 (except for the patients diagnosed with myocarditis) are expert opinions, due to lack of results of large-scale studies. The American College of Cardiology Sports and Exercise Cardiology Section recommendations for returning to sports by athletes after COVID-19 include classification to three groups of patients:

- asymptomatic disease – a gradual return to physical activity after 2 weeks from a positive test result, supervision by a health care team and observation for potential disease symptoms are necessary;
- mild to moderate symptoms, hospitalisation not required – abstaining from physical activity during the disease and for two weeks after resolution of symptoms. After that time, medical assessment and additional tests: troponin concentrations, ECG at rest, echocardiography and, to be considered, cardiac magnetic resonance imaging, exercise test and prolonged ECG monitoring. If no symptoms or abnormalities suggestive of myocardial injury are found in additional tests, a gradual return to sports, under guidance, is possible. In the case of abnormal results, experts recommend further treatment, presented in the guidelines on myocarditis;
- severe symptoms, hospitalisation required – in the case of abnormal troponin concentrations and/or abnormalities in imaging heart tests (performed during the hospitalisation due to COVID-19), following the guidelines on myocarditis is recommended. In other patients, avoiding exercise for at least 2 weeks from resolution of symptoms is recommended, followed by a detailed assessment of the cardiovascular system; further treatment depends on the results of additional tests (described above) [6].

The recommendations on return to physical activity after COVID-19 disease apply mostly to professional players of sport or patients with chronic diseases. On the other hand, the availability of data on the return to physical activity of previously healthy individuals, recreationally or for cardiovascular prevention, practicing sports is limited. Salman et al. proposed a model of risk stratification based on the presence or absence of clinical symptoms [7]. The optimal solution is the recommendation to refer patients after SARS-CoV-2 infection, especially if it required hospitalisation, to a centre offering "post-covid" rehabilitation. In the first step, as part of risk stratification, myocarditis should be excluded. Then, if respiratory, gastrointestinal, neurological or psychological symptoms persist, a more detailed patient assessment in the rehabilitation centre is required. If cardiovascular diseases were present before COVID-19, a cardiac rehabilitation centre may be the target site. Other patients, after a 7-day asymptomatic period, may start a 5-phase return to physical activity, as presented in Table 2.

Table 2. Phases of return to physical activity – developed based on Salman D. et al. [7].

Phase	Recommended activity
Phase 1 (7 days)	Breathing exercises, stretching, balance exercises, gentle walking, light aerobic exercise, 6-8 on the Borg scale
Phase 2 (7 days)	Walking, yoga, light household/garden tasks, light aerobic exercise, 6-11 on the Borg scale, with duration of activity gradually increased by 10-15 minutes, maintaining the same exertion level on the Borg, until activity of 11 on the Borg scale is achieved
Phase 3 (7 days)	Interval exercise, starting with 2 cycles of 5 minutes, e.g. brisk walk, climbing up stairs, jogging, swimming, cycling; moderate aerobic exercise, gradual addition of one interval of 12-14 on the Borg scale, up to 30 minutes of activity, followed by a feeling of complete rest one hour after the exercise
Phase 4 (7 days)	Activities with elements of balance, coordination and resistance exercises, moderate aerobic exercises, 12-14 on the Borg scale, 2 days of exercise per 1 day of rest
Phase 5 (7 days)	Return to regular physical activity, >15 on the Borg scale, if tolerated

If alarming symptoms occur: cough, fever, difficulty with breathing, heart palpitations or anolfactory disorder, regardless of the phase the patient is in, they should stop the exercise, consult a specialist and return to activity after the resolution of the symptoms.

Conclusions

Infection with SARS-CoV-2 virus carries a risk of cardiovascular complications; therefore, it is important to develop detailed recommendations not only for the diagnostics and treatment, but also for return to physical activity after the disease. Previous recommendations are only expert opinions, due to the insufficient number of large-scale scientific studies and the short observation time. The effect of COVID-19 on distant cardiovascular complications, also affecting asymptomatic patients or outpatients, is another important problem, explored in numerous studies.

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REVIEW ARTICLE

IMMUNOPATHOLOGY AND PULMONARY COMPLICATIONS ASSOCIATED WITH SARS-COV-2 VIRUS INFECTION. CAN PLANTS HELP US?

Immunopatologia i powikłania płucne związane z zakażeniem wirusem SARS-CoV-2. Czy mogą nam pomóc rośliny?

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Abstract: During the SARS-CoV-2 virus pandemic, which began in 2019, it has been observed that the vast majority of people have a mild clinical picture or asymptomatic COVID-19. However, approximately 10% of individuals infected with the virus will have a severe, potentially fatal clinical course associated with hyperinflammation, vascular endothelial damage, thrombotic complications and lung injury. To predict and make prognoses about the course of COVID-19, it is necessary to understand the immunologic mechanisms induced after the infection. Sufficient knowledge of the infection immunopathology is essential for the development of effective immunomodulatory therapies. The paper describes selected plant-derived immunomodulators that may exhibit preventive and soothing effects on COVID-19 disease symptoms.

Streszczenie: W czasie trwania pandemii wirusa SARS-CoV-2, która rozpoczęła się w 2019 r., zaobserwowano, że zdecydowana większość ludzi ma łagodny obraz kliniczny lub przechodzi chorobę COVID-19 całkowicie bezobjawowo. Jednak około 10% osób zakażonych wirusem będzie miało ciężki, potencjalnie śmiertelny przebieg kliniczny związany z hiperzapaleniem, uszkodzeniem śródbłonka naczyń, zmianami zakrzepowymi i uszkodzeniem płuc. Do oceny prognostycznej i rokowniczej przebiegu COVID-19 konieczne jest poznanie mechanizmów immunologicznych indukowanych po infekcji. Znajomość immunopatologii zakażenia jest niezbędna dla opracowania skutecznej terapii immunomodulującej. W pracy opisano wybrane immunomodulatory pochodzenia roślinnego, które mogą wykazywać działanie prewencyjne i łagodzące objawy choroby COVID-19.

Key words: lung damage, immunopathology, COVID-19, SARS-CoV-2, phytoimmunomodulators.

Słowa kluczowe: SARS-CoV-2, COVID-19, immunopatologia, uszkodzenie płuc, fitoimmunomodulatory.

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Introduction

In late December 2019, in Wuhan, the first patient infected with SARS-CoV-2 was identified. The new coronavirus, called COVID-19, appeared to be highly contagious and it spread rapidly, giving rise to a pandemic. As the virus demonstrates a potential to mutate, it complicates the search for effective treatments and prophylactic vaccinations which could be used against the infection. According to WHO classification, five variants of concern (VOC) of SARS-CoV-2 have been distinguished: Alpha, Beta, Gamma, Delta and Omicron, as well as a few variants in two lower categories - Variants Of Interest (VOI) and Variants Under Monitoring (VUM). At present, we are experiencing the fifth wave of the pandemic. Despite several treatment algorithms being available, research continues on new preventive and therapeutic strategies.

SARS-CoV-2 structure

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a single-stranded RNA (ssRNA) virus, a member of the betacoronavirus group. It is probably of

animal origin (bats, pangolins), as over 90% of the nucleotide chain is identical, although is it not clear how the infection was transmitted to humans. The virus is spread through droplet transmission and it causes a general systemic disease, COVID-19. In some patients it results in severe pneumonia and acute respiratory distress syndrome (ARDS) that requires mechanical ventilation. The structure of SARS-CoV-2 was compared to that of SARS-CoV-1 and MERS (Middle East respiratory syndrome), demonstrating a similarity of over 70% and 50%, respectively [1, 2].

SARS-CoV-2 is a capsid virus, and its genome consists of a single-strand RNA. Each virion is 60-140 nm in diameter, and it is surrounded by spikes of 9-12 nm. The length of the virus genome is 29867 to 29903 nucleotides, which places it, as is the case with other coronaviruses, among the largest RNA viruses, both considering the genome length and the virion size. The SARS-CoV-2 genome codes non-structural proteins (nsp), necessary for replication, structural proteins and accessory proteins [3].

Similarly to other coronaviruses, SARS-CoV-2 has four structural proteins:

- S (spike) – a fusion protein, i.e. a surface glycoprotein responsible for interaction with the receptor on the cell surface;
- E (envelope) – a protein responsible for virion formation;
- M (membrane) – the main matrix protein of the virus;
- N (nucleocapsid) – a protein that has a protective function for a large RNA molecule and participates in the modification of cellular processes and virus replication.

Cell fusion and replication mechanisms

The four structural proteins of SARS-CoV-2 virus – S, M, N and E – play a significant role in its replication and infectiousness. The N protein stabilises the RNA of the virus, while the S, E and M proteins together create the viral envelope. Moreover, the S protein is responsible for attachment of the virus to the cellular membrane of the host cell, and is the principal immunogene of the virus. The S protein is found on the surface of the viral envelope and it forms characteristic spikes, consisting of two types of glycoproteins, marked as subunits S1 and S2 [4]. The S1 subunit mediates the process of binding with the surface receptor of the host cell, and subunit S2 participates in the fusion with its cellular membrane. In the process of entry into the host cells, the virus uses the S protein to attach to the ACE2 (angiotensin converting enzyme 2) receptor. ACE2 receptors are found mainly in the lungs (type 2 pneumocytes) and in the nasopharynx, intestines, cardiovascular system and CNS. This distribution determines the variety of symptoms of the disease. It has been demonstrated experimentally that the virus may replicate not only in the lungs, but also in the intestinal epithelial cells (enterocytes), kidneys and blood vessels [5]. Inhibition of ACE2 leads to accumulation of angiotensin II, and reduced production of angiotensin 1-7. Most likely directly through the accumulation of angiotensin II, the virus induces expression of TGF-β, which has a profibrotic effect. On the other hand, angiotensin 1-7 demonstrates antiproliferative effects and prevents lung injury. The ACE2 protein is not the only receptor used by the virus for cell fusion [6]. Another important factor in the pathogenesis of CoV-2 is transmembrane serine protease 2 (TMPRSS2), an enzyme considered by researchers to be crucial, together with ACE2, in the process of host cell entry. The TMPRSS2 protease activates the process of cell fusion with the S protein of the SARS-CoV-2 virus and induces syncytium formation [7]. It is suggested that inhibition of TMPRSS2 may be the key treatment strategy in patients with COVID-19 [8, 9].

The SARS-CoV-2 virus may colonise and attack:

- respiratory system – causing, in severe cases, acute atypical pneumonia and ARDS;
- nervous system – causing neurological symptoms, such as headache, nausea, confusion, consciousness disorders and, in severe cases, cerebro-vascular diseases. It causes loss of smell and taste, as it attacks the olfactory and taste receptors – this is one of the most important diagnostic symptoms of infection;
- gastrointestinal system – resulting in diarrhoea and vomiting; studies have demonstrated the presence of the virus in the faeces;
- urinary system – leading to complications such as acute renal injury; studies demonstrated presence of the virus in patients' urine;
- cardiovascular system – it damages vascular endothelium, contributing to acute circulatory and heart failure, it causes lymphopaenia and may trigger: thrombocytopaenia and leukocytosis with elevated CRP and LDH levels; the genome of the virus was found in the cardiac muscle;
- liver – histopathological examination of the organs of 11 dead patients with COVID-19 demonstrated hepatic steatosis in all of them. The liver samples also revealed chronic congestion, various forms of hepatocyte necrosis and nodular proliferation.

Pulmonary damage

Infection with the virus is non-specific, and its course may be asymptomatic or involve mild symptoms, while the primary infection pathway is typically the respiratory system. However, the colonisation factors are not fully understood. The characteristic symptoms vary, depending on the virus variant, but usually they include: flu-like symptoms, high fever, osteoarticular pain, reduced concentration, vomiting, diarrhoea, loss of smell and taste, cough, dyspnoea and reduced blood saturation. Hearing impairment is specific for the Delta variant [12].

The incubation period, i.e. the time from the infection with the virus to the disease onset, is up to 14 days.

After the virus enters the cells, inflammatory mediators are released, and the process, if characterised by high intensity, is referred to as a cytokine storm. The effect of cytokines causes general and local symptoms. In extreme cases, SARS-CoV-2 may lead to general systemic infection (viraemia and sepsis). The following changes occur consecutively in the lungs:

- increased permeability of the alveolar-capillary barrier, for extreme severities it results in pulmonary oedema

and acute respiratory failure;
- injury to the pulmonary alveoli – exfoliation of the damaged pneumocytes and the formation of vitreous membranes;
- advanced inflammatory lesions;
- reconstruction processes that may cause changes in the interstitial architecture and induce interstitial fibrosis, resulting in impaired gas diffusion through the alveolar-capillary barrier.

Simultaneously with the presented changes, thrombosis of the small pulmonary vessels occurs. Decreased production of angiotensin 1-7, a strong inflammatory reaction (cytokine storm), endothelial damage, possibly reduced fibrinolysis, as well as, potentially, the direct effect of the virus on the components of the haemostatic system increasing the thrombogenic potential and leading to thrombosis, primarily in the small pulmonary vessels, both arterial and venous. Thrombosis may also develop in other locations, which in further phases of the disease may result in organ damage [10.

The haemostatic parameters resemble disseminated intravascular coagulation (DIC), although with lesions found mainly in the pulmonary microcirculation, other terms are proposed: pulmonary intravascular coagulopathy or COVID-19 associated coagulopathy (CAC). Thrombi occur also in the venous and arterial vessels of other organs, which may result in their damage. In addition, the incidence of venous thromboembolism is observed more frequently than in other diseases. In some patients the inflammatory lesions also involve the bronchi or even pleura [11].

The molecular and immunological basis of these injuries are presented below.

Complications of COVID-19

The majority of patients recover and return to normal activity after COVID-19. In some cases (approximately 10%), especially where the course of infection is severe, the clinical symptoms may persist for many weeks or even months. After the infection, patients often complain about complications such as: impaired concentration, referred to as post-COVID-19 brain fog, carditis, tachycardia, weakness, increased arterial blood pressure and vasculitis. A small percentage of cases – currently it is unclear how small – is associated with permanent sequelae. Symptoms usually persist after the disease in elderly patients and/or in those with comorbidities. However, even in young patients, previously healthy and initially asymptomatic or with a mild COVID-19, delayed or long-term symptoms may occur [13].

The COVID-19 symptoms typically subside after 2-6 weeks, and the length of this time is determined, among other things, by the severity of the disease. Prolonged recovery may be due to extended viraemia, re-infection, a strong inflammatory reaction or bacterial superinfection. According to WHO, the course of 10-15% cases of COVID-19 is severe, in 5% it is critical, and the mortality rate is 2-3% (CFR – case fatality rate, i.e. the number of deaths per the number of registered disease cases). In a study involving 143 hospitalised patients, after two months following the disease, almost half of the subjects reported reduced effort tolerance [14].

Pulmonary sequelae in COVID-19

As previously mentioned, COVID-19 is a general systemic disease, but the fate of patients is determined primarily by the changes in the lungs. Radiological imaging tests allow us to assess the scope and stage of pulmonary lesions. It should be emphasised that the basic study routinely used for such assessment is a chest X-ray. A chest CT should be limited to the cases where chronic respiratory symptoms and/or lasting, advanced lesions in the parenchyma and/or pulmonary vessels persist. The spectrum of pulmonary lesions in COVID-19 comprises:
- normal radiologic picture of the chest or small lesions with asymptomatic disease;
- signs of atypical, interstitial pneumonia in patients without respiratory failure;
- signs of atypical interstitial pneumonia, more pronounced, at various stages, leading to respiratory failure and, in an extreme form, to ARDS;
- Additional lesions due to bacterial co-infections, pleural involvement or pulmonary embolism. In extensive pneumonia, pneumothorax is sometimes observed.

In evaluating lung dysfunction, it should be noted that the most frequently found abnormality is reduced transfer factor for carbon monoxide, TLco, observed in approximately 40% of patients. It improves within up to 12 months after the disease. The most common abnormality of the mechanisms of ventilation is restriction (approximately 15%), obstruction is observed nearly half as often (approximately 7%) and changes in the radiologic lung picture are also found [15].

The main symptoms of impaired gas diffusion in the lungs, as well as of microthrombosis of the pulmonary vessels, is dyspnoea. With small or moderately intensive changes, it may be only dyspnoea during intensive exercise. As the extension of pulmonary lesions increases, the tolerance of effort decreases until, eventually, dyspnoea at rest occurs, in extreme cases involving respiratory failure. In some patients the symptoms associated with bronchial inflammation are observed. They may be present from the beginning of the disease and persist, with varying intensity, for many weeks or months, or they may occur at a later phase. We may divide them into:
- prolonged bronchitis – dry cough and/or combined with expectoration of mucous or mucopurulent secretion, mostly in the morning; reduced exercise tolerance;
- symptoms resulting from bronchial hyperreactivity and bronchial obstruction – dry cough, often all day long, intensifying at night time, wheezing, paroxysmal dyspnoea at rest, exercise dyspnoea with wheezing and/or dry cough;
- a combination of the above symptoms.

Prolonged bronchitis occurs more often in patients who smoke or have such episodes following previous infections. Symptoms of asthma may occur for the first time or could be previously present, as episodes or for an extended

period of time. In patients with chronic bronchial conditions (COPD, bronchial asthma) the symptoms may exacerbate. Chest pains are observed less often, and some of them are of pleural origin [16].

Immunopathology

Both specific and non-specific immune responses play an important role in SARS-CoV-2 infection. The first phase of infection, related to replication of the virus, causes direct tissue damage due to the proliferation and release of the virus. The damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) released from the infected cells activate the immune response associated with recruitment of T and B cells, monocytes and neutrophils [17]. This is accompanied by the release of proinflammatory cytokines such as tumour necrosis factor alpha (TNF α), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukins (IL) 1, 2, 6, 7, 8, 12, monocyte chemotactic protein-1 (MCP1), macrophage inflammatory protein-1 alpha (MIP) and interferon-γ (IFN). Excessive secretion of these cytokines may result in cytokine storm, which poses a significant danger for the organism and is specific for severe COVID-19 [18]. The virus may also escape immune supervision in the organism [19] by producing proteins that may suppress the antiviral response of the host, e.g. non-structural proteins (nsp), by activating inflammasome, e.g. open reading frame (Orf) 3a protein or by producing proteins antagonistic to INF-1, e.g. open frame proteins 6 and 9b (Orf6, Orf9b). Such a mode of action is observed primarily in oligosymptomatic or asymptomatic COVID-19, where no lung lesions characteristic for the severe course of the disease are found.

Induction of cytokine storm

The organism's response to SARS-CoV-2 infection demonstrates certain similarity to the response to flu infection. Increased antibody-secreting cells (ASCs), follicular helper T cells (TFH), activated CD4+ T cells and CD8+ T cells and IgM and IgG antibodies that bind coronavirus SARS-CoV-2 were detected in the blood. The immune response pattern may be useful for prognosing the course of the disease, developing a vaccine against it or medications to reduce its severity [20].

Other studies demonstrated that SARS-CoV-2 virus infection activates CD4+ T cells in the organism, which later differentiate into Th1 cells and produce GM-CSF. These cytokines induce the production of proinflammatory CD14+CD16+ monocytes with a high expression of IL-6 cytokines, which accelerates the development of pneumonia and contributes to increasing the mortality risk. Considering the fact that in patients with severe COVID-19 a large number of inflammatory infiltrations in the lungs were found, researchers suggest that the previously mentioned abnormal pathogenic Th1 cells and inflammatory monocytes may enter the pulmonary circulation in huge numbers and adversely affect the immune system, causing functional impairment of the lungs or even clinical death. Therefore, the authors of the study suggest that monoclonal antibodies targeting GMCSF or IL-6 may be effective in blocking cytokine storms, offering a promising treatment for severe COVID-19 patients [21].

The main cause of death in COVID-19 patients is acute respiratory distress syndrome (ARDS). One of the principal mechanisms observed in ARDS is uncontrollable systemic inflammatory response in the form of a cytokine (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGF-β etc.) and chemokine (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10) storm, which results in vascular endothelial damage. Endothelial injury in the airways leads to respiratory failure, while endothelial damage in the cardiovascular system may cause intravascular clotting, resulting in multiorgan failure and death [22].

Weakening of the immune system

The SARS-CoV-2 virus infects T, B and NK cells, as well as initiates or promotes lymphocyte apoptosis, observed in the leukogram as lymphopaenia. It explains why in patients infected with SARS-CoV-2 the proinflammatory phase is followed by immune suppression, associated with permanent and significant reduction of the peripheral lymphocyte count, primarily of CD4 and CD8 T cells. The reduced number of T cells in COVID-19 strongly correlates with increased inflammation. In patients with acute disease also a considerable reduction of regulatory T cells [23], responsible for restraining the immune response, is observed. The decreased number of lymphocytes may also be associated with their weakened function, as demonstrated in the studies by Cossarizza et al. 2020 [24] and Diao et al. 2020 [25]. The mechanism in which SARS-CoV-2 infection affects the lymphocyte count is still unknown. It has been established that lymphocytes lack ACE2 expression, so they cannot be infected via this mechanism [26]. A potential mechanism is offered based on a study by Xiong et al., conducted with the use of a bronchoalveolar lavage, in which changes in the expression of the genes associated with apoptosis and P53 signalling pathways were detected, suggesting that the reduction of the peripheral mononuclear blood cells may be due to increased apoptosis [27].

Follow–up examination scheme

Our own observations indicate that in most patients with COVID-19 pulmonary lesions subside after 3-6 months, although it does not always correlate with improved lung function.

The management depends on the severity of the disease (the initial condition and persistence of delayed symptoms) and the extension of pulmonary lesions. After a mild disease, in patients without symptoms or pulmonary lesions, the diagnostic and therapeutic process may be terminated. Although guidelines do not require a mandatory laboratory confirmation of infection in the case of mild disease, patients often perform tests on their own or to satisfy the epidemiological requirements, e.g. to shorten their quarantine. In this group of patients only symptomatic treatment is applied (e.g. analgesic, antipyretic). If symptoms persist in this group of patients, as well as in patients with pneumonia and mild or moderately severe clinical course of the disease, a follow-up assessment is recommended, not later than after three

months. It should include the medical history, a physical examination and a chest X-ray. In patients who had pulmonary embolism it is necessary to assess the effects of the initial treatment and the present risk factors for venous thromboembolism, as well as to verify the indications for further anticoagulation prophylaxis. In patients with severe pneumonia – hospitalised – initial assessment is recommended after 4-6 weeks following hospital discharge and the above evaluation is recommended after approximately three months. If significant abnormalities are found and respiratory symptoms persist after that time, assessment of pulmonary function and extended diagnostics are recommended. Depending on the findings, referring patients to facilities that specialise in interstitial lung diseases or pulmonary hypertension should be considered [28].

Treatment of SARS-CoV-2 infection. Can plants be of help?

Due to the SARS-CoV-2 pandemic, the world of science started to search for substances that could reduce the risk of infection and, in case disease symptoms occur, could reduce their severity and effectively protect against acute COVID-19 (associated with cytokine storm) and a serious lung injury. In the initial phase of the pandemic, various preparations were tested, both pharmacological products and those of natural origin. Initially, antiviral medications seemed to be the best option, e.g. remdesivir, lopinavir, ritonavir or favipiravir [29]. Also tested were antibiotics (e.g. azithromycin) or antimalarial agents (chloroquine and hydroxychloroquine) [30]. In Poland, two therapies of SARS-CoV-2 infections became popular: the first one was based on a medication used in multiple sclerosis, amantadine [31], and the second one involved convalescent plasma transfusions [32]. In the two years of the pandemic, it was possible to assess the effectiveness of many preparations that may significantly reduce the clinical complications of SARS-CoV-2 virus infection. They include antiviral agents (remdesivir, paxlovid), medications based on monoclonal antibodies (casirivimab/imdevimab, bamlanivimab/etesevimab, tixagevimab/cilgavimab) or Janus-activated kinase (JAK) inhibitor (Olumiant) [33].

Most preparations with proven clinical effects in the therapy of SARS-CoV-2 infection are relatively well described in Polish medical literature (e.g. guidelines of the National Consultant in Family Medicine, the Polish Society of Epidemiology and Infectious Diseases or the Agency for Health Technology Assessment and Tariff System). In this study we would like to explore a less frequently discussed subject of a potential use of plant-derived compounds in the therapy of SARS-CoV-2 infections.

Traditional medicine uses many plants whose biologically active components affect the processes of virus entry and its replication in host cells or which stimulate the immune system to produce an effective response to infection. Presented below are two plant species that, due to the composition of biologically active substances and their antiviral properties, could be used at any stage of COVID-19: coneflower (*Echinacea)* and golden root (*Rhodiola*).

Coneflower (*Echinacea*) – belonging to the *Asteraceae* family. Three *Echinacea* species demonstrate therapeutic properties, and they include: *Echinacea purpurea*, *Echinacea pallida* and *Echinacea angustifolia*. Preparations based on *Echinacea* help to reduce the frequency of various infections and the common cold. This is due to the active ingredients present in the plant: polysaccharides, alkaloids, caffeic acid derivatives and proteoglycans, which demonstrate immunomodulating, antiviral, antioxidative and anti-inflammatory properties [34]. Polysaccharides, especially arabinogalactans, activate macrophages and present a cytotoxic effect on neoplastic cells [35, 36]. The active ingredients of *Echinacea* have also antioxidative properties. In the case of flu viruses, studies revealed that administration of coneflower immediately after the onset of disease symptoms significantly reduces the convalescence time and alleviates the clinical course of the disease. Studies confirmed the antiviral effect of coneflower in infections caused by flu viruses H5N1, H1N1 and H7N7. In the case of SARS-CoV-2, using coneflower at different stages of the disease is suggested. Used for prophylaxis, coneflower, due to the presence of kaempferol, quercetin and caffeic acid, blocks the receptors used by the virus to enter the cell [37, 38]. So far, no direct antiviral properties of purple coneflower extracts against SARS-CoV-2 have been demonstrated. However, using supplements based on this plant may be an effective weapon in the treatment of COVID-19, due to their direct effect on the level of cytokines released. It has been shown that coneflower supplementation reduces production of proinflammatory cytokines (primarily IL-1, -6, -8 and TNF-α) that are the main cytokines found in severe COVID with a cytokine storm [39]. Simultaneously, certain studies on subjects receiving coneflower revealed an increase in the concentration of anti-inflammatory cytokines IL-10 and TGF-β, demonstrating strong immunosuppressive properties [40].

Golden root - the *Rhodiola* genus (family *Crassulaceae*) comprises over 200 species, of which over 20 demonstrate medicinal properties. The plants of this genus are used in traditional Asian and European medicine as tonic, adaptogenic, antidepressant and anti-inflammatory agents. Extracts from these plants have also beneficial antineoplastic, antibacterial and immunomodulating properties [41, 42]. *Rhodiola* extracts supplemented for 7 days reduce the infection size in mice infected with *Pseudomonas aeruginosa*. A significant elevation of the blood leukocyte count and their metabolic activity was observed in these mice [43]. Extracts from the *Rhodiola* plants also demonstrate direct antiviral and antibacterial effects against the hepatitis C virus (HCV) and *Mycobacterium tuberculosis* [44, 45]. At present, a few new clinical therapies are being developed, based on the ability of the *Rhodiola* plants to stimulate the immune system. As is the case with coneflower, the positive effects of golden root extracts may be multidirectional. The *Rhodiola* extracts contain kaempferol and quercetin, which – as mentioned above – can block viral attachment to cells. Currently, there is no data on whether golden root extracts can directly affect proliferation of the SARS-CoV-2 virus; however, just like coneflower, they may affect the secretion of pro- and anti-inflammatory cytokines. In 2021, Wang et al.

demonstrated using the *in silico* analysis of pathways that bioactive polyphenols (mainly quercetin) present in *Rhodiola crenulata* extract are strongly correlated with anti-inflammatory response, and they should reduce the secretion of pro-inflammatory cytokines, such as IL-6, IL-1B and TNF-α [46]. Another polyphenol, salidroside, also significantly reduced concentrations of pro-inflammatory cytokines: IL-6, TNF-α, MCP-1, STAT-3 and NF-K-B2 [47, 48].

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COLORECTAL CANCER SCREENING
Badania przesiewowe raka jelita grubego

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Abstract: Colorectal cancer is one of the most common cancers worldwide. Genetic and lifestyle-associated factors affect its development. It is estimated that it takes approximately ten years for a precancerous lesion (adenoma) to progress to malignancy (adenocarcinoma). Colorectal cancer screening enables effective treatment of precancerous lesions and obtaining an early cancer diagnosis, allowing radical treatment and reducing mortality. This article presents the colorectal cancer screening methods available in Poland.

Streszczenie: Rak jelita grubego jest jednym z najczęstszych nowotworów złośliwych na świecie. Na rozwój raka jelita grubego, poza predyspozycją genetyczną, wpływa szereg czynników wynikających ze stylu życia. Szacuje się, że proces rozwoju nowotworu złośliwego od przednowotworowej zmiany (gruczolaka) do zmiany złośliwej (gruczolakoraka) wynosi około 10 lat. Badania przesiewowe (skrining) raka jelita grubego pozwalają na skuteczne leczenie zmian przednowotworowych i rozpoznanie nowotworu we wczesnym etapie rozwoju, co pozwala na radykalne leczenie i zmniejsza umieralność. Celem pracy jest omówienie dostępnych w Polsce badań przesiewowych raka jelita grubego.

Key words: colorectal cancer, screening, screening programme.

Słowa kluczowe: rak jelita grubego, badania przesiewowe, skrining, program badań przesiewowych.

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Introduction

Colorectal cancer (CRC) is third most common malignant neoplasm in the world and its incidence rate continues to grow [1]. In 2020, approximately 2 million new CRC cases were diagnosed across the world, most of them in developed countries and for over 900 thousand patients the disease was fatal [1]. CRC affects men more often than women. A similar trend in incidence rates can also be observed in Poland, where CRC is the third most common malignant neoplasm in men (following prostate cancer and lung cancer) and women (following breast cancer and lung cancer) [2]. In 2019, over 18,000 new CRC cases and over 11,000 deaths were recorded in Poland [2]. Most CRC are located in the colon, while approximately a third of tumours are found in the rectum [1, 2].

Primary prevention

The risk of CRC increases with age and most cases are seen in patients over 45 years of age [1, 2]. The disease rarely affects patients under 40 years of age, but in recent years the incidence in this age group has been increasing, due to lifestyle factors, including alcohol consumption, smoking cigarettes and poor eating habits resulting in obesity, such as excessive consumption of red and processed meat [3-6]. On the other hand, a diet rich in vegetables, fruit and wholegrain products reduces the risk of CRC. A metaanalysis of 21 studies revealed that regular physical activity reduces the risk of CRC, regardless of body weight [7, 8]. A few studies demonstrated the beneficial effects of acetylsalicylic acid (ASA) on the reduction of CRC incidence and mortality rates [9]. The effect is partially due the fact that ASA reduces the risk of adenomatous polyp formation. However, using ASA for prophylaxis is not routinely recommended due to its adverse effects, mainly gastrointestinal bleeding [9]. On the other hand, U.S. Preventive Services Task Force (USPSTF) recommends small doses of ASA (approximately 80 mg a day) in secondary prevention of cardiovascular diseases (CVD) and primary prevention of CRC [10]. These recommendations apply to adults aged 45 to 59 years with a 10% or greater 10-year CVD risk, who are not at increased risk for bleeding and whose estimated life expectancy is at least 10 years [10].

Secondary prophylaxis

Screening tests for colorectal cancer are justified, as the neoplasm poses a significant health problem. It is

characterised by a long pre-neoplastic phase (adenomas and dentate polyps), that when possible might be a cure that, as a result, reduces mortality [11]. Population screening is addressed to the moderate risk group, i.e. the population of women and men aged 50-65 years. Different qualification criteria for screening apply to the population at high risk of the disease due to genetic predispositions: hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome), polyposis syndromes (familial adenomatous polyposis – FAP, Peutz-Jeghers syndrome and others), a positive family history (colorectal cancer in a first-line relative before 50 years of age or two first-line relatives, regardless of the age of onset) and non-specific enteritis (ulcerative colitis, Crohn's disease) [11].

Tests used in the diagnostics of CRC include stool tests, endoscopic procedures and, less often, computed tomography or magnetic resonance colonography (virtual colonoscopy). The choice of appropriate method depends primarily on the analysis of its cost-effectiveness and availability.

Guaiac faecal occult blood test (gFOBT) is a commonly available and inexpensive method of CRC detection, and has been used for years [12]. However, it is characterised by low sensitivity (50-75%) and specificity (50%) in detection of CRC and advanced adenomas [13]. The test results may be affected by the diet and medications used, and it requires the collection of many samples [13].

Faecal immunochemical test (FIT) is a newer method that detects human globin from haemoglobin. Compared to gFOBT, FIT demonstrates higher sensitivity in detecting advanced adenomas, as well as high sensitivity (79%) and specificity (94%) for CRC [13, 14]. It also does not require multiple stool collections – one sample is sufficient, and the diet or medications used do not affect the results. However, the cut-off point for a positive test result remains disputable. The most frequently used value is 10-20 µg Hb/g [15, 16]. Previous findings indicate that FIT, compared to gFOBT, is better accepted by patients and more widely used [14]. Similar observations have been made in the Polish population: the PICCOLINO study, involving 13,000 patients invited to a screening test, demonstrated a higher level of acceptance for FIT, compared to a colonoscopy. It resulted in a higher rate of patients reporting for the test, and it did not affect the outcomes of the screening [17]. According to international screening guidelines, FIT should be repeated every 1-2 years, and if the result is positive, a colonoscopy is required [13].

Clinical studies are being conducted on stool tests using more advanced methods, e.g. multitarget stool DNA test (mt-sDNA test) and gut microbiome test [13, 18].

Apart from the diagnostics for CRC and pre-neoplastic lesions, endoscopic studies (sigmoidoscopy and colonoscopy) offer another great advantage: a possibility to remove potentially carcinogenic polyps and adenomas. The aim of sigmoidoscopy is to visualise the mucosal membrane of the rectum, sigmoid and part of the colon, up to the splenic flexure. The effectiveness of the test in reducing incidence and mortality due to CRC has been confirmed [13]. Compared to gFOBT and FIT, sigmoidoscopy is more sensitive and specific in detecting advanced adenomas and CRC, but it is of smaller value than colonoscopy [13]. The rate of complications associated with sigmoidoscopy is low and they include mostly pain, abdominal distension and local bleeding, while severe complications (intensive bleeding, perforation, death) are extremely rare (0.08%) [19, 20]. Compared to colonoscopy, sigmoidoscopy is a shorter procedure and it is less troublesome for the patient.

Colonoscopy is a standard test in CRC screening, as it enables visualisation of the mucosa of the entire large colon. A positive result of any other screening test: gFOBT, FIT or sigmoidoscopy, is an indication for colonoscopy. Population studies have demonstrated the effectiveness of colonoscopy in reducing the risk of CRC, as well as CRC-related mortality [13, 21-23]. Importantly, the effect is long-lasting, which makes it possible to perform screening colonoscopy in a moderate-risk population every 10 years or even once, depending on the age of the patient [13, 21-23]. Similarly to sigmoidoscopy, the frequency of life-threatening complications associated with colonoscopy, such as intensive bleeding or intestinal perforation, is very low (approximately 0.2% and 0.05%) [24]. Complications are more often observed in elderly patients and in patients who receive endoscopic surgical procedures [24]. The quality of colonoscopy depends on a proper preparation of the patient for the test (assessment of intestinal cleansing according to the Boston Bowel Preparation Scale – BBSP) and on the experience and skill of the endoscopist. The measures of this quality include adenoma detection rate (ADR), caecal intubation rate (CIR) or post-colonoscopy colorectal cancer (PC-CRC, interval CRC) rate within 3 years of the colonoscopy [25, 26].

Due to the increasing incidence among the younger population, the USPSTF and American Cancer Society guidelines currently recommend starting population screening at 45 years of age [13], whereas the European guidelines still recommend starting screening at 50. On the other hand, the upper age limit for testing is not well-defined: usually screening ends for patients aged 75 years or for those whose estimated life expectancy is under 10 years. The guidelines propose endoscopic methods, as well as stool tests and combinations of all these examinations [13]. Despite high costs of screening programmes, the expense is fully justified, as the medical and social costs of treating CRC are much higher [27].

A population study involving over 3 million patients diagnosed with CRC in the years 2000-2016 in 21 European countries demonstrated that in the countries with long-standing CRC screening programmes, regardless of incidence rates, the mortality rates have been reduced [26]. They include Germany (since 1977), Austria (since 1980), Czechia (since 2000), Finland (since 2004) and Great Britain (since 2006) [23]. In all these countries, gFOBT was initially used, whereas at present the main screening methods are colonoscopy and FIT.

CRC screenings take an opportunistic form (patients from a given population visiting health centres at their own

initiative) or an organised form (based on invitations sent to individuals from the target group) [23, 28].

In Poland, the Early CRC Detection Screening Test Programme (STP) based on colonoscopy began in 2000, initially consisting only in opportunistic screening and was addressed to the population aged 50-65 years without gastrointestinal symptoms, individuals aged 40-49 years with a family history of CRC and those aged 25-49 years with a family history of HNPCC or FAP syndromes. In 2012, organised screening was initiated and personal invitations for colonoscopy were sent to individuals aged 55-65 years. Moreover, regional CRC screening programmes are implemented as part of the European social funds framework. The data from the STP indicate that the number of centres conducting screening (in 2019 – 141), the number of invitations sent (in 2017 – 41,868) and tests performed (in 2019 – 69,699 tests) increase with every year, but the rate of patients reporting to colonoscopy is still low (in 2017 – 12.9%) However, following the introduction of STP, the 5-year survival rate in CRC patients increased from approximately 25% in the 1990s to approximately 50% in 2014 [29]. The programme is coordinated by the National Institute of Oncology and detailed information, including the list of centres offering tests, are available on the website www.pbp.org.pl. Until 2020, the STP was financed through the National Programme for Combating Neoplastic Diseases, while currently it is funded through the National Oncological Strategy (NOS) 2020-2030. CRC NOS aims to introduce FIT as a test alternative to colonoscopy (second option for patients who do not consent to colonoscopy), to increase the rate of individuals in the target population who receive CRC screening (colonoscopy at any time or FIT every 2 years) from 18% to 30% by the end of 2024 and to 45% by the end of 2027 to introduce CRC screening tests funded from public resources (National Health Fund) (until the end of 2021 financed from the NOS funds), and until the end of 2028, to introduce a mandatory use of high-resolution endoscopes in colonoscopic examinations [30].

Conclusions

Colorectal cancer is an important health problem in Poland and in the rest of the world. In 2008, the European Parliament declared March as Colorectal Cancer Awareness Month. Screening tests enable early diagnosis of the tumour and treatment of pre-neoplastic lesions. The introduction of FIT as part of screening tests resulted in a higher rate of patients coming for the tests, while it did not affect the screening results. Colonoscopy remains a standard screening procedure in the CRC Screening Test Programme, which significantly improves the survival parameters and should still be developed.

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GENDER RELATED DIFFERENCES IN ACTIVE RENIN, ALDOSTERONE AND BIG ENDOTHELIN CONCENTRATION DURING HEAD-UP TILT TESTING IN PATIENTS WITH ISOLATED VASOVAGAL SYNCOPE

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Abstract: Introduction and objective: Neuroendocrine dysregulation seems to play the main role in liberation of abnormal vasovagal reflex, and the pathomechanism can be gender-related. The aim of the study was to assess changes in concentration of active renin (R), aldosterone (A) and big endothelin (BE) at rest and tilting in patients with vasovagal syncope in reference to gender and result of the head-up tilt test (HUTT). Material and methods: Study involved 133 patients with syncope. In all HUTT were performed. Concentrations of the analyzed hormones were measured at the last minute of the rest, at the 10th minute of tilting and at the end of the test. An aldosterone/renin ratio (A/R) was calculated for each of the phases. Results: HUTT(+) was observed in 87subjects (65%). During tilting a gradual increase of R and A was observed. The highest A/R was present in women HUTT(-) (p=0.040), contrary to non-fainting men in which decrease of A/R was accompanied with the highest absolute values at the end of the test (>18.6 pg/ml and A/R ratio at the end of the test <2.53 revealed to be independent predictors of syncope during tilt. The increase of R concentration during tilting <1.26 fold and the lack of increase of A predicted syncope during passive phase. In men, A concentration at the end of the test <44.0 pg/ml and the increase of A during tilting less than 2.10 fold, independently predicted positive result. None of analyzed parameters was pointed as an independent factor of syncope during passive phase. Conclusions: The activity of the vasoactive hormones is different in women and men and can determine the result of the test and the tolerance to passive tilting.

Key words: vasovagal syncope, head-up tilt test, aldosterone, renin, big-endothelin.

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INTRODUCTION

According to actual guidelines, syncope is defined as „transient loss of consciousness (TLOC) due to cerebral hypoperfusion, characterized by a rapid onset, short duration, and spontaneous complete recovery" [1]. In the general population of patients diagnosed due to the syncope, more than 37% episodes are caused by pathological vasovagal reflex syncope (VVS) [2]. They concern mostly the young persons without structural heart disease. The main diagnostic tool of VVS is detailed anamnesis and head-up tilt test (HUTT) that allows to reproduce VVS in the controlled conditions [3, 4].

Short-term blood pressure regulation is a result of detailed cooperation between humoral and autonomic nervous systems. The increase of sympathetic activity is expressed by increase of catecholamine concentration, activation of a renin – angiotensin – aldosterone system (RAS) [5] and other vasoactive hormones, including endothelin 1 (ET-1) or its precursor big endothelin (BE). Neuroendocrine dysregulation seems to play the main role in liberation of abnormal vasovagal reflex, and the pathomechanism can be different in men (M) and women (W).

Female steroids hormones have significant influence on blood pressure regulation, that's why differences in RAS activity before and after menopause are observed [6].

The primary objective of the study was to assess changes in concentration of active renin, aldosterone and big endothelin at rest and at prolonged tilting in patients with isolated vasovagal syncope in relation to gender.

The additional aim was to determine hormonal parameters that could be useful in prediction a result of the head-up tilt test.

METHODS

Study population

The initial assessment involved consecutive patients presenting symptoms suggesting VVS and qualified for the HUTT after exclusion of other cardiac, neurological or endocrine disturbances. To avoid a potential influence of additional factors all patients with chronic comorbidities (including cardiac pacing) or taking any medicines in the last 8 weeks prior to testing (including nonsteroidal antyinflammatory drugs and hormonal contraceptives)

were excluded from recruitment. Finally 133 persons with isolated VVS were enrolled.

The study was conducted according to Good Clinical Practice guidelines and Declaration of Helsinki, with the approval of local Ethics Committee RNN/170/05/KE. All subjects gave written informed consent to participate in the study.

Head-up tilt test

The diagnostic HUTT was performed on the morning, in slightly darkened room. The participants were asked not to smoke tobacco, not taking alcohol, caffeine or other psychoactives during 12 hours prior to the HUTT. Changes in body position were made with the use of table tilt testing SP-1 the feet supported and the straps placed at the height of the knees and chest. HUTT according to extended Westminster protocol included a 30-minute resting phase and 45-minute tilting at an angle of 60 degrees. If there was a negative response to passive tilting, pharmacological provocation with an aerosol of gliceryl trinitrate (GTN) were administered sublingually in a dose of 0.4mg and tilting continued for a further 15 minutes. The HUTT result was considered positive in the occurrence of syncope or presyncope with sudden hypotension and/ or bradycardia, and was evaluated according to the international VASIS classification [7].

During the HUTT heart rate (HR) and blood pressure (BP) were continuously monitored with the use of Spacelabs Medical 90369 Patient Monitor, and subsequently analyzed offline (HR were calculated as an average of a 30-second period). The used set included the module to noninvasive automatic blood pressure measurement in a 1-minute intervals.

A study group were divided according to the HUTT result (HUTT(+) – positive HUTT; HUTT(-) – negative HUTT) and then according to the phase in which the syncope has occurred (passive vs GTN). Independent analysis for W and M were done.

Hormonal analysis

An active renin (R) and aldosterone (A) concentrations were measured three times: at the last minute of the rest (R1, A1), the 10th minute of tilting (R2, A2) and at the end of the test (syncope or the end of tilting for HUTT(-)) (R3, A3). To avoid a renin crioactivation the blood samples were directly centrifuged in ambient temperature, and then frozen in minus 80 degrees till final laboratory analysis. Immunoradiometric and radioimmunological method to measure of active renin and aldosterone concentration were used respectively.

An aldosterone/renin ratio (A/R) was calculated for each of the phases of the test.

A Big Endothelin (BE) concentration was measured three times: at the last minute of the rest (BE1), the 10th minute of tilting (BE2) and the end of the test (syncope or the end of HUTT(-)) (BE3). Blood samples were immediately placed on ice and centrifuged, and then frozen in mninus 80 degrees till final laboratory analysis with immunoenzimatic method.

For an objective evaluation of variability of parameters assessed in subsequent stages of the tilt test, comparative analysis of the absolute values and also indicators of the dynamics of change (xF), with respect to the reference values were performed: xF(a/b) = F(a)/F(b), where F(a) – the value of the analyzed parameter during phase, F(b) – the parameter value during the phase of being compared.

Statistical analysis

Statistical analysis was performed using StatSoft STATISTICA software and MedCalc 20. Continuous variables were presented mean ± SD and categorical variables as the absolute and relative frequencies (percentages). The distribution and normality of data were assessed by visual inspection and the Kolmogorov-Smirnov test. In the case of continuous variables t-Student's or U Mann-Whitney test was used to determine the significance of differences and the Chi-square test for categorical variables.

To find the relationship between continuous variables the evaluation of the linear correlation of Pearson (or Spearman) was performed.

From the analysis of ROC curves (Receiver Operating Curve) the cut-off values of the discriminative parameters were determined. Then, they were appointed for the final validation with use of univariate and multivariate logistic regression models for each of the sexes independently. A p value of <0.05 was considered significant.

RESULTS

Head-up tilt test result

In group of 133 patients (W 84 (63%); mean age 36.8 ± 16.5 years; modal 22 years) which were enrolled, the positive result of HUTT was observed in 87 subjects (65%). There were no sex differences in relation to the percentage of positive results (p=0.103) nor to the phase of HUTT in which syncope was occurred (p=0.881).

Resting hormonal activity and age

Resting mean values of analyzed hormones were similar in M and W. The trend to lower R and higher BE in W was noted (table 1).

In W significant decrease of R for 0.16 pg/ml for each year was observed (R(pg/ml)=21.31-0.1558*age; p=0.036); this trend was not noted in M. Significant negative correlations of age and A in both groups were observed (W: A(pg/ml)=52.42–0.4639*age; p<0.0001; M: A(pg/ml)=54.78–0.4214*age; p=0.043). Significant positive correlation between age and A/R ratio in W and negative trend in M were also found (W: A/R=1.1+0.0727*age; p=0.039; M: A/R=3.742-0.032*age; p=0.055). Insignificant influence of age for BE was noted.

Hormonal activity during hutt in relation to the result of tilting

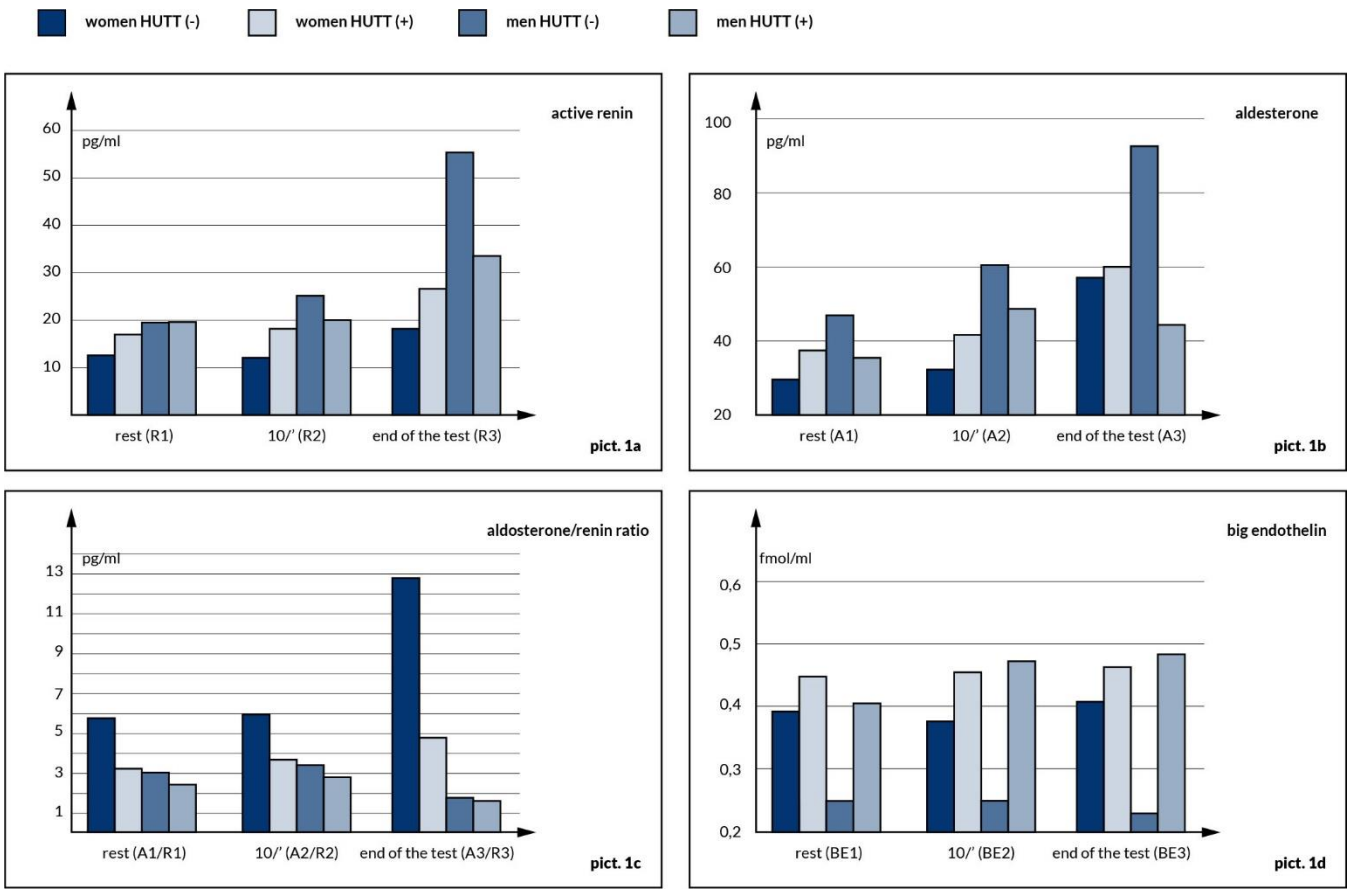

Table 1. Resting hormonal activity in women and men

	Women (n=84)	men (n=49)	p
R (pg/ml)	15.36±11.67	19.47±11.46	0.050
A (pg/ml)	34.70±19.98	40.48±22.48	0.127
A/R	3.88±5.53	2.63±1.74	0.127
BE (fmol/ml)	0.43±0.24	0.35±0.27	0.089

A – aldosterone
A/R – aldosterone/renin ratio
BE – Big Endothelin
R – renin

During tilting a gradual increase of R and A in all subgroups were observed (picture 1a, 1b). The highest absolute values at the end of the test were noted in M HUTT(-) ($p<0.001$) accompanied with decrease of A/R ratio, while the highest A/R ratio was present in W HUTT(-) ($p<0.05$) (picture 1c). M HUTT(-) were also characterized with the lowest BE activity during all the HUTT phases ($p<0.01$) (picture 1d).

In W, R concentration after 10 minutes of tilting (R2), A/R ratio at the end of the test (A3/R3) and coefficient of dynamics of BE concentration from the 10th minute of tilting to the end of the test (xF BE(3/2)) differentiated groups of HUTT(-) and HUTT(+). The R concentration at the end of the test (R3), coefficients of dynamics of R and A concentration from basal to the end of the test (xF R(3/1), xF A(3/1)) and from 10th minute of tilting to the end of the test (xF R(3/2), xF A(3/2)) differentiated subjects in which syncope occurred during passive tilting and after GTN.

In M, positive result of HUTT was related to RAS activity during syncope (R3, xF R(3/1), xF R(3/2), A3, xF A(3/1), xF A(3/2)) and BE activity during tilting (BE2). None of the parameters revealed to distinguish M HUTT(+) that fainted during passive and GTN phase. Significant differences between subgroups, for both sexes independently, are summarize in table 2.

Predictors of positive HUTT

The ROC analysis allowed to identify cut-off values of the parameters presented in table 2. Then, as binary variables, they were incorporated into multivariate logistic regression models. In W, R2 concentration over 18.61 pg/ml (ROC: 0.757; percent of cases correctly classifed: 73.81%) and A3/R3 ratio lower than 2.53 (ROC: 0.708; percent of cases correctly classifed: 71.43%) revealed to be independent predictors of syncope during HUTT (table 3).

In M, A concentration at the end of the test (A3) below 44.05 pg/ml (ROC: 0.753; percent of cases correctly classifed: 75.51%) and the increase of A during tilting (xF A(3/2)) less than 2.10 (ROC: 0.709; percent of cases correctly classifed: 73.47%), independently predicted HUTT(+) (table 4).

Predictors of syncope during passive phase of HUTT

Table 2. Significant differences of hormonal activity during prolonged tilting according to the result of head-up tilt test in females and males groups

	women HUTT(+) (n=60)	women HUTT(-) (n=24)	p
R2 (pg/ml)	18.36±13.83	11.57±7.29	0.025
A3/R3	4.71±12.11	12.71±22.92	0.040
xF BE(3/2)	1.11±0.34	1.93±2.63	0.027
	women HUTT(+) GTN (n=36)	women HUTT(+) passive (n=24)	p
R3 (pg/ml)	33.51±20.63	17.49±11.27	0.001
xF R(3/1)	2.82±2.87	1.3±0.60	0.014
xF R(3/2)	1.97±1.00	1.33±0.77	0.010
xF A(3/1)	1.87±0.86	1.4±0.79	0.036
xF A(3/2)	1.69±0.76	1.29±0.67	0.041
	men HUTT(+) (n=27)	men HUTT(-) (n=22)	p
R3 (pg/ml)	33.40±21.03	54.67±18.76	<0.001
xF R(3/1)	2.23±2.15	3.4±1.53	0.036
xF R(3/2)	1.82±0.97	2.69±1.65	0.024
A3 (pg/ml)	43.09±32.76	92.65±65.02	0.001
xF A(3/1)	1.29±0.71	2.2±1.51	0.007
xF A(3/2)	1.07±0.51	1.72±1.0	0.004
BE2 (fmol/ml)	0.479±0.35	0.251±0.19	0.012
	men HUTT(+) GTN (n=17)	men HUTT(+) passive (n=10)	
	No significant differences		

A – aldosterone
BE – Big Endothelin
HUTT(+) – positive head-up tilt test
HUTT(-) – negative head-up tilt test
GTN – gliceryl trinitrate
R – renin
xF – indicators of the dynamics of change
1 – concentration at rest
2 – concentration after 10 minutes of tilting
3 – concentration at the end of the test (syncope or the end of negative test)
(detailed description of abbreviation was presented in methodology of the study)

In W the increase of R concentration during tilting (xF R(3/2)) below 1.26 (ROC: 0.833; percent of cases correctly classifed: 83.33%) and the lack of increase of A (xF A(3/2) ≤ 1) (ROC: 0.847; percent of cases correctly classified: 86.67%) predicted syncope during passive phase (table 5). In M none of analyzed parameters was pointed as an independent factor of HUTT(+) during passive tilting.

Table 3. Results of univariate and multivariate logistic regression distinguishing women with positive and negative head-up tilt test

Factors	OR	-95% Ci	+95% Ci	p
Univariate analysis				
R2 >18.61 (pg/ml)	6.37	1.37	29.70	0.018
A3/R3 ≤ 2.53	6.00	2.06	17.47	0.001
Multivariate analysis				
R2 >18.61 (pg/ml)	5.77	1.17	28.48	0.031
A3/R3 ≤ 2.53	5.62	1.86	16.93	0.002

A3/R3 – aldosterone/renin ratio at the end of the tilting (syncope or the end of negative test)
R2 – renin concentration at 10th minute of the tilting

Table 4. Results of univariate and multivariate logistic regression distinguishing men with positive and negative head-up tilt test

Factors	OR	-95% Ci	+95% Ci	p
Univariate analysis				
R3 ≤ 37.51 (pg/ml)	10.69	2.74	41.74	0.001
xF R(3/1) ≤ 2.05	9.33	2.53	34.43	0.001
xF R(3/2) ≤ 2.32	4.20	1.22	14.45	0.023
A3 ≤ 44.05 (pg/ml)	9.33	2.53	34.43	0.001
xF A(3/1) ≤ 1.35	6.33	1.81	22.11	0.004
xF A(3/2) ≤ 2.10	21.67	2.48	189.10	0.005
BE2 > 0.40 (fmol/ml)	8.36	1.61	43.27	0.011
Multivariate analysis				
A3 ≤ 44.05 (pg/ml)	7.67	1.65	35.67	0.009
xF A(3/2) ≤ 2.10	11.54	1.14	117.00	0.039

A – aldosterone
BE – Big Endothelin
R – renin
xF – indicators of the dynamics of change
1 – concentration at rest
2 – concentration after 10 minutes of tilting
3 – concentration at the end of the test (syncope or the end of negative test)
(detailed description of abbreviation was presented in methodology of the study)

DISCUSSION

Recurrent syncope is an important diagnostic and therapeutic problem of high prevalence in the general population. The greatest amount of information on the epidemiology of syncope can be found in the Framingham study, with all limitations due to studied population, in which 3.5% of women and 3% of men experienced at least one syncope episode in their lifetime [8]. Due to various conditions and comorbidities the pathomechanisms triggering syncope has not yet been clearly established. There is evidence that its pathophysiological basis in men and women may not be the same [6]. The results of our study show that different activity of RAS and BE in response to tilting can be crucial. It can be explained by the

important role of these hormones in short-and long-term regulation of blood pressure. Changing the position of the body leads to decrease of venous return, decompression of high-pressure baroreceptors of the aortic arch and carotid body, resulting in disinhibition of the sympathetic nerve fibers (type C1) activity. On the other hand, catecholamines released from sympathetic endings are major activators of RAS. In case of functional disorders of any of the components of neurohumoral reflex, hemodynamic imbalance may occur. Unambiguous determination of the cause-and-effect relationship is often impossible to perform.

Table 5. Results of univariate and multivariate logistic regression distinguishing women with syncope occurred during passive tiltng and pharmacological provocation

Factors	OR	-95% Ci	+95% Ci	p
Univariate analysis				
R3 ≤ 14.90 (pg/ml)	5.83	1.87	18.25	0.002
xF R(3/1) ≤ 1.54	8.85	2.48	31.53	0.001
xF R(3/2) ≤ 1.26	25.00	6.25	99.96	<0.001
xF A(3/1) ≤ 1.09	16.00	4.18	61.22	<0.001
xF A(3/2) ≤ 1.00	51.00	9.32	278.96	<0.001
Multivariate analysis				
xF R(3/2) ≤ 1.26	7.45	1.44	38.55	0.017
xF A(3/2) ≤ 1.00	18.91	2.98	120.07	0.002

Age and gender related differences in resting hormonal activity

Analyzing resting hormonal activity we observed the association of the resting concentrations of R with age, but only in W. This phenomenon can be related to more pronounced hormonal and hemodynamic changes in aging females. Pecher-Bertschi et al. [6] reported a different profile of arterial pressure regulation in groups of W before and after menopause. Jacob et al [9] observed higher level of aldosterone and plasma renin activity in women with constitutional hypotension both supine and tilting.

In our study there was no effect of age and gender on the initial concentration of BE, but the significant differences in the activity of the RAS, catecholamines and other vasoactive hormones in relation to age and gender were observed among healthy subjects [6].

RAS dynamics during tilting

Another issue is intensity of activation of the RAS in patients with syncope. In the group of patients prone to hypotension (both young and elderly), there is a subgroup with the initial low plasma renin activity, which does not increase during syncope [7]. Therefore, in our analysis we scoped on the dynamics of hormonal activity. We identified different patterns of neurohormonal regulation in M and W, as well as for passive and GTN provoked syncope.

In response to the tilting we expected the increase of the R and A concentration as that was reported previously [10,11]. Indeed, Grasser et al. [12] noted nearly 3-fold increase in R during tilting, however not accompanied by an increase of A concentration. The authors explained the results by delay in the activation of the RAS axis.

In our study the relation of A and R revealed to be clinically important, especially in W. In W HUTT(+) a growth of R resulted in relatively lower increase in the concentration of A (A/R ratio) in comparison to W HUTT(-)). The detailed analysis showed that high R activity during first 10 minutes of tilting can predict syncope generally (regardless of the phase, table 3), but low final R activity and RAS dynamics favors syncope just during passive phase (table 5). That suggests that high activation of RAS can prevent syncope during passive test but can predispose to VVS after GTN.

In men a reaction of RAS turned out to be also important. In M HUTT(-) significantly higher values of R and A were observed, rising in the course of prolonged tilting. Despite the highest absolute values of both RAS components, the A/R ratio has declined to the value noted in M HUTT(+), in which A, lower just at the beginning, did not increase in response to R. Thus, similarly as for women, the RAS compensatory function was blunted (the strong predictive condition of syncope was the final A below 44.05 pg/ml and only slight increase during tilting). None of the analyzed parameters allowed to identify the test phase in which the syncope would occur.

These results may indicate the presence of additional pathways modulating adrenal response to R in men and women that participate in the syncope triggering. A pathomechanism of hypotension may also be different in case of passive tilting and after GTN admission, what in our study was clearly shown in women, although Nilsson et al. did not observed difference in resting concentrations or at 3 minutes of HUTT after adjustment for age and gender [13].

Role of endothelin in the response to tilting

Another important element of the neurohormonal regulation of blood pressure is ET-1. In our study, we used BE concentration assays. BE is a precursor to ET-1, and thanks to the greater stability of the molecule, the risk of pre-laboratory errors is reduced. The evaluation of BE change during tilting, that we performed, was justified by both: association of endothelial function with RAS and additional independent input of endothelin on cardiovascular hemodynamics. The increase in blood pressure is directly dependent on the endothelin's dose – 2-3-fold increase in the concentration of ET-1 causes a

19% increase in the value of blood pressure. Immediately after intravenous administration of endothelin, a decrease in blood pressure associated with the secretion of nitric oxide and of natriuretic peptides occures, then there is a significant increase in arterial pressure, resulting from the increased vascular resistance, which is maintained for some hours [14]. Sex differences in endothelin 1 levels seems to be due to different transcriptional regulation of endothelin 1 and its metabolism from precursor to converting enzyme activity [15]. Males are also more prone to endothelin-induced vasoconstriction and increases in blood pressure than females [15]. In contrast, women experienced a greater increase in forearm blood flow in response to combined endothelin-A and endothelin-B receptor antagonism, supporting a hypothesis about more prominent role for endothelin-B receptor function in female [16]. However, the observations on endothelin dynamics in response to upright position are discordant. White et al. [17] in a group of 46 healthy subjects reported the increase in ET-1 during prolonged upright but only in those with a negative test result. The opposite results presented Magerkurth et al. [18], who observed significantly higher levels of endothelin in patients with HUTT(+) in both the supine and the upright position compared with HUTT(-). Similarly, Galetta et al. [19] found increased activity of endothelium with progressive peripheral vasodilation and significant bradycardia in patients with induced VVS. Interesting conclusions provided Kaufmann [20] that compared the relationship between the change in blood pressure and endothelin concentrations in healthy subjects, patients with primary autonomic failure, vasovagal syncope and diabetes. In healthy controls the concentration of endothelin increased while pressure was stable. In diabetics and patients with autonomic failure the decrease in blood pressure was not accompanied by the increase in ET-1, whereas in patients with VVS decrease in arterial pressure was associated with an increase in ET-1. Nilsson concluded that higher resting concentration of C-terminal -proET-1 predicts pronounced fall in SBP during first 3 minutes of HUTT [21]. Thus, the relation of endothelin to hemodynamics depends significantly on clinical state of the patient.

Our results revealed the other difference. The BE response to upright position in males and females was not uniform. Only in M the BE concentration differentiated patients according to the result of HUTT. Non-fainting M, in comparison with other subgroups, were characterized by significantly lower BE at all stages of the test. Moreover, the levels of BE in M HUTT(+) were similar as in W. However, in multivariate analysis BE activity did not reach the significant power to be independent predicting factor. It can be explained by strong relationship with RAS that probably dominates the control on vascular hemodynamics during HUTT.

It is difficult to clarify why low endothelial activity protects against syncope. It can be supposed that high BE release is an insufficient compensatory mechanism in case of RAS failure. Due to the prominent role played by the ET system in maintaining cardiovascular homeostasis, coupled with the ability of the ET system to interact with numerous other pathways involved in BP control, more studies are needed to better define how the ET system regulates BP in both males and females. Besides the evaluation of changes in the concentration of BE during prolonged upright also requires further research because our analysis is the first of this kind in patients with recurrent syncope.

Clinical implications

Our results indicate the different relation between renin-aldosterone axis and endothelin in males and females. They suggest modulating action of female hormones on neuroendocrine pathways, water and electrolytes balance [22, 23]. Resting neurohormonal evaluation did not revealed to be useful to predict response to prolonged tilting. However, the differences in dynamic changes of evaluated hormones provide some new insight into gender related differences in provoked fainting. Our observations may have some clinical value. The different response to orthostatic stress may result in a different efficacy of the management of recurrent syncope. The further studies evaluating the effect of the pharmacotherapy guide by the neurohormonal assessment could verify the clinical importance of our results.

Limitations of work

The main limitation of our study is that we did not measured sex hormones and we do not take into account the current phase of the menstrual cycle in W, which can affect the neurohormonal regulation. We also did not measured arterial pressure in the continuous manner (by *beat to beat* method), which may impede the proper classification of the different types of vasovagal response. Thus, the analysis does not include the types of vasovagal reaction that can be characterized by specific neurohormonal patterns.

The methodological limitation can be the fact that we measured BE instead of ET-1. This assumption was justified by very short half-life of ET-1 and its low concentration in young subjects. Clearance of BE is much slower that limits the potential laboratory errors. For this reason we don't have possibility to direct comparing of our results to the other researches performed with using ET-1, what from the other hand can be treated as an innovative, positive aspect of our study.

Another limitation is, paradoxically, the high selectivity of the study group. Therefore our observations should be interpreted carefully for the general population, where fainting is frequently associated with hypertension, diabetes or other cardiovascular diseases.

Conclusions

The RAS and endothelin are involved in the regulation of blood pressure in response to change in body position. The activity of these vasoactive hormones is different in women and men and can determine the result of the test and the tolerance to passive tilting. The observed differences in the activity of RAS and endothelin confirm the important contribution of hormonal dysregulation in the triggering a vasovagal reaction.

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HOSPITALIZATION TIME AFTER LAPAROTOMY, LAPAROSCOPY AND ROBOTIC PROCEDURES IN PATIENTS WITH ENDOMETRIAL CANCER

Porównanie czasu hospitalizacji po zabiegach laparotomii, laparoskopii oraz robotycznych u pacjentek z rakiem endometrium

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Abstract: Comparison of the length of postoperative hospitalization for patients receiving endometrial cancer care by laparotomy, laparoscopy and robotic surgery. Retrospective analysis of the data from patients with operations for endometrial adenocarcinoma who underwent total hysterectomy and adnexal procedures with iliac lymphadenectomy and/or sentinel lymph node evaluation by midline laparotomy, laparoscopy or robotic surgery. The longest average hospitalization time was 5.27 days for the laparotomy group, which was less than half longer than those for the laparoscopic group (2.45 days). The shortest time, at an average 1.96 days, was recorded for the robotic surgery group. Use of minimally invasive techniques significantly shortens the hospitalisation time for patients operated on for endometrial cancer.

Streszczenie: Wprowadzenie i cel: porównanie długości hospitalizacji pozabiegowej pacjentek operowanych z powodu raka endometrium metodą laparotomii, laparoskopii oraz robotyczną. Materiał i metody: analizie retrospektywnej poddano dane pacjentek operowanych z powodu raka gruczołowego endometrium, u których wykonano całkowite wycięcie macicy z przydatkami z limfadenektomią biodrową i/lub oceną węzła wartowniczego drogą laparotomii pośrodkowej, laparoskopii lub chirurgii robotycznej. Wyniki: najdłuższy średni czas hospitalizacji odnotowano w grupie operowanych drogą laparotomii i wynosił on 5,27 doby, ponad połowę krótszy w grupie kobiet operowanych laparoskopowo i wynosił – 2,45 doby. Najkrótszy czas, wynoszący średnio 1,96 doby, odnotowano w grupie poddanych operacji robotycznej. Wnioski: zastosowanie technik małoinwazyjnych skraca znacząco czas hospitalizacji chorych operowanych z powodu raka trzonu macicy.

Key words: laparoscopy, endometrial cancer, robotic surgery.

Słowa kluczowe: laparoskopia, rak trzonu macicy, chirurgia robotyczna, chirurgia robotowa.

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Introduction

Endometrial cancer is one of the most common female genital malignancies – the fourth one after breast, colorectal and lung cancer [1]. In our country, there has been a multi-year increase in the incidence of endometrial cancer, which is in line with the European trend and is inextricably linked to increasing life expectancy and the prevalence of obesity. It is a disease of postmenopausal, obese women, often with an additional history of internal diseases, especially diabetes, hypertension or metabolic syndrome. The incidence of this type of cancer before the age of 40 is rare and does not exceed 4% of all cases. In some patients, it has a genetic basis related to mutations that cause Lynch syndrome [2].

Treatment of endometrial cancer includes surgery, radiotherapy, radiochemotherapy, chemotherapy and hormone therapy, and the proportion of particular treatment methods depends on the clinical stage of the disease according to FIGO (*The International Federation of Gynaecology and Obstetrics*).

In situations where complete cytoreduction (R0) can be achieved, treatment for endometrial adenocarcinoma is based on surgery. The basic procedure involves a simple hysterectomy with removal of the adnexa, without a vaginal cuff. The surgery can be performed by laparotomy, laparoscopy or transvaginal laparoscopy. According to the Polish Society of Oncology, supported by the recommendations of the international societies, ESGO (*European Society of Gynaecological Oncology*) and NCCN

(*National Comprehensive Cancer Network*), minimally invasive techniques are preferred [3, 4, 5]. Data from prospective and retrospective studies confirm both short- and long-term benefits of choosing minimally invasive surgery (MIS) in the treatment of endometrial cancer.

In this study, we have attempted to evaluate different surgical methods (laparotomy, laparoscopy and robotic method) with an assessment of the benefits related to the applied method indirectly by evaluating the duration of postoperative hospitalization.

Material and methods

Patients operated on at the Department of Gynaecology and Gynaecology Oncology of the Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine between 2018 and 2021 were analysed retrospectively. These were patients with a diagnosis of endometrial adenocarcinoma qualified for total hysterectomy and adnexal procedures with or without iliac lymphadenectomy or selective pelvic lymph node dissection in a sentinel node procedure. The procedures were performed by midline laparotomy (TAH), laparoscopy (TLH) or robotic surgery (RS), using the da Vinci surgical robot. The assessment included demographic indicators of women undergoing different types of surgery: age and body mass index. The time for the surgery in each group was calculated: the starting criterion was skin incision for laparotomy procedures and, in the case of laparoscopy and robotic surgery, skin incision before insertion of the first trocar. The criterion for the end of the procedure was the moment when the operator handed over the instruments after suturing the skin after laparotomy and after suturing the skin following removal of the trocars after laparoscopic and robotic procedures. The duration of the hospitalization of patients after each type of surgery was analysed. The time was given in full days from the day of the surgery to the day of hospital discharge.

Results

The assessment involved 134 patients allocated to one of the three groups for further analysis (Table 1). A total of 65 women underwent laparotomy, 20 women laparoscopy and 49 women robotic surgery. The average age in the laparotomy group was 68.36 years, higher than in the group of women that underwent robotic surgery (64.2 years). In the laparoscopy group, the average age of the women was the lowest: 62.37 years. BMI (Body Mass Index) was similar between the groups and was the lowest in the TAH group, 28.8, followed by the TLH group, 28.9, and relatively the highest in the RS group, 29.6. Additionally the comparison included the duration of the procedures. Traditional surgical procedures took the longest (average: 121 minutes), similar to the robotic method (average: 117 minutes). The shortest average operating time was recorded in case of laparoscopy (94 minutes). The greatest differences were observed when determining the length of the patient's hospitalization after surgery. The longest average hospitalization time was 5.29 days in the laparotomy group, which was more than twice that of the laparoscopic group (2.45 days). The shortest time, on average 1.96 days, was recorded in the group having undergone robotic surgery. The differences between the groups were statistically significant. The results are summarised in the tables.

Table 1. Demographic data

Type of surgery	Number of patients	Age of patients (average, age)	BMI (average, kg/m²)
TAH	65	68.36	28.8
TLH	20	62.37	28.9
RS	49	64.2	29.6

Table 2. Results of the statistical analysis

	average	median	SD	p
Time of the surgery (minutes)				
TAH	121	110	40.4	
TLH	94	90	16.7	
RS	117	115	36.0	
TAH vs. TLH				< 0.001
TAH vs. RS				0.583
TLH vs. RS				< 0.001
Hospitalization time (days)				
TAH	5.29	4	3.3	
TLH	2.45	2	1.14	
RS	1.95	2	0.67	
RS vs. TAH				< 0.001
RS vs. TLH				0.003
TLH vs. TAH				< 0.001

T-student test (time of the surgery) and Mann-Whitney test were used for the analysis

Discussion

The randomised GOG LAP2 study published in 2009 showed that laparoscopic surgical staging for uterine cancer is feasible and, above all, safe [6]. The laparoscopy group had a lower postoperative complication incidence rate (21% vs. 14%; P<0.001), a similar rate of intraoperative complications, despite a significantly longer time for surgery (average 204 vs. 130 minutes), and significantly shorter hospitalization time, by an average of 2 days. Follow-up results for the patients covered by the study (average: 59 months) showed a 1.14% difference in recurrence rate between two arms after 3 years. The estimated recurrence rate in the laparoscopy group was 11.4% and in the laparotomy group it was 10.2%. The estimated 5-year overall survival was almost identical in both arms, and amounted to 89.8% [7]. In a randomised trial, Janda et al. confirmed the validity of the choice of minimally invasive techniques in women with stage I endometrial cancer [8]. After 4.5 years of follow-up treatment, the disease-free survival was 81.3% in the TAH group and 81.6% in the TLH group. The difference in disease-free survival rate between the groups was 0.3%. A randomised study by Mäenpää et al. [9] included 99

patients. It compared two techniques in terms of length of the surgery and intraoperative events. The median time of the surgery in the laparoscopy group was 170 minutes (range 126-259) and in the robotic group it was 139 minutes (range 86-197) P < 0.001. There were 5 conversions to laparotomy in the laparoscopy group and none in the robotic surgery group, P = 0.027. There were no differences in the number of lymph nodes removed, bleeding or length of hospitalization after the surgeries. There were 4 intraoperative complications (bladder injury, bowel injury, vascular injury and respiratory failure), all of them in the laparoscopy group. There were no statistical differences with regard to postoperative complications. In their study, Silva e Silva et al. [10], randomised 89 patients aged 47-69 years. The median duration of the entire procedure was 319.5 (170-520) minutes for robotic surgery and 248 (85-465) minutes for traditional laparoscopy. There were no statistical differences in blood loss or length of hospitalization. The conclusions of this work are supported by a number of retrospective studies, and the results of these studies do not support the superiority of either method. In February 2022, a publication attempted to compare minimally invasive surgery (MIS) and laparotomy (TAH). This was a retrospective assessment of 1,382 patients, of whom 684 (49.5%) were operated on by MIS and 698 (50.5%) by open TAH surgery. The MIS involved 233 patients (34%) treated by robot-assisted laparoscopy (RS) and 451 (66%)- by conventional laparoscopy (TLH). After the analysis, disease-free survival (DFS), overall survival (OS) and specific survival related to EC (SS) were significantly higher for MIS compared with TAH. Given that older patients with burdens and more aggressive disease were eligible for laparotomy, homogeneous groups were selected in terms of age, BMI, comorbidities, ASA (American Society of Anaesthesiologists) score, histological type, stage, myometrial infiltration and FIGO stage, and it was found that DFS, OS and SS values were similar in the MIS and TAH groups [11].

The abundant evidence supporting safety of minimally invasive surgeries has been recognised by researchers and has been used in the development of recommendations. However, there is still no conclusive evidence supporting the superiority of either method. The results of our study, consistent with those of other publications, confirm the benefits of minimally invasive surgeries in the surgical treatment of patients with endometrial cancer. The use of these techniques significantly reduces the hospitalization time after the surgery. The demonstration of benefits of robotic surgery over other techniques would require confirmation in large randomised studies.

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MODEL WORK ORGANIZATION ON THE EXAMPLE OF THE COVID-19 UNIT ACCORDING TO THE BPMN 2.0 PROTOCOL

Model organizacji pracy zgodnie z protokołem BPMN 2.0 na przykładzie oddziału leczenia COVID-19

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Abstract: The announcement of the COVID-19 world pandemic has placed modern healthcare systems in a new, unprecedented situation, unencountered in the past century. A rapid introduction of guidelines and standards regarding the reorganization of healthcare systems has been required. The massive occurrence of infections involving respiratory failure involved setting up temporary wards and field hospitals worldwide, in places that were often not prepared for this type of medical activity. The development of forms of conduct in accordance with business procedures (BPMN 2.0), with a clear definition of the scope of responsibility, seems to guarantee the maintenance of an optimal level of safety for all participants in the diagnostic and therapeutic process. The aim of the study is to develop an Emergency Department and COVID-19 Unit functioning model in accordance with the BPMN2.0 business process protocol using Adonis CE platform version 11.0, with its verification based on the system and structure functioning at University Clinical Hospital No. 1 in Lodz. The authors created a standard notation protocol for the process of dealing with a patient in the Emergency Department (ED) / Admission Room and the COVID-19 Unit, and then verified the model based on their own experience, gathered during the creation and running of a temporary COVID-19 Unit with an Isolation Area. The development of treatment algorithms in the field of medicine in accordance with business procedures based on the BPMN 2.0 protocol is the basis for the smooth operation of medical personnel with the determination of the scope of duties and responsibilities. The use of standardized procedures for working with a suspected or confirmed patient with SARS-CoV-2 infection in the area of an ED and COVID-19 Unit when crossing individual risk zones of SARS-CoV-2 infection allows for the maintenance of appropriate epidemiological standards.

Streszczenie: Ogłoszenie ogólnoświatowej pandemii COVID-19 postawiło współczesne systemy ochrony zdrowia w zupełnie nowej sytuacji, niespotykanej w ostatnim stuleciu. Wymagało to adaptacji systemów opieki zdrowotnej i wypracowania nowych rozwiązań na szeroką skalę. Masowe występowanie zakażeń przebiegających z niewydolnością oddechową wiązało się z koniecznością utworzenia oddziałów tymczasowych i szpitali polowych w miejscach, które często nie były przygotowane do tego typu działalności leczniczej. Opracowanie schematów postępowania zgodnego z procedurami biznesowymi (protokół BPMN 2.0), z jasnym określeniem zakresu odpowiedzialności, zdaje się stanowić w takich sytuacjach gwarancję zachowania optymalnego poziomu bezpieczeństwa dla wszystkich uczestników procesu diagnostyczno-terapeutycznego.
Cel pracy: opracowanie modelu funkcjonowania Szpitalnego Oddziału Ratunkowego oraz Oddziału Leczenia COVID-19 zgodnie z protokołem procesów biznesowych BPMN 2.0 oraz zweryfikowanie w oparciu o system i strukturę funkcjonującą w Uniwersyteckim Szpitalu Klinicznym nr 1 w Łodzi.
Materiał i metody: w pracy wykorzystano elementy notacji BPMN 2.0 z wykorzystaniem platformy Adonis CE wersja 11.0. Następnie, stworzone modele zweryfikowano w oparciu o istniejącą infrastrukturę architektoniczno-administracyjną istniejącą w USK nr 1 w Łodzi.
Wyniki: autorzy stworzyli wzorcowy protokół notacji procesu postępowania z pacjentem w Szpitalnym Oddziale Ratunkowym/Izbie Przyjęć oraz Oddziale Leczenia COVID-19, a następnie zweryfikowali model w oparciu o doświadczenia własne, zebrane podczas tworzenia i prowadzenia tymczasowego Oddziału Leczenia COVID-19 z Obszarem Izolacyjnym.
Wnioski: opracowanie algorytmów postępowania w obszarze medycyny zgodnie z procedurami biznesowymi w oparciu o protokół BPMN 2.0 stanowi podstawę do płynnego działania personelu medycznego z wyznaczeniem zakresu obowiązków i odpowiedzialności. Zastosowanie ujednoliconych procedur pracy z pacjentem – podejrzanym lub z potwierdzonym zakażeniem SARS-CoV-2 – w obszarze SOR i Oddziału Leczenia COVID-19 podczas przekraczania poszczególnych stref ryzyka zakażeniem SARS-CoV-2 pozwala na zachowanie należytych standardów epidemiologicznych.

Key words: medical algorithm, COVID-19, Business Process Modelling and Notation.

Słowa kluczowe: algorytm postępowania, COVID-19, protokół postępowania biznesowego.

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Introduction

The first cases of the previously unknown *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2), causing COVID-19, were discovered in Wuhan province (China) in December 2019. As a result of the rapid and global spread of the pathogen, on 11 March 2020 the *World Health Organization* (WHO) declared a global pandemic [1].

During the first wave, the risk of SARS-CoV-2 infection among medical staff was several times higher than in the general population [2] and mortality, especially among doctors and nurses, was particularly high [3]. This resulted from a lack of knowledge of the pathogen's routes of transmission, shortages of personal protective equipment (PPE), inappropriate use of PPE [4] and creation of wards for patients with COVID-19 without sufficient knowledge.

Based on gained experience, WHO guidelines indicate that the most important methods of preventing infections in the general population are, apart from vaccination, wearing a mask that covers the nose and mouth, maintaining a social distance and regular hand disinfection. In the case of medical personnel, the most important method is the appropriate use of PPE [5]. Despite locally implemented procedures, during the first wave, over 7,000 medical staff members worldwide had become infected and died by the end of August 2020 [6]. Numerous opinions suggest that, due to increased availability of free movement between continents via air transport, similar pandemics may become a cyclical phenomenon [7].

In the absence of generalised recommendations for the organisation of the work of temporary infectious disease wards and the care for patients with a severe form of the disease, it becomes necessary to establish a sound protective strategy for medical personnel and to introduce appropriate, well-defined procedures and responsibilities. Describing the management algorithms according to the functioning protocols of *Business Process Modelling and Notation* version 2.0 (BPMN 2.0) makes it possible to distribute clearly formulated scopes of duties and responsibilities [8].

The aim of this paper was to develop a model for management of patients with suspected or confirmed SARS-CoV-2 infection, in the area of the Hospital Emergency Department and the COVID-19 Unit, according to the BPMN 2.0 business process protocol, and to verify the model based on the system implemented at University Clinical Hospital No. 1 in Lodz.

Method

The authors of this paper used elements of BPMN 2.0 notation with the Adonis CE platform version 11.0. The created models were then verified against the architectural and administrative infrastructure existing at University Clinical Hospital No. 1 in Lodz.

Results

The resulting process models of patient management in the hospital's Emergency Department (ED) / Admission Room (AR) and the COVID-19 Unit (CVU) are presented in Figures 1, 2, 3.

In University Clinical Hospital No. 1 in Lodz, due to architectural and spatial constraints (the building is listed in the voivodeship register of historic monuments, reg. no: A/106 of 1971-01-20) the creation and separation of the "modular" nature of the ward was not possible. In order to adapt the available infrastructure to the needs of caring for COVID-19 patients, the following organisational changes were made (Fig. 4):

- the south wing of the hospital was separated to manage patients with confirmed SARS-CoV-2 infection (COVID +),
- a separate entrance was established for medical staff and for patients reporting to the ED,
- within the circulation path above the ED area (ground floor), the previously functioning wards were converted into an Isolation Area (IA) located on the first floor and the CVU on the second floor,
- the lift connecting the wards was put out of use for the remaining wards,
- in the same wing on -1 level, one of the CT scanners was dedicated to the examinations of patients admitted to the CVU,
- a separate circulation path (yellow) was created from the staircase connecting the above-mentioned floors to the outside, for patients leaving the hospital building after being discharged from the IA and CVU.

Patients arriving at the hospital for emergencies were initially directed to the TRIAGE area located in a tent in front of the ED entrance. Then, after initial assessment, second-generation antigenic screening tests were performed. Until the results of the screening test were available, the patient remained in the so-called "red zone" of the ED. Other areas of the ED, including the operating theatre and the resuscitation and recovery room, were included in the yellow zone. In case of a strong clinical suspicion of COVID-19 and the need for hospitalisation and treatment initiation, a nasopharyngeal swab was taken for a RT-PCR test and the patient was referred to the IA to await the result. After a positive test result and indications for continued hospitalisation, the patient was transferred to the CVU.

Based on the decision of the Governor of Lodz Voivodeship, a multidisciplinary medical and nursing team comprising physicians from various specialities (internal

medicine, pulmonology, cardiology, gastroenterology, neurology, anaesthesiology, surgery, laryngology) was established to work in the CVU. The team was then trained in the use of PPE. The training was extended to all staff, including support staff (nursing assistants, ward attendants, health carers) performing daily duties in the yellow and red zones.

Risk zones

The designation of three infection risk zones allowed the de-escalation of applied disinfection procedures and PPE saving. The zones are described below and are reflected in the presented CVU architecture and created process diagrams.

Green zone

The staff in the clean zone were mainly exposed to potential infection from other members of the medical team, and therefore the continuous use of masks without an exhalation valve was recommended. All patients' medical records, including medical recommendations, had to be kept in the green zone. Medicaments administered to the patients on a permanent basis were prepared in the clean zone.

Separate social rooms for nursing and medical staff were used for resting and eating, with the recommendation that meals should be eaten by one person at a time and that the number of people in one room should be kept to a minimum. In the social rooms, air was purified by flow-through plasma purifiers.

Yellow zone

Anyone staying in the potentially contaminated zone, such as the ED area, the area of the hospital's circulation paths between the IA and the CVU and the common areas of the IA, should use appropriate PPE. In addition, mechanical and gravity ventilation should be switched off throughout the ventilation duct and the rooms should frequently ventilated by opening windows. Doors to common areas must be kept closed and the air must be purified of bioaerosols by means of flow-through air purifiers. When using HEPA filter air purifiers, it is essential to remember that the filter must be changed regularly. Plasma purifiers do not require replacement, but their particulate filters must be mechanically cleaned. Objects (including patients' personal belongings) in the yellow zone should be regarded as potentially contaminated. They should not be brought into the green zone without a due quarantine or disinfection period. Floors and flat surfaces on circulation paths and in rooms in the yellow zone should be regularly disinfected. There are no recommendations in the literature on the frequency of disinfection, with some authors recommending routine disinfection 1-2 times a day [9]. The yellow zone rooms should also be disinfected, if possible, every time a patient with confirmed SARS-CoV-2 infection has passed through them.

Red zone

In the contaminated zone, mechanical ventilation must be completely switched off. In Poland, it is usually not possible to maintain negative pressure in patient rooms and ensure individual isolation of patients within the areas of temporary infectious disease wards. However, such measures are strongly recommended if the technical conditions in the hospital allow it. Negative pressure can also be used during transport of the patient via common circulation paths [10]. A cheaper alternative may be barrier tents. Medical records should not be stored in the red zone. Only life-saving drugs and those requiring preparation immediately prior to administration are prepared in the red zone if preparation in the clean zone would constitute a delay resulting in a risk to a patient's life.

Special situations

Some COVID-19 patients suffer from end-stage renal failure or require dialysis for acute renal failure. In this case study, the problem was solved by setting up a separate dialysis round for CVU patients as the last dialysis round of the day. The dialysis unit is then disinfected. Patients should be transported through uncontaminated circulation paths in the barrier tents. At the end of the transport procedures, these paths should be decontaminated.

The need for imaging examinations of COVID-19 patients requires development of a procedure for transporting the patient to the CT scanner. The CT scanner room should be equipped with an oxygen system or oxygen cylinder for ventilation during examinations. At University Clinical Hospital No. 1 in Lodz, one CT scanner was intended for examinations of potentially infected patients. In the absence of such facilities, every effort should be made to establish separate times for performing scheduled CT examinations and a procedure for transporting a ventilated patient via common circulation paths.

Collection of waste and belongings from deceased patients poses another logistical challenge. All waste leaving the ward should be treated as contaminated and placed in double bags for disposal. Items of deceased patients given to the family should be placed in double plastic bags and quarantined for 5 days before being unpacked at home. The family of a deceased person should receive information on the handling of belongings.

If possible, a list of COVID-19 Unit consultant specialists should be established, so that as few staff members as possible are forced to enter the red zone. In surgical medicine centres, a separate procedure should be established in the operating theatre for urgent procedures in COVID-19 patients.

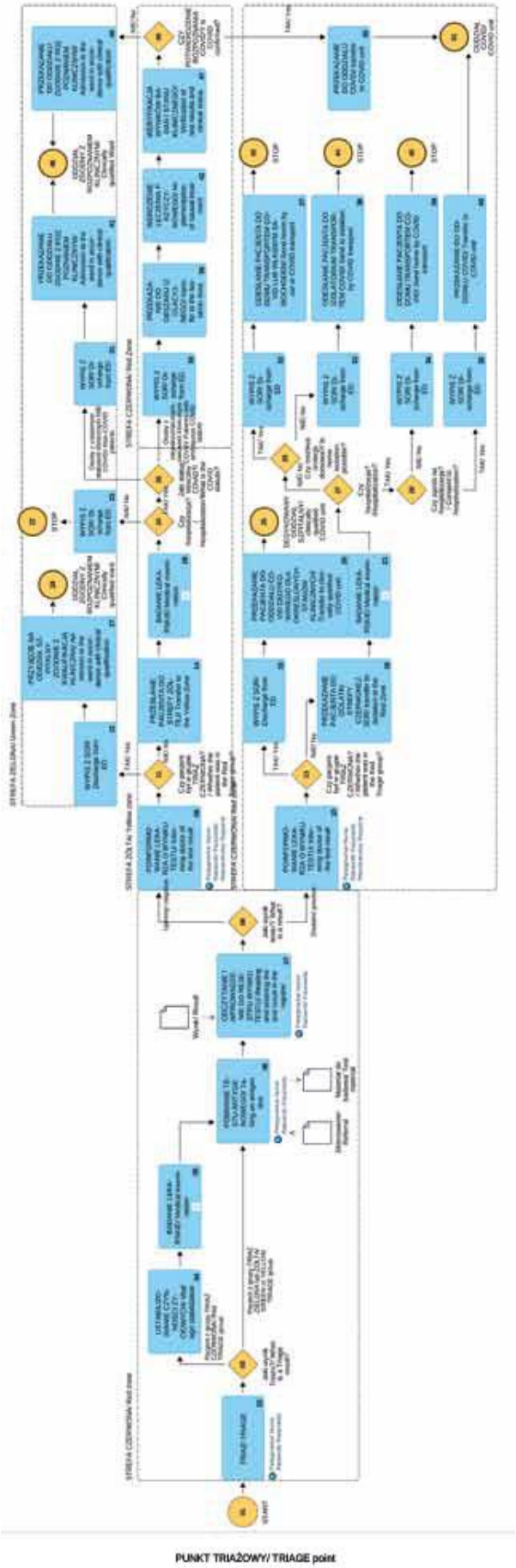
PUNKT TRIAŻOWY/ TRIAGE point

Fig. 1

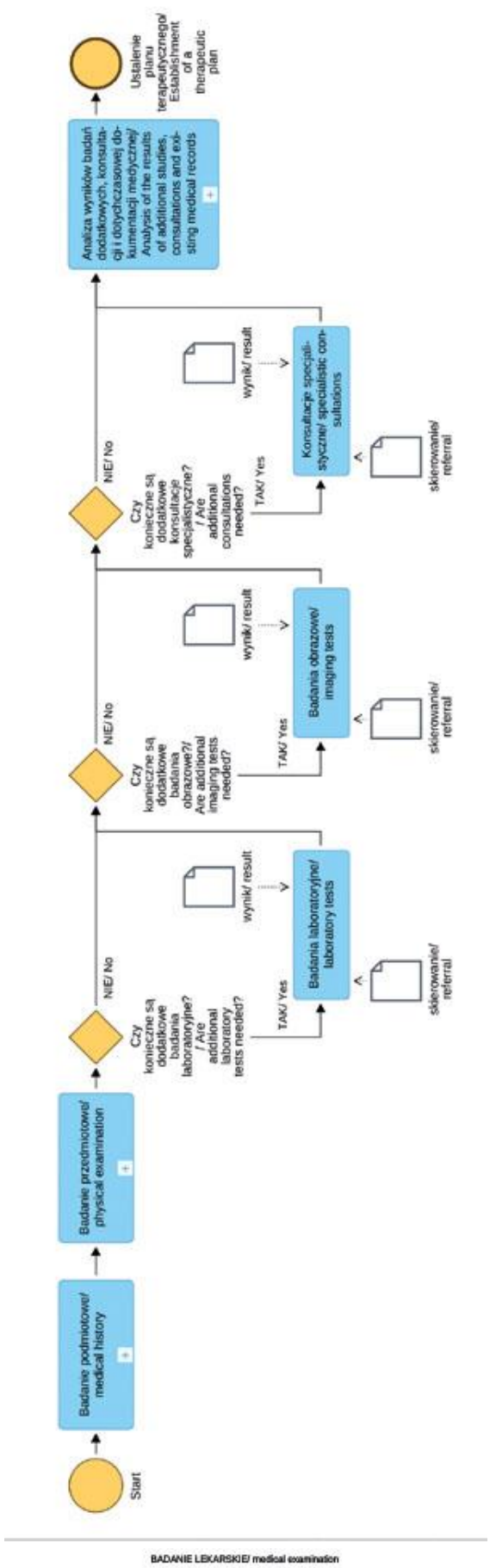
Start
Badanie podmiotowe/ medical history
Badanie przedmiotowe/ physical examination
Czy konieczne są dodatkowe badania laboratoryjne? / Are additional laboratory tests needed?
TAK/ Yes
NIE/ No
Badania laboratoryjne/ laboratory tests
wynik/ result
skierowanie/ referral
Czy konieczne są dodatkowe badania obrazowe?/ Are additional imaging tests needed?
Badania obrazowe/ imaging tests
Czy konieczne są dodatkowe konsultacje specjalistyczne? / Are additional consultations needed?
Konsultacje specjalistyczne/ specialistic consultations
Analiza wyników badań dodatkowych, konsultacji i dotychczasowej dokumentacji medycznej/ Analysis of the results of additional studies, consultations and existing medical records
Ustalenie planu terapeutycznego/ Establishment of a therapeutic plan
BADANIE LEKARSKIE/ medical examination

Fig. 2

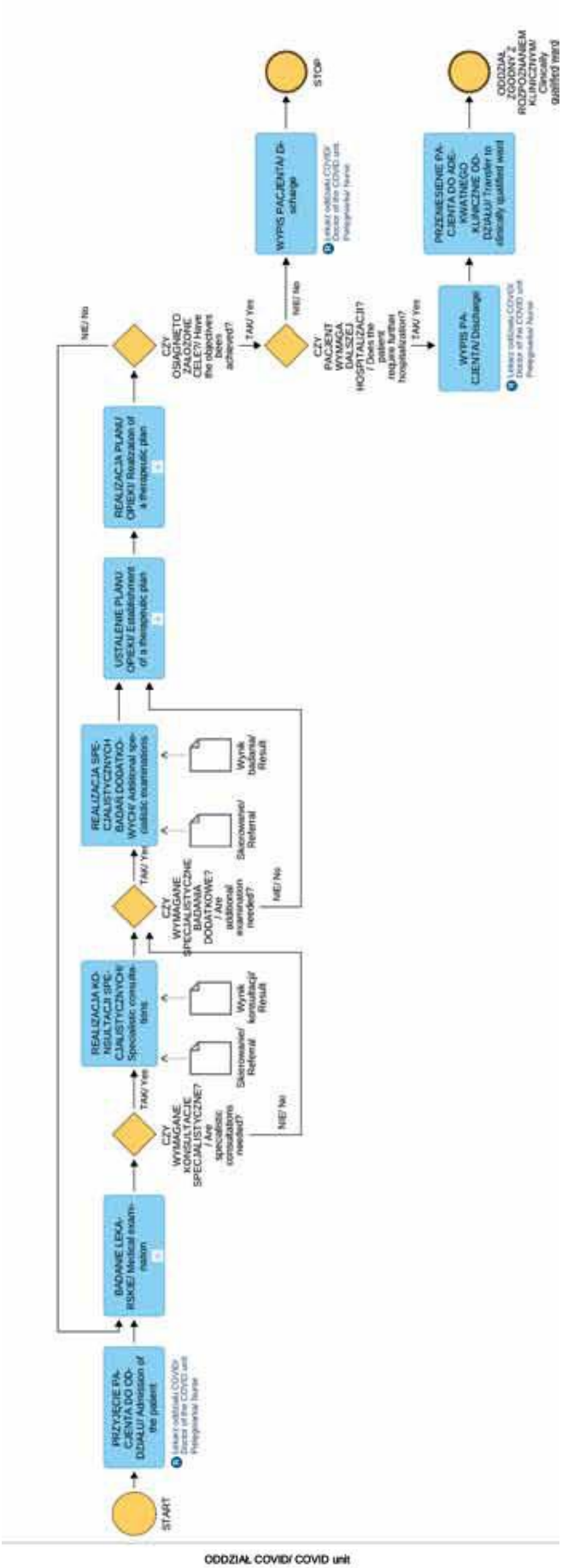
START
PRZYJĘCIE PACJENTA DO ODDZIAŁU/ Admission of the patient
BADANIE LEKARSKIE/ Medical examination
CZY WYMAGANE KONSULTACJE SPECJALISTYCZNE? / Are specialistic consultations needed?
TAK/ Yes
NIE/ No
Skierowanie/ Referral
REALIZACJA KONSULTACJI SPECJALISTYCZNYCH/ Specialistic consultations
Wynik konsultacji/ Result
CZY WYMAGANE SPECJALISTYCZNE BADANIA DODATKOWE? / Are additional examination needed?
Skierowanie/ Referral
REALIZACJA SPECJALISTYCZNYCH BADAŃ DODATKOWYCH/ Additional specialistic examinations
Wynik badania/ Result
USTALENIE PLANU OPIEKI/ Establishment of a therapeutic plan
REALIZACJA PLANU OPIEKI/ Realization of a therapeutic plan
CZY OSIĄGNIĘTO ZAŁOŻONE CELE? / Have the objectives been achieved?
CZY PACJENT WYMAGA DALSZEJ HOSPITALIZACJI? / Does the patient require further hospitalization?
WYPIS PACJENTA/ Discharge
Lekarz oddziału COVID/ Doctor of the COVID unit, Pielęgniarka/ Nurse
PRZEMIESZCZENIE PACJENTA DO ODDZIAŁU KWARANTANNY/ Transfer to the quarantine ward
ODDZIAŁ ZAKAŹNY Z ROZSZERZONYM KLINICZNYM/ Specialty quarantine ward
WYPIS PACJENTA/ Discharge
STOP
ODDZIAŁ COVID/ COVID unit

Fig. 3

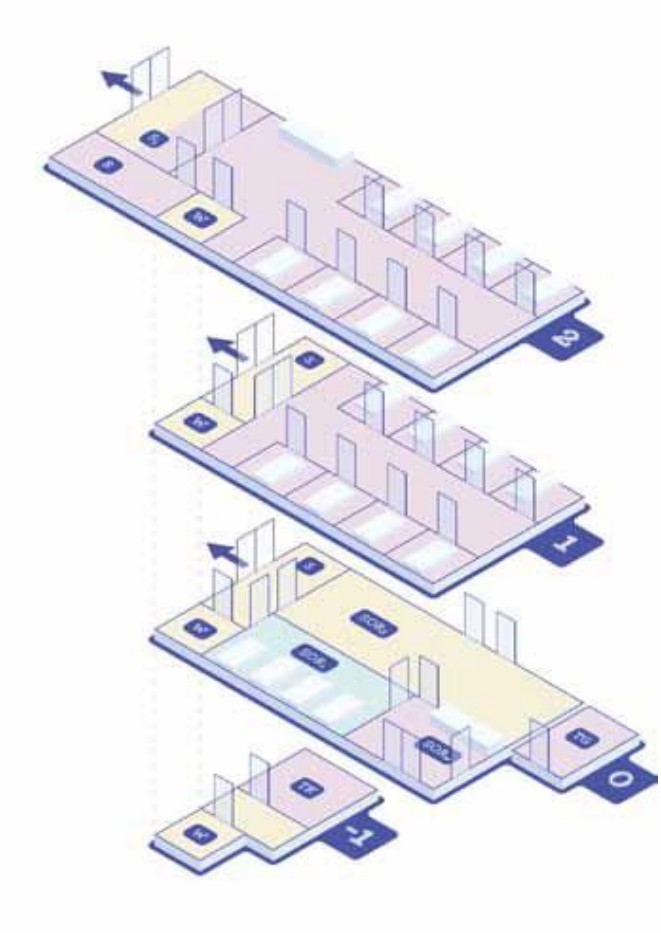
4
3
2
1
0
-1
ROOM
ROOM
ROOM

Fig. 4

Transferring patients in sluice rooms requires separate analysis. Care should be taken to ensure that staff taking a patient to the green zone use PPE appropriate for the yellow zone and bring their own bed. Beds and trolleys used in the red and yellow zones should not be taken out of these zones without being disinfected first. When transferring a patient from the contaminated zones, personal belongings should be packed in a plastic bag and should not be opened during the 5-day quarantine period.

Discussion

At University Clinical Hospital No. 1 in Lodz, the first case of SARS-CoV-2 infection was identified in April 2020, while CVU was established by order of the Governor of Lodz Voivodeship on 26 October 2020.

The aim of this paper was assessment of feasibility of using formal graphical notations designed for business process modelling [8] to describe the administrative procedures related to patient care in the ED area and a dedicated hospital ward in terms of SARS-CoV-2 infection. The guiding idea was to establish a conduct path, a sequence of events, decisions made on the basis of the acquired information and possible modifications of the process flow depending on the flexibility of the clinical situation.

The sequence of events described in the process is based on the principle of incremental information at successive stages and the corresponding management. The level of detail of the described steps can be discussed. As one of the first attempts of this type, it was conceived as a compromise between graphic clarity and lengthy descriptions. Ultimately, one may be tempted to prepare even more detailed descriptions and processes covering sequences of several activities, approaching the level of a workstation manual.

Another critical element of the paper was showing the "crossing points" between the zones. This was an extremely important, and yet the weakest, element of epidemiological protection, as at these points and in specific situations agents may migrate, causing the transmission of pathogens. Their proper identification, describing the events in which they can occur and describing the regimes that accompany crossing of the zones allows for better risk management and preparation of the environment for the activities performed to overcome such a sluice room, a buffer.

As mentioned above, the creation of an algorithm for the management and selection of patients with suspected and confirmed SARS-CoV-2 infection aimed at reducing the risk of epidemic outbreaks on the premises of University Clinical Hospital No. 1 in Lodz. At the same time, based on the governor's decision, a multidisciplinary medical and nursing team was established to work in the CVU. The reason for assigning physicians of different specialities to work in the CVU was the possibility of limiting external consultations to the necessary minimum, so that mostly people with developed sanitary habits remained in the red zone.

In terms of preventing the transmission of pathogens through medical staff, training in the use of sanitary procedures and PPE played a particularly important role. Staff training should emphasise the procedures associated with resuscitating patients in a contaminated area, including the absolute necessity of using PPE due to the particular exposure to bronchial secretions during the resuscitation procedures.

Process management is, on the one hand, a relatively new management approach in health sector organisations and, on the other hand, almost every organisation, based on a culture of knowledge and repetition of functions performed, produces and implements processes, often not using that name. Following ISO 9001:2008, a process is understood as: "set of interrelated or interacting activities, which transforms inputs into outputs. Often the output from one process directly forms the input to the next" [8]. Outputs should be understood as the result, and inputs the start of a process under certain conditions. The literature provides different definitions of processes, analysing them from different levels. For the purpose of this paper, the definition quoted above was relied upon and the well-known BPMN notation was used. Since the models of notation are not directly intended for medical events, but as the authors understand it, they can contribute to a better depiction of the cause-effect sequences, based on the general assumptions of the notation. Priority has been given to the comprehensibility and readability of the graphics rather than orthodox adherence to the rules of notation. In the authors' opinion, this is our own well-grounded modification to the notation. It should be mentioned that intense works were being conducted to create a variant of the notation that covered the needs and specifics of medical descriptions even more precisely.

Current trends in the assessment of process management systems incorporate, and we could even dare say that they seek, unification of process management through knowledge management [11]. The diagrams presented in this paper should be perceived as dynamic processes that, by using the knowledge of those involved in their implementation, can plasticise their course. It meets a demand for flexibility and distinguishes them from strict procedures, which by definition limit interpretative diversity. Procedures are the background and their execution is triggered by situations described by the process, but the process can trigger different procedures depending on the needs, knowledge and experience. Process management understood in this way, applying the analysis of the course of actual events to the process depicted, enables evaluation and better adaptation of the process to an ever-changing reality, while providing a basis of comparison of the "*as is*" and "*to be*" conditions. It provides a framework for developing and evaluating change directions. The authors believe in the usefulness and operability of the knowledge presented in this way, utilising, on the one hand, achievements of process management tools and, on the other, enabling a flexible approach to process implementation. Studying actual processes, sequences of events, decisions made and performed activities can allow improvements in the model and/or improvement of the actual functioning. It will always be feasible comparison with all the consequences of

improving efficiency, measurability of the course and optimisation of the undertaken activities.

Summary

The development of treatment algorithms in the field of medicine in accordance with business procedures based on the BPMN 2.0 protocol is the basis for the smooth operation of medical personnel with the determination of the scope of duties and responsibilities. The functioning of an infectious disease ward intended for the treatment of COVID-19 patients requires reorganisation of the work of the entire hospital, with development of dedicated procedures and training of medical staff in the use of PPE – appropriate to the work zone. The use of standardized procedures for working with a suspected or confirmed patient with SARS-CoV-2 infection in the area of ED and CVU when crossing individual risk zones of SARS-CoV-2 infection allows the maintenance of appropriate epidemiological standards.

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ACUTE PERIPHERAL BALANCE SYSTEM DAMAGE IN THE COURSE OF VESTIBULAR NEURITIS

Ostre obwodowe uszkodzenie narządu równowagi w przebiegu zapalenia neuronu przedsionkowego

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Abstract: The aim of the study was to present a case of acute peripheral damage to the balance system in the course of vestibular neuritis. A 32-year-old female was admitted to the Department of Otolaryngology, Laryngological Oncology, Audiology and Phoniatrics - II Department of Otolaryngology, of Lodz Medical University, due to a sudden onset of severe vertigo with a sensation of spinning of her surroundings, nausea as well as vomiting lasting several hours. She had never experienced such symptoms before. She also did not report any other ailments. She had experienced no hearing disorders or tinnitus, and she had not been treated so far due to these ailments. ENT (ear, nose, throat) examination, audiological and otoneurological diagnostics, imaging (CT scans of the head) and laboratory tests were performed. The patient was diagnosed with acute peripheral damage to the balance system in the course of right-sided vestibular neuritis. Symptomatic treatment was applied: piracetam and vinpocetine. Individually selected kinesiotherapy consisting of habituation exercises together with balance and coordination exercises (performed by the patient, if possible, several times a day), was recommended after reducing the vegetative symptoms. In conclusion, the aetiology of vestibular neuritis is not fully explained in most cases. Researchers typically advocate viral theory, although the disorders of vestibular nuclei blood supply maybe emphasized. The clinical picture is usually similar and involves sudden, rapid symptoms, resulting from a labyrinthine deficit, often with complete loss of its function.

Streszczenie: Celem pracy było przedstawienie przypadku ostrego obwodowego uszkodzenia narządu równowagi w przebiegu zapalenia neuronu przedsionkowego. Pacjentka, lat 32, przyjęta do Kliniki Otolaryngologii, Onkologii Laryngologicznej, Audiologii i Foniatrii - II Katedry Otolaryngologii, Uniwersytetu Medycznego w Łodzi w trybie pilnym z powodu silnych zawrotów głowy z uczuciem wirowania otoczenia, nudnościami oraz wymiotami utrzymującymi się od ok. kilkunastu godzin. Wcześniej zawroty u niej nie występowały. Innych dolegliwości nie zgłaszała. Zaburzenia słuchu oraz szumy uszne nie występowały i dotychczas z powodu tych dolegliwości nie była leczona. Przeprowadzono badanie ORL, diagnostykę audiologiczną, otoneurologiczną oraz obrazową (TK głowy) oraz badania laboratoryjne. Rozpoznano ostre obwodowe uszkodzenie narządu równowagi w przebiegu zapalenia nerwu przedsionkowego po stronie prawej. Zastosowano leczenie objawowe: Piracetam, Vinpocetine. Po zmniejszeniu dolegliwości wegetatywnych zalecono indywidualnie dobraną kinezyterapię - ćwiczenia habituacyjne oraz równoważne i koordynacyjne, wykonywane przez pacjentkę - w miarę możliwości - kilka razy dziennie. Wnioski: Etiologia zapalenia neuronu przedsionkowego w większości przypadków jest nie do końca wyjaśniona. Naukowcy opowiadają się za teorią wirusową, ale podkreślano także znaczenie zaburzeń ukrwienia okolicy jąder przedsionkowych. Obraz schorzenia jest zawsze podobny, dominują nagłe burzliwe objawy. Wiążą się one z występującym deficytem błędnika często z całkowitym wypadnięciem jego funkcji.

Key words: acute peripheral vertigo, balance system, vestibular neuritis.

Słowa kluczowe: ostre obwodowe zawroty głowy, narząd równowagi, zapalenie neuronu przedsionkowego.

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Introduction

Vestibular neuritis, known as *neuronitis vestibularis*, is the third most common cause of peripheral vertigo. The disease entity was first described in 1909 by Ruttin. Its aetiology is not fully understood and the likely cause is a viral disease (activation of latent herpes simplex virus type 1 – HSV1). Autoimmune reactions triggered by viral replication led to inflammation and eventually nerve demyelination.

The syndrome predominantly affects young people and there are no gender-specific differences. It may present as single paroxysmal vertigo or as a persistent syndrome, disappearing after about two weeks. The clinical picture is dominated by very severe, suddenly occurring systemic vertigo, described as a spinning sensation combined with nausea and vomiting. Patients develop balance disorders and nystagmus. In addition to the history, videonystagmography is performed and the diagnosis is based on abolished or weakened excitability of one labyrinth in the caloric test. However, despite the dramatic onset (vestibular shock), the prognosis is favourable.

Case study

Patient C.E., age 32 (medical history no. 19-17948), admitted to the Department of Otolaryngology, Laryngological Oncology, Audiology and Phoniatrics - II Department of Otolaryngology, Medical University of Lodz under the emergency procedure for treatment of sudden vertigo.

The patient reported severe vertigo with a sensation of spinning of her surroundings, nausea and vomiting persisting for several hours. She had not previously experienced vertigo. She also did not report any other ailments. She experienced no hearing disorders or tinnitus, and she had not been treated so far due to these ailments. The patient did not report any chronic diseases. She was not taking any permanent medication. She had not undergone surgery. She had no addictions.

Her history revealed an upper respiratory tract infection with concomitant herpes in the upper lip of her mouth two weeks before.

An ENT (ear, nose, throat) examination, laboratory tests, audiological and otoneurological diagnostics and also imaging (CT scans of the head) were performed.

On the day of admission, she had a 3rd degree horizontal

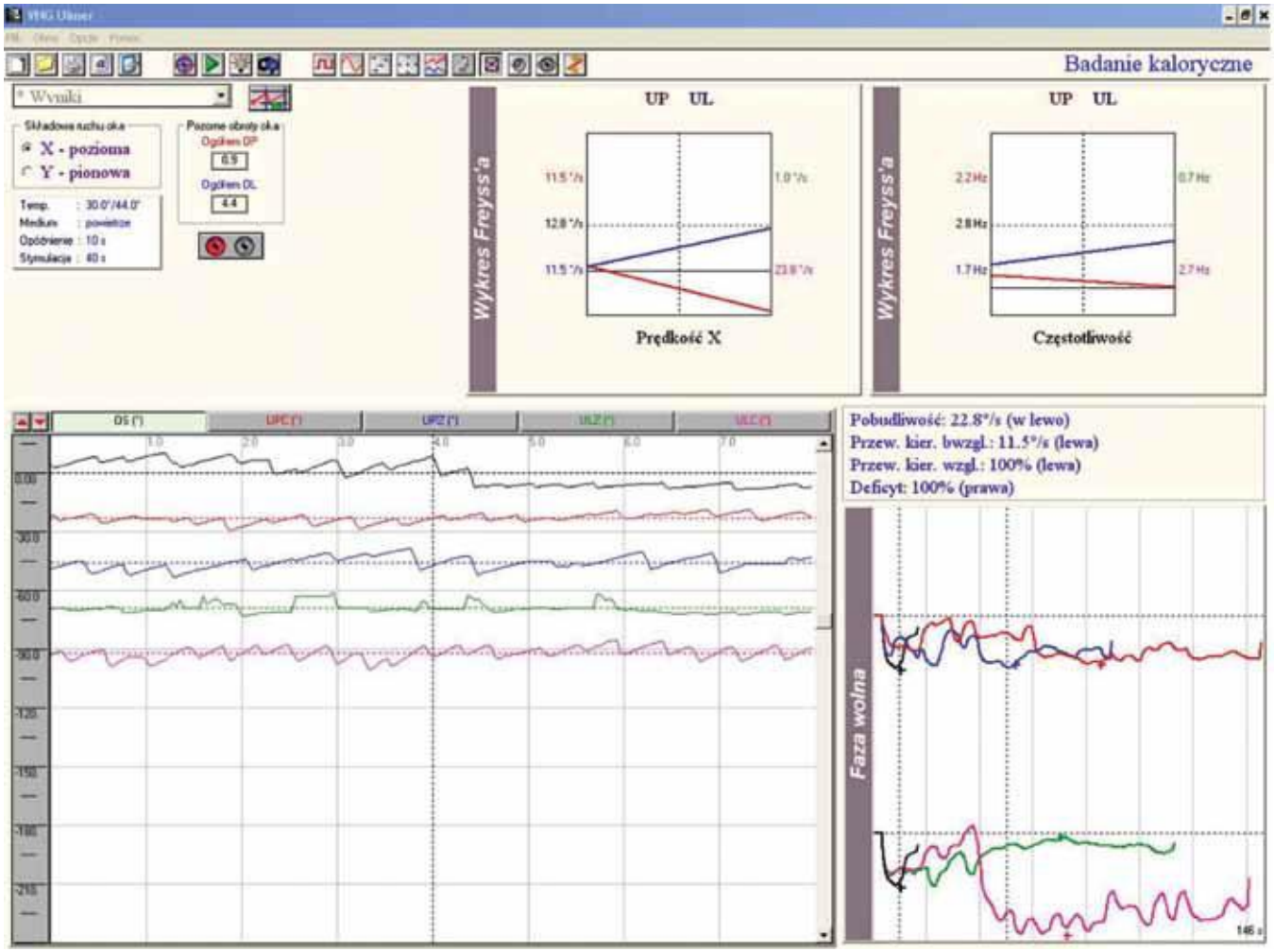

Fig. 1. *Fitzgerald-Hallpike caloric test result.*

rotatory spontaneous left-beating nystagmus. To assess the vestibulospinal reflexes, two tests were performed on the second day of hospitalisation: Romberg's test and Unterberger's test. They revealed a directional predominance (incidence) to the right.

Videonystagmography recorded spontaneous horizontal rotationary left-beating nystagmus 12.8°/s, upward beating nystagmus 8.7°/s and positional nystagmus. The Fitzgerald-Hallpike caloric test revealed an uncompensated total right-sided 100% labyrinthine deficit, with a relative directional predominance of 100% to the left (Fig. 1).

Apart from this, no neurological abnormalities were recorded. Pure-tone threshold audiometry and tympanometry along with reflexes were within norms.

The results of additional examinations indicated a slightly elevated glucose level – 6.97 (4.1-5.5 mmol/l) and CRP - 8.0 (0.0-6.0 mg/l).

A CT scan result of the brain was within norms.

The symptomatic treatment was delivered: Memotropil 20% (12 g/60 ml) x 1 flac. intravenously, Cavinton (0.01 g/2 ml) x 1 amp. intravenously, Natrium Chloratum (0.9%) x 250 ml, 1 pc intravenously (2 x daily).

On the basis of clinical observation, audiological and otoneurological examinations, and the exclusion of other causes of the complaints, the patient was diagnosed with acute peripheral damage to the balance system in the course of right-sided vestibular neuritis.

From the third day of hospitalisation, a gradual reduction in the complaints was observed, and second-degree spontaneous nystagmus and positional nystagmus were recorded. Individually selected kinesiotherapy consisting of habituation exercises together with balance and coordination exercises (performed by the patient, if possible, several times a day), was recommended after reducing the vegetative symptoms.

The patient was discharged from the clinic on day six in good general and local condition, with the recommendations: Memotropil 1.2 g; 2 x 1 tabl. (1-1-0), Cavinton 0.005 g; 3x1 tabl. (1-1-1). The patient remains under the care of the otolaryngology clinic.

Summary

Vestibular neuritis is diagnosed in patients of all age groups, although middle-aged patients predominate. The aetiology of vestibular neuritis in most cases is not fully elucidated. The aetiology of vestibular neuritis is not fully explained in most cases. Researchers advocate viral theory [1, 2], but the disorders of vestibular nuclei blood supply may not be emphasized. The clinical picture is usually similar and involves sudden, rapid symptoms, resulting from the labyrinthine deficit, often with the complete loss of its function [1, 2, 3,].

Regardless of the aetiology of the syndrome, symptomatic treatment is usually recommended [3], and if a viral background (*Herpes* virus) is suspected, acyclovir is additionally used. Most symptoms resolve after 1-6 weeks (average 3 weeks), although canal paresis may persist much longer [4, 5, 6]. Regardless of the duration of the syndrome, after inpatient treatment the patients should remain under the care of an otolaryngology outpatient clinic, and their condition and compensation process should be monitored with specialised investigations, such as videonystagmography, VHIT and stabilometric platform tests. In about 95% of cases, vestibular neuritis is a once-in-a-lifetime syndrome and recurrences are very rarely observed.

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NEUROPROTECTIVE TREATMENT IN TRAUMATIC BRAIN INJURY – PRESENT OR FUTURE?

Leczenie neuroprotekcyjne w pourazowym uszkodzeniu mózgu - przyszłość czy teraźniejszość?

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Abstract: Traumatic brain injury (TBI) poses a challenge to modern medicine due to its high incidence, varied clinical presentation, and the need for interdisciplinary treatment, often including intensive care, surgery, pharmacological therapy, nutritional therapy, and rehabilitation. Despite advances in the treatment of severe TBI, many patients require long-term care and do not regain self-reliance. For this reason, new therapies are still under evaluation, including neuroprotective treatment, which could improve prognosis after brain injury in both the early and late therapy stages. This paper presents a case of a patient with prolonged unconsciousness due to severe TBI, quadriparesis, and post-traumatic epilepsy despite intensive therapy and early rehabilitation, whose condition significantly improved after the use of a neurotrophic drug (cerebrolysin) and extensive rehabilitation with occupational therapy. Multidisciplinary management and multimodal therapy including neuroprotective treatment in TBI, is a promising management strategy that may contribute to therapeutic success.

Streszczenie: Pourazowe uszkodzenie mózgu (TBI) stanowi wyzwanie dla współczesnej medycyny z powodu znacznej częstości występowania, zróżnicowanego obrazu klinicznego oraz konieczności interdyscyplinarnego postępowania często obejmującego intensywną terapię, leczenie chirurgiczne, farmakologiczne, żywieniowe i rehabilitację. Pomimo postępu w uzyskiwanych wynikach leczenia ciężkich TBI wciąż wielu chorych wymaga długotrwałej opieki i nie odzyskuje pełnej samodzielności. Z tego powodu trwają badania nad nowymi terapiami, w tym nad leczeniem neuroprotekcyjnym, które mogłoby poprawić rokowanie po urazie mózgu zarówno na wczesnym, jak i późnym etapie leczenia. W artykule przedstawiono opis przypadku chorego z ciężkim TBI przebiegającym z długotrwałymi zaburzeniami świadomości, niedowładem czterokończynowym, padaczką pourazową pomimo wczesnej intensywnej terapii i rehabilitacji, którego stan znacząco poprawił się po zastosowaniu leku o działaniu neurotroficznym i neuroprotekcyjnym (cerebrolizyny) w połączeniu z intensywną rehabilitacją i terapią zajęciową. Multidyscyplinarne postępowanie oraz terapia multimodalna będąca połączeniem zróżnicowanych metod leczenia, w tym neuroprotekcyjnego w TBI, jest obiecującą strategią postępowania, które może znacząco przyczynić się do sukcesu terapeutycznego.

Key words: traumatic brain injury, neuroprotective treatment, cerebrolysin, short-term and long-term prognosis.

Słowa kluczowe: pourazowe uszkodzenie mózgu, leczenie neuroprotekcyjne, cerebrolizyna, rokowanie krótkoterminowe i odległe.

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Introduction

The sequelae of craniocerebral injuries, especially those suffered in traffic accidents, are a serious problem for modern medicine. Traumatic brain injury (TBI) is a major cause of death and long-term or permanent disability. It leads to high economic costs for healthcare systems and forms a global health problem. According to the National Institute of Public Health, traffic accidents are the third most common external cause of deaths and are estimated at approximately 10/100,000 of the population (2014) [1]. They contribute to premature mortality and are the leading cause of death in young people (men <39 years old and women <24 years old). In 2014 alone, nearly 13,000 people died as a result of injuries suffered in accidents (CSO data). For each accident fatality, there are on average 220 total injuries, 15 post-traumatic disabilities of varying degrees and 30 hospital admissions [2]. Despite a general trend towards decreasing mortality from traffic accidents, the death rate in Poland is about 72% higher than in the EU. According to data from the National Health Fund, in 2020 the incidence of hospital admissions for sequelae of head injuries sustained from various causes, was 18.9/100,000 for patients treated conservatively, and 12.2/100,000 for those requiring neurosurgical intervention [3]. In the largest Trauma Centre in Mazovia, at the Military Institute of Medicine in Warsaw, 351 patients were hospitalised or received consultations in the ED in 2021 due to the sequelae of head injuries suffered as a result of accidents. In the United States, over 1.5 million craniocerebral injuries were reported in 2013, of which more than 55,000 casualties died. The estimated cost of treating TBI in the USA is approximately USD 80 billion annually.

Clinical course of TBI

Over 80 % of craniocerebral injuries suffered in traffic accidents result in severe sequelae, necessitating long-term hospitalisation and rehabilitation. TBI is not a single pathophysiological event occurring at the time of injury, but a complex, continuous and often chronic disease process. The pathology of craniocerebral trauma may be divided into primary trauma dependent on external factors (with soft tissue damage, calvarial fracture, foci of parenchymal contusion, intracranial haematomas and diffuse axonal injury) along with secondary trauma, which is a secondary consequence of multi-organ injury or primary central nervous system (CNS) injury and usually leads to cerebral oedema that exacerbates the sequelae of the primary injury [4]. The type and severity of the traumatic factor leading to closed injury determines the extent of injury in the cranial cavity. Parenchymal contusion results from direct injury, or is influenced by the acceleration and deceleration forces on the brain (causing, for example, damage to locations distant from the site of injury in a rebound mechanism as a result of inertial displacement of the brain within the cranial cavity), or results from cavitation (rupture of the brain due to negative pressures generated during the injury). Diffuse axonal injury usually occurs in a rebound mechanism; the damage is diffuse and usually localised within the corpus callosum, brainstem and brain lobes. The lesions are usually microscopic and initially computed tomography (CT) images may be normal or show small, focal, diffuse, pinpoint haemorrhages. Cerebral contusion, diffuse axonal injury and damage to small blood vessels usually occur together.

Secondary injuries can develop minutes, months or even years after the primary injury. It follows biochemical and pathophysiological disorders that can lead to brain cell damage and/or death and subsequent atrophy. Mechanisms of secondary damage include: deregulation of signalling pathways and ionic homeostasis, mitochondrial dysfunction, release of neurotransmitters (e.g. excitotoxic glutamate) and initiation of inflammation and other complex immune responses (Fig.1) [5, 6]. The neurochemical cascade results in the formation of toxic and pro-inflammatory oxidative metabolites, free radicals, prostaglandins and cytokines, which lead to peroxidation of membrane lipids, apoptosis in neurons, damage to the blood-brain barrier and neurovascular unit and, consequently, increased vascular permeability and development of brain oedema [7]. Associated increases of intracranial pressure may contribute to local hypoxia, ischaemia or secondary intracranial haemorrhages happening at the same time or later on, increasing the complexity of these disorders and determining the variability of the symptoms and clinical course [8, 9].

The clinical picture of TBI includes a wide spectrum of symptom, from short-term unconsciousness, prolonged coma, to vegetative state or death. Axial symptoms of TBI, such as consciousness disorders, disorientation or subsequent cognitive deficits, behavioural or affect disorders may be caused directly by the brain injury, result from impaired autoregulation of flow and decreased cerebral perfusion, and from their deferred sequelae [10]. Depending on the consequences of the injury, we can distinguish several clinical stages, which are the basis for the diagnosis of moderate to severe brain injury (Tab. 1).

Table 1. clinical stages of post-traumatic brain injury

Phase 1.	Loss of consciousness or coma, not present in a mild craniocerebral injury.
Phase 2.	Periodic disorientation, memory dysfunction and behavioural disorders (post-traumatic amnesia).
Phase 3.	Resolution of initial motor disruption and improvement in cognitive function.
Phase 4.	Establishment of a neurological deficit, compensation for permanent damage.

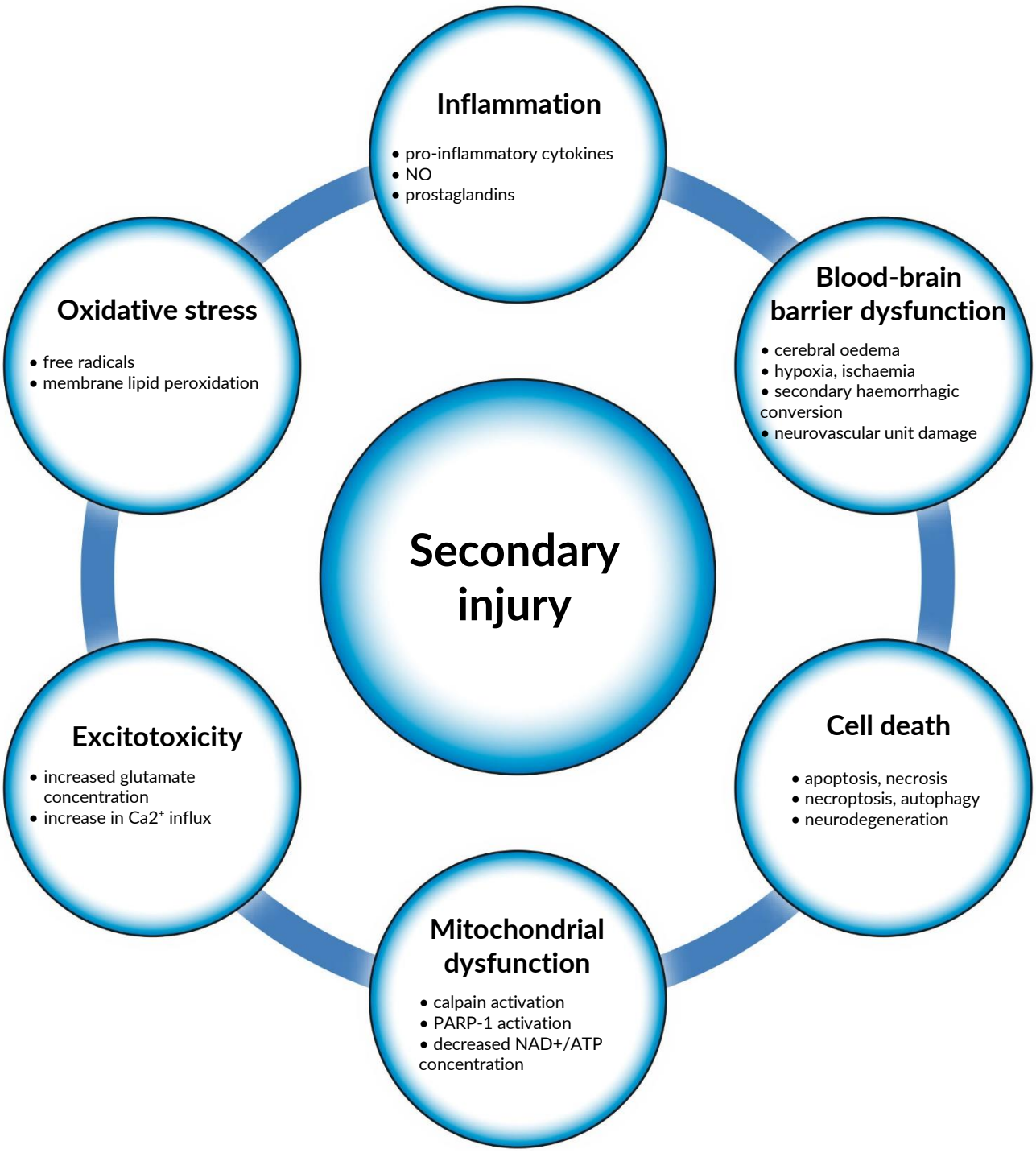

Fig. 1. *Diverse mechanisms of secondary injury after craniocerebral trauma.*

The duration of different clinical stages depends on the suffered injury and ranges from less than a day to many days or weeks after the injury. In moderate to severe TBI, the first phase is prolonged and includes varied quantitative consciousness disorders, including coma, stupor and pathological sleepiness. In extreme cases, this period may last for many weeks or become permanent. The most common symptoms in phase two are disorientation, delirium, behavioural disorders or prolonged impairment of episodic perceptual visual-spatial and auditory-verbal memory and retrograde amnesia, spanning up to weeks or months preceding the injury. This can cause psychomotor agitation of the patient and may pose a danger to the patient and/or to medical staff [11]. Other common disorders include a range of cognitive deficits including: attention, concentration, verbal fluency, verbal activity, intellectual performance, as well as depression and sleep disorders [12].

Due to the variable prognosis of TBI, various predictive and prognostic models are used based on the severity of the clinical condition on admission or initial CT imaging

characteristics [13]. This is usually achieved using the *Glasgow Coma Scale* (GCS), duration of consciousness disorders and presence of post-traumatic amnesia [14]. The mortality rate among patients assessed at baseline with a 6-7 GCS score is 50% and reaches 90% among patients with a 3 GCS score. Severity of the craniocerebral injury is defined as:

- mild damage – with a ≥13 GCS score, brief loss of consciousness and/or brief retrograde amnesia,
- moderate damage – with 9-12 GCS score, loss of consciousness for up to 24 h, retrograde amnesia from 1 to 24 h,
- severe damage – with a ≤8 GCS score, loss of consciousness for more than 24 h, retrograde amnesia lasting more than 1 day [15]. In the group of patients with severe damage, 60% have multiple organ injuries, 25% of whom require surgical treatment for various injuries.

The Marshall Scale (or the complementary newer Rotterdam Score) helps to determine prognosis based on assessment of radiological changes in the cranial cavity from the CT scan, by determining the compensatory capacity of the brain depending on the type and size of the post-traumatic lesions, as well as images of the ventricular system, the basal cisterns of the brain and the degree of displacement of the central brain structures (tab. 2) [16]. In the Marshall scale:

- Grade I – includes no visible intracranial pathology,
- Grade II (diffuse traumatic brain injury) – when midline shift is 0 to 5 mm, basal cisterns remain visible, and there are no lesions of high or mixed density >25 cm3,
- Grade III (diffuse brain injury with oedema) – when midline shift is 0 to 5 mm, basal cisterns are compressed or completely lost, no high or mixed density lesions >25 cm3,
- Grade IV – when midline shift is >5 mm, no high or mixed density lesions >25 cm3,
- Grade V – in the case of a post-traumatic lesion causing a mass effect and requiring surgical evacuation,
- Grade VI – in the case of any post-traumatic lesion causing the mass effect, with a high or mixed density >25 cm^3, and not surgically evacuated.

Grade III is associated with a 34% mortality rate, while for grade IV it exceeds 50%.

In recent years, there have been significant advances in management strategies for patients with TBI. Treatment of primary injuries focuses primarily on preventing secondary damage and the occurrence of complications. Patients with craniocerebral injuries require comprehensive care from the entire multidisciplinary treatment team. Patients with severe TBI, in coma (≤8 GCS score) and eligible for urgent craniotomy due to intracranial brain haemorrhage causing a mass effect are usually admitted to intensive care units in specialised neurosurgical centres.

Consequences of TBI

As a result of improvements in the quality of acute period care and increased survival rates, many patients with severe CNS damage require long-term multidisciplinary treatment and, above all, multidirectional rehabilitation. Chronic sequelae of TBI include: seizures (often after moderate to severe TBI), hydrocephalus, deep vein thrombosis (incidence reaches 54%), limb spasticity, dysphagia, sphincter disorders, gait abnormalities and chronic post-traumatic encephalopathy with varying cognitive impairment. Within the first year after TBI, patients are more likely to die from epilepsy, sepsis, pneumonia, and gastrointestinal disease than other persons of similar age, sex and race [17].

Epileptic seizures usually take the form of focal or generalised convulsions while consciousness disorders alone are rare. The prevalence of post-traumatic epilepsy is 5-19%. Risk factors include chronic alcoholism, old age at the time of injury and a history of epilepsy. If a patient with TBI has had a seizure, the likelihood of another seizure is more than 50%. This group of patients is more likely to have in-hospital pneumonia, acute respiratory distress syndrome, acute kidney injury and increased intracranial pressure. Patients with seizures tend to have poorer hospital treatment outcomes, with higher rates of referral to social care centres [18].

Post-traumatic aggression is common after TBI and occurs in approximately 25% of patients. Risk factors include depression and young age at the time of injury.

Table 2. Rotterdam score for assessment of craniocerebral trauma by CT scan

Brain base cisterns	Brain shift beyond the midline	Epidural haemorrhage	Intraventricular or subarachnoid haemorrhage	mortality after 6 months
0: normal	0: absent or ≤5 mm	0: absent	0: absent	1: 0% 2: 7% 3: 16% 4: 26% 5: 53% 6: 61%
1: compressed	1: displacement >5 mm	1: present	1: present	
2: invisible				

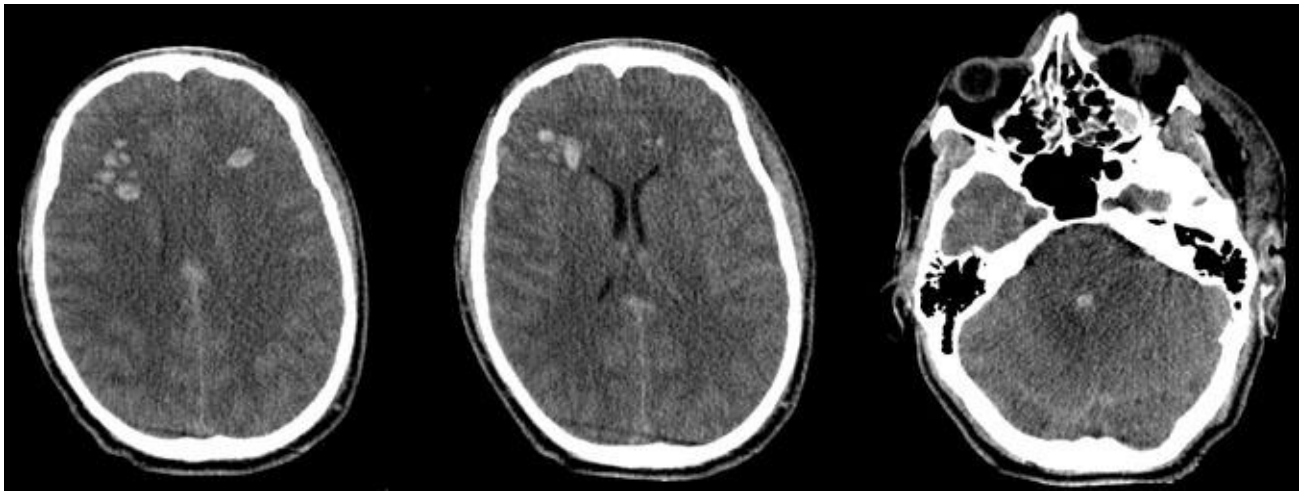

Fig. 2. *An initial CT scan on admission shows multiple haemorrhagic foci of the frontal lobes, a trail of blood along the cerebellar tentorium, cerebral oedema, reduced pericerebral fluid space and loss/compression of the sulci, signs of lateral ventricular stenosis, narrowing of the brain base cisterns and bleeding into the fourth ventricle.*

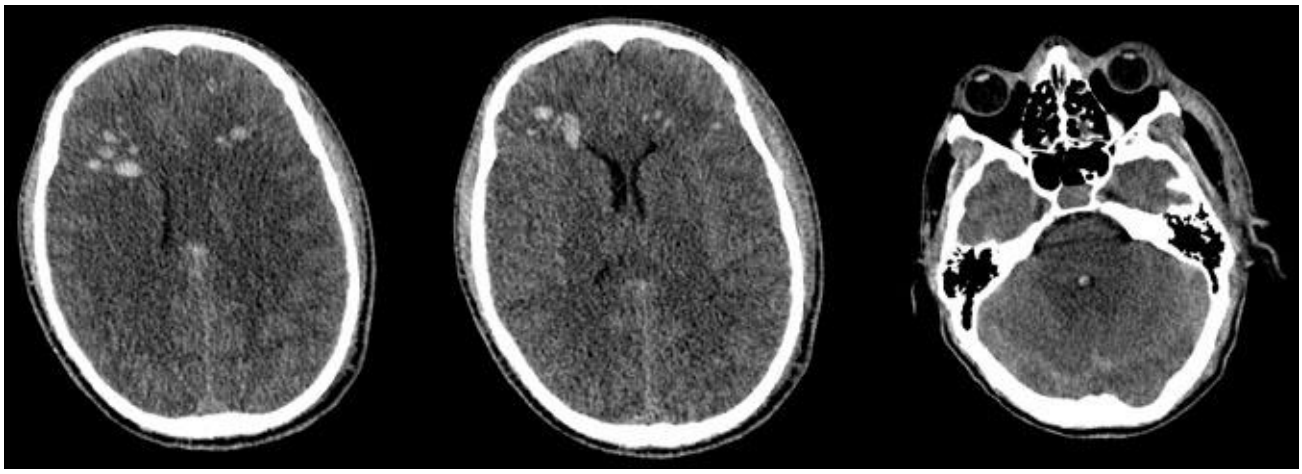

Fig. 3. *A follow-up CT brain scan 24 hours after the injury shows the foci of haemorrhagic contusions of the frontal and temporal lobes, presence of blood in ventricle IV, along the cerebellar tentorium, signs of cerebral oedema with reduction of the sulci and pericerebral fluid space and oedema of both cerebellar hemispheres with stenosis of the lateral ventricles, stenosis of the brain base cisterns and bleeding into the fourth ventricle.*

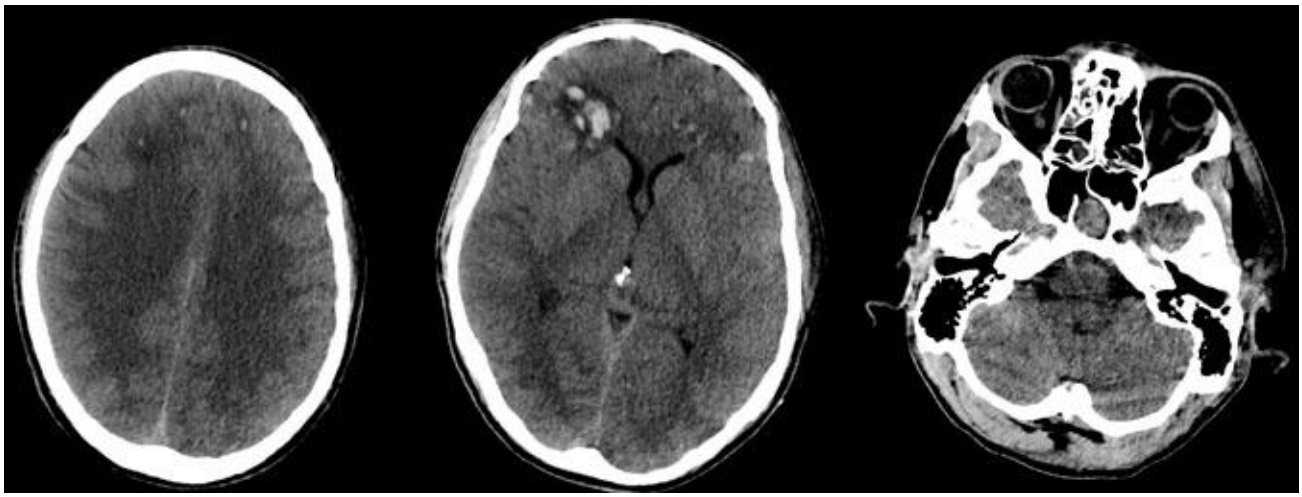

Fig. 4. *A follow-up CT brain scan 3 days after injury shows foci of haemorrhagic contusions of the frontal and temporal lobes with signs of malacia of brain tissue in the haemorrhagic areas. Signs of cerebral oedema with reduction of the sulci and pericerebral fluid space. Regression of stenosis of the lateral ventricles and brain base cisterns. No blood is found in the fourth ventricle.*

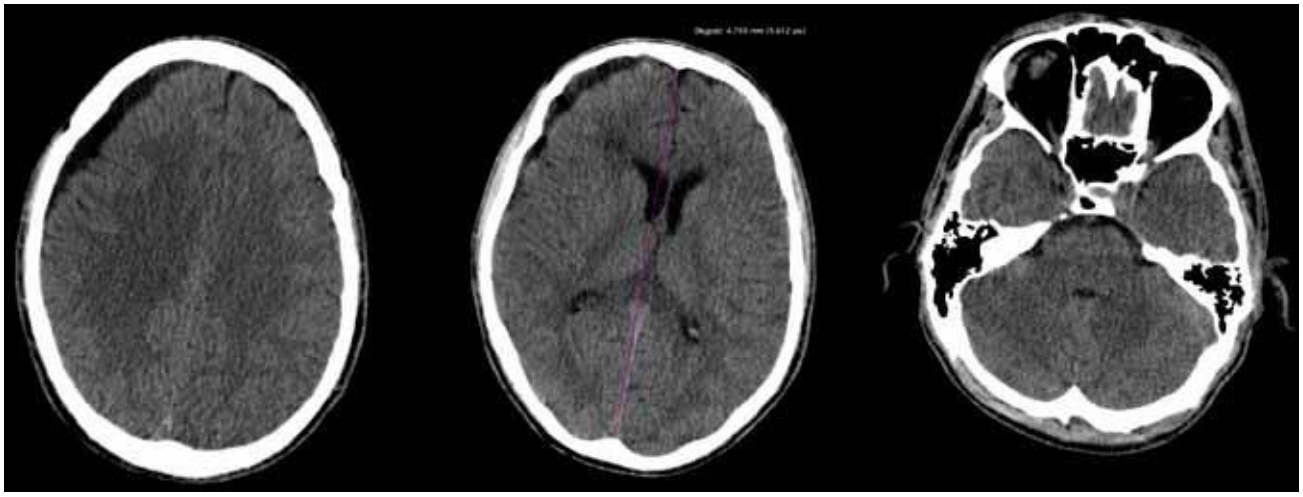

Fig. 5. *A follow-up CT brain scan 30 days after the injury shows presence of a hydrocele along the right frontal lobe with a 4.7-mm shift of the medial structures to the left side, regression of cerebral oedema with appearance of the sulci and prominent brain base cisterns. Ventricular system of normal width. No blood is found in the fourth ventricle.*

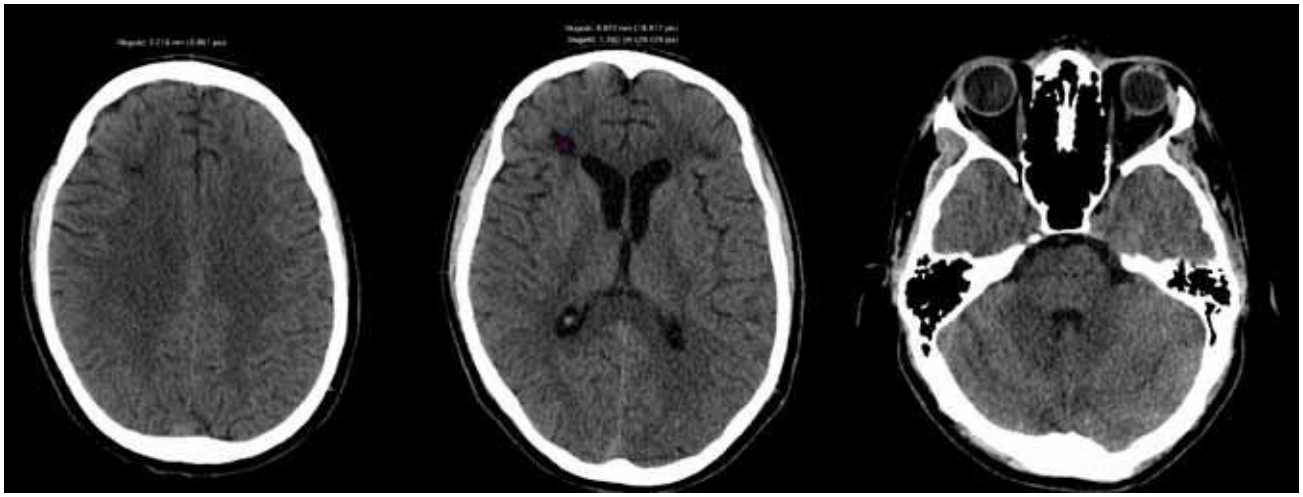

Fig. 6. *A follow-up CT brain scan during hospital discharge shows the presence of a trace hydrocele along the right frontal lobe (3.2 mm thick), without any shift in the medial structures, normal width of the sulci and pericerebral fluid space and brain base cisterns. Malacic cavitation after a haemorrhagic focus in the right frontal lobe. Ventricular system of borderline width (anterior horns of lateral ventricles = 6-7 mm), normal fourth ventricle and normal brain base cisterns.*

Causes of aggressive behaviour may be related to the patient's pain, reduced ability to communicate, insomnia, infection, electrolyte imbalance, adverse drug reactions or endogenous psychotic disorders [19]. Chronic TBI symptoms also include cognitive function decline, post-traumatic cerebrasthenia: depression, anxiety disorders, emotional dysregulation, insomnia, post-traumatic headaches (usually tension-type) and abuse of psychoactive substances or pain medication. Development of these chronic symptoms can also occur in patients who have suffered only a minor trauma. In a study by Kraus et al. involving 235 patients, the most commonly reported complaints 6 months after mild TBI were fatigue (43%), weakness (43%), memory disorders (40%), pain (36%) and vertigo (34%) [20]. Another study on a similar group found that at the end of the 6-month follow-up period, approximately 83% of patients reported at least one physical complaint [21]. Long-term follow-up also showed that a history of TBI is a risk factor for neurodegenerative diseases, e.g. amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, probably due to induction of inflammation and/or accumulation of pathological protein aggregates, including amyloid beta, in the brain [22, 23]. Long-term physical, cognitive and behavioural impairment also limit the patient's reintegration into society and return to work.

Pharmacological treatment of TBI

In addition to neurosurgical treatment, patients after TBI are now routinely administered pharmacological treatment, which mainly consists of an adjunctive therapy aimed at prevention, early detection and treatment of secondary damage. The effectiveness of this therapy is limited by the multiple pathological and pathogenetic mechanisms that occur as a result of the injury [24]. Therefore, new and more effective pharmacological treatment methods for acute

phase TBI and methods reducing the risk of distant complications are still being sought, especially in patients with predominant consciousness disorders [25].

Despite the promising results of preclinical trials and attempts to apply non-pharmacological (e.g. hypothermia) and pharmacological treatment (e.g. calcium channel blockers, corticosteroids, N-methyl-D-aspartate receptor antagonists, antioxidants, magnesium sulphate, stem cells) with presumed differential neuroprotective effects, their efficacy or safety in large clinical trials has not yet been conclusively proven and they are not routinely recommended after TBI in humans [26, 27]. The reasons for this are complex and include:

- limited mechanisms of action – e.g., it has been shown in animal models that single-target drugs probably do not exert significant effects during either neuroprotection or neurorehabilitation phases,
- methodological errors related to study design and planning – e.g. too low a drug dose, lack of a control group or inadequate sample size, heterogeneity of the population and lack of a standardised rehabilitation programme and consideration of the impact of chronic diseases,
- under-sensitive methods of assessing patient status, which do not capture clinically relevant functional impairment, or varying endpoints and duration of clinical follow-up [28].

One of the challenges for modern neuroprotective strategies in recent years has therefore become identifying and targeting treatments for specific mechanisms involved in the complex cascade of secondary damage. Recent interventions tend to target the mechanisms of neuroplasticity involving complex processes of neuronal reorganisation, including the formation and activation of inactive synapses, growth of dendrites, axons, recruitment of new anatomical pathways with functions similar to those of injured ones. Collected data from previous preclinical studies indicate that mammalian brains have the capacity for structural and functional plasticity and partial regeneration, which may positively influence the return of functions lost after an injury and which can be stimulated pharmacologically. Diverse mechanisms involving the promotion of angio-, neuro-, oligodendro- and axonogenesis are involved in these processes, resulting in a beneficial effect on neurological status after TBI [29]. However, the confirmation of optimal pharmacological management of TBI in humans is not fully established – there is a need for further observational and clinical studies, as well as individualised management.

Case study

A 21-year-old man, a driver of a passenger car, was transported to the ED of the Military Institute of Medicine by Air Ambulance due to multi-organ injuries sustained as a result of a traffic accident: a head-on collision between two passenger cars with several roll-overs, during which the man fell outside the vehicle. He was unconscious at the scene. He was assessed by the ambulance team and received a 6 GCS score, with respiratory failure requiring mechanical ventilation by conicotomy due to craniofacial injuries preventing intubation. He was cardiovascularly stable. On admission to the ED of the Military Institute of Medicine, a trauma-scan CT was performed, revealing multiple haemorrhagic foci of the frontal and parietal lobes, haemorrhage into the fourth ventricle, signs of cerebral oedema, reduced pericerebral fluid space, signs of lateral ventricular stenosis and stenosis of the brain base cisterns (Rotterdam score 2, Marshall score III), multiple fractures of the facial bones and slight signs of pulmonary contusion (Fig. 2). There was no evidence of damage to the C-LS spine or other internal organs. On physical examination, there were signs of superficial trauma to the limbs, shoulders and hips. In the ED, the patient was put into an induced drug coma and was examined by a maxillofacial surgeon and a neurosurgeon, who found no indication for surgical intervention. The patient's family history indicated that he was previously healthy and had not undergone chronic treatment. He was admitted to the Intensive Care Unit, where mechanical ventilation was maintained and intensive therapy including antibiotic therapy, supportive treatment, nutrition and rehabilitation was initiated. A follow-up CT brain scan after 24 h and on day 3 after the injury showed multiple foci of haemorrhagic cerebral contusions, presence of blood in the third and fourth ventricles, and signs of cerebral oedema (Figs. 3, 4). He did not require neurosurgical intervention. On day 8 of hospitalisation, mechanical ventilation was discontinued – at a later stage of treatment the patient breathed independently and efficiently through a tracheostomy. Despite stabilisation of vital signs during the ICU stay, the patient remained permanently unconscious (6-7 GCS score) with periodic significant motor agitation, requiring sedative medication.

On day 19, the patient was transferred to the Department of Neurology of the Military Institute of Medicine for further treatment. On admission: in severe general condition, unconscious (7 GCS score: eye opening: score of 1, verbal contact: score of 2, motor response: score of 4), pupils P=L with preserved response, bilateral pyramidal syndrome with severe spastic quadriplegia (MRC 1/5). During hospitalisation, there were multiple incidents of focal seizures secondary generalised to tonic-clonic seizures, refractory to initial antiepileptic treatment (valproic acid, Levetiracetam, Phenytoin, Topiramate). On initial EEG: abnormal recording with a recorded clinic and electrographic seizure in the left temporal region with significant generalised basic transition deceleration and single generalised sharp waves over the left and right hemispheres (Fig. 7a). Satisfactory control of the epileptic seizures and improvement of the EEG recording was only achieved with high doses of antiepileptic drugs (valproic acid at a dose adequate to achieve concentrations in the upper therapeutic range, Levetiracetam 2 g/daily, Topiramate 0.8 g/daily). A follow-up CT head scan showed regression of the haemorrhagic foci but still persistent cerebral oedema (Fig. 5). Despite normalisation of vital signs and electrolyte parameters, absence of infection, ongoing pharmacological treatment (analgesic, anxiolytic), nutritional treatment and rehabilitation, the patient's condition improved only a little – the patient remained in a state of minimal consciousness (10 GCS score), with no response to verbal stimuli, periodic visual tracking,

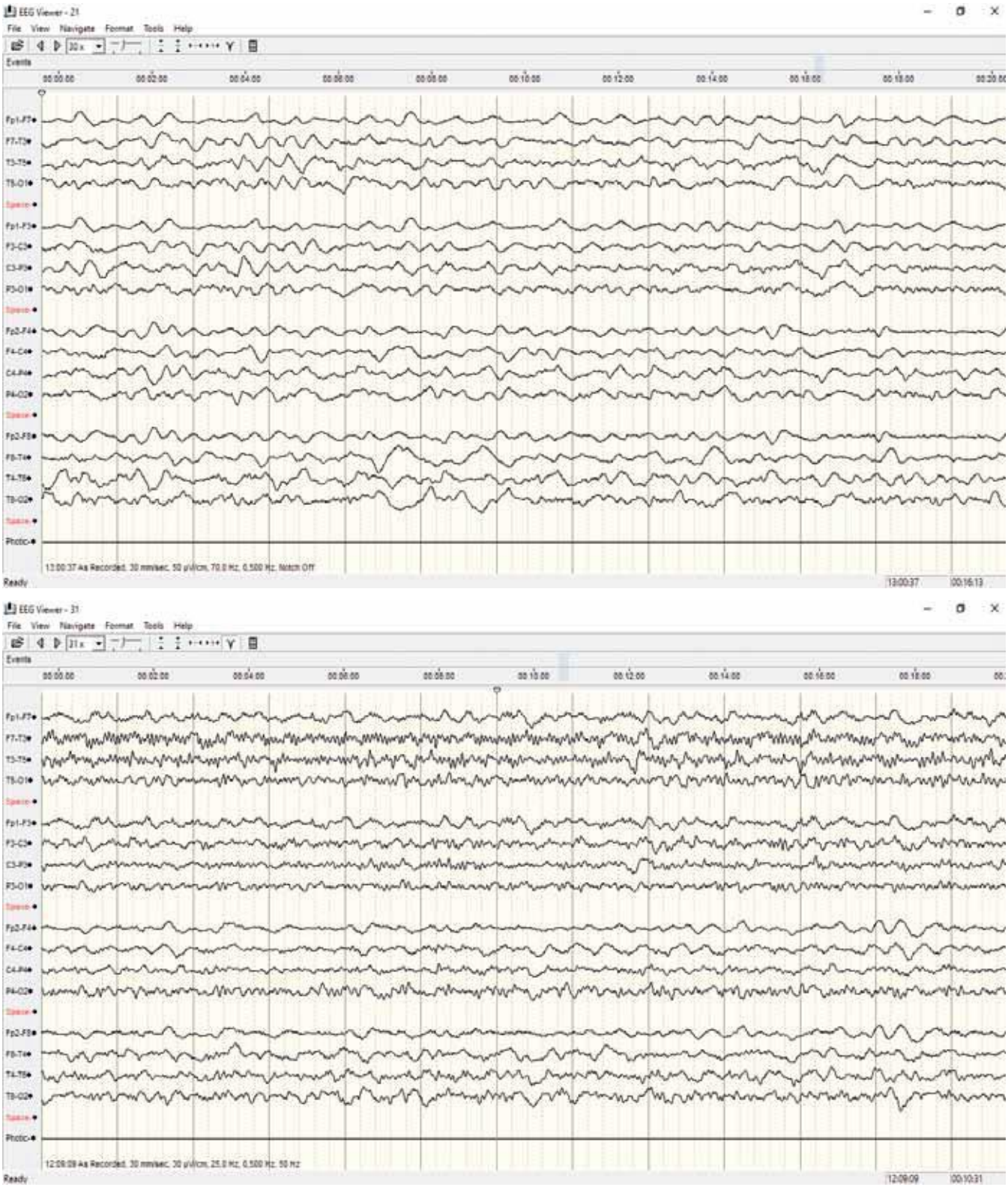

Fig. 7. *EEG recording. (A) initial – apparent lack of spatial differentiation and generalised slowing to delta and theta waves in the background rhythm, (B) during cerebrolysin treatment - improved recording with spatially differentiated basic transition, with alpha waves present in the posterior regions and slowing mainly in the frontal regions.*

localisation of pain stimuli, and severe spastic quadriplegia persisted. Frequent psychomotor agitation with predominant motor restlessness (despite ensuring physical comfort and limiting exposure to external stimuli).

In week 5 of hospitalisation, a decision was made to start *Cerebrolysin* in a daily dose of 30 ml administered over the following 28 days in 1-hour intravenous infusions (the drug was administered in 250 ml of 0.9% NaCl). After several days of treatment, clinical improvement was observed with

a reduction in consciousness disorders (14 GCS score), frequency of motor excitation incidents and with reduction of limb paresis. Attempts were made to sit the patient on the bed, but he could not maintain a sitting position independently and remained dependent on other persons. In addition, the doses of antiepileptic drugs were reduced. The EEG recording further improved (Fig. 7b). The patient began to respond to a voice and periodically follow simple commands; visual fixation time increased. The frequency of multimodal sensory stimulation was increased. The patient maintained emotional contact. However, the clinical picture was dominated by signs of frontal syndrome.

In week 7 after the injury, the patient was transferred to the Department of Rehabilitation, Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine, where Cerebrolysin treatment was continued and intensive bedside rehabilitation followed by rehabilitation in the exercise room was carried out. Initially at the participatory level, the patient remained without verbal contact, with persisting profound spastic limb paresis, requiring assistance with most daily activities, including self-care, body hygiene and position changes. During the following 2 weeks of treatment and intensive rehabilitation (carried out in the morning and afternoon) including therapy with a neuropsychologist, neurologist, occupational therapist, the patient's condition improved. Verbal-logical contact also improved, although the patient was still disoriented with regard to time and space. Limb paresis decreased (MRC 3). At the activity level, the patient was able to change position in bed independently, maintain a sitting position and transfer to a wheelchair and back to bed with the help of another person. Follow-up CT showed further improvement in the radiological picture (Fig. 6). After a total of 2 months of rehabilitation, the patient was discharged home with good verbal-logical communication, oriented with regard to place and space, with retrograde memory spanning the pre-accident period, with persistent discrete frontal lobe syndrome with reduced criticism and talkativeness and slight limb paresis. Independent within the room, he independently performed most activities of daily living, including toilet use.

Discussion

In Poland, almost 3 million people a year suffer injury in various types of accidents, of which more than 384,000 require hospitalisation and 192,000 suffer a disabling injury. The presented case of a patient after severe TBI demonstrates a beneficial therapeutic effect achieved by combining Cerebrolysin therapy with standard treatment and intensive rehabilitation.

Cerebrolysin is a low-molecular-weight (<10 kDa), antigen-free mixture of neuropeptides and free amino acids with multidirectional beneficial effects in TBI and other diseases with acute or chronic brain injury, including stroke, vascular and Alzheimer dementia, demonstrated in preclinical models and clinical observations [30]. The action of Cerebrolysin is based on the diverse mechanisms of cytoprotection, neuroregeneration and neuronal and synaptic plasticity. Neurotrophic factors contained in cerebrolysin (including brain [BDNF] and ciliary neurotrophic factor [CNTF], nerve growth factor [NGF], orexins, enkephalins) penetrate the blood-brain barrier, initiate the intracellular Shh signalling pathway, leading to activation of transcription proteins, the expression of endogenous neurotrophins and inhibition of excitotoxicity and oxidative stress processes [31].

Cerebrolysin stabilises the blood-brain barrier and microcirculation, reduces the risk of cerebral oedema, secondary haemorrhage of brain injury foci and ischaemic necrosis, beneficially influences the microglia function and inhibits the formation of neurotoxic compounds, including free radicals [32, 33]. Preclinical TBI models have shown that Cerebrolysin stimulates endogenous repair processes and exhibits cytoprotective effects through involvement in glutamatergic, GABAergic and cholinergic transmission [34]. This multimodal mechanism of action and influence on several components of the pathological cascade are beneficial in both TBI and CNS vascular and neurodegenerative diseases. Cerebrolysin is currently recommended by numerous scientific societies (including the Section of Vascular Diseases of the Polish Neurological Society, the European Academy of Neurology and the European Federation of Neurorehabilitation, the German Society for Neurorehabilitation) for adjunctive treatment in early post-stroke rehabilitation, especially in patients with moderate to severe stroke [35, 36]. It significantly improves the cognitive function in patients with dementia and, regardless of indication, has a very good safety profile at broad doses of 10-50 ml/day [37, 38].

The clinical efficacy of Cerebrolysin therapy in TBI has been the subject of several studies, which included patients with different neurological status, different time since injury and which used varying doses of the drug (10-50 ml/daily) and length of therapy (5-30 days) [39]. The largest retrospective cohort study to date, on a group of 615 patients, showed improvements in consciousness and functional status (based on the *Glasgow Outcome Score* GOS and modified Rankin Scale, respectively) compared to a control group [40]. Another retrospective study of 129 patients after severe TBI found a beneficial effect of treatment with a dose of 10 ml/daily for 30 days on functional status improvement at 3 and 6 months [41]. A meta-analysis of eight studies with different methodologies showed that patients treated with Cerebrolysin (n=112) achieved a favourable GOS score three times more often than the controls (odds ratio, OR 3.01; 95% CI 1.7-5.1; $p = 0.003$) and that cognitive improvement was significantly more likely than in the controls (OR 3.4; 95%CI 1.8-5.2; $p < 0.001$) [42]. Survival of Cerebrolysin-treated patients did not differ from that of the control group.

Favourable results were obtained in an analysis involving two prospective, randomised placebo-controlled trials in 185 patients after moderate to severe TBI (6-12 GCS score) receiving cerebrolysin at a dose of 50 ml/daily for 10 days (with subsequent two additional treatment cycles of 10 ml/daily for 10 days) [43]. The study showed a statistically significant improvement in neurological status, cognitive function and working memory as determined by differentiated assessment scales (including GCS, MMSE, Barthel Scale, Stroop Test) at 30 and 90 days post-injury.

Similarly, a systematic review of Cerebrolysin studies showed, based on random effects analysis, that administration of Cerebrolysin, as an adjunct to standard care, significantly improved functional status (on the GOS scale) and on the Rankin Scale [44]. An interesting study of 23 patients with haemorrhagic brain contusion, not requiring craniotomy, found that Cerebrolysin administration within 24 hours of TBI was associated with a significant improvement in cognitive function [45].

An interesting clinical observation of the patient case that we have discussed demonstrated an improvement in EEG recordings after cerebrolysin administration. Similar observations were shown by Alvarez et al. who, in an open-label study involving 20 post-TBI patients with significant disability, including a vegetative state, found improvement in clinical status and EEG recordings in patients receiving Cerebrolysin with a significant reduction in abnormal slow ~~free~~ bioelectric brain activity (*delta* and *theta* waves) and an increase in *alfa* and *beta* wave activity [46]. A randomised trial to evaluate efficacy of a "cocktail" of neuroprotective drugs with different mechanisms of action in patients with prolonged consciousness disorders after TBI using Cerebrolysin and Amantadine is being conducted [47]. In a recently published retrospective study, it was shown that this combination therapy may be more effective than monotherapy [48].

Conclusions

The use of Cerebrolysin is becoming increasingly promising in the treatment of patients with traumatic brain injury. The case presented here and the available clinical trial data indicate that intravenous Cerebrolysine combined with intensive rehabilitation results in an improved chance of functional recovery after TBI. However, there is a need for further research to determine optimal patient selection and regimen for such treatment.

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POST-TRAUMATIC DIAPHRAGMATIC HERNIA - DIAGNOSIS AND TREATMENT

Pourazowa przepuklina przeponowa - diagnostyka, leczenie

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Abstract: The article deals with the problem of treating a post-traumatic diaphragmatic hernia in a 56 year-old female. There is no developed standard of conduct in the case of a massive loss of the diaphragm. According to current medical knowledge, clinical symptoms of diaphragmatic hernia are an indication for surgical treatment. Immediate surgery does not raise any doubts in case of haemorrhage, bowel obstruction or other urgent indications. However, some patients may benefit from postponement of surgical treatment. Nutritional treatment, metabolic and water-electrolyte management as well as development of a surgical strategy can reduce the risk of complications and mortality.

Streszczenie: Artykuł podejmuje problem zaopatrzenia pourazowej przepukliny przeponowej, która wystąpiła u 56-letniej pacjentki po urazie wielonarządowym. Nie istnieje wypracowany standard postępowania w sytuacji wystąpienia szerokiego ubytku przepony. Zgodnie z obowiązującą wiedzą medyczną obecność przepukliny przeponowej z towarzyszącymi objawami klinicznymi stanowi wskazanie do interwencji chirurgicznej. Nie budzi wątpliwości konieczność natychmiastowej operacji w przypadku potwierdzenia krwotoku, niedrożności lub innych wskazań pilnych. Natomiast odroczenie operacji u części chorych może przynieść znaczące korzyści. Przedoperacyjne leczenie żywieniowe, wyrównanie metaboliczne i wodno-elektrolitowe oraz opracowanie strategii postępowania chirurgicznego obniżają ryzyko powikłań i śmiertelności chorych.

Key words: diaphragmatic hernia, trauma.

Słowa kluczowe: przepuklina przeponowa, uraz.

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Introduction

Acquired diaphragmatic hernias may be consequences of both blunt trauma (69.8%) and penetrating trauma (30.2%) [1]. The common and characteristic element in both instances is a high force of trauma. In the victims of traffic accidents, diaphragmatic trauma occurs in up to 5% of patients following multiorgan injury [2], mostly in motorcyclists and cyclists. The incidence of diaphragmatic hernia is low, as the condition occurs in 0.8-1.6% of trauma patients. The vast majority of patients are men (diaphragmatic trauma is found in men four times more often than in women, which is associated with a higher exposure of men to trauma factors). Approximately 79% of diaphragmatic injuries are found in the left dome, the rest are found in the right side of the diaphragm, where the liver absorbs more of the trauma force [3]. Less than 2% of diaphragmatic injuries are bilateral [4]. The pathomechanism of the visceral displacement to the chest is closely related to reduction of the pressure gradient between the pleural and peritoneal cavities [5].

Diagnostics

The diagnostic algorithm includes medical history and physical examination, as well as laboratory test panel and imaging tests for a trauma patient. Symptoms suggestive of diaphragmatic injury include absence of vesicular murmur on lung auscultation, dyspnoea, respiratory destabilisation and signs of pneumothorax. The ultrasound examination may present intestinal peristalsis in the pleural cavity. The final diagnosis is based on a chest and abdominal tomography confirming visceral displacement to the chest due to the diaphragmatic injury.

Management

The recommended management involves the surgical repair of the defect [6]. The type of surgical intervention depends on patient's general status and the risk of

entrapment of the hernia sac content.

Urgent indications for a surgical procedure:

- suspected haemorrhage into the abdominal cavity or chest;
- mechanical gastrointestinal obstruction;
- ischaemia, necrosis of the intestinal loops in the hernial sac;
- acute respiratory failure;
- other organ injuries that are independent factors for surgical intervention.

If urgent surgical intervention is required, the decision to operate should not be delayed, as in the cases involving intestinal obstruction in the hernial sac the mortality rate increases rapidly from approximately 10-40% to 40-57% [7].

Repair methods

Surgical access may be obtained by thoracotomy or laparotomy. The abdominal access provides the benefit of examining the peritoneal organs for trauma-induced damage. In some cases, minimally invasive methods (video-assisted thoracoscopic surgery - VATS, laparoscopy) are useful as, with sufficient surgical experience, they often allow the surgeon to perform a complete repair of the diaphragm without the need to extend the scope of the procedure.

The diaphragmatic hernia gate may be closed by simple suturing or using plastic materials. Prosthetic material is used typically in old hernias, treated a long time after the trauma. In these cases, fibrosis and loss of elasticity of the scar tissue surrounding the hernial sac prevent effective and permanent closing of the gate. Post-traumatic defects are typically a few centimetres in diameter, which makes approximation of the diaphragmatic tissue difficult. In order to avoid tension and to minimise the risk or recurrence, larger than standard mesh sizes are preferable. Contractually, the gate and at least five centimetres around it should be covered. Primary suturing of the hernia opening, without using plastic material, provides numerous advantages. It allows the avoidance of complications associated with leaving a graft, such as infections, agglutination or increased locoregional effusion – a potential source of intra-abdominal abscesses.

Case study

A 56-year-old female patient was admitted to the ED of the Military Institute of Medicine due to multiorgan injury following a traffic accident – a cyclist hit by a car. The main injury was multiple fracture of the pelvis. The patient's general condition on admission to the ED was severe. She was intubated and put on a respirator. A FAST ultrasound test did not confirm any pathologies. A trauma scan CT confirmed a few haemorrhagic foci in the CNS. Moreover, the test confirmed lung contusion and displacement of the left hepatic lobe and stomach to the chest (Fig. 1, Fig. 2, Fig. 3). The examination also revealed a multiple fracture of the lateral mass of the sacral bone on the left, bilateral fracture of the pubic rami, fracture of the proximal head of the right tibia and fibula, extending to the joint surface, fracture of the medial and lateral malleoli of the right lower limb.

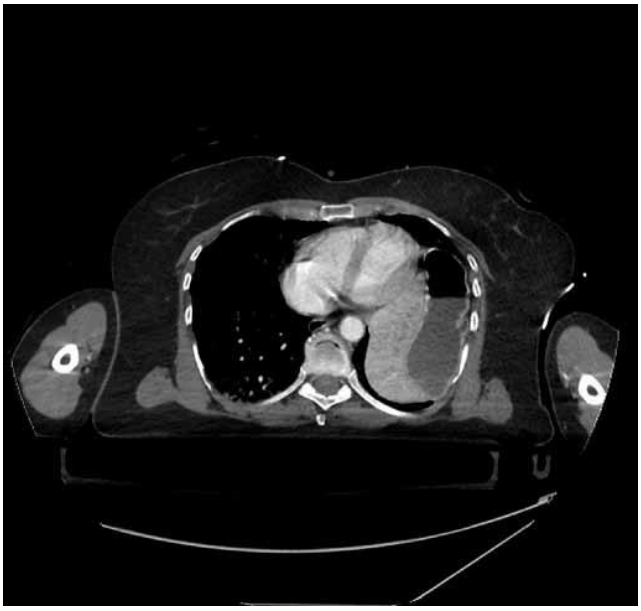

Fig. 1. *Trauma scan CT. Displacement of the left hepatic lobe and stomach to the chest (Institute of Medical Radiology, Military Institute of Medicine).*

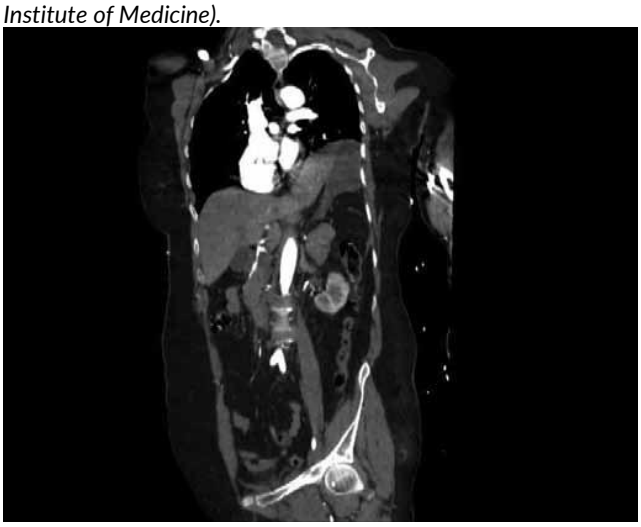

Fig. 2. *CT: stomach filled with gas in the inferior pole of the left lung (Institute of Medical Radiology, Military Institute of Medicine).*

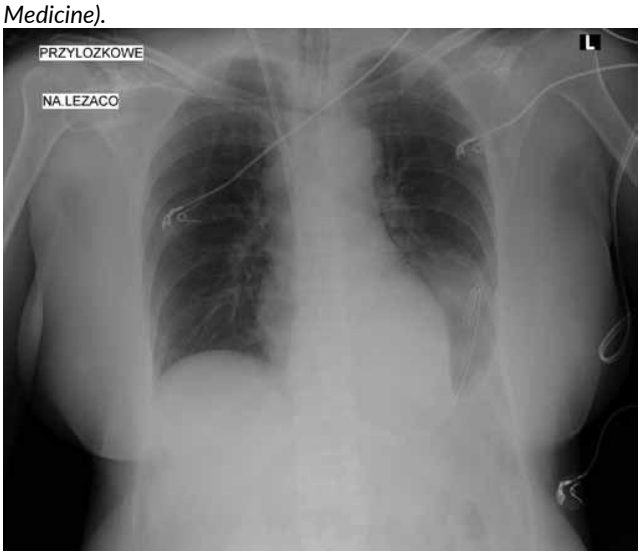

Fig. 3. *Thoracic X-ray. Reduced transparency in the inferior pole of the right lung – diaphragmatic hernia (Institute of Medical Radiology, Military Institute of Medicine).*

Following the initial treatment at the ED, the patient was transferred for further treatment at the Department of General and Thoracic Surgery. Following multispecialty consultations, surgical treatment was postponed until the patient's general condition became more stable. The sprained right ankle joint was repositioned.

At the beginning of hospitalisation at the Department of general Surgery and Thoracic Surgery, the patient's condition did not allow the performance of complicated surgical procedures. She presented symptoms of shock and required continuous circulatory stimulation with pressor amines. Due to acute renal injury, haemodialysis was performed regularly. Blood-related products were transfused. Eventually, following intensive care, the patient's general condition stabilised. After the analysis of the risk of complications associated with diaphragmatic hernia, an expedited exploratory laparotomy was performed. The real threat of respiratory failure, pneumonia, mediastinitis and intestinal obstruction were considered strong medical indications for a surgical intervention.

Before the surgery, nutritional treatment was introduced for optimal metabolic preparation of the organism of the patient. On the 20th day of hospitalisation, a laparotomy through a medial incision was performed. A 15 cm injury in the dome of the diaphragm was found intraoperatively, with displacement of the stomach and greater omentum into the thorax. The organs were repositioned and the diaphragmatic defect was closed with a simple suture. The treatment was continued after the surgery (Fig. 4). The wound healed completely, and the patient was transferred for further orthopaedic treatment.

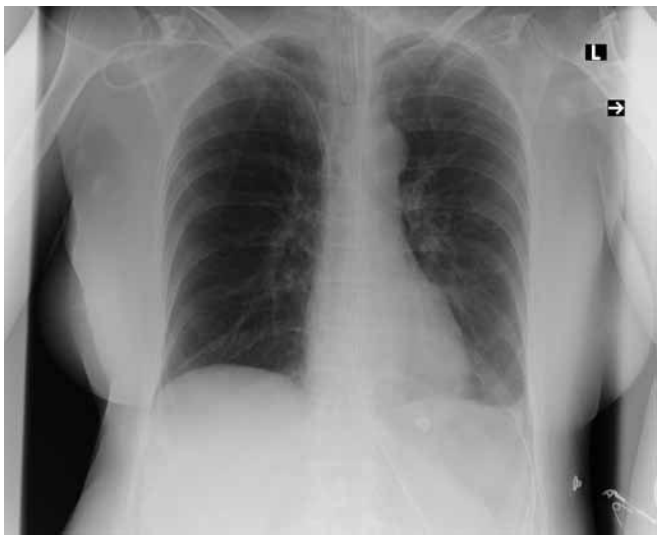

Fig. 4. *X-ray. Status post diaphragmatic repair (Institute of Medical Radiology, Military Institute of Medicine).*

Conclusions

Not all cases of diaphragmatic hernia are life-threatening. In some patients, a misdiagnosis may result in a chronic condition which may be oligosymptomatic. In such cases, the pathology is detected during routine radiologic diagnostics or a search for the causes of non-specific systemic symptoms. In such situations the patient is qualified for an elective hernia repair, which minimises the risk of complications and death. In the therapy planning process, the principle of optimal patient preparation should be followed. In a critical situation, the DAMAGE CONTROL strategy provides the time required to conduct the necessary actions, but a definitive repair of the defect should not be delayed unnecessarily.

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ORAL KAPOSI SARCOMA COMPLICATED BY CENTRAL NERVOUS SYSTEM TOXOPLASMOSIS IN A LATE DIAGNOSED HIV PATIENT

Mięsak Kaposiego zlokalizowany w jamie ustnej u pacjenta z późno zdiagnozowanym hiv i toksoplazmozą ośrodkowego układu nerwowego

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Abstract: Kaposi sarcoma is a malignant neoplasm that most often develops on the basis of an HIV infection. It is considered one of the indicator diseases of AIDS. Its diagnostics can be challenging in patients without other symptoms indicating immunodeficiency. A 35-year-old patient was admitted to the clinic with an advanced tumour of the oral cavity after a two-year observation period. One year earlier he underwent an excisional biopsy; however, the result of the histopathological examination did not raise any suspicions of neoplastic growth. At the time of admission, he suffered from severe dehydration and malnutrition, and reported pain when consuming food or fluids. Kaposi's sarcoma was suspected, and the diagnosis of HIV was confirmed. A CT of the central nervous system showed signs of toxoplasmosis. Early diagnosis of HIV and efficient cooperation between various medical professionals can lead to improved diagnostics for HIV and indicator diseases of AIDS, and therefore better treatment outcomes.

Streszczenie: Mięsak Kaposiego to złośliwy nowotwór najczęściej rozwijający się na podłożu infekcji HIV. Jest zaliczany do chorób wskaźnikowych AIDS. Diagnostyka tego nowotworu może nasuwać trudności u pacjentów bez innych objawów wskazujących na niedobór odporności. Rok wcześniej u pacjenta pobrano wycinek z tej zmiany jednak wynik badania histopatologicznego nie nasuwał podejrzeń rozrostu nowotworowego. W momencie przyjęcia do kliniki pacjent był odwodniony i wyniszczony zgłaszał ból przy przyjmowaniu płynów i pokarmu. Postawiono podejrzenie zmian o typie mięsaka Kaposiego i potwierdzono zakażenie HIV. Ponadto w badaniu TK OUN stwierdzono zmiany sugerujące toksoplazmozę ośrodkowego układu nerwowego. Wczesna diagnostyka zakażenia HIV i współpraca między lekarzami wielu specjalności może prowadzić do usprawnienia procesu diagnostycznego i lepszych wyników leczenia HIV oraz chorób wskaźnikowych.

Key words: Kaposi sarcoma, oral neoplasm, HIV, toxoplasmosis.

Słowa kluczowe: mięsak Kaposiego, nowotwór jamy ustnej, HIV, toksoplazmoza.

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Introduction

Kaposi's sarcoma (KS) is a neoplasm associated with human herpesvirus 8 (HHV8) and HIV infection. Since the introduction of antiretroviral therapy, the frequency of KS in patients infected with HIV decreased significantly, especially among those with a well-controlled infection [1].

Diagnosis of Kaposi's sarcoma may be difficult, especially in patients without a history of diseases suggesting immunodeficiency. When KS is the first complication that motivates a patient unaware of a HIV infection to seek medical assistance, late presenter definition, developed by the European Late Presenter Consensus Working Group,

should be applied [2]. Late diagnosis is associated with a poorer prognosis in this group of patients.

The introduction of Combined Antiretroviral Therapy (cART) changed our views on HIV infection, currently considered to be a chronic disease. At present, HIV patients have almost the same life expectancy as the general population. European studies still demonstrate a 10-times higher risk of death in the first year following the diagnosis for patients diagnosed at the AIDS stage. This discrepancy is closely associated with later initiation of antiretroviral therapy [3]. One of the most common diseases in a late presenter is CNS toxoplasmosis, a re-activation of a latent invasion of *Toxoplasma gondii* – a globally prevalent, intracellular parasitic protozoan. Acute *Toxoplasma gondii* infection is usually subclinical in most people with normal immunity.

Case report

A 35-year-old patient was admitted to the Department of Infectious Diseases, Tropical Diseases and Hepatology, Medical University of Warsaw, due to a tumour of the upper gum, tongue and palate, growing for approximately 2 years (Fig. 1 and Fig. 2). Two years earlier, he informed his dentist about the growing lesion in his oral cavity, but the growth was limited and did not cause concerns for the patient.

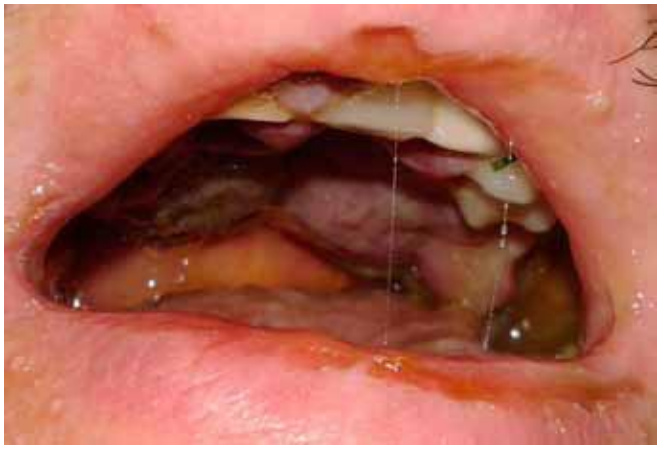

Fig. 1. *Kaposi's sarcoma on the hard palate.*

After a year, the patient returned to the same dentist with chronic pain and increased size of the tumour, now invading the tongue, gums and the palate. After a few months, he was admitted to the surgical department with symptoms of dehydration and cachexia, where he received a head and neck computed tomography scan, revealing a multifocal lesion. The diameter of the largest foci was 16 mm. Moreover, lymph nodes were found bilaterally enlarged up to 12-13 mm.

The patient received the first HIV test in his life and, as the result was inconclusive, he was transferred to the Department of Infectious diseases, Tropical Diseases and Hepatology of the Medical University of Warsaw. Further tests confirmed HIV infection. HIV-1 RNA viraemia was 396,121 copies/ml, and the CD4+ T cell count was 83 cells/µl (normal range for CD4+ lymphocytes: 420-1490 cells/µl). Combined antiretroviral therapy (cART) was applied: emtricitabine, tenofovir disoproxil, darunavir and ritonavir. Simultaneously, parenteral nutrition was introduced.

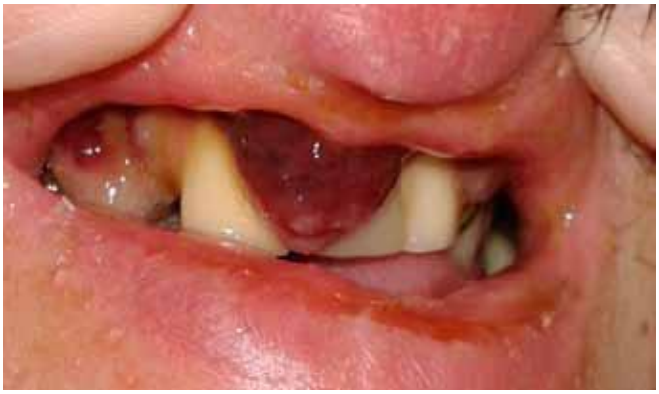

Fig. 2. *Kaposi's sarcoma invading the gums.*

Follow-up tests to verify CD4+ levels and HIV-1 RNA viraemia were scheduled for the 30th day of hospitalisation. During the hospitalisation, a re-assessment of the histopathological test was requested.

A magnetic resonance imaging of the head and neck revealed progression of the lesion in the oral cavity compared to the CT study performed at the surgical department. In addition, the examination demonstrated specific areas of hyperintense signals, suggestive of an active infection of the central nervous system by *Toxoplasma gondii*. Despite a negative result of serological blood tests (IgG, IgM) for toxoplasmosis, the patient was treated according to CDC recommendations. Over the next few days, the general condition of the patient stabilised. On day 18 of hospitalisation, his condition worsened suddenly. Seizures, nystagmus, respiratory arrest and cardiac arrest occurred. Despite the therapeutic efforts, the patient died.

Considering the clinical picture and the administered antiretroviral medications, death due to immune reconstitution inflammatory syndrome (IRIS) was suspected. A few days later, the result of histopathological re-assessment of the biopsy performed a year before was delivered, confirming the diagnosis of Kaposi's sarcoma.

Discussion

Kaposi's sarcoma is frequently located in the head and neck area, so any abnormal mass in this location should be an indication for an HIV test.

Early detection of HIV infection is crucial. A British study demonstrated that in patients who started cART therapy when the CD4+ lymphocyte count was lower than 100 cells/µl the life expectancy was approximately 15 years shorter than in those who started the treatment when their CD4 count was between 200 and 350 cells/µl. Patients who started therapy in an advanced stage are also exposed to a higher risk of HIV-associated diseases, which increases the cost of medical care [4, 5, 6].

According to the Polish AIDS Society, immune reconstitution inflammatory syndrome (IRIS) is defined as an inflammatory reaction to infectious or non-infectious factors, associated with excessive or impaired immunological response due to the start of the cART therapy. The clinical diagnostic criteria for the syndrome include temporal relationship between IRIS and cART, atypical clinical symptoms, rapid reduction of HIV-1 RNA viraemia, increased CD4+ T cell count, skin reaction appearance, exclusion of other causes of the symptoms. We were not able to confirm all the criteria required to diagnose IRIS in our patient. We did not have the results of follow-up HIV-1 RNA viraemia or the CD4 lymphocyte count – the tests were scheduled for the 30th day of hospitalisation. The patient presented with two main factors of high risk of IRIS: low (<100 cells/µl) lymphocyte count and high HIV-1 RNA viraemia >100 000 copies/ml [7]. Due to the gradual improvement in the patient's condition following cART, IRIS was suspected as a potential cause of death.

In immunodeficient patients cerebral toxoplasmosis may develop as a reactivation of latent cysts in the brain [8]. Introducing cART significantly reduced the incidence of cerebral toxoplasmosis in AIDS patients [9]. A study conducted in a Danish population demonstrated a significant reduction in the risk during the period when cART has been available (1997-2014) compared to the pre-cART era (1995-1996) after a year from an HIV diagnosis. The same study revealed a reduction by 78% in the mortality due to cerebral toxoplasmosis in the first 3 months from the diagnosis and a reduction by 98% in the consecutive years [10]. It shows the importance of an effective system of early detection and treatment of AIDS co-existing with cerebral toxoplasmosis.

Depending on the number and location of brain lesions, the most common symptoms of cerebral toxoplasmosis include fever, headache and mental disorders. Visual impairment, seizures, impaired cranial nerve function and sensory disorders may also occur. Typical findings in computed tomography and magnetic resonance imaging in patients with cerebral toxoplasmosis include numerous ring-enhancing lesions, like those found in the magnetic resonance of the central nervous system of our patient. They are usually located in the white matter, basal ganglia, corticomedullary junctions or periventricular regions [11]. With this type of lesion, differentiation between cerebral toxoplasmosis and cerebral lymphoma should be considered. A certain diagnosis of cerebral toxoplasmosis in patients with severe immunodeficiency is possible, usually based on typical lesions in the imaging tests of the central nervous system (PCR sensitivity of 50%) [7]. The results of serological tests of blood and cerebrospinal fluid are very difficult to interpret and even negative results cannot exclude the disease. We experienced similar difficulties with our patient. The final diagnosis was based on both the MRI findings and the full clinical picture.

Conclusions

Late diagnosis of HIV infection is very often associated with the co-presence of indicator diseases, whose course may significantly worsen the prognosis. Immune reconstitution inflammatory syndrome may be another factor exacerbating the clinical symptoms and its occurrence poses a significant diagnostic and therapeutic challenge. Broader HIV testing should also include all patients with focal lesions.

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EUROPE'S LEADING GYNAECOLOGICAL ONCOLOGY GATHERING: ESGO 2021 PRAGUE CONGRESS REPORT

Sprawozdanie Z XXII Kongresu Europejskiego Towarzystwa Ginekologii Onkologicznej

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Abstract: Report from the 22nd Congress of the European Society of Gynaecologic Oncology, held on 23-25 October 2021 in Prague.

Streszczenie: Sprawozdanie z XXII Kongresu Europejskiego Towarzystwa Ginekologii Onkologicznej, który odbył się w dniach 23-25 października 2021 r. w Pradze.

Key words: endometrial cancer, ovarian cancer, gynaecological oncology.

Słowa kluczowe: rak endometrium, rak jajnika, ginekologia onkologiczna.

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On 23-25 October 2021, Prague hosted the Congress of the European Society of Gynaecological Oncology (ESGO) - the leading scientific event in the field of gynaecological oncology in its broadest sense. It was attended by a team of physicians from the Department of Gynaecology and Gynaecological Oncology of the Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine.

The location of the congress was significant, since Prague is home to an excellent Centre for Gynaecological Oncology headed by Prof. David Cibula.

The scientific committee of the congress consisted of eminent specialists, including personalities in the fields of gynaecological oncology, general surgery, clinical genetics, immunology and radiology. It demonstrated a multidisciplinary approach to oncology patients.

The session was opened by Prof. Philippe Morice from the Gustave-Roussy Institute in France, who is the President of ESGO. Other experts were: Prof. Nicole Concin (President Elect of ESGO), Prof. Jonathan Ledermann (Vice President of ESGO), Prof. Anna Fagotti, Prof. Giovanii Scambia, Prof. Denis Querleu as well as Prof. Andreas du Bois (a long-standing activist and former President of ESGO), who was honoured at the congress with a Distinguished Lifetime Achievement Award for his scientific work and activity in the development of gynaecological oncology. Experts from outside Europe included Prof. Pedro T. Ramirez and Prof. Nadeem Abu-Rustum from the United States and Prof. Catagay Taskiran from Turkey.

The congress comprised many scientific and discussion panels, which was a great opportunity not only to deepen medical expertise, but also to exchange experiences and hold many interesting discussions. The scientific sessions were divided thematically according to the type of cancer in question, covering issues of prevention, in-depth diagnosis, surgical and complementary treatment and broadly defined oncology patient care. Topics included endometrial, cervical, ovarian, vulvar and vaginal cancers. Discussion also centred on international guidelines and principles of cooperation in the treatment of gestational trophoblastic disease. The scientific sessions were accompanied by workshops and scientific meetings among adepts starting their training in gynaecological oncology.

Prof. Daniela Fisherova together with Prof. David Cibula led a very interesting and valuable session on advanced ultrasound diagnosis in preoperative staging of ovarian and cervical cancer. Prof. Fisherova, an expert in ultrasound cancer diagnosis, performed real-time examinations, which were broadcast in the conference hall during the congress.

Detailed discussions concerned genetic aspects of cancer, the increasing importance of genetic testing in cancer diagnosis and its impact on therapeutic decisions. During the congress, it was repeatedly emphasised how important it is to complement existing diagnostic schemes and treatment algorithms with examinations that determine specific mutations accompanying malignant tumours. These examinations are indispensable in modern, interdisciplinary and highest in global-level oncology patient care. A comprehensive lecture on this topic was delivered by Prof. Frederic Amant from the University of Leuven in Belgium, who analysed in detail the topic of incorporating molecular classification into therapeutic decisions in the treatment of endometrial cancer.

Incorporation of modern diagnostic methods involving genetic testing into clinical practice, and especially into clinic-specific regimens, allows prestigious accreditation of the European Society of Gynaecological Oncology. Much attention was also given to modern complementary treatment methods, which included introducing immunological treatment for a select group of patients.

Several sessions chaired by Prof. Jan Persson from Sweden were devoted to the increasing use of da Vinci robotic techniques in modern surgical treatment of endometrial cancer.

An interesting addition to the widely discussed topics in gynaecological oncology was a workshop on telemedicine and its use in modern approaches to patient treatment and training of medical staff.

During the congress, a multifaceted model of care for patients undergoing treatment for female genital cancers was presented, based on the latest scientific reports. The updated joint guidelines were presented of the European scientific societies (ESGO – ESTRO – ESP) on endometrial cancer and the ESGO recommendations on ovarian cancer. Wide-ranging discussions were dedicated to methods and diagnostic pitfalls in gynaecological oncology. Much attention was also paid to complications after surgical treatment and the special challenges of the obese patient.

During the session, it was repeatedly emphasised how large and demanding challenge oncological diseases are. At the same time, stress was placed on the need for interdisciplinary cooperation and continuous education and qualification.

20 YEARS OF BIOLOGICAL THREAT IDENTIFICATION AND COUNTERMEASURE CENTER OF THE MILITARY INSTITUTE OF HYGIENE AND EPIDEMIOLOGY (MIHE) - AREAS OF ACTIVITY AND ACHIEVEMENTS

20 lat ośrodka diagnostyki i zwalczania zagrożeń biologicznych wojskowego instytutu higieny i epidemiologii (wihe) -kierunki działania i osiągnięcia

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Abstract: This work involves a summary of the activities of the Biological Threats Identification and Countermeasure Centre, from its creation until now. The unique contribution of the Centre for the Polish Armed Forces resulting from the synergy of research and development, advisory and expert activities as well as clinical and environmental diagnostics in the area of biological agents of weapons of mass destruction has been underlined. Over the past 20 years, BTICC has accumulated highly experienced specialists with the knowledge, skills, competences and experience, as well as unique research infrastructure which has allowed it not only to become an important player for the military health service or CBRN (chemical, biological, radiological, nuclear) defence system, but also to carry out important scientific projects or advisory works for domestic or foreign stakeholders.

Streszczenie: Praca obejmuje podsumowanie działalności Ośrodka Diagnostyki i Zwalczania Zagrożeń Biologicznych od jego powstania do chwili obecnej. W szczególny sposób uwzględniono unikalny wkład ośrodka dla Sił Zbrojnych RP wynikający z synergii aktywności: badawczo-rozwojowej, doradczo-eksperckiej oraz diagnostyki klinicznej i środowiskowej w obszarze czynników biologicznych broni masowego rażenia. W ciągu 20 lat w ODiZZB skumulowano wysokiej klasy specjalistów dysponujących wiedzą, umiejętnościami, kompetencjami i doświadczeniem oraz unikalną infrastrukturę badawczą, co pozwoliło nie tylko być istotnym zapleczem dla wojskowej służby zdrowia czy obrony przed bronią masowego rażenia, ale także realizować istotne prace naukowe lub doradcze na rzecz partnerów krajowych lub zagranicznych.

Key words: biological weapon, WIME, Biological Threats Identification and Countermeasure Centre, research and development works.

Słowa kluczowe: broń biologiczna, WIHE, Ośrodek Diagnostyki i Zwalczania Zagrożeń Biologicznych, prace badawczo-rozwojowe.

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Genesis, objectives and tasks of the Biological Threats Identification and Countermeasure Centre (BTIaCC) in Puławy

The traditions of BTIaCC date back to 1934, when the Military Veterinary Research and Training Centre was established in Warsaw. After World War II, a unit with the same name was formed and located in Puławy in 1949. In 1967, the centre was renamed the Military Research and Scientific Centre of the Veterinary Service, which

specialised not only in the protection of food from contamination by weapons of mass destruction (wmd), epidemiology or epizootiology, but its special field of interest was also medical and veterinary microbiology [1, 2]. In 1990, as a well-known scientific institution focused on solving problems in military protection related to zoonoses and food hygiene, the Veterinary Research Centre was incorporated into the Military Institute of Hygiene and Epidemiology.

The centre's achievements in research into anthrax spores or botulinum toxins aroused interest not only at home but also abroad. In 1994, at the request of the USA, the institution was visited by a delegation from the *US Army Edgewood Research Development and Engineering Center* (ERDEC). This was an unprecedented event only three years after dissolution of the Warsaw Pact, and it also formed part of the reorientation of Polish foreign policy and the process of accession to NATO. In the same year, Poland joined the *Partnership for Peace – PfP)* programme. During the visit, ERDEC representatives: Richard Smardzewski, PhD and Peter Stopa became acquainted with the tasks and achievements of the centre, and proposed cooperation in the field of defence against biological weapons. As a result of this visit, in the following year a delegation from WIME (Col. Prof. Jerzy Mierzejewski and Col. Prof. Michał Bartoszcze) participated in a scientific conference organised by ERDEC. The paper by Professor Mierzejewski on the key role of General Kazimierz Sosnkowski, a representative of the Second Republic in the League of Nations, in establishing a ban on the use of bacteriological weapons under the Geneva Protocol of 1925 (the so-called *"Polish addendum"*) enjoyed exceptional interest. When the position of WIME commandant was assumed by Colonel Professor Krzysztof Chomiczewski, an important event took place, namely the signing of an international agreement with the USA on the exchange of information in the field of defence against biological weapons on 13 December 1996, allowing further development of many different forms of cooperation [3]. They included a joint field training exercise at the Dugway Proving Ground in 1998, during which Professors K. Chomiczewski and M. Bartoszcze familiarised themselves with the organisation and conduct of testing a device for detecting biological agents during an aerosol attack. In another joint laboratory exercise, at the Salmon Test Facility USA in 1999, representatives of the centre, composed of: Agata Bielawska, Urszula Szymajda and Michal Bartoszcze, received high ratings from the exercise leadership for their ability to identify bioterrorist agents [3, 4].

Bioterrorist threats after 11 September 2001

The subsequent years of cooperation with the US partners resulted in the implementation of a number of experience-sharing projects in the area of rapid response to biological agents. In 1999-2002, working closely with the leadership of the Military Health Service, the centre played a key role in the creation of a national defence system against biological weapons and bioterrorism in the Polish Armed Forces. Established within the structures of the Military Centres of Preventive Medicine (MCPM), the mobile Biological Reconnaissance Teams (BRTs) were supposed to be capable of responding in the areas of their responsibility. Thanks to having competent personnel, they were prepared to identify, collect suspicious material at the scene, perform initial identification, secure and transport samples for confirmatory tests. The teams were equipped with all the necessary tools, reagents and personal protective equipment. The focal and unifying point of the BRTs and MCPM network was a reference, microbiological stationary laboratory, capable of providing definitive confirmation of potential biological hazards, located at the Puławy centre.

Fig. 1. *Commemorative plaque in the Biosafety Level-3 laboratory (Puławy).*

The emergence of the threat related to the new face of biological terrorism and sending of "anthrax letters" proved the prospectiveness of the assumptions made before and the usefulness of the created structures. A very important moment for the centre in Puławy was the decision to build and equip the first Biosafety Level-3 microbiological laboratory in Poland (*BSL-3*), which was later given the name Dr. Peter Stopa (Fig. 1), distinguished for initiating cooperation with the USA. The establishment of this facility was possible thanks to the commitment of the management of the National Security Bureau, an investment grant from the Committee for Scientific Research and cooperation with the US Embassy in Warsaw. The undertaking was supported on the US side by the head of the Embassy's *Office of the Defense Cooperation*, Colonel Peter Podbielski, and his deputy, Betty Dent. The USA equipped the laboratory with: thermocyclers for PCR, microbiological safety chambers, ELISA analysers, flow cytometer, centrifuges, incubators, HEPA filtration system, sterilisation equipment, etc. Launching the laboratory was preceded by working visits to CAMR in Porton Down (UK) and an audit by US experts, who concluded that the laboratory in Puławy meets all the criteria required for this type of facility and was ready for operation.

In the spring of 2002, the centre was visited by the Minister of National Defence, Jerzy Szmajdziński. At the same time, the previous name was also changed to the current one:

Biological Threats Identification and Countermeasure Centre of WIME. After the ceremonial opening on 26 April 2002, the BSL-3 laboratory actively joined the fight against the anthrax threat and created opportunities for research into live *in vitro* cultures with particularly dangerous biological agents of the third threat group (e.g. *Y. pestis*, *F. tularensis*, *E. coli* EHEC, *C. botulinum*). Valuable works were carried out on isolation of *B. anthracis* spores from animal cemeteries [5], luminometric detection of anthrax spores [6] and the effects of biocidal agents on anthrax spores [7, 8].

Cooperation with the USA continued, and an important step in improving the diagnostic readiness and scientific development of the staff was obtaining of DARPA (*Defense Research Project Agency*) research fellowships by the centre's employees (Agata Bielawska, Urszula Szymajda, Beata Osiak, Dorota Żakowska, Marcin Niemcewicz, Jerzy Gaweł) at the prestigious Institute of Molecular Biology and Medicine at the US University of Scranton. The centre's staff gained experience and extensive knowledge in the identification of microorganisms by molecular biology methods, including various varieties of PCR (*polymerase chain reaction)*. After their return, they added to the scientific and professional potential of the centre (Fig. 2). Over time, they also published a number of valuable scientific articles [9-12] and doctoral dissertations.

The international events of 2003 were another test of the developed system. For the purposes of foreign missions, the centre developed a concept for equipping and operating a mobile laboratory capable of performing a full microbiological reconnaissance using the PCR techniques of the RAPID system and allowing the identification under field conditions of 12 biological agents with bioterrorist potential. The laboratory was also able to perform clinical or environmental microbiological tests, depending on the needs of the contingent. The first crew of the mobile laboratory during the Second Gulf War was composed mostly of officers from the Puławy centre [13].

Taking into account the bilateral benefits of Polish-American cooperation, the agreement with the USA (Information Exchange Annex IEA-A-03-PL-1688) was extended in May 2004. It entailed research, equipment testing, procedures, reagents and techniques contributing to the defence against biological agents. A technical commissioner for the project on the Polish side was the centre's representative, Professor Michał Bartoszcze, and on the US side, the above-mentioned Doctor Peter Stopa. Thanks to extension of the agreement, the centre's representative, Major Aleksander Michalski, completed unique continuing education courses in the USA (Fig. 3) in *biosafety*, *biosecurity* at the U.S. Army Medical Research Institute of Infectious Diseases (2005) and the Eagleson Institute in the USA (2012).

At this time, a number of challenging tasks were carried out at the centre, using both experienced microbiologists, US-trained staff and employed biotechnologists, who were

Fig. 2. *Analysis of the results of electrophoretic separation of PCR reaction products in the Laboratory of Molecular Biology (Puławy, 2004).*

trained extensively in microbial identification techniques using molecular biology methods and cloning techniques, including heterologous expression and separation of immunogenic proteins [14, 15].

As a culmination of the many years of cooperation with the USA was the visit by Ambassador Victor Ashe (2005), who personally familiarised himself with impressive results of the centre's cooperation with the US Army in the area of defence against biological weapons, rapid diagnostics, the participation of representatives of the centre in allied missions in Iraq and the results of staff training in the USA.

Fig. 3. *Training under BSL-4 conditions (USA, 2005).*

Threat of viral diseases and influenza pandemic

Eradication of the smallpox virus on a global scale and underestimation of influenza virus potential meant that the threat of viral agents was not properly appreciated by health services. This situation changed when news emerged of a dangerous disease, the highly pathogenic avian influenza (HPAI A/H5N1), in 2003 globally and in 2006 in Poland. A global outbreak of a new strain of so-called swine flu (A/H1N1) in 2009 permanently changed the attitude towards viral threats. As early as in 2006 conceptual work was undertaken at the centre to set up and launch a new organisational unit – a modern virology laboratory for research into particularly dangerous viruses. Again, thanks to cooperation with the USA and inclusion of the centre in the US *Global Emerging Infections Surveillance and Response System* (GEIS), grants were obtained in 2008-2013 to renovate and equip the Biosafety Level-2 laboratory (BSL-2), which was officially opened in 2010 in the presence of representatives of the Ministry of National Defence and the US embassy. The launch of the laboratory has enabled continuation and implementation of the influenza virus monitoring project, with a particular focus on strains with pandemic potential, in the Polish Armed Forces at home and abroad. A major achievement was the application, for the first time on such a large scale, of multidirectional molecular analysis of the aetiological factors of influenza-like illnesses and the scale of bacterial, viral and bacterial-viral infections [16]. The study demonstrated that it was not influenza viruses, but seasonal coronaviruses and enteroviruses, that were most frequently detected in symptomatic individuals.

An important step demonstrating the good skills of the new organisational unit was participation of the centre's virology team (in 2009-2011) in a project by the National Centre for Research and Development aiming to detect *in vitro* the most active non-nucleoside compounds against different strains of influenza virus. Subsequently (in 2013-2014), thanks to a SPUB grant from the Ministry of Science and Higher Education, HEPA filtration and vacuum control systems were installed in the laboratory and the effluent decontamination system was upgraded, transforming it into a Biosafety Level-2 and 3 laboratory complex (BSL-3+ with a *"shower out"* option). This allowed for re-undertaking seroprevalence and environmental research on the presence of dangerous Hanta viruses, aetiological agents of haemorrhagic fever with renal syndrome, in Poland [17]. In Podkarpackie, our team demonstrated for the first time in Poland the occurrence of Dobrawa, Puumala viruses in an animal reservoir in very close proximity to the Tula virus already detected in central Poland. It is worth noting that many years earlier, scientists from Puławy (Prof. J. Mierzejewski, Prof. M. Bartoszcze) and Professor Józef Knap from Warsaw, were the first to draw attention to the threats from these viruses in Poland [18].

In the following years (2016-2020), projects implemented at the centre under the "Mil-NPZ" - National Health Programme aimed at identifying arthropod-borne viral threats on military training grounds and sites where Polish contingents were stationed abroad.

During 20 years of the centre's functioning, its representatives have initiated and supported efforts to establish a Biosafety Level-4 laboratory in Poland to work with the most dangerous haemorrhagic viruses and smallpox [19]. The lack of such a facility represents a significant security gap in the diagnosis of highly dangerous diseases. To date, however, no such laboratory has been established in Poland, and the nearest are in the Czech Republic, Germany and Belarus.

Fig. 4. *Training of sample teams (Puławy, 2012).*

National and international expert and training activities

The undertaking of specialised tasks by the centre led to the gaining of knowledge and competence by its staff. A natural consequence was the sharing of experience with other services. Topics of particular importance were related to epidemiology of wmd biological agents, sampling, field and laboratory analysis, biosafety and personal protective equipment. There were usually one-off, thematic or cyclical training courses for the personnel of the Voivodeship Occupational Health Centre, the Epidemiological Response Centre of the Polish Armed Forces or the Central Centre for Contamination Analysis, as well as for the units of the Ministry of Internal Affairs – State Fire Service, Government Security Bureau, and Central Bureau of Investigation (Fig. 4). Acquisition and improvement of skills by the centre's staff members in the field of environmental diagnostics resulted in their involvement in the process of certification of teams of the Multinational Chemical, Biological Radiological and Nuclear Defence Battalion of the 12th NATO SO set at the Drawsko Pomorskie training ground in terms of sampling and detection of biological agents.

The centre has also organised scientific conferences of national and international scope. These included meetings on modern diagnostic methods (2002), or on defence against bioterrorism (*"Protection against bioterrorism"*) in 2003, 2004, 2005 and 2006. The latter promoted new methods for dealing with mass emergencies. Meetings devoted to terrorist incidents (jointly with the Military Institute of Medicine Warsaw in 2007), preparation of hospitals for mass incidents and the methods of response of services to biological incidents (together with the 1st Military Teaching Hospital with Polyclinic in Lublin in 2009) enjoyed great interest. Conferences participated by foreign specialists provided an opportunity to learn about global trends in crisis management. Organised presentations of equipment and technologies designed to deal with large-scale emergencies (mass disinfection of people and the environment) gave a boost to the national defence industry.

Another event summarising the departmental research work on cloning immunogenic protein genes [15, 20] was the 2014 vaccinology workshop "Vaccines against particularly dangerous infectious diseases". The theses concerning the availability of vaccines have been confirmed during the current pandemic.

The unique expertise and experience of the centre's staff was an important competitive fact, and enabled participation in expert and support projects. The BIO3R project (*Bioterrorism – Readiness*, *Response*, *Resilience*), I-022639, was implemented in 2007-2008, aiming at developing procedures to deal with different scenarios in the use of biological agents. From 2010 to 2014, the Puławy centre was the leader of the European Commission's biosafety assistance project for Ukraine *"Bio-Safety and bio-security improvement at the Ukrainian Antiplague station (UAPS) in Simferopol"* – project no. IFS/2010/248-957. This project was not completed due to the annexation of Crimea. Further EU assistance projects were procured through the European Network of Chemical, Biological, Radiological and Nuclear Risk Mitigation Centres of Excellence (*CBRN CoE*). Together with partners from Italy, Spain, France and Slovakia, the executed projects focused on: improving biosafety in laboratories in the Middle East (CoE project no. 25 BIO-OPERATE from 2013-2016), improving the response capacity of emergency services to a WMD incident in the Middle East (CoE no. 34 from 2013-2017), and improving hazardous waste management in the Caucasus (CoE no. 65 CABICHEM from 2017-2020). A major challenge for the centre was its participation in an educational project of the European Commission together with the University of Lodz, the Military Institute of Chemistry and Radiometry, INSEMI (Slovakia), the Bureau of Anti-Terrorist Operations of the Police Headquarters and the Police Academy in Szczytno to organise a postgraduate course for security coordinators of defence against weapons of mass destruction (*dawmd)*. Between 2016 and 2018, dozens of participants from across the EU completed the course, and the resulting educational materials and textbooks will be available for use in future editions of these studies. The aim of the European Commission's ongoing international project, "Mall-CBRN", is the identification of inadequacies, needs and strengthening the *dawmd* protection of large format stores. The project involves not only commercial partners, but experts from the scientific world as well as state services competent for *dawmd*.

Regardless of project implementation, the centre's experts have been appointed to participate in numerous advisory bodies: the Ministry of Defence, the National Security Bureau and NATO advisory committees. Representatives of the centre (M. Bartoszcze, M. Niemcewicz, A. Michalski) represented the Military Healthcare Service in the NATO COMEDS advisory groups (*Force Health Protection*, *BioMedAdC*), NATO *Land Group 7 Expert Subgroup on Sampling and Identification of Biological*, *Chemical and Radiological Agents* and HFM OO5 Group. An important contribution of the centre for raising awareness of biological threats at higher levels of administration, responding to threats, identifying optimal solutions, improving the system and creating international

cooperation was the involvement in 2003 of specialists from WIME (Prof. K. Chomiczewski and Prof. M. Bartoszcze from the centre) by the head of the National Security Bureau in the Team for Biological Threat Assessment at the National Security Bureau headed by Professor Stanisław Majcherczyk. The team was very active in the fight against biological threats, organising a number of conferences and meetings, especially on the fight against influenza, also on the basis of the centre in Puławy.

A major international event was the global staff training exercise, *"Atlantic Storm"* (2005) in Washington DC. There, representatives of individual countries carried out an interactive bioterrorism scenario in the area of response to smallpox eradication. One of the consultants of the Polish delegation, headed by a former prime minister, Professor Jerzy Buzek, was Professor M. Bartoszcze – the Head of BTIaCC. The exercise highlighted the vulnerability of the countries to this eradicated and almost forgotten disease, and their unwillingness to provide mutual aid and their limited stocks of vaccines. The conclusions from the exercise were the impetus to re-evaluate plans to respond to such an attack and to develop and produce new, safe vaccines or find effective drugs against this virus. In 2010, Professor M. Bartoszcze was appointed by the head of the National Security Bureau to the prestigious team of experts recommending areas of defence against biological weapons to the Strategic National Security Review and the first of its kind in Europe – the White Book on National Security.

Representatives of the centre have been repeatedly involved in the work and reporting on the Biological and Toxin Weapons Convention (BTWC). Participation of the centre's representative (A. Michalski) in the 2011-2018 expert group at the Ministry of Foreign Affairs was an important voice in the discussion on supplementing national *biosafety/ biosecurity* regulations [21]. The group aimed at developing legal mechanisms implementing the recommendations of the BTWC review conferences, especially the provisions of UN Security Council Resolution 1540 of 2004 in the field of biological agents (e.g. rationing, control of use, standards of physical protection and personal security, reviewable qualitative and quantitative records).

Period of development and implementation of modern technologies

- **Molecular diagnostics**

Molecular diagnostic techniques, as rapid and precise methods for the detection and identification of particularly dangerous agents, have been used from the beginning in the detection of *wmd* agents. Research work at the centre initially involved methods based on the DNA polymerase chain reaction (PCR) technique with analysis of the products by electrophoresis. Later, the centre used RFLP (*Restriction Fragment Length Polymorphism*) [9] and REP-PCR (*repetitive sequence polymorphism*) [10] methods to differentiate *Bacillus cereus* group strains. It conducted research and tests on the use of advanced PCR- ELISA, PCR-CLEIA (*chemiluminescence enzyme immunoassay*) [21] or the analysis of PCR products using a flow cytometer [22]. In later years, it used more modern *real-time* PCR technology, including the multiplex reactions. This allowed subtyping at the level of species, subspecies and the study of the virulence of isolates. Optimisation of *real-time* PCR detection and molecular identification began with the *B. anthracis* anthrax, belonging to the homogeneous *Bacillus cereus* group, which implied the need for differentiation from other species [23]. In this respect, it is worth highlighting the pioneering work carried out on the application of macro-restriction analysis for the differentiation of this group. The PFGE (*puls field gel electrophoresis*) method proved successful in differentiating related bacteria of the *Bacillus cereus* group [11]. Another method successfully used to differentiate this group is MLST (*multilocus sequence typing*) [24]. In subsequent years, an algorithm was developed for molecular identification of toxigenic cholera vibrio (*V. cholerae)* strains by means of a conserved gene sequence, allowing the differentiation of toxigenic (cholera-causing) strains and differentiation of serotype O1 into biotypes (*El Tor* or *classical*), and for the differentiation of the O1 group from O139 [25].

An important research topic was the optimisation of the detection and differentiation of the aetiological agent of Q fever (*C. burnetii).* The analysis of the pathogen occurrence in the farm and livestock environment (livestock and arthropods, ticks and environmental swabs), proved the effectiveness for the developed molecular diagnostic methods. The MTS (*multispacer typing*) differentiation method based on sequencing of *intergenic spacers* was used for the obtained isolates [26].

High specificity and sensitivity were obtained in molecular diagnosis of *F. tularensis* with hybridising probes and using FRET. In addition, for differentiation of four subspecies of tularemia bacilli (type A, type B and *F. tularensis* subsp. *mediasiatica* and *F. tularensis* subsp. *novicida*), duplex reactions targeting different gene regions were used. The methods developed at Puławy are a convenient diagnostic tool for determining the degree of virulence of a detected pathogen and, under certain conditions, also its source [27].

Recent years have seen advances related to *next generation sequencing* (NGS). Funds from *European Defense Agency* (EDA) projects has enabled acquisition of the MiSeq (Ilumina) compact next-generation sequencer based on SBS (*sequencing by synthesis*) technology and, more recently, the MinION (*Oxford Nanopore Technologies*) nanopore sequencer. These two platforms perfectly complement each other's features, allowing advanced genetic and phylogenetic analyses of bacteria and viruses, which has been successfully used in the study of new variants of the SARS-CoV-2 virus.

- **Collection of *wmd* biological agent strains**

Over the years, through isolation from environmental or clinical samples and exchange, the centre has gathered a unique collection of bacterial and viral strains. This

collection is not only used to improve diagnostic techniques and control tests, but it is an important reference point for research into new isolates or epidemiological investigation using molecular biology techniques in the event of biological weapons or bioterrorist attacks. The collection may be used in testing new antiviral or antibacterial substances, developing new diagnostic tests (specificity tests) or testing the efficacy of new biocidal substances. The centre's potential in this area has been discounted since 2009 through its participation in three multinational projects of the European Defence Agency (*Database of B Agents*, *European Biodefense Laboratory Network* – EBLN). Their aim was to characterise the strain collections held by selected institutions in each country by molecular biology (including *MLST*, *MLVA*, *SNP* or even *WGS*) and phenotypic methods. The acquired data, research results and information on individual bacterial and viral strains are collected in a collaboratively developed database on the digital Bio-Numerics platform. The set of features of a given strain, characterising the biological agent, e.g. sequences, profiles, constitutes its unique feature – its "fingerprint". This information can be very useful in potential uses for comparative purposes, when the agent is detected in the environment or in clinical trials. Comparison with the database may lead to some suspicions or clues about the origin of the agent. The projects also aim at exchanging experiences and best practice, as well as standardising methods for laboratory detection, identification and characterisation of agents. The exchange of such important information, hitherto mutually unavailable between European countries, strengthens the EU defence capabilities and the speed of response in the event of the use of biological weapons.

- **Comparative exercise**

Inter-laboratory proficiency tests are important, not only in the field of medical diagnostics (e.g. PolMicro), but also in the detection and identification of *wmd* agents. Acquisition of externally validated skills by laboratory teams is the key to responding effectively in real-life situations involving dangerous bacteria, viruses and toxins. As the only scientific institution of our country, the centre has been participating in the NATO-SIBCRA *"round-robin"* session on biological agents since 2000. These are dedicated to improving and testing the ability to identify biological agents sent to individual countries in the form of coded samples.

In November 2014, an exercise to investigate the alleged use of biological weapons within the framework of the UN Secretary-General's mechanisms was organised by the Robert Koch Institute and the German Foreign Ministry in Berlin in conjunction with the UN Office for Disarmament Affairs (UNDOA). The Department of Security Policy of the MFA designated WIME as our country's representative at the event. A task force sub-team, the field laboratory, was formed, and the WIME director, Colonel Janusz Kocik PhD, was appointed to have close command over the exercise. Over 70 participants and observers from different countries in the world took part in the exercise, in addition to sampling teams from Denmark and Portugal, field laboratories from Poland and Canada, epidemiological investigation teams from Norway and representatives of Interpol and WHO. The exercise was an important part of the countries' preparation for a biological crisis.

Further proficiency exercises are carried out thanks to the centre's participation in the European network of virology laboratories (ENIVD network and, since 2015, EVD LabNet). In 2017, these were neurotropic viruses (TOSV, WNV, TBEV, USUV), in 2018 yellow fever virus (YFV) and in 2019 Orthopox viruses (Cowpox-CWPX, Monkeypox-MPXV or Vaccinia-VACV). The Robert Koch Institute (Germany) has been hosting the EQAEb (*External Qualified Assurance Entity)* exercise since 2018 within the UNSGM RefBio project. The centre participated in RKI proficiency tests on: plague bacillus (2018), Orthopox viruses (2018) and SARS CoV-2 virus (2020). The centre has regularly participated in exercises for diagnostic proficiency and to verify existing diagnostic schemes and procedures.

- **Bacteriophages and phage enzymes**

With the increasing emergence of strains of multidrug-resistant bacteria worldwide, it has become important to search for alternatives to antibiotics. Since 2002, when the Bacteriophage Laboratory in Puławy was launched, the tracking of bacteriophages and their associated lytic enzymes (phagolysins) has been an important scientific contribution of the centre in the development of novel therapies. The laboratory has taken on the challenge of searching for, characterising in detail and collecting lytic bacteriophages against clinically relevant drug-resistant bacteria, as well as being potential bioterrorism agents. Particular attention has been given to bacteriophage endolysins and the potential for biofilms to combat phages and recombinant enzymes. The work related to bacteriophages lytic towards *Bacillus anthracis* (2014-2022) allowed participation in the international project *"Anthrax Environmental Decontamination Network – Marie Curie Actions"*. The project aims at comparing the lysins encoded by phages lytic against *B. anthracis* obtained by cloning and evaluating their lytic activity against vegetative cells of anthrax strains and other *Bacillus cereus* bacteria, but especially against endospores, which are difficult to control in an effective and environmentally safe way [28]. The search for phagolysins, active against wound-infecting bacteria, such as *Staphylococcus aureus* MRSA (methicillin-resistant), was of interest to American DARPA, which funded research at the centre (2009-2011). This prestigious project aimed at isolating and selecting virulent bacteriophages active against MRSA and obtaining a purified, biologically active staphylococcal endolysin that lyses MRSA strains with the possibility of its practical further clinical application.

- **Cooperation in the field of medical and military engineering**

Possessing a collection of *wmd* biological agents and experience in their *in vitro* culture meant that the centre's

cooperation with partners carrying out development work in the field of military and medical engineering was an important point of its activity. The partnership concerned development of new equipment for the detection and identification of biological agents, as well as establishment of the field laboratories themselves.

Cooperation with the Military Institute of Chemistry and Radiometry on the MIDAS project and creation of the Mobile Laboratory for Defence Against Weapons of Mass Destruction (2004-2005) allowed the development of a state-of-the-art wheel set enabling identification of biological agents in hermetic conditions close to the battlefield. This effort was recognised in Kielce in 2006 with the Grand Prix award at the 14th International Defence Industry Exhibition. Between 2005 and 2008, the centre collaborated with the Institute of Optoelectronics at the Military University of Technology on the FABIOLA project. The institute constructed a medium-range LIDAR (*Light Detection and Ranging*) detector for remote detection of biological contamination in the air during a biological aerosol attack. The centre participated in the creation of a database of biophysical fluorescence spectra – determinants of bioaerosols, covering agents of high public health risk, essential for hazard discrimination. The use of polyaniline fibres for detection and even identification of biological agents was recognised by DARPA, which funded research (2009-2011) for the project: *"Nanobiodetector for spore forming bacteria"*. In cooperation with Professor Jerzy Langer's team from Adam Mickiewicz University in Poznań, significant progress was made in distinguishing bacterial vegetative forms from spores with the use of the nanobiodetector [29].

A unique project was "SFORA Mobile Laboratory for Sampling and Identification of Biological Threats" (2011-2014), by the National Research and Development Centre. The participation of as many as 12 consortium members, as well as the synergy of knowledge and competences from the fields of engineering, construction and both natural and medical sciences, enabled unprecedented development of purely domestic technologies. The project manager was Colonel J. Kocik PhD, and the centre in Puławy played a very important role in development of the concept and in testing. The result of the project was a modern prototype of a field Biosafety Level-3 laboratory based on a 30' container, with a gas decontamination system for the interior and a self-loading system. The laboratory was integrated with a robot (UGV) capable of remotely collecting suspected contamination samples in the field and having the capacity for initial hazard identification, thanks to its mounted biological contamination detectors (Fig. 5). Further analysis was planned in an airtight laboratory, which was equipped with a device capable of precise identification of pathogens by molecular biology methods, including sequencing. It is worth emphasising that this project allowed the perfecting of national solutions: the above-mentioned polyaniline detectors, as well as an original portable *real-time* PCR thermocycler, an automatic nucleic acid isolation kit, an electrophoretic analyser (Wrocław University of Technology) [30], and a chemical and biological agent analyser (Military Institute of Chemistry and Radiometry). Molecular biology consortia (A&A Biotechnology) developed optimised reagents for the manufactured instrumentation, and the centre developed a diagnostic scheme as well as probe and primer sets. The achievements of this project were recognised with the following awards: Golden Laurel of Innovation in the competition of the Ministry of Science and Higher Education for the best innovative products and the Defender award at the XXII MSPO Kielce 2014 for outstanding technical solutions. Good experience from cooperation with the Military Institute of Chemistry and Radiometry has allowed for implementation of current work on the immune detector. The aim of the work, funded by the Ministry of Defence and started in 2019, was to produce a biodetector for the detection of *B. anthracis* spores, which would enable automatic identification in modern battlefield and operational activities of various services (police, National Fire Service, national security services, Internal Security Agency and others). The designed nanobiodetector will operate on the principle of a double-antibody sandwich enzyme-linked immunosorbent assay – *ELISA*, using a carrier-membrane, with antibodies specific for *B. anthracis* spores.

Fig. 5. *SFORA Mobile Sampling and Biological Hazard Identification Laboratory (Puławy, 2014).*

- **Translational medicine – gene therapies**

In 2016, the departmental "Kościuszko" programme was announced, aimed at Polish scientists from abroad who would like to return to the country and start cooperation with defence R&D institutions. Establishing cooperation between the centre in Puławy and Leszek Lisowski, PhD, from the Children's Medical Research Institute (Sydney, Australia) opened up new possibilities for the virology laboratory. An innovative research area has become the use of viral vectors (*recombinant adeno-associated virus* (rAAV))

targeting liver cells in the treatment of genetically determined metabolic diseases. Identifying the molecular mechanisms of an intracellular block of hepatocyte transduction would allow more efficient delivery of the therapeutic transgene. To explain them, a platform for the creation of AAV vectors was developed, the mechanisms of interaction between the liver and AAV were studied and recombinant vectors capable of transducing human hepatocytes with high efficiency were sought. Cooperation with the best laboratories allowed the transfer of knowledge, methodologies and experience. Reciprocal internships and apprenticeships, as well as exchanges of scientific personnel, enabled the rapid implementation of genetic recombination and directed evolution techniques, leading to the creation of differentiated libraries (*error prone PCR*, *primerless PCR*, *DNA shuffling*), library testing, sequence analysis, techniques for working with cell lines or induced stem cells (iPSCs) as hepatocyte models [31].

Times of the COVID-19 pandemic

The SARS-CoV 2 coronavirus pandemic created a new focus related to COVID-19 diagnostics. Having received information about the new variant of the SARS virus, thanks to exchange of information within EVD LabNet and EVA (*European Virus Archive)*, in January 2020 BTIaCC acquired relevant reactors and response controls within the framework of cooperation, and in line with development of the situation related to the COVID-19 pandemic, it became a priority for the centre to provide diagnostics for the Polish Armed Forces. In accordance with the decision of the Minister of National Defence, on 2 March 2020 the centre in Puławy began conducting analyses of biological material for the presence of the SARS-CoV-2 virus. Soon, thanks to the Ministry of National Defence, it was retrofitted with a Cobas 6800 automatic analyser, which significantly increased throughput to 1,400 tests per day. In July 2020, the centre was visited by the Minister of National Defence, Mariusz Błaszczak. As part of emphasising the role of military laboratories in counter-epidemic activities, he indicated the leading participation of BTIaCC in Puławy. Since November 2021, the centre, using its previous experience and competence in sequencing and analysis, has been involved in the SARS-CoV-2 coronavirus genome sequencing programme. This technique allows monitoring the variability of the genetic material (mutation detection), enriching the international GISAID database with information on SARS-CoV-2 virus variants present worldwide. Assessing the frequency of the occurrence of new coronavirus variants with greater infectivity and virulence, and characterising SARS-CoV-2 virus strains, will accelerate the process of identifying and assessing significance of new variants, allowing a more precise analysis of the current epidemiological situation and an effective fight against the pandemic.

Summary

The Biological Threats Identification and Countermeasure Centre of the Military Institute of Hygiene and Epidemiology is a scientific institution that makes unique contribution to the development of medical, biological and veterinary sciences. In terms of the topics and performed tasks, the centre has no equivalent among departmental or civilian organisations. The uniqueness of the BTIaCC results from the synergy of three important functions: scientific, diagnostic and expert-training in the area of biological agents (bacteria, viruses and toxins) of weapons of mass destruction. Participation in international diagnostic exercise maintains readiness and proficiency in the identification and differentiation of dangerous pathogens. The centre has a unique collection of strains of biological agents (viruses and bacteria of risk groups 2 and 3) on a national scale, thanks to which the work performed at the centre performs not only cognitive, but also applied functions. In light of scientific advances, contemporary threats of pandemics, bioterrorism and bioweapons all call for comprehensive measures to address them. In addition to the diagnosis of clinical specimens, associated with a medical diagnostic laboratory, the centre must be able to identify *wmd* biological agents in environmental samples. To achieve this, rapid sequencing methods must be developed, taking into account sequencing under field conditions during an outbreak. This is possible thanks to unique infrastructure, trained personnel and modern testing equipment.

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MILITARY INSTITUTE OF MEDICINE LAUNCHES TRAINING FOR MEDICAL PERSONNEL AT THE ROBOTIC SURGERY CENTER

WIM uruchamia szkolenia personelu medycznego w centrum chirurgii robotycznej

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Abstract: The Robotic Surgery Center (CCHR) launched in 2020 at the Military Institute of Medicine performs surgical procedures with the use of the da Vinci surgical robot. It allows the performance of extremely complex surgical procedures using minimally invasive techniques. Within the CCHR, the institute is also creating a training centre for new robotic platform personnel, covering Poland and Eastern European countries. From September 2022, training courses will be launched for staff assisting operators during surgery with the da Vinci system.

Streszczenie: W uruchomionym w WIM w 2020 r. Centrum Chirurgii Robotycznej wykonywane są zabiegi operacyjne z wykorzystaniem robota operacyjnego da Vinci. Umożliwia on przeprowadzenie niezwykle skomplikowanych procedur operacyjnych przy użyciu minimalnie inwazyjnych technik. W ramach CCHR Instytut tworzy również ośrodek kształcący nowy personel platform robotycznych i obejmujący swoim zasięgiem Polskę i kraje Europy Wschodniej. Od września 2022 r. uruchomione zostaną szkolenia dla personelu asystującego operatorom przy zabiegu z wykorzystaniem systemu da Vinci.

Key words: da Vinci, surgical robotics, on-line surgery, Robotic Surgery Center of the Military Institute of Medicine.

Słowa kluczowe: da Vinci, robotyka chirurgiczna, operacje on-line, Centrum Chirurgii Robotycznej WIM.

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We are talking to Colonel Jacek Doniec, MD, PhD, the Head of Robotic Surgery Center of the Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine, on development of robotics in Poland and worldwide, the potential of the WIM Centre for Robotic Surgery and the training of medical staff in the da Vinci surgical robotics system.

What is a robot? What conditions must something meet to be called a robot?

The word "robot" comes from the Czech word "robota" meaning hard work. It was first used in 1920 by Karel Čapek in his drama "R.U.R.", referring to an artificially produced, simplified version of a human designed for hard work. An early and important concept of the robot was its telepresence developed in the 1950s. It is the feeling that you perform an activity in one place while being in another. Initially, they were master-slave manipulators used in hazardous human environments – in such systems, one device (master) overrides and manages or controls another device (slave). In the 1980s, the vision of using remote surgery on the battlefield emerged, financed by the US Defense Advanced Research Projects Agency

Fig. 1. *Col. Jacek Doniec, MD, PhD, Head of Robotic Surgery Center of the Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine.*

Fig. 2. *The permanent team of the Robotic Surgery Centre of the Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine trained to perform any type of surgery.*

(DARPA). It gave the impetus for developing robots suitable for modern surgical practice.

What was the first robot in medicine?

Robotics is a fairly young branch of medicine. The world's first surgical robot was the Arthrobot, designed to assist during orthopaedic surgery – it responded to the surgeon's voice and helped to position the patient's legs in a convenient position for the surgery. It was first used in Vancouver, Canada, in 1983. Since then, we have witnessed the development of simple systems assisting a surgeon in a specific type of surgery. They were not universal platforms, but were strictly tailored to one typical activity during the surgery.

In 1998, the Cleveland Clinic in the USA presented the Surgical Robotic System, which consisted of a control centre and three arms mounted on the operating table. It was used for the first full endoscopic robotic procedure, which was oviduct reanastamosis.

When did the greatest development in robotics happen?

At the beginning of the 21st century there were two robotic systems for potential applications in various surgical specialities. They were the Zeus system by Computers Motion and the less advanced da Vinci system by Intuitive Surgical. A tumultuous merger of these two companies accelerated development of the universal da Vinci robotic platform, which has now seen four generations of robots in more than 20 years, and has inspired the development of other robotic platforms.

How has the development of surgical robotics been in Poland?

In our country, the da Vinci robot was first presented in 2009 during the Congress of the Society of Polish Surgeons. Not much later, the first specimen was purchased for the Provincial Specialist Hospital in Wrocław, but due to the high cost of surgery, its use had been low for years. Another da Vinci robot appeared in Poland in 2016, but the following year there were already four units. Since then, the development of robotics in Poland has accelerated significantly. Currently, an increasing number of surgeries assisted by the robotic system are being recorded every quarter of the year.

In 2020, the Robotic Surgery Center was established at the Military Institute of Medicine with the first da Vinci robot. How has this development affected surgical potential of the Military Institute of Medicine?

Obviously, the development of robotics could not avoid the Military Institute of Medicine. In the case of a centre with state-of-the-art diagnostic and medical facilities, with extensive experience in telemedicine gained during NATO peacekeeping missions, failure to implement breakthrough technology would be inconceivable. The robot arrived at our Institute in the autumn of 2020. We were the 17th Polish centre with a da Vinci robot, and after only a year and a half of operation, the Military Institute of Medicine has become one of the most important and largest robotic surgery centres in Poland. We are now the only one with a total of six certified specialities for robotic surgery. These include general surgery, gynaecology, urology, laryngology, cardiac surgery and thoracic surgery. Our aim is to introduce further

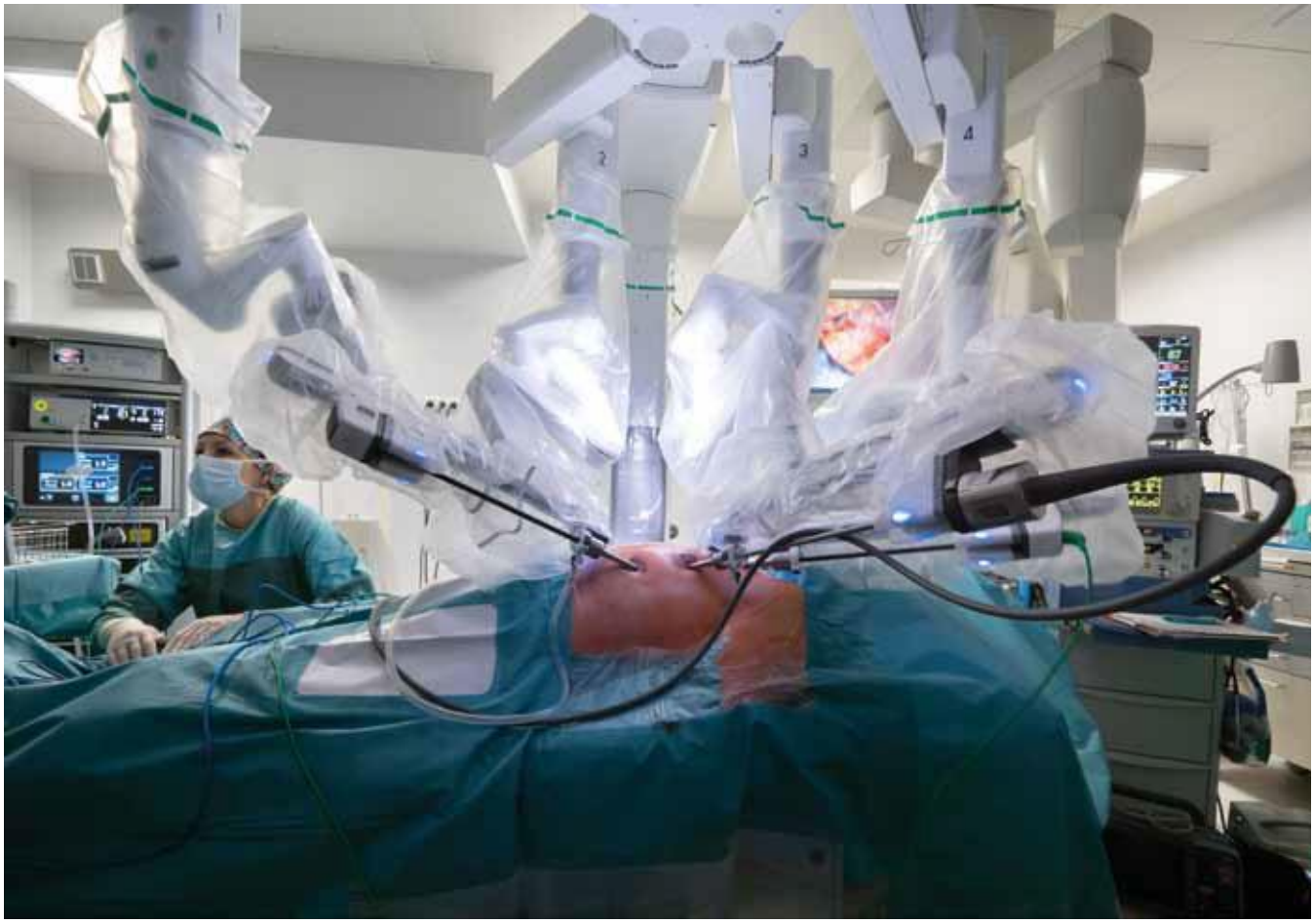

Fig. 3. *The da Vinci robot during cardiac surgery at the Robotic Surgery Center of the Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine.*

specialities in order to make the most of the opportunities offered by this cutting-edge technology.

Why is the da Vinci robot so innovative? How can it benefit patients, physicians and the healthcare system?

The entire development of robotic surgery is primarily focused on the patient's benefits. The surgical use of the da Vinci robot means that a patient can undergo a minimally invasive, highly precise procedure with minimal tissue traumatisation. This significantly translates into reduced post-operative pain, shorter hospitalisation and, as a result, quicker full recovery after surgery. A physician performs the procedure in a comfortable position and has extremely agile and precise instruments at their disposal, which further eliminates the risk of hand tremor. In addition, the image that the operator sees is three-dimensional, at 10x magnification, stabilised and self-adjustable in an intuitive way. Unfortunately, the healthcare system does not yet fully appreciate the benefits for the patient in the long term, but rather focuses on the cost of the procedure itself, and these, as for any innovative system, are high.

The second da Vinci system will soon be implemented in the Military Institute of Medicine and training sessions for physicians will begin. Who can take part in them and what will they learn?

When establishing the Robotic Surgery Center, the philosophy of the Military Institute of Medicine was based on a focus on the patient, the benefits for the patient from robotic surgery and not on the procedure itself. A large number of specialities and the increasing demand for robotic surgery necessitated the purchase of the second system. Continued development and experience gained are promoting our centre to become a training centre for robotic surgery. We are already a centre for case observation visits. The next step is the training of personnel assisting operators during the surgery, for both physicians and nurses, to finally become a regular training centre with certification rights. This training begins in September 2022. We can already see the benefits of using the robotic system. Every physician, even not taking active part in the procedure, says that, thanks to the possibilities of precise preparation and extraordinary visualisation, one can "rediscover human anatomy".

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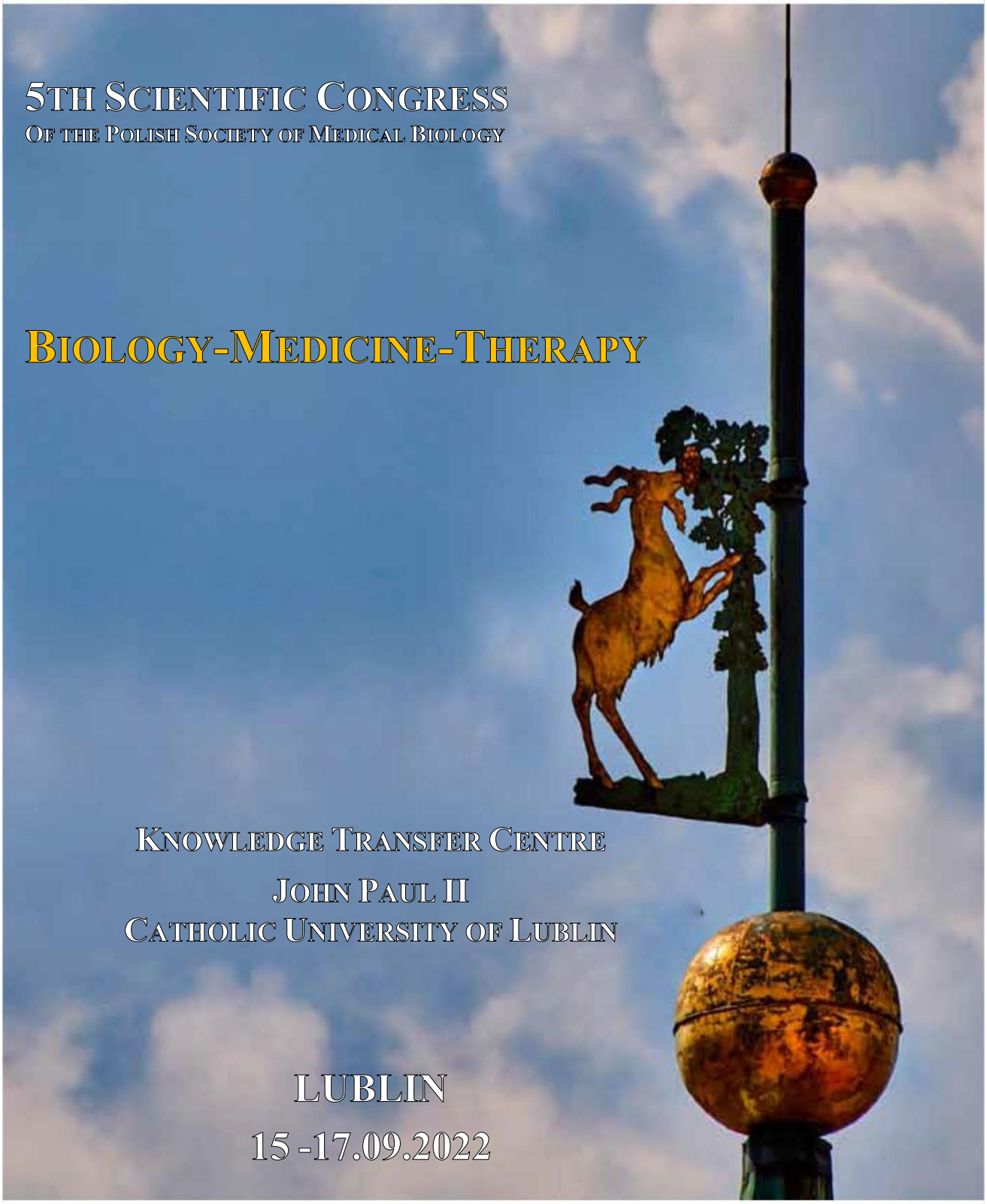

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The Robotic Surgery Center of the Military Institute of Medicine (Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine) invites you to training courses for personnel assistance in procedures using the da Vinci surgical system

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