2018, vol. 96 No. 1

# MILITARY PHYSICIAN

SCIENTIFIC JOURNAL OF THE MILITARY INSTITUTE OF MEDICINE
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# MILITARY PHYSICIAN


## Military Physician

**Quarterly**
**Official Organ of the Section of Military Physicians at the Polish Medical Society**
**Oficjalny Organ Sekcji Lekarzy Wojskowych Polskiego Towarzystwa Lekarskiego**
**Scientific Journal of the Military Institute of Medicine**
**Pismo Naukowe Wojskowego Instytutu Medycznego**

Published since 3 January 1920

Number of points assigned by the Polish Ministry of Science and Higher Education (MNiSW) – 6





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Practical Medicine Publishing House / Medycyna Praktyczna
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**Print**
TECHNET, Kraków

Circulation: 700 copies

Price PLN 14

**ISSN 0024-0745**





For many years, “Military Physician” has been indexed in the Polish Medical Bibliography (Polska Bibliografia Lekarska), the oldest Polish bibliography database.

The primary version of the "Military Physician" quarterly is its electronic version (www.lekarzwojskowy.pl)

The journal is financed by the Military Medical Chamber

**Translation, proofreading and DTP of the English version by Skrivanek Sp. z o.o.**

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3) References should be presented according to the order they appear in the text. If the article has no more than four authors, all of them should be named, if there are more - the maximum of the first three, followed by "et al.". References should be numbered using the keyboard, please do not use automatic numbering. Examples of citations:

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# Animus Fortis (Courageous Spirit) award

I have taken the liberty to turn to military physicians, readers of the “Military Physician”, with a request to disseminate information about the award and to propose candidates to the Head of the Military Institute of Medicine on account of the establishment of the Animus Fortis award (Courageous Spirit) at the Military Institute of Medicine. The award reflects the best traditions of the health services and aims to honour people and institutions having a particular influence on positive changes to and the reputation of the emergency services.

The Animus Fortis award is under the auspices of the Prime Minister. The award committee comprises 13 members, including the representation of the Minister of National Defence, Minister of the Interior and Administration and the Minister of Health. The winning candidates will be awarded the “Paramedic Monument” statue (designed by Edward Wittig, shown on the cover of this “Military Physician”) and a certificate, and will take place once a year and consist of two categories:

- **individual** – “Personal courage” for a medical rescue worker who in the year preceding the award excelled in their position; the winner can be a person on duty or employed by the institution to save human lives (Ministry of National Defence, Ministry of the Interior and Administration etc.);
- **institutional** – for the representation of institutions which significantly and positively influenced changes facilitating the increased effectiveness of life saving; the winner can be the person performing the function in an institution, who implements or participates in broadly defined rescue tasks.

The award regulations and application forms are available at: www.wim.mil.pl/aktualnoci-topmenu-19/medycyna-w-mundurze/2782-nagroda-wim-dla-ratujacych-zycie

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Head of the Military Institute of Medicine

Maj. Gen. Prof. Grzegorz Gielerak, MD PhD

# Enhanced recovery after colorectal surgery at the Department of General, Oncological, Metabolic and Thoracic Surgery, Military Institute of Medicine

## Protokół ERAS u chorych operowanych z powodu raka jelita grubego w Klinice Chirurgii Ogólnej, Onkologicznej, Metabolicznej i Torakochirurgii WIM

**Maciej Walędziak**
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**Abstract.** The idea behind ERAS (Enhanced Recovery After Surgery) was to create a protocol that allows the optimisation of perioperative care. According to the recommendations of an international team of specialists, the ERAS protocol reduces perioperative stress, and helps to maintain optimal postoperative physiological functions and enhance mobilisation after surgery. The ERAS protocol was introduced to the Department of General, Oncological, Metabolic and Thoracic Surgery, Military Institute of Medicine, Central Clinical Hospital of the Ministry of National Defence in Warsaw in 2015 as part of a randomized prospective study on a group of patients operated on for colorectal cancer. The ERAS protocol includes patients' preoperative education, optimized analgesia with restricted fluid intake, avoidance of hypothermia, minimizing of opioid doses, early mobilization and fast realimentation in the postoperative period. Numerous test results explicitly show fewer postoperative complications and shortened stays in hospital in the ERAS group as compared to the control group, which indicates that the ERAS protocol should now be considered as a standard for perioperative care.
**Key words:** colorectal cancer, ERAS protocol, perioperative care

**Streszczenie.** Ideą ERAS (enhanced recovery after surgery – kompleksowa opieka okołooperacyjna dla poprawy wyników leczenia) jest stworzenie odpowiedniego protokołu optymalizacji postępowania okołooperacyjnego. Zgodnie z wytycznymi opracowanymi przez międzynarodową grupę specjalistów protokół ten pozwala na zredukowanie stresu okołooperacyjnego, utrzymanie prawidłowych funkcji fizjologicznych i szybkie uruchomienie chorych po operacji. Protokół ERAS był stosowany w Klinice Chirurgii Ogólnej, Onkologicznej, Metabolicznej i Torakochirurgii Wojskowego Instytutu Medycznego CSK MON w Warszawie w 2015 roku jako element randomizowanego prospektywnego badania w grupie operowanych z powodu raka jelita grubego. Do założeń protokołu zaliczają się: przedoperacyjna edukacja, zoptymalizowane znieczulenie z ograniczoną podażą płynów, unikanie hipotermii, minimalizowanie dawek opioidów, wczesne uruchomienie i realimentacja po operacji. Wyniki wielu badań jednoznacznie wskazują na występowanie mniejszej liczby powikłań pooperacyjnych i skrócenie pobytu w szpitalu u operowanych prowadzonych według protokołu ERAS niż w grupie kontrolnej, co wskazuje na celowość uznania protokołu ERAS za standard postępowania okołooperacyjnego.
**Słowa kluczowe:** protokół ERAS, nowotwory jelita grubego, opieka okołooperacyjna

Delivered: 08/06/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 10-13


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## Introduction

Over the last decades, the progressive development of minimally invasive techniques, especially laparoscopy, has contributed to many improvements in surgical treatment results. Recovery is influenced not just by surgical techniques, but also by a multi-faceted approach to patients under perioperative care.

The previous conservative perioperative care methods involved the uncomfortable preparation of patients for surgery and long fasting during the postoperative period. This both delayed and inhibited recovery, especially in patients undergoing surgery for cancer.

Towards the end of the previous century, it was shown that success depended on more than the surgical techniques, as it also included the entire course of care during the perioperative period.

Initially, the multi-faceted approach used to improve treatment results of patients undergoing surgery was described as *fast-track surgery* [1]. The idea behind "fast track" included: preoperative education, minimally invasive surgery, optimised analgesia with due consideration to maintaining the normovolaemia and restricted fluid intake, avoiding hypothermia and hypovolemia, effective analgesic therapy with minimised opioid doses, fast rehabilitation and mobilisation after surgery, early oral alimentation during the postoperative period and avoiding the use of feeding or drainage tubes. In 2001, another important step in the development of this line of approach was achieved by a team established in London. A working group was established, which was made up of: Kenneth Fearon (Edinburgh, Great Britain), Henrik Kehlet (Copenhagen, Denmark), Arthur Revhaug (Tromsø, Norway), Maarten von Meyenfeldt and Cornelis de Jong (Maastricht, Holland) and Olle Ljungqvist (Stockholm, Sweden). These researchers thoroughly analysed the literature concerning perioperative care plans. As a result, they developed guidelines based on *evidence-based medicine* (EBM), later called the ERAS protocol (Enhanced Recovery After Surgery) The implementation of this was to improve surgical treatment results. At a later date, the same group of researchers transformed into an international scientific association called the Enhanced Recovery After Surgery Society for Perioperative Care (ERAS Society), which prepares perioperative protocols for many surgical disciplines [2, 3].

The acronym ERAS has no official equivalent in the Polish language, but is translated as *szybki powrót do zdrowia po operacji* [4, 5], essentially "quick return to health after an operation" [6].

The perioperative care based on the ERAS protocol reduces the stress connected with surgery, facilitates the return of the physiological functions of the body and accelerates the recovery period after surgery.

Many current research works reveal a significant improvement in treatment results after using care plans based on the ERAS protocol. Such perioperative care brings positive outcomes, including a decreased number of perioperative complications, shortened hospital stays and reduced number of repeat hospitalisations following surgery [7-9].

## Aim

The aim of the research was to compare the treatment results of patients operated on for colorectal cancer. This included patients who were provided with a perioperative care plan based on the ERAS protocol, and patients provided with traditional perioperative care.

## Material and methods

In 2015 and 2016, having been given a favourable assessment by the Bioethics Committee of the Military Institute of Medicine (40/WIM/2015 of 17 June 2015), 15 randomly selected patients operated on for colorectal cancer at the Department of General, Oncological, Metabolic and Thoracic Surgery, Military Institute of Medicine, were provided with perioperative care based on the ERAS protocol. The plan included preparation for surgery, the course of analgesia and surgery, and early postoperative care.

**The main assumptions of the plan based on the ERAS protocol are:**

- Inform the patient about the suggested treatment in an approachable manner
- Stop drinking alcohol and smoking 4 weeks before surgery
- Take moderate physical exercise 4 weeks before surgery to increase physical endurance
- Start nutritional intervention with oral food supplements at least 2 weeks before surgery and a low-fibre diet 3 days before surgery
- Stop colon cleansing before surgery (no oral laxatives or enema)
- A regular diet up to 6 hours and clear fluids up to 2 hours before surgery, and high carbohydrate food products (400 ml 12.5% maltodextrin) 2 hours before surgery
- Stop administering short- and long-acting sedatives directly before surgery
- Use antithrombotic prevention in the form of properly adjusted compression stockings and low-molecular-weight heparins (LMWH) up to 28 days after surgery
- Administer proper antibiotic prophylaxis [10]
- Prepare the operative site properly
- Use the analgesia protocol for immediate awakening
- Perform epidural administration for traditional surgery
- Regional or local analgesia in laparoscopy and morphine in a pump administered independently by the patient (*patient controlled analgesia* – PCA)

- Prevent postoperative nausea and vomiting *(postoperative nausea and vomiting* – PONV); additional prophylaxis of patients who according to the Apfel score have >1 (variables with assigned points according to the Apfel scale: female sex, non-smoker, opioid intravenous treatment, history of postoperative nausea and vomiting or of motion sickness)
- Minimally invasive surgery (laparoscopy) of longitudinal cut
- Avoid feeding tubes (apart from gastric emptying after intubation)
- Avoid intraoperative hypothermia using heating appliances (electric blankets) and the intraoperative administration of warm infusion liquids
- Well-balanced intraoperative fluid therapy (avoiding intraoperative fluid overload, maintaining normotension with vasopressors)
- Early oral alimentation after surgery (drinking fluids on day zero after regaining conscience) and stop intravenous fluid administration on the first day after surgery)
- Do not leave drains in the peritoneal cavity after surgery
- Remove urinary catheter (on the first/second day after surgery)
- Avoid postoperative bowel obstruction
- Avoid using postoperative opioid treatment; analgesic treatment mainly with local and regional analgesia, non-steroid anti-inflammatory drugs (NSAIDs) and PCA
- Early return to a regular diet: a mixed diet from the first day after surgery, full diet on the second day after surgery; taking oral food supplements from zero day
- Postoperative blood sugar level control and restoring normoglycemia
- Early rehabilitation, mobilisation on day zero

A control group included patients treated simultaneously and of similar age, operated on due to colorectal cancer of similar location, using traditional perioperative plan.

Both groups assessed in terms of sex and age, nutritional status, hospitalisation period (time spent at a hospital after surgery), surgery type, surgical approach and complications during the thirty days after surgery. The results were prepared using the SAS software, University Edition (SAS Institute Inc., Cary, NC, USA). Variables in the statistical analysis used the Mann–Whitney–Wilcoxon test. Statistical significance was determined at <0.05.

## Results

From July 2015 to March 2016, fifteen patients undergoing surgery were observed in a study group and fifteen patients in a control group.

In the study group, women constituted 33.3% and men 66.6%; average age was 64.4 (±16). At the time of the surgery, the nutritional status based on body mass index (BMI) was 25.6 (±5.4) kg/m$^2$ on average. Performed 8 right hemicolectomies and 7 anterior resections of the rectum, 13 of which were laparoscopic. During a 30-day observation period none of the patients required reoperation and repeated hospitalisation at the Department of General Surgery, Military Institute of Medicine.

In the control group, women constituted 40% and men 60%; average age was 70.4 (±6.37). At the time of the surgery, the nutritional status based on body mass index (BMI) was 29.6 (±6) kg/m$^2$ on average. A total of 5 right hemicolectomies, 4 sigmoidectomies, 3 anterior resections of the rectum, 2 left hemicolectomies and 1 colectomy were performed, 8 of which were laparoscopic. During a 30 day observation period, 3 of the patients required reoperation, and 1 patient died due to postoperative complications.

Time spent at a hospital after surgery was 3 days in the study group (Q1:3, Q3:4) and was statistically significantly shorter (p =0.0007) than in the control group, which was 6 days (Q1:5, Q3:16).

## Discussion

The study and control groups were compared in terms of sex, age and BMI.

There were no postoperative complications confirmed in the study group, whereas 3 patients from the control group required reoperation, and 1 of them died. The patient in an early postoperative period developed wound dehiscence, then anastomotic leakage and septic shock as a result of which she died. Due to the abscess wound, the second patient was re-operated on and treated using negative-pressure wound therapy. The third patient developed a strangulated inguinal hernia during the postoperative period; the patient underwent reoperation and small intestine resection.

At present, when the results as well as treatment costs are assessed, it can be said, based on contemporary literature and our own results, that following the ERAS protocol significantly reduces the incidence of intraoperative complications and decreases the hospitalisation period, thus reducing treatment costs and, also, shortening the social dysfunction period of patients even after surgical treatment of advanced colorectal cancers [11-14].

## Conclusions

Perioperative care following the plan based on the ERAS protocol considerably shortened the hospitalisation period and statistically decreased the number of postoperative complications leading to reoperation.

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# Lipid peroxidation, total antioxidant status, and glycemic control in patients with type 2 diabetes mellitus

## Peroksydacja lipidów, całkowity potencjał antyoksydacyjny i kontrola glikemii u pacjentów z cukrzycą typu 2

**Wiesław Piechota,[1] Paweł Krzesiński,[2] Agnieszka Woźniak-Kosek,[1] Magdalena Wojtowicz,[1] Małgorzata Dzierżanowska,[1] Sławomir Literacki[1]**

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**Abstract.** Oxidative stress promotes atherogenesis in diabetes. The aim of our study was to assess whether increased lipid peroxidation and/or antioxidant depletion occurs in diabetic patients, and to establish whether these processes are interrelated and correlated with glycemic control. In 135 patients with type 2 diabetes and 64 non-diabetic controls we determined the lipid peroxidation products in the plasma (LPO), serum total antioxidants (TAS), glycated hemoglobin ($HbA_{Ic}$) fructosamine, glucose, lipids (total cholesterol, triglycerides, and HDL cholesterol) and apolipoproteins A-I and B. LPO (sum of malondialdehyde and 4-hydroxynonenal) was significantly elevated in diabetics (1.34 ±0.51 *vs* 1.02 ±0.37pmol/L; p <0.001). Serum total antioxidant status (TAS) remained unchanged in diabetes. Neither LPO nor TAS correlated with glycated hemoglobin HbAlc, fructosamine, or fasting glucose. No correlation was observed between LPO and TAS. HDL-cholesterol and apolipoprotein A-I were decreased in diabetics. Our results showed increased lipid peroxidation in type 2 diabetes without measurable antioxidant depletion and without any association with glycemic control. Furthermore, the results suggest that peroxidation and glycation may operate independently as atherosclerosis promoters in diabetes.
**Keywords:** lipid peroxidation, glycation, antioxidants, diabetes

**Streszczenie.** Stres oksydacyjny sprzyja aterogenezie w cukrzycy. Celem badania byto ustalenie, czy u pacjentów z cukrzycą typu 2 występuje nasilona peroksydacja lipidów osocza i/lub zmniejszenie stężenia antyoksydantów w surowicy oraz czy procesy te są wzajemnie powiązane i skorelowane z kontrolą glikemii. U 135 pacjentów z cukrzycą typu 2 i 64 osób bez cukrzycy oznaczono produkty peroksydacji lipidów w osoczu (LPO), całkowity potencjał antyoksydacyjny (TAS) w surowicy, odsetek hemoglobiny glikowanej ($HbA_{Ic}$), stężenie fruktozaminy, glukozy, lipidy (cholesterolu całkowitego, triglicerydów i HDL-cholesterolu) oraz apolipoproteiny A-I i B. LPO (suma malondialdehydu i 4-hydroksynonenalu) były znacząco zwiększone u pacjentów z cukrzycą (1,34 ±0,51 *vs* 1,02 ±0,37µmol/l; p <0,001). Całkowity potencjał antyoksydacyjny osocza (TAS) nie ulegał zmianie w cukrzycy. Ani LPO, ani TAS nie były skorelowane z odsetkiem $HbA_{Ic}$ (%) oraz stężeniami fruktozaminy i glukozy na czczo. Nie zaobserwowano korelacji między LPO i TAS. Stężenia cholesterolu HDL i apolipoproteiny A-I były zmniejszone u chorych na cukrzycę. Osoczowe stężenie produktów peroksydacji lipidów w cukrzycy typu 2 było zwiększone, ale bez wpływu kontroli glikemii i całkowitego potencjału antyoksydacyjnego, który był niezmieniony. Uzyskane wyniki wykazują zwiększenie peroksydacji lipidów w cukrzycy i sugerują, iż peroksydacja i glikacja mogą działać niezależnie jako czynniki sprzyjające rozwojowi miażdżycy w cukrzycy.
**Słowa kluczowe:** peroksydacja lipidów, glikacja, antyoksydanty, cukrzyca

Delivered: 31/05/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96(1): 14-18


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## Introduction

Atherogenesis is distinctly accelerated in people with type 2 diabetes [1-3]. This fact cannot be fully explained by the quantitative lipid changes occurring in diabetes because they are frequently not strongly pronounced [4]. Therefore, it has been postulated that qualitative lipoprotein changes occur, namely glycation and peroxidation, which render lipoproteins more atherogenic [5, 6]. Oxidized lipoproteins, especially oxidized LDL, are known to have a variety of biological actions promoting atherosclerosis [7]. They exert a cytotoxic effect on endothelium and they are easily taken up by macrophages [8], which triggers a complex cellular response, mediated by cytokines and growth regulatory factors, leading to plaque formation [9]. In diabetics the production of lipid peroxides may be increased due to the oxidative stress of diabetes [10]. The increased generation of reactive oxygen species seems to be connected with the glycation of proteins and glucose autooxidation [11]. Thus the intensity of lipid peroxidation may correlate with the degree of glycemic control.

Malonyldialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are aldehydic lipid peroxidation products. Their concentration in the plasma is regarded as a measure of lipid peroxidation intensity [12, 13]. Plasma levels of lipid peroxidation products in diabetics have been reported to be elevated [14, 15], or normal [16]. Some authors found MDA concentrations in the plasma to be associated with glycated hemoglobin [17], while others did not confirm any correlation between peroxidation and glycation [18, 19]. These discrepancies may be attributed to differences in plasma antioxidant levels in the study groups.

High levels of antioxidants may prevent lipids from peroxidation by scavenging free oxygen radicals. This process may in turn lead to depletion of the antioxidants present in the plasma. A number of antioxidant scavengers, including glutathione, ascorbate [20] and a-tocopherol are reduced in the plasma of diabetics [21].

The aim of our study was to assess whether there was evidence of increased lipid peroxidation and/or antioxidant depletion in diabetics, and to establish whether these processes, if they occur, were interrelated and, in addition, correlated with glycemic control.

## Materials and methods

A total of 135 type 2 diabetes subjects and 64 non-diabetic control subjects were studied. There were 80 males and 55 females in the diabetic group. The mean age of the diabetic group was 62.5 ±9.6 years (mean and standard deviation), range 39-83 years. The duration of diabetes was on average 9 years (1-39 years). A total of 88 were treated with oral hypoglycemic agents, 37 with insulin, 5 with the oral agent and insulin, and 5 with diet alone. There were 17 smokers in the diabetic group. The control group consisted of 64 apparently healthy subjects undergoing routine yearly medical check-ups, without diabetes according to the World Health Organization criteria of 2 hours with plasma glucose levels below 11.1 mmol/l. There were 38 males and 26 females in this group. The members of the control group were also matched for body mass index; their age (59.9 ±8.0 years) was not significantly different from that of the diabetic group.

Blood samples were collected after an overnight fast, by venipuncture into glass tubes containing K3ED-TA and tubes without anticoagulant. Small volumes of the K3EDTA samples were used for glycated hemoglobin determination, and all the remaining samples were centrifuged at 2000 g for 15 minutes at a temperature of 4-80°C to obtain plasma and serum.

Lipid peroxidation products (LPO) were determined in the plasma by a method described by Esterbauer and Cheeseman [22] using the Bioxytech® LPO 586 reagent set from Oxis International, Inc., Portland, USA. The procedure chosen determined both malonylodialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Serum antioxidants were determined using the Total Antioxidant Status (TAS) reagent set from Randox, Ardmore, UK. In this assay, ABTS® (2,2'-azino-di-/3-ethylbenz-thiazoline sulphonate) is incubated with a peroxidase (metmyoglobin) and H202 to produce the radical cation ABTS® +, which has a relatively stable blue-green colour measured at 600 nm. Its production is suppressed by antioxidants in the added sample, proportionally to their concentration. Total cholesterol, triglycerides, and glucose were measured by standard enzymatic methods with an Integra automatic analyzer (Roche Diagnostics, Basel, Switzerland). The same analyzer and Roche cassette reagents were used to assay the glycated hemoglobin ($HbA_{1c}$ [immunoturbidimetric method]), fructosamine, HDL-cholesterol (direct method), apoA-l and apoB (immunoturbidimetry). LDL- cholesterol was calculated according to the Friedewald formula [23].

The data were expressed as means and standard deviations. The Mann-Whitney U test was used to assess differences between the two groups. Spearman correlation coefficients were calculated for selected variables. The statistical analyses were performed using Statistica for Windows, Release 12 (StatSoft, Inc, Tulsa, OK, USA).

**Table 1. Clinical and biochemical characteristics of the type 2 diabetes subjects and control subjects**
**Tabela 1. Kliniczna i biochemiczna charakterystyka pacjentów z cukrzycą typu 2 i grupy kontrolnej**

| Variables | Controls (n = 64) mean ±2SD | Patients (n = 135) mean ±2SD | Statistical significance |
|---|---|---|---|
| age (years) | 59.9 ±8.4 | 62.5 ±9.6 | N.S. |
| BMI | 26.9 ±2.9 | 27.6 ±4.0 | N.S. |
| duration of diabetes (years) | | 9.0 (1-39)* | |
| $HbA_{1c}$ (%) | 5.37 ±0.24 | 7.64 ±0.86 | <0.001 |
| fructosamine (µmol/L) | 219 ±17 | 289 ±67 | <0.001 |
| fasting glucose (mmol/L) | 4.96 ±0.51 | 8.00 ±3.46 | <0.001 |
| MDA +HNE (µmol/L) | 1.02 ±0.37 | 1.34 ±0.51 | <0.001 |
| TAS (mmol/L) | 1.58 ±0.10 | 1.60 ±0.15 | N.S. |
| total cholesterol (mmol/L) | 5.16 ±0.87 | 5.32 ±1.12 | N.S. |
| triglycerides (mmol/L) | 1.62 ±0.84 | 1.71 ±0.87 | N.S. |
| LDL-cholesterol (mmol/L) | 2.90 ±0.85 | 3.15 ±0.95 | N.S. |
| HDL-cholesterol (mmol/L) | 1.52 ±0.39 | 1.39 ±0.37 | <0.05 |
| ApoA-I (g/L) | 1.53 ±0.25 | 1.44 ±0.27 | <0.05 |
| ApoB (g/L) | 1.07 ±0.20 | 1.09 ±0.21 | N.S. |

* Mean and range
N.S. - not significant

## Results

The clinical and biochemical characteristics of the study groups are presented in Table 1. The groups were matched for age, sex and BMI. The degree of glycemic control in diabetic patients was reflected by the levels of HbAlc, fructosamine and fasting glucose. At least half of the diabetic patients were not satisfactorily controlled according to the criteria of the International Diabetes Federation [24].

Lipid peroxidation products, measured as a sum of MDA and HNE, were distinctly and significantly elevated in the diabetic subjects. Total antioxidant status (TAS) remained unchanged. Neither lipid peroxidation products nor TAS correlated with the indices of glycemic control, i.e. $HbA_{1c}$, fructosamine, and glucose. No correlation was observed between MDA + HNE and TAS. There were no statistically significant differences in lipid peroxidation products and TAS between men and women with diabetes (1.37 ±0.57 *vs* 1.30 ±0.42 µmol/L, and 1.59 ±0.16 *vs* 1.61 ±0.14 mmol/L respectively). TAS positively correlated with the subjects' age (Spearman r =0.254; p <0.01) whereas MDA + HNE did not show any association with age. Neither lipid peroxidation products nor TAS correlated with BMI, total cholesterol, LDL-cholesterol, triglycerides, HDL-cholesterol, apoB, or apoA-I in the diabetic group. There were no significant differences in lipid peroxidation products and TAS between diabetic smokers and diabetic non-smokers (1.26 ±0.47 *vs* 1.36 ±0.53 µmol/L, and 1.60 ±0.16 *vs* 1.59 ±0.15 mmol/L respectively).

Total cholesterol, LDL-cholesterol, triglycerides and apolipoprotein B concentrations did not differ significantly in comparison to the control group. HDL-cholesterol and apoA-I were reduced in the diabetic patient group. Among the lipoprotein constituents triglycerides correlated with the degree of glycemic control indices: fasting glucose, and $HbA_{1c}$; apoB and cholesterol correlated significantly only with glucose (Table 2).

## Discussion

The elevated level of lipid peroxidation products in the plasma of diabetic patients indicate that increased lipid peroxidation is a biochemical abnormality of type 2 diabetes. Our finding is in agreement with most earlier reports [14, 15, 25-27]. However, some authors report the opposite results [16], perhaps due to different methods used to determine the products of lipid peroxidation [12]. More recently, elevated MDA levels were also reported in patients with metabolic syndrome, as well as increased triglyceride and glucose levels [28].

We did not find significant correlations between the products of lipid peroxidation and the levels of lipids and apolipoproteins B and A-I in the serum. It seems that lipid peroxidation may depend more on the intensity of reactive oxygen species generation than on availability of the lipid "substrate", which is usually abundant. Our finding confirms that of Nourooz-Sadeh et al. [29] but not that of Nacitarhan et al., who reported higher MDA levels in diabetics with hyperlipidemia [30].

**Table 2. Spearman correlation coefficients between biochemical variables in subjects with type 2 diabetes**
**Tabela 2. Współczynniki korelacji Spearmana pomiędzy parametrami biochemicznymi u pacjentów z cukrzycą typu 2**

| Variables | $HbA_{1c}$ | Fructosamine | Fasting glucose |
|---|---|---|---|
| MDA+HNE | 0.057 | 0.054 | 0.029 |
| TAS | 0.145 | 0.046 | 0.078 |
| total cholesterol | 0.097 | 0.103 | 0.184* |
| triglycerides | 0.252" | 0.016 | 0.325*" |
| LDL-cholesterol | 0.070 | 0.092 | 0.106 |
| HDL-cholesterol | 0.028 | 0.016 | 0.107 |
| ApoA-I | 0.064 | 0.019 | 0.003 |
| ApoB | 0.110 | 0.052 | 0.287*" |
| *p <0.05 | | | |
| **p <0.01 | | | |
| ***p <0.001 | | | |

Peroxidation may occur in the blood but it is assumed that most of it takes place in the subendothelial space with the active participation of macrophages and other cells of the artery wall [7, 31, 32]. The normal total antioxidant status (TAS) found in the serum of the diabetic subjects suggests that blood may not be the most favorable milieu for intensive lipid peroxidation. The normal antioxidant activity of the serum in diabetics was reported earlier by Ozdemirler et al. [33]. Abnormally low antioxidant status were found by other researchers in people with type 2 diabetes [34, 35]. These discrepancies may have been a result of putative differences in the antioxidant content of diets in the studied populations. The lack of a significant correlation between TAS and the concentration of lipid peroxidation products in the subjects suggest that plasma antioxidants may be sufficient to counterbalance any free radical activity remaining without measurable change.

Production of lipid peroxides is assumed to be favored by glycation [36]. However, we were not able to show any correlation between MDA and HNE and glycated hemoglobin, fructosamine and fasting glucose. One of the reasons behind this may be the fact that MDA and HNE are the end products of a very extensive oxidative lipoprotein modification [37] and perhaps the production of less modified lipoproteins would be more dependent on glycation [32]. Although contrary to the expectation, there was no apparent dependence of lipid peroxides levels on glycemic control. Perhaps there is a low threshold, above which further glycation of lipoproteins does not influence their oxidation. Such a threshold may result from the permeability of endothelium. Perhaps the size of the modified lipoprotein is more significant than the mere fact of modification. The percentage of small LDL particles is raised in diabetes [38]. These particles are known to be more susceptible to oxidation [39], probably due to their easier penetration into the subendothelial space [40]. Lipid peroxidation is a complex and multifactorial process [41], while glycation is mainly dependent on hyperglycemia. Recently Harmon et al. have not shown any statistically significant correlation between oxidised LDL (oxLDL) and HbA1c in a Navajo Indian population with a very high frequency of diabetes [42].

The only lipid change in the diabetes subjects was a significant reduction in HDL cholesterol, which is a frequent phenomenon in diabetes. A low level of HDL cholesterol is sometimes ascribed to functional disturbances resulting from apoA-I glycation and consequent impairment of HDL-associated enzyme activities, which leads to the reverse cholesterol transport defect [43]. Here the disturbance did not appear to be functional because the level of apo-AI was also reduced.

Our results showed poor association between the levels of the lipoprotein constituents and glycemic control in the diabetic subjects. Only triglicerides, apoB, and total cholesterol correlated significantly with the degree of glycemic control (fasting glucose or HbA1c). Glycation of apoB in LDL was postulated to reduce its catabolism through the classical LDL-receptor causing LDL retention and increasing the probability of its oxidation in the blood [44, 45]. In diabetes the percentage of glycated apoB is higher than in normoglycemia [46]. The retention of apoB may not necessarily occur due to the alternative route of glycated LDL catabolism, namely the scavenger receptor pathway [47].

In summary, we have shown that type 2 diabetes mellitus is associated with increased lipid peroxidation products in the plasma and, at the same time, normal total antioxidants in the serum. Lipid peroxidation did not correlate with total antioxidant status or the degree of metabolic control. There is a possibility that lipoprotein peroxidation, glycation and the changes in their composition may operate more or less independently as atherosclerosis promoters.

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# Polymorphism in amino acid position 571 of the TOP2A gene in patients with ovarian cancer treated with PLD

## Ocena polimorfizmu pojedynczego nukleotydu w pozycji 571 aminokwasu genu *TOP2A* u pacjentek z rakiem jajnika leczonych liposomalną doksorubicyną

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**Abstract.** Pegylated Liposomal Doxorubicin (PLD) is one of the basic chemotherapeutics used in the treatment of ovarian cancer in second and subsequent line therapies. Seeking appropriate prognostic and predictive factors to select the most effective treatment method is essential. The aim of this study was to evaluate the predictive and prognostic significance of polymorphism in amino acid position 571 of the T0P2A (topoisomerase II alpha) gene. A series of 67 patients with diagnosed ovarian cancer, treated with PLD at the Oncology Clinical Hospital of the Military Medical Institute between March 2006 and December 2014, who fulfilled the study criteria, underwent retrospective analysis. Upon careful screening of the NCBI SNP database (http://www.ncbi.nlm.nih.gov), the authors found that the rs144 622 532 gene variant was of great significance. The median PFS was 11.58 months, while the median OS was 18.88 months. No polymorphism was found in any of the paraffin blocks from the patients. All the patients proved to be homozygous. The lack of the polymorphism in amino acid position 571 of the T0P2A gene had no predictive or prognostic values in patients with ovarian cancer treated with PLD.
**Key words:** Liposomal Doxorubicin, ovarian cancer, single nucleotide polymorphism

**Streszczenie.** Wstęp. Pegylowana liposomalną doksorubicyną (PLD) jest jednym z podstawowych chemioterapeutyków stosowanych w leczeniu chorych na raka jajnika w drugiej lub kolejnej linii. Poszukiwanie czynników prognostycznych i predykcyjnych w celu doboru najskuteczniejszego sposobu leczenia jest niezbędne. Celem badania była ocena znaczenia predykcyjnego i prognostycznego polimorfizmu pojedynczego nukleotydu w pozycji 571 aminokwasu genu *T0P2A* (topoizomerazy IIα). Materiał i metoda. Retrospektywnej analizie poddano kolejnych 67 chorych z rozpoznanym rakiem jajnika leczonych PLD w Klinice Onkologii WIM w okresie pomiędzy marcem 2006 a grudniem 2014 roku, spełniające kryteria włączenia do badania. Po dokładnym przejrzeniu bazy NCBI SNP (www.ncbi.nlm.nih.gov) uznaliśmy, że największe znaczenie ma wariant genu rs144622532. Mediana czasu wolnego od progresji choroby wynosiła 11,58 miesiąca, zaś czasu całkowitego przeżycia 18,88 miesiąca. W żadnym z bloczków pochodzących od chorych nie potwierdzono polimorfizmu. Wszystkie pacjentki okazały się homozygotami. Wnioski. Brak polimorfizmu w pozycji 571 aminokwasu dla genu *T0P2A* nie ma żadnej wartości predykcyjnej ani prognostycznej u chorych z rakiem jajnika leczonych PLD.
**Słowa kluczowe:** polimorfizm pojedynczego nukleotydu, rak jajnika, liposomalną doksorubicyną

Delivered: 05/06/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 19-24


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## Introduction

Liposomal doxorubicin is an anthracycline antibiotic. The mechanism of anthracycline antibiotic activity consists in inhibiting topoisomerase I and II. These medications play an important role in the treatment of neoplasms, especially in neoadjuvant and adjuvant chemotherapies for breast cancer as well as in palliative treatment following generalisation of neoplastic disease. The results of numerous clinical studies have demonstrated the effectiveness of anthracycline antibiotics in the therapy of gynaecological neoplasms [1]. Pegylated liposomal doxorubicin is the basic chemotherapeutic used as a second line treatment for ovarian cancer. It appears that a certain group of patients may benefit from this type of therapy more than others. It is necessary to determine the predictive and prognostic factors for this treatment to precisely distinguish the groups of patients for whom it is the most advantageous. Controlled clinical trials involving 474-672 [2-4] patients with ovarian cancer compared the use of second-line PLD plus topotecan or PLD plus carboplatin versus paclitaxel plus carboplatin (Calypso), or the combination of PLD and trabectedin vs PLD in monotherapy. The study comparing PLD and topotecan demonstrated that overall survival was 3 months longer in the PLD group, which was statistically significant. Significant benefits of this treatment were recorded in patients with ovarian cancer responding to platinum derivatives. However, is has not been established which factor determines the response to PLD treatment in the course of ovarian cancer. Individual studies regarding breast cancer reveal a correspondence between the deletion in the *TOP2A* gene and the response to treatment.

Topoisomerase II, similarly to topoisomerase I, has many important functions in the processes of transcription, replication, recombination and detection of DNA damage. It is found in all living organisms with a double-helical DNA, and its role is to reduce the torsional stress during unwinding of the double DNA helix.

Expression of topoisomerase IIα (topo IIα) is the highest in the G2-M phase of the cell cycle. Doxorubicin, by creating complexes with topo IIα and DNA, prevents re-binding of the separated DNA strands, thus contributing to apoptosis of the proliferating cells [5]. Experience resulting from studies on breast cancer indicates a significant role of topo IIα in the treatment based on anthracycline antibiotics.

Referring to previous publications, Jarvien et al. [6] demonstrated *in vitro* that sensitivity to topoisomerase inhibitors depends on the degree of *TOP2A* expression in the neoplastic breast cancer cells. The sensitivity to topoisomerase IIα inhibitors is directly proportional to the topo IIα content in the cells. In breast cancer, amplification of the *TOP2A* gene results in overexpression of the protein, and increased sensitivity to topoisomerase inhibitors, whereas deletion is associated with reduced expression of the protein, which generates primary resistance to topoisomerase IIα inhibitors.

Di Leo et al. performed a meta-analysis of five large-scale studies [7]. One of them was conducted in Canada, and the remaining four in Western Europe. Their aim was to establish the predictive value of gene amplification for HER2 in 3,452 patents with breast cancer, and the predictive value for *TOP2A* in 3,102 patients with breast cancer treated with anthracyclines or a CMF regimen (cyclophosphamide, methotrexate and 5-fluorouracil). The authors divided the patients into four groups, according to the presence or absence of steroid and HER2 receptors. In patients with *TOP2A* amplification or deletion, a clear advantage of CMF treatment over anthracyclines was observed, both regarding the time to disease progression, and overall survival time.

The results of clinical studies on ovarian cancer conducted to determine the role of topoisomerase IIα are based on smaller groups of subjects [8, 9]. Chekerov et al. [10] performed an immunohistochemical analysis of pathological material from 62 patients with ovarian cancer, including 34 patients who had not received any chemotherapeutic treatment. The authors obtained varied *TOP2A* expression in the stromal and central part of the tumour, depending on the presence or absence of previous chemotherapeutic treatment. Higher *TOP2A* overexpression in the previously treated patients was observed in the stromal tumour cells, whereas in the subjects who had not received chemotherapy, in the central part of the tumour.

The above observations inspired us to seed a relationship between the expression of topoisomerase IIα and the response to PLD therapy. In this study we focused only on the significance of deletion, determined through the assessment of polymorphism at the amino acid position 571 of the *TOP2A* gene. The importance of *TOP2A* amplification and the expression of topo IIα will be presented in future studies.

Genetic polymorphism is a repetitive phenomenon, consisting of the presence of different variants of the same gene, chromosome and phenotype [11, 12]. Single nucleotide polymorphism (SNP) consists of a substitution of one nucleotide for another. In the human genome, it affects on average 1 in 1,000 base-pairs. Polymorphisms may be synonymous ("silent"), i.e. the change in the nucleotide does not affect the amino acid sequence of proteins, and non-synonymous, changing the amino acid sequence of proteins. In the human genome, non-synonymous single nucleotide polymorphisms nsSNPs constitute about 10% of all coding polymorphisms [13]. Approximately 30% of

nsSNPs have further consequences. Polymorphisms are associated with insertions, deletions, conversions or re-arrangements.

Molecular changes in the form of genetic polymorphism may potentially be as important as prognostic and/or predictive factors.

Belotte et al. [14] analysed seven different SNPs in 143 women aged 19-80 years old. The control group comprised 94 women, including 18 healthy volunteers. A total of 53 women were not carriers of the BRCA1/2 suppressor gene (breast and ovarian cancer susceptibility gene 1/2) mutation, although due to the family history they were considered at high-risk of ovarian cancer, while 23 women were carriers of the BRCA1/2 mutation, and also had a family history of the disease. In the studied group 49 patients had ovarian cancer; 13 of them were *BRCA1/2* mutation carriers, 34 were not, and in 2 subjects the test results for the mutation were not available. The results of SNP analysis indicate that catalase (CAT) polymorphism, associated with reduced catalase activity, is a predictor of shorter survival. The difference in the median was 48 months, and its statistical significance was confirmed. For other polymorphisms the differences were not as significant. Ahn et al. demonstrated that the *33CC* variant of the CAT gene (homozygous CC), associated with increased catalase activity, reduces the risk of breast cancer by 17% compared to variants 330CT (heterozygous CT) and 330TT (homozygous TT) [15].

## Aim of the study

The aim of the study was to determine the usefulness of a single nucleotide polymorphism in topoisomerase IIα at amino acid position 571 [16] in patients with ovarian cancer treated with PLD.

## Material and methods

### Patient characteristics

The retrospective study involved a group of 67 consecutive patients treated at the Military Institute of Medicine due to ovarian cancer or primary peritoneal cancer, who received PLD in monotherapy as a first, third, fourth or further line of treatment in the years 2006-2014. Only the patients whose tumour tissue samples were available in the Pathomorphology Division of the Military Institute of Medicine were eligible for the study. The group characteristics are presented in Table 1. The study was approved by the Bioethics Committee of the Military Institute of Medicine.

In a group of 67 patients, the first-line therapy in 60 cases was paclitaxel with platinum, 4 patients received carboplatin in monotherapy, 1 patient was treated with TEC chemotherapy (paclitaxel with epirubicin and cisplatin), 1 received GC chemotherapy (gemcitabine and cisplatin), and for 1 patient the information about the first-line treatment could not be found. PLD was used as the second-line therapy in 27 patients, as the third-line treatment in 25 patients, while the other 15 patients received PLD in further therapy.

### Statistical analysis

The statistical analysis was conducted with the use of Statistica for Windows version 10.0 by StatSoft, and it involved the analysis of progression-free survival (PFS) and overall survival (OS) with the Kaplan-Meier method, as well as univariate analyses to determine the effect of the examined variables on the progression free survival and the overall survival. A log-rank test was used to select a preliminary list of significant prognostic factors.

### Detection of polymorphism

In order to detect a single nucleotide polymorphism, pieces measuring 3 x 3 mm were placed in Eppendorf tubes, then TE buffer, SDS and proteinase K were added and the mixture was incubated for 1 hour at 56°C and 900 revolutions per minute. Next, the incubation temperature was changed to 95°C for another 15 minutes at 900 rpm. Subsequently, the buffer was added to the lysis and incubated for 20 minutes at 70°C and 900 rpm. The obtained material was centrifuged and placed in a new tube. In the next stage, magnetic beads and isopropanol were added, and the mixture was incubated for 10 minutes at room temperature and 1,000 rpm. The tubes were placed in magnetic tripods, so that the magnetic beads covered in the material were on the tube walls. The fluid was removed from the tubes, and the beads were rinsed three times with rinsing buffer. After the final rinsing, they were left to dry. Subsequently, the buffer was added to the elution and incubated for 5 minutes at 70°C and 700 rpm. The obtained liquid containing DNA was poured in a new tube for an RT-PCR (real-time polymerase chain reaction) assay. On a special plate, the diluted standard solutions, negative control and tested material were place in their proper wells. The plate was placed in the RT-PCR device, and after 180 minutes the results were taken from the standard curve and the samples. Based on the RT-PCR results, the tested material was diluted, a plate was prepared, as well as a reactive mixture containing a marked rs144622532 *TOP2A* probe, and the mixture was pipetted onto the plate. The tested samples, positive control and negative control were placed in their proper wells, and the plate was placed in the programmed device. After the reaction, the readings were obtained, including numerical data and diagrams. The presence of normal homozygotes (CC) was considered to demonstrate a lack of polymorphism. Any result for polymorphism confirms the absence of deletion in the given region.

**Table 1. Study group characteristics**
**Tabela 1. Charakterystyka badanej grupy**

| | |
|---|---|
| Number of patients | n = 67 |
| Age, median, range (years) | 67 (33-82) |
| General status acc. to WHO | |
| 0 | 21% (14/67) |
| 1 | 72% (48/67) |
| 2 | 7% (5/67) |
| Platinum sensitivity | |
| Sensitive | 70% (47/67) |
| Resistant | 30% (20/67) |
| Histological grade | |
| 1 | 3% (2/67) |
| 2 | 30% (20/67) |
| 3 | 31% (21/67) |
| Unspecified | 36% (24/67) |
| FIGO stage | |
| I | 6% (4/67) |
| II | 0% |
| III | 79% (53/67) |
| IV | 15% (10/67) |
| Histological type | |
| Serous | 57% (38/67) |
| Endometrioid | 24% (16/67) |
| Mucinous | 3% (2/67) |
| Clear cell | 3% (2/67) |
| Unspecified | 13% (9/67) |
| Scope of cytoreductive surgery | |
| Exploratory laparotomy | 4.5% (3/67) |
| Primary optimal (<1 cm) | 31.5% (21/67) |
| Delayed optimal (<1 cm) | 24% (16/67) |
| Primary suboptimal (≥1 cm) | 28% (19/67) |
| Delayed suboptimal (≥1 cm) | 12% (8/67) |
| Histological grade | |
| 1 | 3% (2/67) |
| 2 | 30% (20/67) |
| 3 | 31% (21/67) |
| Unspecified | 36% (24/67) |
| Previous therapy with topotecan | |
| Yes | 66% (44/67) |
| No | 34% (23/67) |
| Previous therapy with gemcitabine Yes | 78% (52/67) |
| No | 22% (15/67) |
| Line of PLD chemotherapy | |
| 2 | 40% (27/67) |
| 3 | 37.5% (25/67) |
| 4 | 13.5% (9/67) |
| 5 | 6% (4/67) |
| 6 | 1.5% (1/67) |
| 7 | 1.5% (1/67) |

## Results

This study examined the presence or absence of polymorphism at amino acid position 571 of topoisomerase IIα. Normal homozygotes (genotype CC) were found in all 67 (100%) patients (genotype CC) (Tab. 2, Fig. 1); so no polymorphism at amino acid position 571 of the *TOP2A* gene was found. Therefore, it was impossible to determine whether the presence or absence of the polymorphism was associated with the response to therapy with liposomal doxorubicin. The absence of polymorphism also excluded the existence of deletion in a given section.

Out of 67 patients treated with liposomal doxorubicin, the best response to caelyx, according to RECIST 1.1 criteria, and based on an imaging test, was a partial response in 12 patients, a total response in 1 patient, disease stabilisation in 30 patients, disease progression in 20 patients, while no data were available in 5 patients regarding the best response. The median progression-free survival for the entire group was 11.58, and the overall survival was 18.88.

## Discussion

In the presented study no single nucleotide polymorphism in *TOP2A* was found. This result may be due to the actual absence of this polymorphism, or it may result from using only one molecular probe for the polymorphism. The lack of relationship between the polymorphisms in the topo IIα-coding gene and the effectiveness of PLD-based chemotherapy indicates that the mechanisms generating resistance to the therapy or responsible for its effectiveness may be entirely different.

However, there are studies suggesting that the role of polymorphisms is significant. Krivak et al. [17] demonstrated that in patients with ovarian cancer who presented genotype CC in codon C8092A of the *ERCC1* gene the progression-free survival (PFS) and overall survival (OS) were longer than in patients with other genomes.

Following this study, Moxley et al. [18] decided to examine the presence and effect of polymorphisms in the *ERCC1* and *MMS19* genes in the tissues of patients with platinum-sensitive and platinum-resistant ovarian cancer, stage III and IV. They obtained material from 107 patients, including 45 subjects sensitive to platinum derivatives, and 62 with platinum-resistant disease. In codone 18 of the *ERCC1* gene 34 patients presented genotype TT, 39 had genotype TC, and 25 demonstrated genotype CC. The shortest survival (both PFS and OS) was reported in the group of patients with genotype CC, but the statistical significance was not confirmed. In codone C8092A of the *ERCC1* gene the shortest survival was also observed in patients with genotype CC, and the survival of patients with genotype AA was significantly longer: OS by nearly 14 months, and PFS by over 15 months, with p = 0.06.

**Table 2. Results. Incidence of SNP**
**Tabela 2. Wyniki. Częstość występowania SNP**

| Total number of patients | Number of CC homozygotes | Percent |
|---|---|---|
| 67 | 67 | 100 |

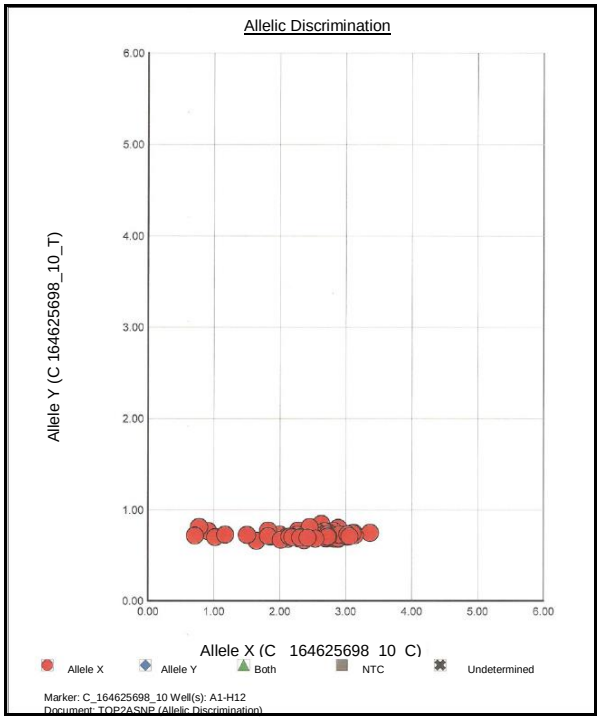


**Figure 1.** Allelic (CC homozygotes) discrimination
**Rycina 1.** Rozkład występowania alleli (homozygoty CC)

In the *MMS19* gene three different polymorphisms were studied initially, but eventually the study focused on two of them, as no homozygote was found in the third one. In rs 2236575 SNP no differences were observed in PFS and OS between homozygotes and heterozygotes, and the study was not statistically significant, while in rs872106 SNP the PFS was significantly longer in the groups with genotype GG, compared to CG or CC (statistically significant, without differences in OS).

Recently many studies have focused on polymorphisms in neoplastic diseases, and the relationships between their presence and the development of a neoplasm, as well as their treatment.

Following the reports that polymorphism in the MiR-196a-2 locus plays an important role in the neoplasms of the lungs, breast, oesophagus and stomach, Zhi-Shuan Song et al. decided to examine its importance in ovarian cancer. The study involved 479 patients operated on due to ovarian cancer, and a control group of 431 healthy women without a genetic burden. The PCR method and rs11 614913 probe were used in the study. Genotype TT was found in 111 patients, genotype CT in 247, genotype CC in 121 patients, whereas in the control group the values were 142, 203 and 86, respectively. Genotypes CT and CC were found more often in the studied group compared to the control. The risk of ovarian cancer is considerably higher in patients with genotype CC than in those with genotype GT or TT.

The above studies present strong indications that the presence of polymorphism may significantly affect the probability of developing a neoplasm.

There are publications demonstrating that the presence of a given polymorphism may determine the response to antineoplastic treatment in different neoplasms. In non-small cell lung cancer the analysis of MDR1 polymorphisms revealed that three out of the six selected haplotypes are responsible for increased resistance to cisplatin (the basic chemotherapeutic agent used to treat this tumour), and that they are related with an increased risk of gastro-intestinal complications compared to other variants [19].

The theories concerning the polymorphism of *ERCC1* gene vary. Two SNP of this gene were described. One study demonstrated that the presence of at least one of them corresponds to a reduced amount of protein and a longer survival for patients with non-small cell lung cancer (NSCLC) treated with cisplatin [20], whereas another study revealed shorter survival in this group of patients [21].

There is no one standard management for second-line treatment of patients with platinum-resistant ovarian cancer, and the third-line treatment of patients with platinum-sensitive ovarian cancer. It appears that in the studies comparing PLD and topotecan or other medicines, patients benefit from the PLD treatment. A therapy with PLD could be further justified if the predictive factors for the response to the treatment were known, allowing one to select the appropriate group of patients for this therapy.

It appears that in breast cancer the overexpression of topoisomerase IIα is an important factor determining the sensitivity to anthracyclines, especially with concurrent amplification of *HER2* gene. In breast cancer the role of *TOP2A* gene deletion is also described (although not extensively), and it may be indirectly caused by SNPs.

## Conclusions

Single nucleotide polymorphism at amino acid position 571 was not found in any of the 67 patients with ovarian cancer, thus its importance cannot be determined. However, it should be emphasised that testing gene polymorphisms with the use of a single probe is associated with a high risk of error, which could explain the lack of a successful outcome in this study.

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# Influence of antimicrobial prophylaxis on the incidence and course of infectious complications in haematopoietic stem cell transplant patients - comparison of rifaximin and ciprofloxacin

## Wpływ profilaktyki przeciwbakteryjnej na częstość występowania i przebieg powikłań infekcyjnych u chorych leczonych przeszczepieniem komórek krwiotwórczych - ocena porównawcza rifaksyminy i cyprofloksacyny

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**Abstract.** Haematopoietic stem cell transplantation is an effective therapy in many haematological diseases. The objective of the study was to compare the influence of antimicrobial prophylaxis with unabsorbable rifaximin and systemic ciprofloxacin on infectious complications in hematopoietic stem cell transplant patients. As an antibiotic prophylaxis, ciprofloxacin was given to 40 patients, and rifaximin to 31. Frequency of fever episodes, bacteremia, sepsis, pneumonia, fungemia, number of antibiotics used and duration of their use, frequency of bacterial resistance to ciprofloxacin and presence of multidrug resistant bacteria were compared. A significant reduction in the duration of antibiotic treatment was observed in the ciprofloxacin group (7 *vs* 10 days). With bacteremia, the rate of resistance to ciprofloxacin was 30% in the rifaximin group and 100% in the ciprofloxacin group. Multidrug resistant bacteria were reported more often in the ciprofloxacin group. The other parameters of infectious complications were comparable in both groups. The efficacy of rifaximin and ciprofloxacin in the prophylaxis of infectious complications seems to be comparable in the study group. The high resistance to ciprofloxacin in the study group could reduce the preventive effect of prophylactic ciprofloxacin.
**Key words:** antimicrobial prophylaxis, ciprofloxacin, hematopoietic stem cell transplantation, rifaximin

**Streszczenie.** Wstęp. Przeszczepienie komórek krwiotwórczych jest procedurą leczniczą skuteczną w wielu chorobach hematologicznych. Cel. Celem pracy było porównanie wpływu profilaktyki przeciwbakteryjnej niewchłanialną z przewodu pokarmowego rifaksyminą i działającą ogólnoustrojowo cyprofloksacyną na powikłania infekcyjne u chorych leczonych przeszczepieniem komórek krwiotwórczych. Materiał i metody. 40 chorych otrzymało cyprofloksacynę, a 31 rifaksyminę w profilaktyce przeciwbakteryjnej. Porównano częstość występowania gorączki, bakteriemii, posocznicy, zapalenia płuc, fungemii, liczbę stosowanych antybiotyków i czas ich stosowania, częstość występowania oporności na cyprofloksacynę oraz obecności szczepów wielolekoopornych. Wyniki. W grupie z cyprofloksacyną istotnie krócej stosowano antybiotyki (7 vs 10 dni). W przypadku bakteriemii w grupie z ciprofloksacyną stwierdzono 100% oporność na ciprofloksacynę, a w grupie z rifaksyminą - 30%. Bakterie wielolekooporne stwierdzano częściej w grupie z cyprofloksacyną. Pozostałe oceniane parametry były w obu grupach porównywalne. Wnioski. Skuteczność rifaksyminy i cyprofloksacyny w profilaktyce powikłań infekcyjnych w ocenianej grupie chorych wydaje się porównywalna. Duża oporność bakterii na cyprofloksacynę u badanych osób mogła być czynnikiem ograniczającym skuteczność profilaktyki cyprofloksacyną.
**Słowa kluczowe:** przeszczepienie komórek krwiotwórczych, profilaktyka przeciwbakteryjna, cyprofloksacyną, rifaksyminą

Delivered: 07/08/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 25-31


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## Introduction

High-dose chemotherapy followed by haematopoietic stem cells is a recognised method of treatment in many haematological diseases, as well as in certain solid tumours [1]. This treatment is associated with a high risk of organ complications due to the high doses of chemotherapeutics. The complications lead to infections; particularly dangerous are those caused by endogenous bacteria [2]. High-dose chemotherapy locally damages the gastrointestinal mucosa, which, combined with neutropenia, may result in serious general systemic complications, including sepsis. Therefore, decontamination of the gastrointestinal tract may play an important role in the prophylaxis of bacterial complications in patients receiving high-dose therapy and the transplantation of haematopoietic cells. It has been demonstrated that in patients with prolonged neutropenia treated with chemotherapy, prophylactic fluoroquinolones reduce the frequency of infectious complications [3]. However, they increase the risk of developing multi-drug resistance compared to the patients who did not receive such prophylaxis [3, 4]. Rifaximin is a gastrointestinal antibiotic with a broad spectrum of antimicrobial activity, it does not pass into the bloodstream, and it is not associated with the risk of inducing drug-resistance [5, 6]. Its effectiveness in the decontamination of the gastrointestinal tract of patients with hepatic encephalopathy prompted our research in patients receiving haematopoietic stem cell transplants [7].

Researchers at the Bone Marrow Transplantation Centre of the Military Institute of Medicine developed a study to determine which antibacterial prophylactic antibiotic: ciprofloxacin or rifaximin, could best improve the course of the post-transplantation period, and reduce the number of infectious complications. In the medical literature there are no reports about the use of rifaximin in patients undergoing haematopoietic stem cell transplants. This medication will probably be used for the first time in this group of patients.

## Aim of the study

The aim of the study was to assess the usefulness and to compare the effect of antibacterial prophylaxis with non-absorbable rifaximin, and general systemic ciprofloxacin on the incidence and course of infectious complications in patients undergoing haematopoietic cell transplant.

## Material and methods

The study involved consecutive patients admitted between June 2012 and July 2014 to the Bone Marrow Transplantation Centre at the Department of Haematology and Internal Diseases, Military Institute of Medicine, who gave their consent to participate in the programme. The study was planned as a prospective, controlled, interventional study with the use of methods applied in daily clinical practice. The Bioethical Committee provided their approval. The patients were divided into two groups, according to the medical history number assigned at their registration with the Main Admission Unit (even vs odd numbers).

In the period between June 2012 and July 2014, 71 patients were qualified for the study. They all received high-dose chemotherapy with haematopoietic stem cell transplantation. The high-dose chemotherapy was administered in conditioning regimens appropriate for the primary diagnosis. Forty patients received additionally ciprofloxacin, used routinely in the Centre at that time (control group), and 31 patients received rifaximin as an antibacterial prophylaxis.

The mean age of the patients using rifaximin was 48.2 years (range of 22-69 years), and 56 years for those using ciprofloxacin (range of 29-69 years). The patients receiving ciprofloxacin were significantly older ($p = 0.016$).

There were no differences regarding sex or type of transplantation between the groups.

In both groups the greatest number of patients were those with myeloma multiplex. Considering the incidence of all the diseases, one analysis did not reveal a significant difference between the groups ($p = 0.065$). The high-dose chemotherapy regimens were varied, and adjusted to the primary disease. At the moment of transplantation, all the patients were in remission.

The prophylactic antibiotic was administered from the first day of high-dose chemotherapy until the development of transplant tolerance (i.e. the total neutrocyte count of >500/μl in 3 consecutive days) or the occurrence of fever.

Fever was defined as an increase in body temperature to >38°C. The occurrence of fever and the number of days of fever were reported. Body temperature was measured using a touch thermometer, and each abnormal reading was verified using an alcohol thermometer, taking the measurement in the armpit.

Each time a fever occurred, the patient's blood samples were taken from the central catheter and from a peripheral vein. If the blood sample culture was positive, bacteraemia was recorded.

In the case of bacteraemia, the resistance of the cultured bacteria to fluoroquinolones (including ciprofloxacin) and/or multi-drug resistance were recorded, if found.

In the case of clinical indications, also the following were obtained: urine cultures, stool cultures, rectal swabs for alarming pathogens (apart from the baseline swab for alarming pathogens at admission), swabs from the tip of the removed central catheter, and swabs from the inflammatory lesion in subcutaneous tissue. Positive results of these cultures were reported.

Another reported parameter was sepsis, if the patient with fever required the administration of pressor amines. The blood cultures were not always positive in the case of sepsis.

From the moment the fever developed, patients received empirical broad-spectrum antibiotics. If the positive blood culture was confirmed, followed by an

antibiogram test, the antibiotics were switched to targeted ones, or the previous therapy was continued (when the cultured bacteria demonstrated sensitivity to the antibiotic used). The duration of the antibiotic therapy was recorder (excluding prophylaxis), as well as their quantity (excluding those used prophylactically).

In the case of prolonged fever, blood samples were collected for fungal cultures, as well as for determining the antigens against yeast and aspergillus infections. The positive results and the duration of antifungal therapy were reported (except for fluconazole used as prophylaxis).

Cases of radiologically confirmed pneumonia were also recorded (X-ray, CT).

## Statistical methods

The descriptive statistics included arithmetic mean, median and the minimal and maximal values (range) for the quantitative variables, as well as the numerical and percentage values for the qualitative variables. The normality of the quantitative variable distribution was verified using the Shapiro-Wilk test, and the equality of variances between the groups was analysed using Levene's test. The significance of the differences between two groups independent of a quantitative variable was verified using Student's t-test, or the Mann-Whitney U-test in the cases where the criteria for the t-test could not be met. If the variances were unequal, then Welch's test was used instead of Student's t-test. The significance of differences between two groups independent of a quantitative variable was analysed using Pearson's chi-squared test, while if the expected number in a given field of the contingency table was <5, Fisher's exact test was used. Results with P values of less than 0.05 were considered statistically significant. All calculations were conducted using the Statistica software (Statsoft Inc, USA) version 12.

## Results

Table 1 presents comparative characteristics of the patients participating in the study, while Table 2 demonstrates the observed infectious complications in both studied groups. Fever was found in 24 patients from the rifaximin group, and in 27 from the ciprofloxacin group; the differences were not statistically significant. Similarly, there were no differences between the groups regarding fever duration. No statistically significant differences between the groups were observed with respect to bacteraemia, fungaemia, sepsis, pneumonia and multiple organ failure. In each group other antibiotics, apart from the prophylactic ones, were required, but their number was similar. The patients using prophylactic ciprofloxacin received antibiotics for slightly shorter periods of time compared to those using rifaximin (7 vs 10 days, respectively; $p = 0.045$).

Table 3 presents the characteristics of the studied groups regarding bacteria resistance to ciprofloxacin and multidrug resistance in those patients with bacteraemia. In the case of bacteraemia, nearly 31% of the patients receiving prophylactic rifaximin demonstrated resistance to ciprofloxacin and multidrug resistance. In the group of patients using prophylactic ciprofloxacin, the resistance of bacteria to ciprofloxacin was 100%, and multidrug-resistant pathogens were found in 80% of the subjects. The difference between the groups, both in the resistance to ciprofloxacin and multidrug resistance, was statistically significant. More cases of drug resistance were found in the subjects receiving prophylactic ciprofloxacin.

**Table 1. Group characteristics**
**Tabela 1. Charakterystyka chorych**

| | Rifaximin | Ciprofloxacin | Total | *P-value* |
|---|---|---|---|---|
| Number of patients | 31 | 40 | 71 | |
| Age | | | | |
| Mean | 48.2 | 56.0 | 52.6 | 0.016 |
| Median | 49.0 | 57.5 | 57.0 | |
| Range | 22.0-69.0 | 29.0-69.0 | 22.0-69.0 | |
| Sex | | | | |
| Female | 11 (35.5%) | 17 (42.5%) | 28 (39.4%) | 0.549 |
| Male | 20 (64.5%) | 23 (57.5%) | 43 (60.6%) | |
| Type of transplantation | | | | |
| Autotransplantation | 28 (90.3%) | 37 (92.5%) | 65 (91.5%) | 1.000 |
| Allotransplantation | 3 (9.7%) | 3 (7.5%) | 6 (8.5%) | |
| Primary disease | | | | 0.065 |
| AML | 2 (6.5%) | 2 (5%) | 4 (5.6%) | |
| HL | 6 (19.4%) | 2 (5%) | 8 (11.3%) | |
| DLBCL | 1 (3.2%) | 2 (5%) | 3 (4.2%) | |
| MCL | 1 (3.2%) | 3 (7.5%) | 4 (5.6%) | |
| MM | 11 (35.5%) | 26 (65.0%) | 37 (52.1%) | |
| PCL | 1 (3.2%) | 0 (0.0%) | 1 (1.4%) | |
| FL | 1 (3.2%) | 1 (2.5%) | 2 (2.8%) | |
| AITL | 1 (3.2%) | 1 (2.5%) | 2 (2.8%) | |
| MDS | 0 (0.0%) | 1 (2.5%) | 1 (1.4%) | |
| EGCT | 2 (6.5%) | 1 (2.5%) | 3 (4.2%) | |
| TGCT | 4 (12.9%) | 1 (2.5%) | 5 (70.4%) | |

AML - acute myeloid leukaemia, HL – Hodgkin lymphoma, DLBCL – diffuse large B cell lymphoma, MCL – mantle cell lymphoma, MM – myeloma multiplex, PCL – plasma cell leukaemia, FL – folicular lymphoma, PTCL – peripheral T cell lymphoma, AITL – angioimmunoblastic T cell lymphoma T, MDS – myelodysplastic syndrome, EGCT – extragonadal germ cell tumour, TGCT – testicular germ cell tumour

## Discussion

High-dose chemotherapy with haematopoietic stem cell transplantation is a therapeutic method associated with a high risk of general systemic complications, including infections. The risk is primarily due to the consequences of a high-dose treatment: temporary impairment of the immune mechanisms (myelosuppression) and a damaged gastrointestinal mucosal barrier, which may result in life-threatening infections. In order to reduce the complications associated with the transplantation procedure, antiviral, antifungal and anti-pneumocystis prophylaxis is used. The use of antibacterial prophylaxis is still controversial, especially in patients treated with autoHSCT (haematopoietic stem cell transplantation). Fluoroquinolones, due to the broad spectrum of activity, especially against Gram-negative bacteria, are most frequently considered as an antibacterial prophylactic treatment in patients treated for haematological disease when the expected time of neutropenia exceeds 7 days [8]. Decontamination of the gastrointestinal tract with fluoroquinolones lead primarily to the reduction of aerobic Gram-negative bacteria, without affecting the anaerobic flora [9]. There are few studies supporting unequivocally the need for antibacterial prophylaxis in the group of patients treated with haematopoietic stem cell transplantation; the majority of them are retrospective studies. A few studies demonstrate a reduced incidence of neutropenic fever and bacteraemia after the use of prophylactic fluoroquinolone in the patients undergoing autologous stem cell transplantation compared to those who did not receive such prophylaxis [10-12]. In addition, the effect of prophylactic fluoroquinolones on reducing the mortality or morbidity rates was not demonstrated [13]. Tabarraee et al. found that the prophylactic use of ciprofloxacin had no effect on the incidence of neutropenic fever in patients treated with auto HSCT, compared to a lack of such prophylaxis. However, they demonstrated a reduced duration of neutropenic fever, incidence of bacteraemia, length of hospitalisation, and number of units of transfused blood platelets in favour of prophylactic ciprofloxacin [14].

**Table 2. Group characteristics in terms of infectious complications and treatment**
**Tabela 2. Charakterystyka grup pod względem powikłań infekcyjnych i leczenia**

| | Rifaximin (N=31) | Ciprofloxacin (N=40) | Total (N=71) | *P-value* |
|---|---|---|---|---|
| Bacteraemia | 13 (41.9%) | 11 (27.5%) | 24 (33.8%) | 0.202 |
| Positive cultures (excluding bacteraemia) | 8 (25.8%) | 4 (10.0%) | 12 (16.9%) | 0.078 |
| Fungaemia | 1 (3.2%) | 2 (5.0%) | 3 (4.2%) | 0.712 |
| Sepsis | 3 (9.7%) | 3 (7.5%) | 6 (8.5%) | 1.000 |
| Pneumonia | 5 (16.1%) | 1 (2.5%) | 6 (8.5%) | 0.079 |
| Multiple organ failure | 3 (9.7%) | 1 (2.5%) | 4 (5.6%) | 0.311 |
| Fever | 24 (77.4%) | 27 (67.5%) | 51 (71.8%) | 0.357 |
| Days of fever (median/range) | 2 (0-18) | 1.5 (0-12) | 2 (0-18) | 0.218 |
| Days of antibiotic therapy (median/range) | 10 (0-39) | 7 (0-28) | 8 (0-39) | 0.045 |
| Number of antibiotics used (median/range) | 3 (0-10) | 2 (0-8) | 3 (0-10) | 0.073 |
| Days of hospitalisation (median/range) | 17 (11-42) | 19 (14-31) | 18 (11-42) | 0.789 |
| Death | 1 (3.2%) | 2 (5%) | 3 (4.2%) | 0.712 |

In the case of patients treated with alloHSCT, international guidelines recommend the use of prophylactic fluoroquinolones [8, 15, 16]. However, the recommendations are based on studies involving patients treated due to various haematological diseases. Sometimes the patients receiving alloHSCT were not included in the studies, or their number was very limited. In a retrospective study involving 47 patients, German authors demonstrated that alloHSCT therapy without a fluoroquinolone or fluconazole prophylaxis is possible. They did not reveal an increased incidence of blood stream infections (BSI), invasive fungal disease (IFD) or day 100 mortality compared to the data reported for the patients who received such prophylaxis [17]. Simondsen et al. conducted a retrospective analysis of almost 100 patients receiving alloHSCT; half of the group were treated with fluoroquinolones, whereas the other half were not. Among the patients who received prophylactic antibiotics there were fewer episodes of neutropenic fever, fewer positive cultures collected during the fever, and the duration of the antibiotic therapy was shorter. However, the differences were not statistically significant. No difference between the groups was observed regarding resistance to fluoroquinolones [18].

**Table 3. Group characteristics in terms of bacterial resistance to ciprofloxacin and multidrug resistance among patients with bacteraemia**
**Tabela 3. Charakterystyka grup pod względem oporności bakterii na cyprofloksacynę oraz oporności wielolekowej wśród chorych z bakteriemią**

| | Rifaximin (N=13) | Ciprofloxacin (N=10) | *P-value* |
|---|---|---|---|
| Resistance to ciprofloxacin | 4 (30.8%) | 10 (100.0%) | <0.001 |
| Multi-drug resistance | 4 (30.8%) | 8 (80.0%) | 0.019 |

Prophylactic use of fluoroquinolones is associated with the development of bacterial resistance to this group of antibiotics, and growth of multi-drug resistant strains [4, 19].

In the presented study, the prophylactic antibiotic therapy was applied to reduce the number of infectious complications, according to previous guidelines. The antibiotic used most frequently for this indication in the Transplantation Centre at the time of the study was

ciprofloxacin. The risk of the development of multi-drug resistant bacteria due to a systemic therapy with prophylactic fluoroquinolones prompted the use a local, gastrointestinal-selective antibiotic. The effectiveness of rifaximin in decontamination of the gastrointestinal tract in patients with hepatic encephalopathy dictated the choice of this antibiotic. No data were found in the literature regarding the use of rifaximin in the above mentioned group of patients.

In our study, the effectiveness of rifaximin and ciprofloxacin in patients treated with HSCT was similar. The incidence of neutropenic fever and bacteraemia did not differ significantly between the groups (77.4% vs 67.5%, p = 0.357, and 41.9% vs 27.5%, p = 0.202, respectively), and the outcomes were comparable to those reported in the literature for patients treated with prophylactic fluoroquinolones [13, 14, 18]. In addition, no differences were observed between the groups regarding other documented infections (other positive cultures, pneumonia, sepsis, fungaemia). The number of antibiotics used (excluding those administered as prophylaxis) was also similar in both groups of patients (3 vs 2, p = 0.073). The demonstrated difference in the number of days of an antibiotic therapy was borderline statistically significant (p = 0.045), in favour of ciprofloxacin (10 vs 7 days). The patients in the ciprofloxacin group were significantly older than those in the rifaximin group, which could indirectly suggest that ciprofloxacin is more beneficial.

Significant differences were demonstrated in the resistance of the bacteria cultured from the blood of patients. The bacteria isolated from the blood of patients receiving ciprofloxacin were 10% resistant to this medicine, whereas those of patients receiving rifaximin, in the case of bacteraemia, the resistance to ciprofloxacin was 31% (p < 0.001). In the patients receiving ciprofloxacin, multi-drug resistant pathogens were isolated from blood significantly more often (30.9% vs 80%, p = 0.019); most frequently this was ESBL+. In both groups Gram-negative bacteraemia dominated (85% in the rifaximin group, and 63% in the ciprofloxacin group), which is typical for haematology departments. It is estimated that Gram-negative bacteria contribute to nearly 70% of cases of the bacteraemia detected in haematology departments [20]. The occurrence of multi-drug resistant (MDR) pathogens contributed to worse outcomes in patients with haematological diseases [21, 22].

A wide use of fluoroquinolones, both in prophylaxis and in empirical therapy, resulted in increased bacterial resistance to this group of antibiotics, thus reducing their effectiveness, and promoting the development of multi-drug resistant pathogens [23-25].

There are reports of the limited effectiveness of fluoroquinolone therapy in the centres where the resistance of bacteria to these antibiotics is over 30%. In these cases, resignation from the prophylactic therapy with fluoroquinolones should be considered [23].

## Conclusions

The effectiveness of rifaximin and ciprofloxacin in the prophylaxis of infectious complications in the described group of patients appears to be similar. The high resistance of bacteria to ciprofloxacin in the studied group might have reduced the effectiveness of ciprofloxacin-based prophylaxis. Further studies on the prophylactic use of these antibiotics are required, preferably including a control group (placebo or no intervention), and following a local assessment of resistance to fluoroquinolones.

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# Calcium-phosphate metabolism disorders in children with faulty posture

## Zaburzenia gospodarki wapniowo-fosforanowej u dzieci z wadami postawy

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**Abstract.** Faulty posture is one of the most common health problems in the paediatric population. Its aetiology and pathogenesis are still poorly recognized. The aim was to assess calcium-phosphate (Ca-P) metabolism in children with faulty posture. A retrospective analysis was performed of the medical data of patients hospitalized in the paediatric department between February 2015 and November 2016. The study involved a group of 30 children with faulty posture found during a physical examination. The control group consisted of 30 children with a negative history of skeletal system problems and Ca-P balance disorders. The groups were compared in terms of serum Ca-P metabolism parameters and urine excretion of crystalloids. We found lower concentrations of the examined parameters (Ca, P, Mg, ALP, vitamin $D_3$) in the serum of the study group children than the control group. Statistically significant higher values of 24-hour urine calcium output measurements in the study group were observed. The coincidental occurrence of faulty posture and urolithiasis was noted in 40% of the cases. The results confirm the role of Ca-P metabolism in shaping correct posture in the paediatric population. Regular control of Ca-P parameters in the serum and urine accompanied by urinary tract monitoring for deposits is necessary in any case of faulty posture in children.
**Key words:** calcium-phosphate metabolism, children, faulty posture

**Streszczenie.** Wstęp. Jednymi z najczęściej występujących problemów zdrowotnych u dzieci są wady postawy. Etiologia i patogeneza tej grupy chorób to zjawiska wciąż jeszcze mało poznane. Cel pracy. Ocena zaburzeń gospodarki wapniowo-fosforanowej (Ca-P) u dzieci z wadami postawy. Materiał i metody. Analizie retrospektywnej poddano dokumentację dzieci hospitalizowanych na oddziale pediatrycznym w okresie od lutego 2015 do listopada 2016 roku. Grupę badaną stanowiło 30 dzieci ze stwierdzoną w badaniu przedmiotowym wadą postawy. Do grupy kontrolnej wybrano 30 pacjentów, u których przed rozpoczęciem badania nie podejrzewano zaburzeń w zakresie układu ruchu ani odchyleń parametrów gospodarki Ca-P Grupy porównano w zakresie stężeń parametrów gospodarki Ca-P w surowicy krwi oraz wydalania krystaloidów z moczem. Wyniki. Stwierdzono mniejsze wartości oznaczanych parametrów laboratoryjnych (Ca, Mg, P, ALP, witaminy $D_3$) w surowicy krwi w grupie badanej w porównaniu z grupą kontrolną. W grupie badanej obserwowano istotnie statystycznie większe wartości w zakresie wydalania jonów wapnia w dobowej zbiórce moczu. Odnotowano 40% koincydencję wad postawy z kamicą układu moczowego. Wnioski. Otrzymane wyniki potwierdzają rolę gospodarki Ca-P w kształtowaniu się postawy populacji pediatrycznej. Konieczne jest monitorowanie jej parametrów oraz ocena układu moczowego pod kątem występowania złogów u dzieci z wadami postawy. **Słowa kluczowe:** wada postawy, gospodarka wapniowo-fosforanowa, dzieci

Delivered: 28/08/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 32-37


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## Introduction

Postural defects are among the most frequent health problems affecting children and adolescents [1]. The data regarding the incidence have a high variance. The studies assessing the health of children and adolescents demonstrate that postural defects constitute 10-80% of disorders in school-age children [1]. Such high discrepancies result from a lack of unified diagnostic criteria and from the individual character of posture formation [2].

The correctness of posture may be assessed for the first time in children when they can stand up on their own. This process results in the formation of lumbar lordosis and thoracic kyphosis. In the second and third year of life, the posture is characterised by a protruding abdomen and slightly marked spinal curve. At 4-7 years, increased lumbar lordosis and flattening of the abdomen may be observed. At school age, there are two critical periods contributing to the formation of postural defects. The first one is the early-school period, associated with lifestyle changes (enforced sitting position for several hours), and the other is the puberty leap period, when body growth increases [2, 3].

It is estimated that postural defects that may be considered significant affect approximately 10-15% of children [1, 2]. The most frequent one is juvenile idiopathic scoliosis, found in approximately 0.47-5.2% of children [4, 5].

However, presently we are witnessing an increasing number of posture-related disorders, especially in the paediatric population of highly-developed countries. It is directly related to a sedentary lifestyle, limited physical activity, and incorrect eating habits [2].

Mineralisation of the ossicular system during a child's development depends on the proper supply of calcium and phosphorus. The lack of a balanced diet, especially in the period of increased growth, adversely affects the calcium-phosphate metabolism, thus leading to reduced bone mass [6, 7]. Vitamin $D_3$ also plays an important role in maintaining a balanced calcium-phosphate metabolism, and its blood serum concentrations are directly and positively correlated with the degree of bone mineralisation [8, 9].

However, there are few scientific studies available regarding the effect of disturbed calcium-phosphate metabolism on the incidence of postural defects in children and adolescents [9].

## Aim of the study

The aim of the study was to assess the calcium-phosphate metabolism in children with postural defects.

## Material and methods

The study was conducted as a retrospective analysis of the medical records of children hospitalised at the Department of Paediatrics, Paediatric Nephrology and Allergology of the Military Institute of Medicine between February 2015 and November 2016.

The study group involved 30 children, including 20 girls (67%) and 10 boys (33%), diagnosed with postural defects during a physical examination. The mean age in this group was 13 ± 3 years. The control group comprised 30 randomly selected patients of the department (15 girls and 15 boys), who did not reveal any signs of musculoskeletal disorders or abnormal parameters of calcium-phosphate metabolism before the beginning of the study. The mean age was 8 ± 5 years.

In the studied group, 16 subjects were diagnosed with scoliosis, 1 with excessive kyphosis, 1 with excessive lordosis, and 3 with a complex defect comprising excessive kyphosis and excessive lordosis. In 9 subjects other postural defects were observed. In the control group, following the study design, no spinal disorders were found.

The results of the laboratory tests (blood serum concentrations of calcium, phosphorus, magnesium, alkaline phosphatase and vitamin D) were analysed in detail, as well as the results of crystalloid excretion in the daily and three-hour urine collection (daily excretion of calcium, phosphorus and magnesium, and crystallisation indicators: calcium/creatinine ratio, magnesium/creatinine ratio, and phosphorus/creatinine ratio). In each case the results of an abdominal ultrasound examination were examined, for deposits in the urinary tract.

During hospitalisation, the decisions regarding the scope of diagnostic tests were made on individual basis. Not all the parameters analysed in the study were examined in every subject.

The statistical analysis of the obtained results was performed using StatSoft, Inc. (2014) STATISTICA (data analysis software system), version 12. Before the analysis, the data were initially verified using the normality distribution chart, and finally with the Kolgomorov-Smirnov test and the Lillefors normality test. Due to the lack of conformity of selected variables with the normal distribution, non-parametric tests that do not require normal distribution were used for the statistical analysis of these variables. Student's t-test was used to assess the variables with normal distribution. To analyse correlations for the variables without a normal distribution, the Spearman's rank coefficient was derived, and for the variables with normal distribution, the Pearson's linear correlation coefficient was calculated. Results with P values of less than 0.05 were considered statistically significant.

## Results

In both the study group and the control group, abnormal blood serum concentrations of calcium-phosphate metabolism parameters were observed, as well as the excretion of ions both in the 3-hour and daily urine collection. Comparison between the two groups allowed the significance of the deviations to be assessed.

In the subjects with faulty posture the blood serum levels of all the analysed laboratory parameters (Ca, Mg, P, ALP, vitamin $D_3$) were lower than in those without musculoskeletal disorders. However, statistically significant differences were observed only between the concentrations of calcium phosphorus and magnesium (Tab. 1, Fig. 1).

Regarding the crystallisation indicators, the excretion of calcium ions in the daily urine collection was statistically significantly higher in the studied group, whereas the magnesium/creatinine ratio in the 3-hour urine collection was statistically significantly lower in the control group.

No significant differences were found between the groups in the daily excretion of phosphorus, magnesium, calcium/creatinine and phosphorus/creatinine ratios (Tab. 2-3, Fig. 2).

The presence of urolithiasis was also determined in both groups. It was found in 12 subjects with faulty posture, whereas in the subjects from the control group the abdominal ultrasound examinations did not reveal any stones. Subjects with urolithiasis in the study group constituted 40% of the observed population.

## Discussion

The aetiology and pathogenesis of postural defects have been studied by researches for many years. There are numerous theories about the origin of faulty posture, but no unequivocal conclusions regarding the development of this group of disorders have been formulated. Currently, a multifactorial aetiology is considered the most probable [10, 11]. Scientific research focuses on juvenile idiopathic scoliosis as the most frequent disorder found in children and adolescents [5].

Some studies emphasise the role of melatonin as the causative factor in the development of juvenile idiopathic scoliosis. This hypothesis is supported by a number of studies on animals in which the removal of the pineal gland resulted in scoliosis [12, 13]. Other studies demonstrated increased concentrations of platelet calmodulin and calmodulin in the bioptates of the paravertebral muscles on the convex side of the curve in patients suffering from scoliosis [14]. Osteoprotegerin, which modifies the differentiation of osteoclasts and osteoblasts, may be another potential causative factor. Both calmodulin and osteoprotegerin directly affect the cellular calcium-phosphate metabolism, and potentially may be responsible for spinal deformation observed in adolescents [14]. A few studies emphasise the role of vitamin $D_3$ [9, 10], growth factors or thrombospondins [7, 15, and 16]. Some publications discuss the effect of sex hormones, especially oestrogens, on the bone metabolism in children. The described disorders of the endocrine system contribute to the status typical for women in the postmenopausal period, suffering from osteoporosis [6, 14, 17].

The results of this study confirm the presence of calcium-phosphate metabolism disorders in children with faulty posture. Small, but significant differences in blood serum concentrations of the calcium and phosphorus ions, and in calcium ion excretion in the daily urinary collection in healthy children compared to those with postural defects confirm the increased processes of skeletal remodelling in the study group. Lysis of osteoid hydroxyapatites may result in hypercalcaemia, which reduces the secretion of parathormone. PTH deficiency limits the synthesis of vitamin $D_3$, responsible for absorption of calcium ions from the gastrointestinal tract, and reabsorption of calcium in the kidneys. It may result in a dangerous situation, where intensive metabolism of the bone matrix reduces the synthesis of PTH and vitamin $D_3$, as well as absorption of calcium ions [6, 7] (Tab. 4).

**Table 1. Comparison of serum Ca-P metabolism parameters in the groups**
**Tabela 1. Porównanie wartości parametrów gospodarki Ca-P w surowicy krwi w grupach**

| Biochemical parameter in blood serum | Normal range | Mean value in the control group | Mean value in the study group | p | $N_{Control}$ | $N_{Study}$ | SD Control group | SD Study group |
|---|---|---|---|---|---|---|---|---|
| Ca | 8.6-10.2 mg/dl | 10.01 | 9.83 | 0.05 | 27 | 30 | 0.34 | 0.35 |
| Mg | 1.6-2.6 mg/dl | 2.18 | 2.09 | 0.05 | 27 | 27 | 0.16 | 0.15 |
| P | 2.6-4.5 mg/dl | 4.77 | 4.37 | 0.03 | 27 | 30 | 0.74 | 0.63 |
| ALP | <320 j/l | 246.75 | 207.45 | 0.25 | 16 | 22 | 81.18 | 116.83 |
| Vitamin $D_3$ | 30-50 ng/ml | 29.06 | 27.05 | 0.47 | 19 | 29 | 9.13 | 9.61 |

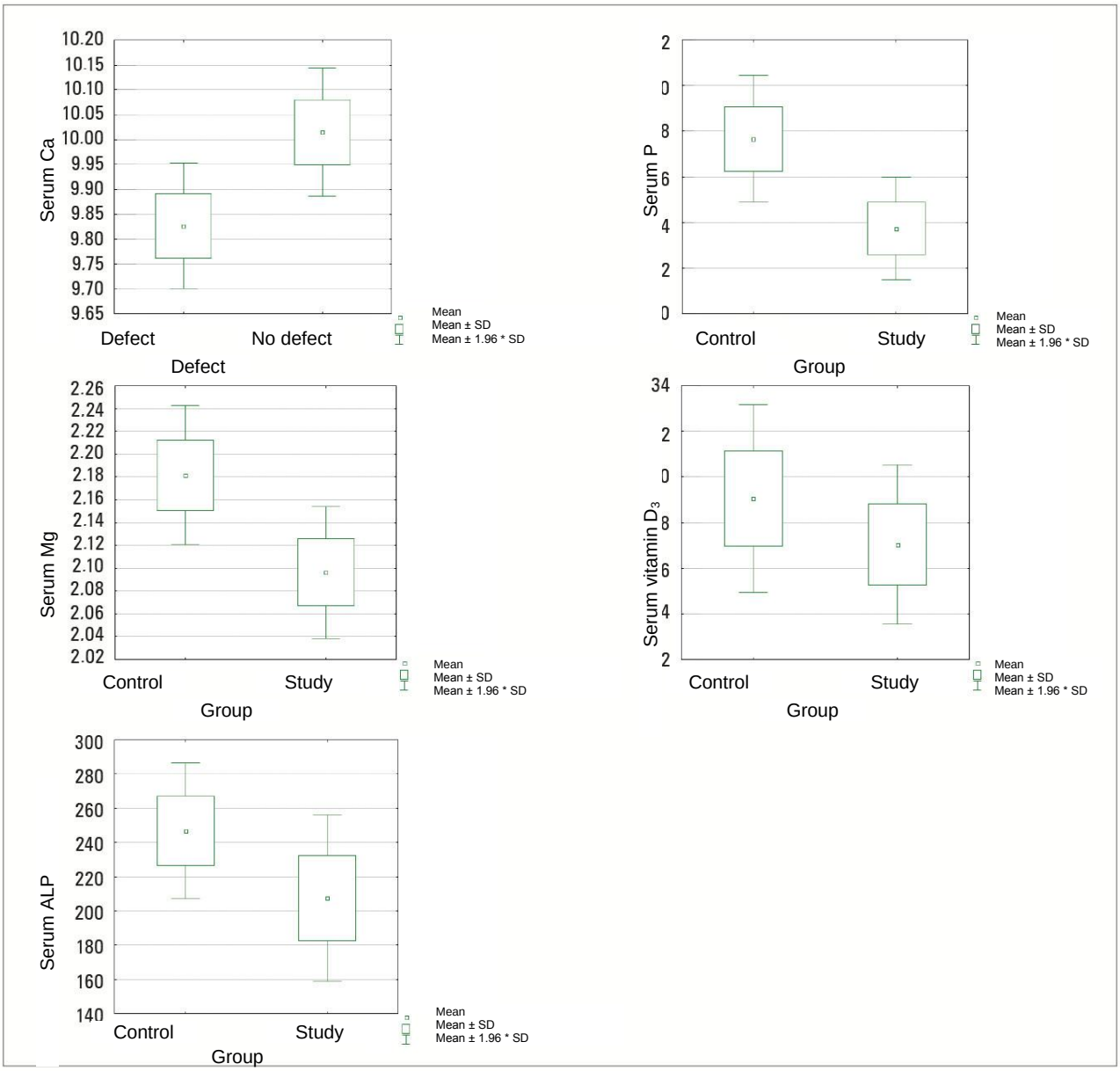


**Figure 1.** Results of the comparison of serum Ca-P metabolism parameters in the groups
**Rycina 1.** Wyniki porównania wartości parametrów gospodarki Ca-P w surowicy krwi w grupach

The coincidence of faulty posture and urolithiasis is discussed extensively in the literature [18-20]. The following factors predispose this group of children to an increasingly frequent incidence of kidney stones: limited physical activity, immobility, anatomic defects resulting in limited urinary flow or in urinary retention, more frequent comorbidities, and pharmacotherapy. In a retrospective study conducted by P. Ramachandre et al. in a group of approximately 12 thousand paediatric patients, the frequency of this coincidence was 1.4%, while in patients without postural defects it was 0.24% [20].

**Table 2. Results of the comparative analysis of ion excretion in 24-hour urine output measurements in the study and control groups**
**Tabela 2. Wyniki analizy porównawczej wydalania jonów w dobowej zbiórce moczu w grupie badanej i kontrolnej**

| Ion | Normal range (mg/kg/d) | Mean in the study group | Mean in the control group | P | $N_{study}$ | $N_{control}$ | SD Study group | SD Control group |
|---|---|---|---|---|---|---|---|---|
| Ca | 1-4 | 2.83 | 1.70 | 0.01 | 27 | 24 | 1.71 | 1.27 |
| Mg | >0.8 | 1.96 | 2.19 | 0.42 | 27 | 24 | 0.88 | 1.14 |
| P | 15-20 | 11.46 | 11.99 | 0.73 | 27 | 24 | 4.62 | 6.47 |

SD – standard deviation

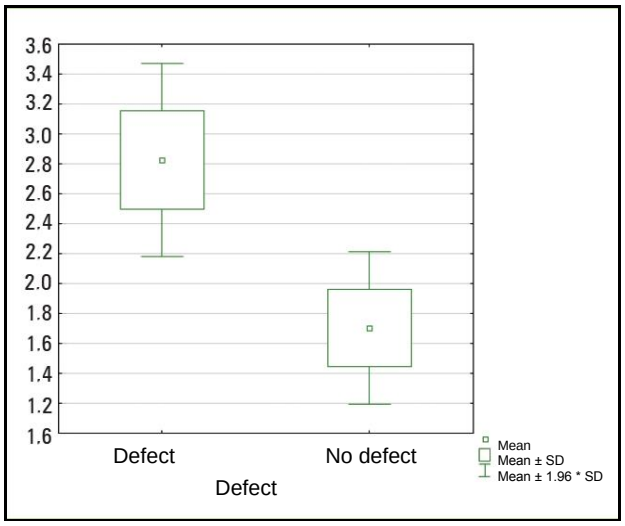


**Figure 2.** Calcium excretion comparison in the groups
**Rycina 2.** Wynik porównania wartości wydalania jonów wapnia w grupach

The results obtained in this study (frequency of concurrence of 40%) may be slightly higher than in population studies, due to the nephrological character of our establishment (Department of Paediatrics of the Military Institute of Medicine specialises in the monitoring and treatment of urolithiasis), and support the need for ultrasound examinations of children with postural defects.

**Table 3. Comparative analysis of ion excretion rates in 3-hour urine samples in the groups**
**Tabela 3. Wyniki analizy porównawczej wydalania krystaloidów w 3-godzinnej zbiórce moczu w grupach**

| P index | P | Study | Control |
|---|---|---|---|
| Ca/creatinine | 0.231 | 29 | 24 |
| Mg/creatinine | 0.004 | 29 | 24 |
| P/creatinine | 0.163 | 29 | 24 |

Another aspect of the presented coincidence is the potential common metabolic background of both diseases. Hypercalciuria, observed significantly more often in the study group, may be a consequence of the intensive bone remodelling, but also a direct cause of deposit formation in the urinary tract. This phenomenon requires further research.

The observed calcium-phosphate metabolism disorders in children with spinal deformities raise the difficult question: which of the observed abnormalities lead to postural defect, and which result from the existing processes of pathological bone remodelling?

**Table 4. Simplified calciotropic hormone involvement in the regulation of the calcium and phosphate metabolism [7]**
**Tabela 4. Uproszczony udział hormonów kalciotropowych w regulacji stanu gospodarki wapniowo-fosforanowej [7]**

| Hormone | Serum concentration | | | Absorption from the gastrointestinal tract | | | Mobilisation from the bones | | | Reabsorption in the kidneys | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ca | $PO_4$ | Mg | Ca | $PO_4$ | Mg | Ca | $PO_4$ | Mg | Ca | $PO_4$ | Mg |
| PTH | ↑ | ↓ | ↑ | **0** | 0↑ ? | ↑ | ↑ | ↑ | ↑ | ↑ | ↓ | ↑ |
| $1,25(OH)_2D_3$ | ↑ | ↑ | ↓ | ↑ | ↑ | ↑ | ↑**s** | ↑**s** | ↑**s** | ↑ | ↑**f** | ↓ |
| calcitonin (s) | ↓ | ↓ | ↓ | ↑ | **0** | **0** | ↓ | ↓ | **x** | ↓ | ↓ | ↓ |

s - only with high hormone levels, f - only with physiological hormone levels, ↑ - increase, ↓ - decrease, 0 - no evidence of direct effect, x - no data in the literature, ? - inconsistent literature data

One of the limitations of this study is the fact that many other factors affecting calcium-phosphate metabolism were not analysed. Apart from calciotropic hormones (PTH, calcitonin, $25(OH)D_3$, $1a,25(OH)_2D_3$, $1a,24,25(OH)_3D_3$, $24,25(OH)_2D_3$), other factors affecting bone remodelling, retention or release of calcium and phosphates include substances like: hormones (primarily androgens and oestrogens), ions, local mediators of factors engaged in the immune processes, and vitamins [6, 7]. Only a comprehensive analysis of all the parameters of calcium-phosphate metabolism, conducted in the course of extensive research, could provide answers to the question regarding the causes and effects of the observed study abnormalities.

Our study was also limited by the difference in mean age between the study group and the control group. However, although the normal ranges for the analysed parameters may be different for younger children, in the case of patients of school age this trend was practically negligible. It can therefore be assumed that the reference ranges for the concentrations of the analysed substances in the blood serum and for excretion of ions in the urine were identical in both groups.

Due to the limited number of subjects in both groups, further prospective studies involving higher number of children are required to formulate unambiguous conclusions.

## Conclusions

Normal calcium-phosphate metabolism plays an important role in the process of formation of the skeletal system in children. Its disturbance may result in abnormal bone mineralisation, and, as a consequence, in postural defects. The parameters of the calcium-phosphate metabolism should be monitored in children with faulty posture, especially the serum concentrations of calcium, phosphorus and vitamin $D_3$, as well as excretion of calcium in urine.

Supplementation of vitamin $D_3$ should be considered in this group of patients; however, the dose needs to be individualised according to the total balance of the calcium-phosphate metabolism in a given patient.

Close co-operation between paediatric orthopaedists, paediatricians and paediatric endocrinologists is required to enable careful monitoring and optimal treatment of patients with postural defects.

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# Aggressive behaviour of veterans as a consequence of war trauma - a case study

## Gniew weterana jako następstwo traumy wojennej - opis przypadku

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**Abstract.** The article presents the course of treatment and psychotherapy of a 38 year old veteran of the Polish War Contingent, who took part in military missions in Lebanon and Afghanistan (sixth rotation). The patient had a traumatic experience, i.e. he was shot on the helmet. Since then he has had a history of long-term and intensifying behavioural changes, manifesting in aggressive behaviour and violence. The authors retrace the traumatic experience frame by frame, and discuss the psychological mechanism of dissociation. The article describes the attempts at war trauma psychotherapy, which were demanding both for the patient and the clinical psychologist/psychotherapist. The article tries to explain aggressive behaviours in veterans, based on the identification with the aggressor defence mechanism. At the end, the authors give a few remarks on the clinical experience in PTSD diagnosis in veterans with a high level of aggressive behaviour and anger.
**Key words:** aggression, anger, PTSD, war trauma

**Streszczenie.** W artykule przedstawiono historię leczenia oraz przebieg psychoterapii 38-letniego weterana Polskich Kontyngentów Wojskowych, uczestnika misji w Libanie i Afganistanie (VII zmiana), u którego kilka dni po przeżyciu traumatycznego doświadczenia (postrzał kulą w hełm) doszło do długotrwałej i nasilającej się zmiany w zachowaniu pod postacią zachowań agresywnych i przemocy. Śledząc klatka po klatce przebieg zdarzenia traumatycznego, autorzy omawiają mechanizm psychiczny dysocjacji. Opisują trudną próbę psychoterapii traumy wojennej zarówno po stronie pacjenta, jak i psychologa klinicznego/psychoterapeuty. W omówieniu podjęto próbę wyjaśnienia zachowań agresywnych u weteranów misji wojennych w oparciu o mechanizm obronny identyfikacji z agresorem. Na koniec autorzy dzielą się doświadczeniami klinicznymi w rozpoznawaniu zespołu stresu pourazowego u weteranów, u których występuje wysoki poziom zachowań agresywnych i gniewu. **Słowa kluczowe:** agresja, gniew, PTSD, trauma wojenna

Delivered: 30/11/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 38-41


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## Introduction

Anger and aggression are the problems most frequently reported by Polish veterans returning from missions in Iraq and Afghanistan. This article is a continuation of a series of publications regarding aggression as part of post-traumatic stress disorder (PTSD), presented in *Military Physician* in 2016, and in *Tułaczka Odyseusza*. *Powrót z wojny do domu* [*Odyssey. Returning home from war*] [6].

## Case study

This article presents a case of a 38-year-old professional soldier with the rank of sergeant, a veteran of missions in Lebanon and Afghanistan. He was previously hospitalised five times in the Department of Psychiatry, Combat Stress and Psychotraumatology of the Military Institute of Medicine with a diagnosis of adjustment disorder with signs of PTSD, and signs of mixed personality disorders. The patient is married and has three daughters. He lives with his family in a town in southern Poland.

During the last hospitalisation at the Department of Psychiatry, Combat stress and Psychotraumatology, the patient was still an active soldier. A head MRI test excluded any organic basis for the aggressive behaviour. The patient had no history of alcohol abuse or use of psychoactive substances.

The patient comes from a working-class family. He was the youngest child, with a sister three years older. The patient had a strong emotional connection with his mother, who died due to an oncological disease when he was 19 years old. He says that "Nothing was the same" after his mother's death. He regrets not having had the courage to keep his mother company in her last days. He refers to it as "desertion at the most important moment of his life". The patient's father is retired. Since the return from Afghanistan, the patient's contacts with his sister and her family are occasional and superficial. In his family house the mother was the leader. She worked as a guard. She was the first one to show him a gun, which she used as part of her work, when he was 6 years old.

In 2000, the patient was deployed on a mission to Lebanon, and then to Afghanistan in 2010. During the latter, he experienced a traumatic event: for approximately an hour he was under enemy fire. He was shot on the helmet, but the bullet did not pass through it. Initially, the patient was completely confused, and did not understand what was happening. "As if I couldn't understand what I saw, what happened." At that moment, he was experiencing derealisation, which is a symptom of dissociation. Dissociation is the most powerful method of avoiding pain; a protective mechanism occurring when trauma cannot be prevented, mentally processed or avoided. If the body cannot escape, at least the mind and soul can [7].

Some time after the shooting, while the patient was running towards the helicopter near the houses of an Afghan village, he felt "weak at his knees", as if "his legs were simply cut off". This is a symptom of somatoform dissociation, with symptoms of conversion in the form of balance disorders. They have been formerly described in psychiatric literature as "hysterical astasia-abasia": neurological disorders, usually transient and reversible, in which no neural damage can be demonstrated [1].

At that moment, he fell to the ground, felt a strong pain in the back, and was afraid that he had been shot. Everything around him seemed unreal. The patient experienced temporal and spatial confusion (another sign of dissociation). He remembers that later he tried to crawl, still under enemy fire, to the helicopter that was hovering near the village, waiting to collect the soldiers and take them to the base. At that point the patient felt complete helplessness, and a lack of strength to fight for survival. "It was a feeling of fear that I'm done, and that all the power had left me. I thought that I was alone. I was powerless". He feared he would not crawl to the helicopter in time. When he got on board, he cried. "I realised I was so close to dying". Many authors consider the need to escape, and the realisation that it is impossible, to be the essence of trauma [7].

The experience of a traumatic event triggers excessive, pent-up emotions, which are later released in aggressive behaviours and violence. A few days after this event the patient, upon his own request, worked with the American army to help with the transport of Afghan people suspected of collaboration with terrorists. He abused these people. "I hit and kicked them so hard, that they were falling onto the walls. A colleague told me to stop and asked if I couldn't see that they were scared. I answered: "And I'm not f.... scared? Don't I have a family?". The patient was considered so psychotic in his treatment of those people, that following the suggestions of the American soldiers, he was removed from this activity. Later, until the end of the mission in Afghanistan, he used any opportunity to demonstrate brutal behaviours towards Afghan people. He says that the more fear he saw in their eyes, the more powerful and secure he felt. This is a classic protective mechanism of identification with the aggressor, and is discussed in more detail below. He remained in Afghanistan until the end of the mission.

After his return to Poland, he began demonstrating frequent aggressive behaviours, initially at the place of work and then at home, towards his family. He was verbally and physically aggressive towards soldiers. He did not control his outbursts of aggression, was vulgar, and threw objects at soldiers. At home the patient behaved similarly, although here the level of verbal and physical aggression was higher. The youngest daughter, aged under two years old, reacted with a protective reflex, hiding her face in her hands, when she saw her father coming home. The patient destroyed objects and furniture. During a fight with his wife, he stopped himself with difficulty from hitting her on the head with his fist. When agitated, he could stab a knife into a kitchen unit while his wife was standing near. The patient would shout at his daughters: "You little Afghan c...s, I'll blow your f.... heads off!"

## Hospitalisation and therapy

The patient was previously hospitalised five times, the first time was in 2013. He was discharged at his own request after only a two-week stay on the ward. He visited the Department of Psychiatry, Combat Stress and Psychotraumatology again in 2014. At that time, his aggressive behaviour intensified. He demonstrated unwillingness to participate in psychotherapeutic activities. He was discharged to be admitted to the Department of Psychiatry, Combat Stress and Psychotraumatology for individual therapy, but he did not arrive for the planned treatment on the arranged date.

He revealed the details of the traumatic event from Afghanistan and the spectrum of PTSD symptoms only during his third hospitalisation, in mid-2015. The psychological diagnostics of PTSD was extended to include CAPS IV (Clinician-Administrated PTSD Scale), which has been the model PTSD assessment for 20 years. The results of the CAPS IV test confirmed the axial symptoms of post-traumatic stress disorder, such as excessive agitation and reliving (regardless of the

patient's will, the memories of the event from Afghanistan "invaded" his consciousness in the form of intensive images, sounds and smells). Each time that the memory of the traumatic event was activated during an individual conversation, the patient said: "I can smell the specific scent of that earth, it's a tragedy". The analysis of the patient's memories indicated that the memory trace of the event from Afghanistan was recorded and functioned in the system of emotional memory, and in the situational memory that can trigger intrusion and contains the records of perceptive processes associated with a traumatic event [5]. Whenever a Medical Air Rescue helicopter landed on the hospital airport, the patient experienced the invasion of memories of the event. He avoided anything that might remind him of that experience. With the patient's consent, an individual therapy was implemented, continued during the next long hospitalisation in the winter of 2015/2016.

For the past few years since the return from Afghanistan, the patient often had a recurring dream in which he was standing motionless and scared in an Afghan village, and could not move to protect himself or escape, which is probably a direct reflection of the traumatic event, when "his legs were as if cut off". After some time of individual therapy, the patient had the same dream, in the same Afghan village, but this time he could move ("my body listened to me"), and he could protect himself, regaining a symbolic sense of power and control. The night before he was discharged from the hospital, the patient had a nightmare in which he saw the brutally battered bodies of Afghan people, whom he approached and shot in the heads from a close distance, in the same spot where he had been shot on his helmet. Both the patient and the therapist considered it an introduction to further therapeutic work, and the problems they will have to face during the next planned hospitalisation: the war trauma from the perspective of the victim, as well as the perpetrator. Apparently the patient was not ready to deal with it, as he did not arrive on the arranged admission date at the hospital.

## Discussion

During the war, many veterans learned that revealing emotions other than hostility or aggression is a sign of weakness that may cost them their lives. Anger and rage help them survive in combat. When they face helplessness, paralysing fear and their own mortality, they cope with it by inducing rage. It protects them from fear and helplessness. It appears that nothing is scarier for a person than the feeling they have no influence on their life, and depend on random luck or the enemy's mercy. The basic protective mechanism behind it is identification with the aggressor, described by Anna Freud. She considers identification with the aggressor to be one of the most powerful methods of dealing with danger [2]. Identification means internalisation of other characteristics by developing a likeness to his person. In the case of identification with the aggressor, the individual using this mechanism internalises the aggressive traits of another person. By becoming similar to the aggressor, the individual can gain from him at least a minimum of a protective presence. This paradoxical relation is observed, for example, in children who experienced trauma in the family environment, but also in adult victims of interpersonal abuse in the developmental period (e.g. relation with the spouse, employer, but also prison guards in the case of prolonged incarceration), when the victim can find no one else to be the object, has a strong tendency to look for relief in another member of a social group. Such a distorted perception of the aggressor often results in a tendency for victimisation, i.e. entering new, risky relations, and repeated hurting [1].

The clinical experience with veterans hospitalised in the Department of Psychiatry, Combat Stress and Psychotraumatology demonstrates that the violence they frequently present is a part of the flashbacks, when the veteran is unaware of his actions or against whom they are directed. Flashbacks are dissociative episodes, lasting between a few seconds to a few hours, during which an individual acts as when experiencing the traumatic event [4]. Many life partners of the veterans hospitalised in our department were beaten at night, when the veteran had nightmares. During these nightmares veterans may kick, hit, thrust themselves or strangle their partners. Sometimes they sleep with a knife under the pillow, or force the entire family to crawl on the floor at night to avoid a suspected danger. If the acts of violence occur only during the nightmares, and at daytime there are no acts of aggression, it may be an isolated sign of PTSD. However, impulsive, uncontrollable aggression may be considered a sign of PTSD aggression, while if it is planned and intended, often instrumental, it is indicative of other mental disorders.

## Conclusions

The protective mechanism of identification with the aggressor helps to understand aggressive behaviours and violence in the veterans of Polish Military Contingents during psychotherapy of war trauma.

Impulsive, unplanned aggression may be related to PTSD, while intended and planned aggression relates to other mental disorders.

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# Extensive radicular cyst in the mandible – a case report

## Rozległa torbiel korzeniowa żuchwy – opis przypadku

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**Abstract.** This paper presents a case study of a patient with an extensive radicular cyst in the mandible. Radicular cysts that are particularly extensive should be differentiated from the various other types of cysts, benign tumours - especially ameloblastomas, cyst alike lesions, some forms of fibrous dysplasia or central granuloma. In the majority of cases those lesions are asymptomatic, therefore periodic radiological diagnostics of patients, especially those with general burden, are advisable in order to exclude potential outbreaks of infection. Based on the available literature, full radiological, clinical, and histopathological diagnostics were made of the lesion, and a non-standard therapeutic procedure was applied in the later stage. Enucleation treatment was suspended until proper bone reconstruction took place, which avoided the need for massive surgery.
**Keywords:** cystectomy, cystotomy, decompression, enucleation, marsupialization, radicular cyst

**Streszczenie.** W pracy przedstawiono przypadek pacjenta z rozległą torbielą korzeniową żuchwy. Torbiele korzeniowe, zwłaszcza rozległe, należy różnicować z innymi typami torbieli, guzami łagodnymi, szkliwiakami, centralnymi ziarniniakami olbrzymiokomórkowymi, niektórymi postaciami dysplazji włóknistej oraz guzami złośliwymi. Zmiany te najczęściej są asymptomatyczne, dlatego ważna jest okresowa diagnostyka radiologiczna, zwłaszcza u pacjentów z obciążeniami ogólnymi, w przypadku których wskazane jest wykluczenie ognisk infekcji zębopochodnej. Opierając się na dostępnym piśmiennictwie, pełnej diagnostyce radiologicznej, klinicznej i histopatologicznej zmiany, w dalszym etapie zastosowano niestandardowy schemat postępowania terapeutycznego. Odroczono zabieg enukleacji do czasu znamiennej odbudowy kości, co pozwoliło uniknąć rozległego zabiegu chirurgicznego.
**Słowa kluczowe:** torbiel korzeniowa, dekompresja, marsupializacja, cystotomia, cystektomia, enukleacja

Delivered: 05/06/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 42-47


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## Introduction

The radicular cyst (RC) is the most frequently found cyst of the maxillo-facial area, and the most common odontogenic cyst [1, 2]. It is an inflammatory lesion that, following proper surgical treatment, does not typically recur. If the associated teeth are removed without the cyst (or if the cyst is not completely enucleated), it may grow further, causing a reduction in the bone tissue, eventually reaching a large size, partially due to its asymptomatic development.

What is the etiopathogenesis of odontogenic inflammatory cysts? Two factors are important in their origin: survival of the epithelial Malasseza cells, and an inflammatory factor (usually teeth with necrotic pulp). The development of RC and the atrophy of the surrounding bone tissue are due to proliferation and metabolism of the epithelium lining the cyst, and the pressure difference between the inside and outside of the lesion [3].

The treatment of choice of a radicular cyst is enucleation, i.e. complete removal of the capsule by extirpation or excochleation [1, 2]. It may be combined with resection of the tooth root after the endodontic treatment involving simultaneous retrograde filling of the root canal or tooth extraction. In the latter case, the cyst capsule is removed through the dental alveolus or via extra-alveolar extraction by elimination of the bone lamella, usually from the oral vestibule. In the case of extensive RC (when enucleation is associated with the risk of pathological fracture), a two-stage method is an option: decompression in the first stage (cystotomy,

Partsch I method), to reduce the size of the cyst, and enucleation in the second stage (cystectomy, Partsch II method) [2].

## Case study

A 50-year-old man visited the Clinic of Cranio-Maxillofacial Surgery of the Military Institute of Medicine due to pain in the left temporomandibular joint area, persisting for 6 days. The patient reported moderate, transient pain, increasing during meals, and alleviated by analgesics (NSAIDs). He lost the teeth in the left lateral section approximately 15-17 years before. Vincent's symptom was not observed. The patient had not used the removable lower denture for a few years. He had a history of arterial hypertension and type 2 diabetes.

The extraoral physical examination revealed elevation of the left cheek. An intraoral examination demonstrated distension of the mandibular shaft and branch on the left, without pain upon palpation, and with a perceptible fluctuation. The patient had no teeth in the lateral section on the side of the lesion. A limited scope of mandibular abduction was observed, with second degree trismus.

A pantomogram was performed and revealed an extensive, cystic lesion in the left mandible. The diagnostics were completed with computed tomography, which demonstrated the scope of the lesion, covering the entire thickness of the mandible, resulting in atrophy of the cortical layer (in some sections it was invisible: interrupted or significantly reduced). The lesion, extending from the mandibular notch to the middle of the left mandibular shaft, was of approx. 27 x 35 mm in the transverse plane, and approximately 51 mm in the vertical dimension. Atrophy of the coronoid was also observed.

Considering the size of the cyst, a biopsy was performed to enable differential diagnosis to exclude lesions such as ameloblastoma, KCOT (keratinizing cystic odontogenic tumour) or other odontogenic tumours. Under block and infiltration anaesthesia with a 2% solution of lidocaine and an 0.5% solution of bupivacaine with adrenaline, a lenticular incision was made in the mucosal membrane over the lesion (approx. 2 cm in diameter), through which a significant amount of opalescent, amber fluid effused. The cystic cavity was left open to enable drainage. The obtained material (a fragment of the capsule) was sent for histopathological analysis, which provided a microscopic diagnosis of *radicular cyst.*

The above procedure also allowed to decompress the lesion, and was the first phase of a two-stage therapy. The applied therapy involved patient's visits 2-3 times a week for 2 months after the procedure for rinsing of the cystic cave with an 0.05% solution of chlorhexidine, and was instructed to rinse his oral cavity at home as well. Subsequently, the frequency of visits was reduced to 3-4 times a month for the following year. Follow-up radiological tests (OPG, CBCT) were performed after 4, 9, 17 and 20 months following the procedure. Regression of the lesion was observed, as well as an increase in bone density, accompanied by increased bone trabeculation. The outline of the cyst began to disappear, and no clear osteosclerotic case was found, which indicated spontaneous fading.

The final procedure of removing the remaining cystic capsule was conducted 20 months after the decompression. Under block and infiltration anaesthesia, with a 2% solution of lidocaine and an 0.5% solution of bupivacaine with adrenaline, a lenticular incision was made on the mucosal membrane of the left mandibular shaft, around the formed fistula. The cystic capsule was enucleated whole, and the obtained material was sent for histopathological analysis. The obtained results confirmed the initial diagnosis.

Due to the observed spontaneous autoregeneration of the mandibular bone, the deficit was not reconstructed with an autogenous transplant or bone substitute material.

Presently, the patient dose not report any pain. The scope of the mandibular abduction is normal.

## Discussion

The primary treatment of a radical cyst consists in a total removal (cystectomy, enucleation). This method is used and recommended by the majority of specialists, as it provides predictable outcomes. However, some authors postulate more conservative treatment, i.e. decompression. Asutay et al. examined the evolution of cystic lesions (of >3 cm in diameter) in the mandibular bones of 40 patients, using radiological examination. They compared CBCTs performed during the diagnostics, and 6 months after decompression. The results indicated the size of the pathological lesion decreased in all the patients [4].

Despite the sceptical approach of many authors to the treatment of radicular cysts with conventional endodontic methods, there are experienced clinicists who report success with such therapy in periapical lesions of up to 20 mm in diameter [5]. However, it has not been explained why some RC are susceptible to this treatment, while others are not [1].

In the presented case, due to the large size of the RC, its removal was associated with a high risk of damage to the neurovascular bundle, or pathological fracture of the mandible (considering the limited possibility of using prophylactic miniplates due to the significant thinning of the cortical layer of the mandible). Treatment involving decompression seemed to be the most beneficial.

There is a risk of neoplastic transformation of the remaining lining of the radical cyst. According to Araujo et al., the frequency of odontogenic cysts transforming into PIOSCC (primary intraosseous squamous cell carcinoma) is 0.13-2%, and most cases apply to the mandible. In their analysis, out of 116 lesions that developed into PIOSCC, 70 were radical/residual cysts, 19 were dentigerous cysts, 16 were KCOT, and 10 were other types [6].

Schlieve et al. attempted to determine if the histopathological results based on the material collected

during decompression differed from the final results after the delayed cystectomy. In their study they analysed the medical history of 25 patients with histopathological diagnosis following the decompression of a benign cystic lesion, and after its radical removal at least 9 months later. The histological diagnosis of odontogenic cysts established after decompression and after enucleation was the same in 88% of cases [7].

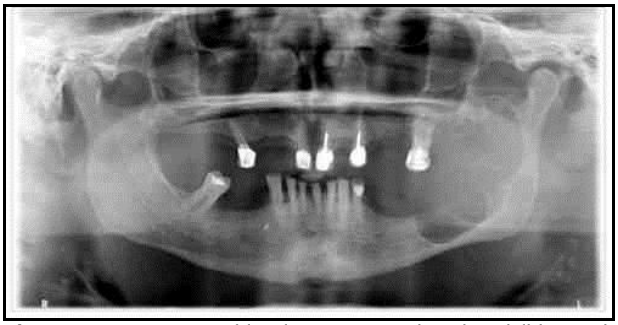

**Figure 1.** Pantomographic picture presenting the visible cystic lesion in the corpus mandibulae on the left side. Pre-treatment condition.
**Rycina 1.** Zdjęcie pantomograficzne z widoczną zmianą torbielowatą trzonu żuchwy po stronie lewej. Stan przed leczeniem.

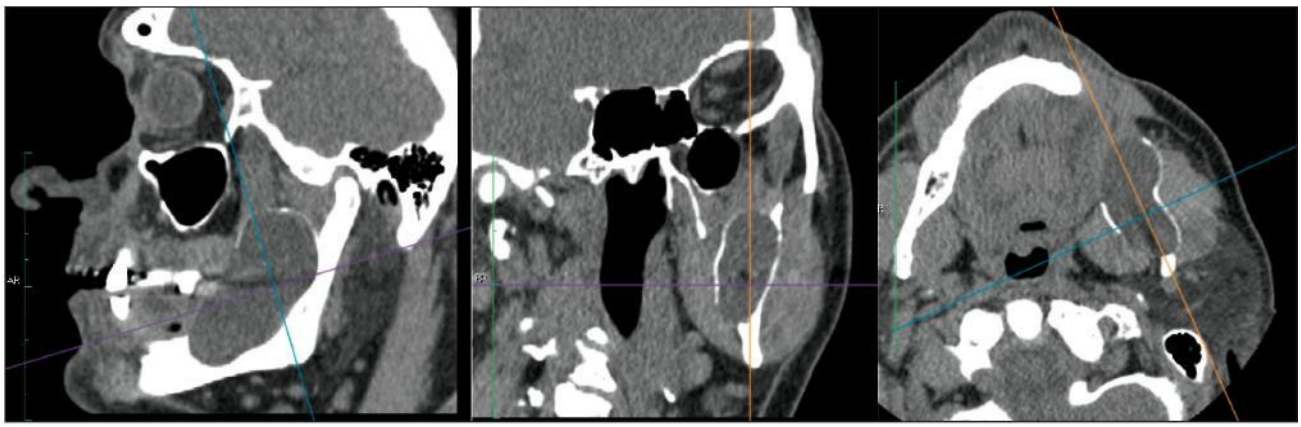

**Figure 2.** CT profiles with visible radicular cyst. Pre-treatment condition.
**Rycina 2.** Przekroje TK z widoczną torbielą korzeniową. Stan przed leczeniem.

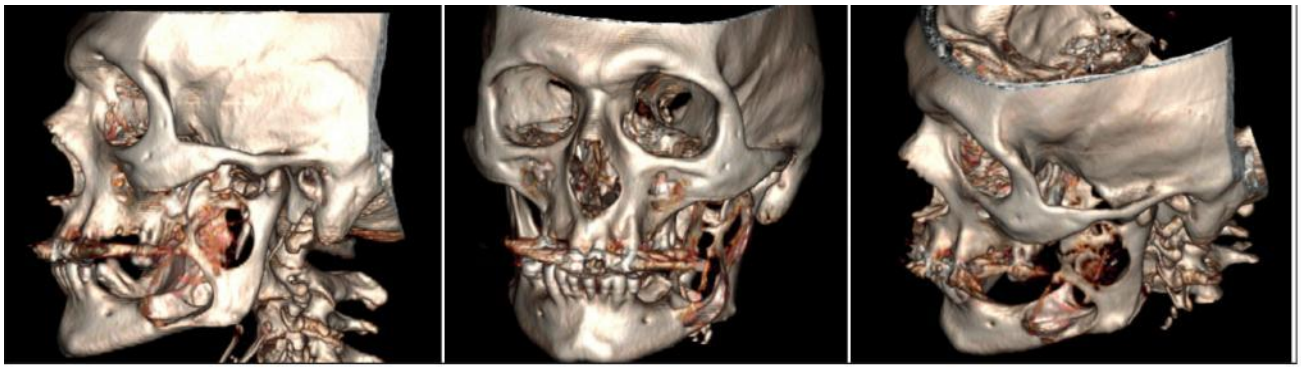

**Figure 3.** 3D reconstruction of CT examination
**Rycina 3.** Rekonstrukcja 3D badania TK

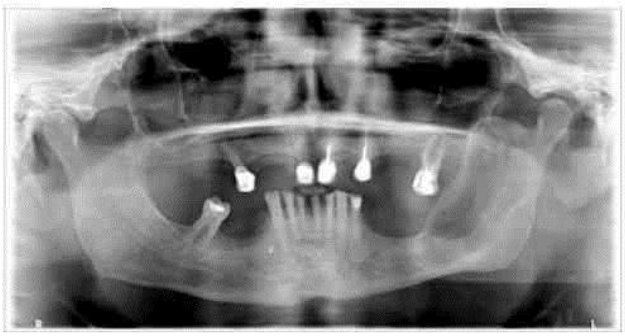

**Figure 4.** Pantomographic picture taken 4 months after decompression
**Rycina 4.** Zdjęcie pantomograficzne wykonane 4 miesiące po zabiegu dekompresji

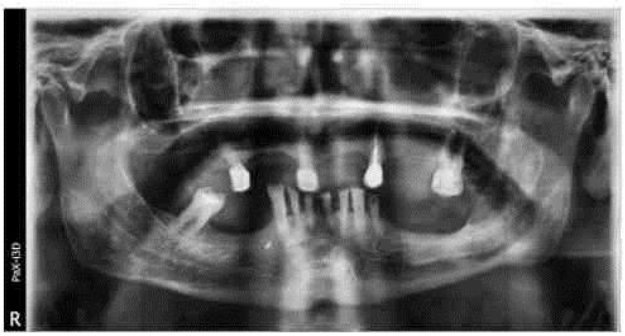


**Figure 5.** Pantomographic picture taken 20 months after decompression
**Rycina 5.** Zdjęcie pantomograficzne wykonane 20 miesięcy po zabiegu dekompresji

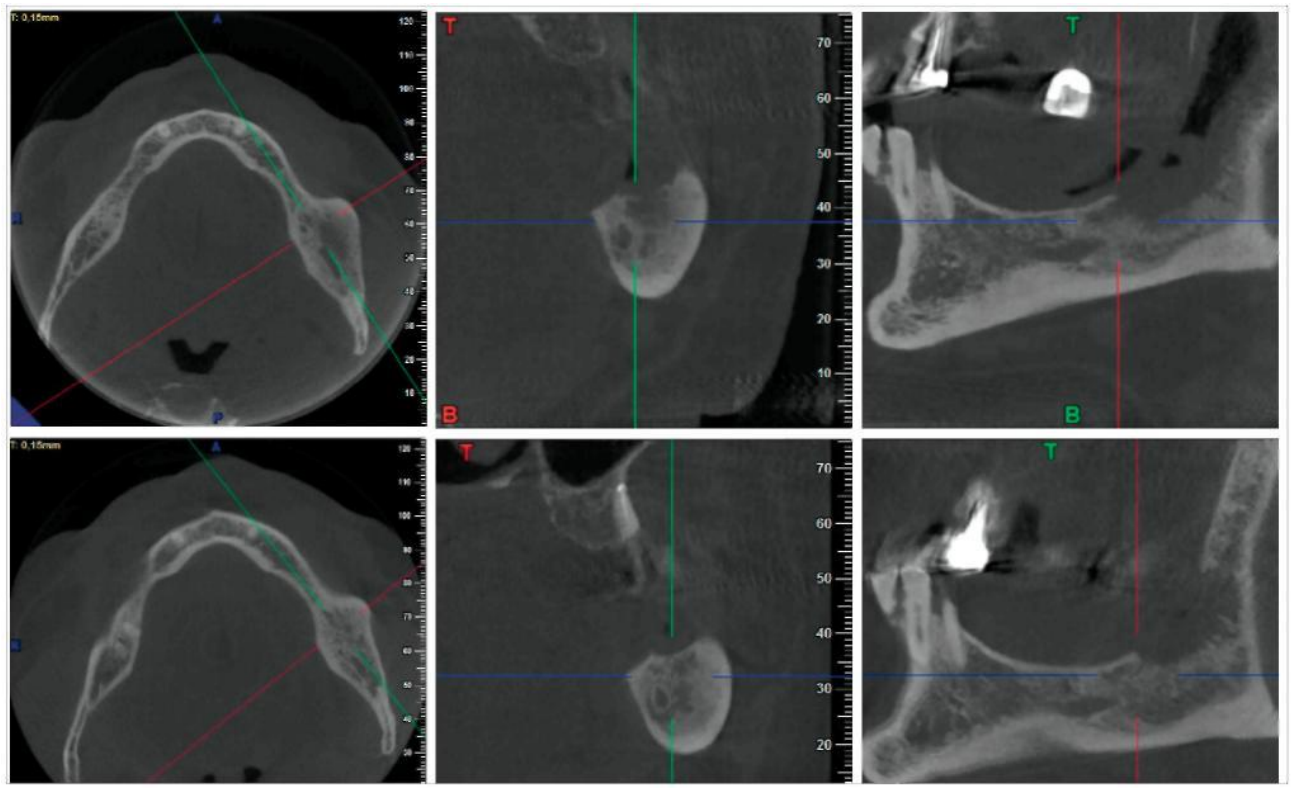


**Figure 6.** Comparison of CT profiles taken 9 months (upper series) and 17 months after decompression (bottom series)
**Rycina 6.** Porównanie przekrojów TK wykonanych 9 miesięcy po zabiegu dekompresji (seria górna) oraz 17 miesięcy po zabiegu (seria dolna)

In summary, histopathological diagnosis following cystotomy or following cystectomy is in most cases identical. Therefore the therapy involving initial decompression with histopathological diagnosis and delayed enucleation is associated with a good prognosis. Based on regular radiological tests and follow-up observations, the decision to delay cystectomy in the presented case provided good outcomes. Close contact with the patient enabled potential modification of the treatment in the case of symptom exacerbation.

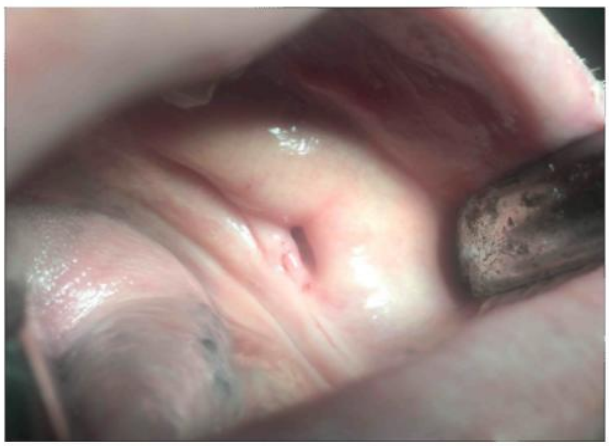

**Figure 7.** Exhaled outlet of the cyst cavity
**Rycina 7.** Wynabtonkowane ujście jamy torbieli

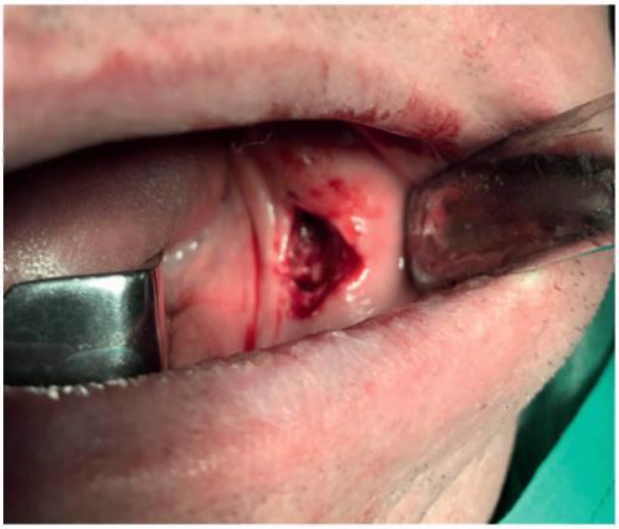

**Figure 8.** Postenucleation condition of cyst capsule
**Rycina 8.** Stan po wyłuszczeniu torebki torbieli

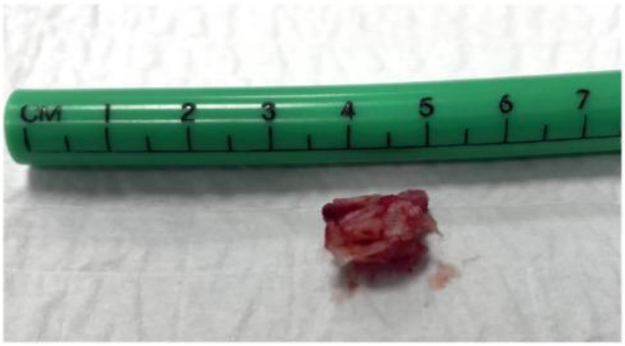


**Figure 9.** Cyst after enucleation
**Rycina 9.** Wyłuszona torebka torbieli

Another important question is the need to fill the bone deficit after the removal of the RC. According to observations by Hren and Miljavec, based on the analysis of the pantomograms of 33 patients treated due to radical and residual cysts, the bone density level after 12 months following enucleation was 97% for lesions of 20-30 mm, and 84% for lesions of 30-50 mm [8]. Therefore, augmentation of the cystic cavity after such procedures is usually not required. Closing of the deficit with a mucosal-periodontal flap enables proper clot formation, and, subsequently, its transformation into regular bone structure [9]. In the case of decompression the mechanism of bone reconstruction is different. Reduction of the pressure created by the cyst enables the newly created bone tissue to fill the defect. This was demonstrated in the studies by Zhao et al., who used marsupialisation to decompress cysts in 53 patients with KCOT [10].

In the presented case the high regeneration rate allowed the augmentation procedure to be avoided.

## Conclusions

In the therapy of large radical cysts in the mandibular bone, a two-stage treatment can be successfully implemented. It allows the considerable reduction in the number of peri- and post-operative complications, such as iatrogenic damage to the mandibular neurovascular bundle, damage to the extraosseous structures, pathological fracture of the mandible, and late complications associated with a disturbed temporomandibular joint function. The two-phase method also significantly limits the scope of the final procedure, the cystectomy may eliminate the need for general anaesthesia, minimise the post-surgical complaints, and considerably shorten the convalescence period.

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# Intestinal malrotation - a case report

## Nieprawidłowy zwrot jelit - opis przypadku

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**Abstract.** Intestinal malrotation is a congenital defect of the gastrointestinal tract. The illness is usually diagnosed in the neonatal period or infancy. The most common symptoms are vomiting, abdominal pain and duodenum obstruction. These symptoms are caused by the presence of Ladd's bands. The Ladd's procedure is the most frequent surgical treatment in intestinal malrotation.
**Keywords:** intestinal malrotation, Ladd's bands

**Streszczenie.** Nieprawidłowy zwrot jelit jest jedną z wad wrodzonych przewodu pokarmowego. Objawy występują zwykle w okresie noworodkowym lub niemowlęcym. Najczęstszymi objawami klinicznymi są wymioty, dolegliwości bólowe ze strony jamy brzusznej i objawy niedrożności wysokiej. Powyższe objawy są związane z obecnością wrodzonych zrostów pasmowatych (włókna Ladda). Operacja Ladda jest najczęściej wykonywanym zabiegiem chirurgicznym przy nieprawidłowym zwrocie jelit. **Słowa kluczowe:** nieprawidłowy zwrot jelit, pasma Ladda

Delivered: 30/06/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 48-49


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## Introduction

Intestinal malrotation is a congenital defect of the gastrointestinal tract. It is observed in 1 in 500 live births. Clinical symptoms usually occur in the neonatal period or infancy. However, some patients do not demonstrate clinical symptoms, and intestinal malrotation may only be observed during imaging studies or a laparotomy/laparoscopy carried out for another reason. The most common symptom is a significant obstruction of the gastrointestinal tract, caused by the pressure of the Ladd's bands on the duodenum. Contrast-enhanced examination of the upper gastrointestinal tract is the most precise and useful additional test [1, 2].

## Case study

A 20-year-old patient visited the emergency department due to bile vomiting and strong abdominal pain persisting for 2 days.

The pain increased and was not alleviated by NSAIDs or muscle relaxants. The patient had a history of similar symptoms for a few years, but they were never equally pronounced, and usually subsided after several hours.

The patient denied any changes in the bowel movement or the presence of blood in the stool; there was no family history of gastrointestinal diseases. The patient did not have any chronic diseases, and did not have any regular treatment.

The physical examination revealed a slight abdominal distension, with diffused pain, without peritoneal symptoms or pathological resistance.

Laboratory tests did not demonstrate any significant abnormalities.

A contrast-enhanced intestinal passage examination was ordered, but as the test could not be performed on an emergency basis, contrast-enhanced CT was performed instead. The test revealed a swirl in the right upper abdomen, possibly consistent with intestinal malrotation in the duodenal area, with stomach distension. The patient was qualified for urgent surgery.

During the procedure, a significant obstruction of the gastrointestinal tract was demonstrated, in the course of congenital band-like adhesions (Ladd's bands), and signs of malrotation of the intestinal loops. Ladd's procedure

was performed: the small intestinal mesenteric torsion was decompressed, the periduodenal adhesions were freed, and an appendectomy was conducted.

No complications were observed in the post-operative period. The patient was discharged in a good general and local condition. After two weeks she returned to the surgical clinic at the hospital for a follow-up and removal of the sutures. She did not complain about pain, the wound was properly healed, and the sutures were removed.

## Discussion

Intestinal malrotation is any disturbance of the normal midgut rotation in the foetal period. It is a congenital defect of the gastrointestinal tract, observed in 1 in 500 live births. Clinical symptoms usually occur in the neonatal period or infancy, in over half of the patients in the first month. Intestinal malrotation may also be associated with other congenital defects, such as duodenal or intestinal atresia, or umbilical or diaphragmatic hernia [2, 3].

In adult patients the clinical symptoms may be absent, but if they occur, they take the form of chronic, acute abdominal pain, vomiting, and symptoms of a significant gastrointestinal obstruction. The above symptoms may be due to the presence of Ladd's bands (abnormal peritoneal bands), which press on the intestine or cause intestinal rotation [4, 5].

A physical examination may reveal abdominal distension, tenderness upon palpation, reduced or absent peristalsis, and sometimes also peritoneal symptoms [6, 7].

The most useful diagnostic tests include imaging studies, such as a contrast-enhanced examination of the upper gastrointestinal tract, plain abdominal X-ray, abdominal ultrasound and computed tomography. The contrast-enhanced examination of the upper gastrointestinal tract is considered the most precise and useful test in intestinal malrotation; in this examination the duodenum may be corkscrew-shaped or hook-shaped. On an emergency basis, an ultrasound or CT test are often more available. An ultrasound test may reveal a whirlpool effect due to the superior mesenteric vein and mesentery wrapping around the superior mesenteric artery, whereas CT scan may demonstrate the right-sided position of the small intestine, and a left-sided position of the caecum, as well as reversed position of the superior mesenteric vessels [7, 8].

In patients who do not present any clinical symptoms, where intestinal malrotation is demonstrated during imaging diagnostics performed for other reasons, the most suitable management is still subject to discussion (surgical treatment vs observation).

If the symptoms occur, the patient is qualified for the Ladd's procedure. This consists in separating the band-like adhesions (Ladd's bands) between the duodenum and the large intestine, prophylactic appendectomy, and extending the mesenteric base to prevent any recurrence of the malrotation [4, 7, 9].

In the presented case the patient experienced numerous episodes of abdominal pain. As part of the imaging diagnostics, a contrast-enhanced abdominal CT was performed, which revealed intestinal rotation in the duodenal area, and stomach distension. The patients was qualified for an urgent surgery; the Ladd's procedure was performed, and the patient tolerated the operation well. No complications were observed in the post-operative period.

## Conclusions

Intestinal malrotation is a congenital defect rarely detected in adult patients; however, it should be taken into consideration during differential diagnostics in patients with significant gastrointestinal obstruction.

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# 67-year-old female with variant angina complicated by myocardial infarction

## Dławica naczynioskurczowa powikłana zawałem serca u 67-letniej kobiety

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**Abstract.** Variant angina is a form of stable coronary artery disease. The essence of the disease is a spontaneous spasm of the coronary arteries. The symptoms are paroxysmal. In most cases, the prognosis is good, but serious complications may occur, like a myocardial infarction, arrhythmia or sudden cardiac death. In spite of the recurring nature of the disease, a myocardial infarction is very rare (<0.5% a year). This article presents a case of a patient with myocardial infarction in the course of variant angina.
**Key words:** myocardial infarction, spasm of coronary arteries, variant angina

**Streszczenie.** Dławica piersiowa naczynioskurczowa jest postacią stabilnej choroby wieńcowej. Istotą choroby jest samoistny skurcz tętnic wieńcowych. Objawy mają charakter napadowy. Rokowanie w większości przypadków jest dobre, choć zdarzają się poważne powikłania w postaci zawału serca, zaburzeń rytmu serca oraz nagłej śmierci sercowej. Pomimo nawracającego charakteru dolegliwości zawał serca w przebiegu dławicy odmiennej występuje bardzo rzadko (<0,5% w ciągu roku). Poniższy artykuł przedstawia przypadek pacjentki z zawałem serca w przebiegu dławicy odmiennej.
**Słowa kluczowe:** dławica piersiowa naczynioskurczowa, skurcz tętnic wieńcowych, zawał serca

Delivered: 24/09/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 50-53


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## Introduction

Variant angina (Prinzmetal's angina) is a relatively rare disease, described for the first time in 1959. Previously, it was classified as acute coronary syndrome. According to the current 2013 ESC guidelines it is a form of a stable angina pectoris. The clinical course may exacerbate in the case of a complete and prolonged vascular contraction, resulting in a transmural myocardial infarction. As in STEMI, due to atherosclerotic lesions, a sudden cardiac arrest may occur as a consequence of disturbed heart rhythm. Calcium channel blockers, which affect the primary mechanism of vascular contraction, are used in the treatment of the disease. Presented below is the case of a patient presenting with complications in the course of Prinzmetal's angina.

## Case study

A 67-year-old patient was admitted to the Department of Internal Diseases and Cardiology due to palpitations persisting for 3 days, associated with non-specific, continuous chest pain, intensive sweating and dyspnoea at rest. The patient reported a long history of arterial hypertension, chronic ischaemic heart disease, permanent atrial fibrillation, status post-ischaemic cerebral stroke and advanced degenerative disease of peripheral joints.

The physical examination at admission revealed peripheral oedema extending to the knee joints, irregular heartbeat of approximately 90 beats/min, and crackles at the base of both lung fields.

The laboratory tests did not demonstrate significant abnormalities, such as increased concentration of the enzymes associated with heart muscle damage. The thoracic imaging presented signs of chronic stasis in the pulmonary circulation.

The echocardiogram revealed regional contractility disorders in the form of hypokinesis of the anterior interventricular septal wall in the basal and middle segments, as well as a reduced ejection fraction of 45%.

A coronarographic examination was performed, demonstrating paramural lesions in the epicardial arteries. The therapy with bisoprolol, perindopril, furosemide, spironolactone, acetylsalicylic acid and clopidogrel at standard doses was continued. During the procedure there were difficulties with moving the guidewire through the brachial artery due to its significant constriction. On the same day, before noon, the patient complained about a strong chest pain, associated with blood pressure reduction to 70/40 mm Hg, and a heartbeat reduced to 40 beats/min. An electrocardiogram revealed a new ST elevation and a Pardee wave in the leads over the inferior wall, and a regular and slow (approx. 40 beats/min.) heart rhythm. Another echocardiogarm was performed to exclude dissection of the main artery wall or the presence of pericardial fluid indicating a dissection of a coronary vessel. Since the echo did not demonstrate new lesions, a control coronarography was performed through the femoral artery, and revealed a massive contraction of the right coronary artery (Fig. 1), resulting in a critical constriction of the posterior descending artery, unresponsive to the intracoronary administration of nitroglycerin and papaverine.

Simultaneously, due to a complete atrioventricular block, an endocavitary electrode was introduced for temporary cardiac stimulation. The laboratory tests performed the following day revealed an elevated concentration of ultrasensitive troponin to 100 ng/ml, whereas the normal range is 0.06 ng/ml. The patient was diagnosed with ST-elevation myocardial infarction due to variant angina. The introduced therapy included double antiplatelet therapy, unfractionated heparin, and dihydro- and non-dihydropyridine calcium channel blockers at titrated doses (diltiazem 120 mg, amlodipine 10 mg). In the next days the patient reported a sense of well-being, the chest pain did not recur, and the vital parameters were normal (Fig. 2.). A haemodynamically efficient atrial fibrillation rhythm was restored, with a ventricular complex frequency of approx. 70/min, which justified the removal of the endocavitary electrode. In the coronarographic examination performed a few days later, the previous changes were absent. In the following days the patient's general status remained stable, and she was discharged from the hospital.

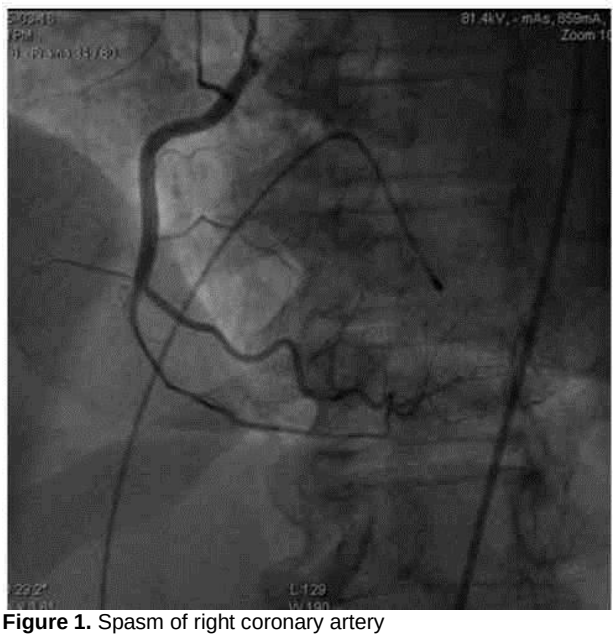

**Figure 1.** Spasm of right coronary artery
**Rycina 1.** Skurcz prawej tętnicy wieńcowej

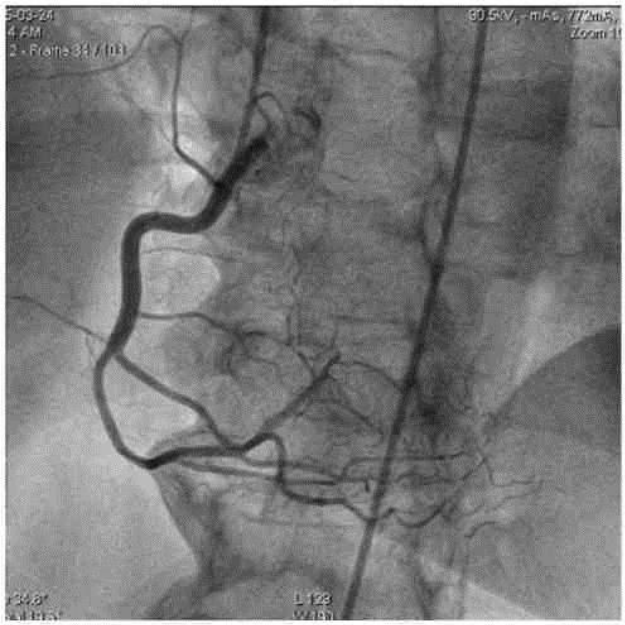

**Figure 2.** Right coronary artery after spasm abated
**Rycina 2.** Ustąpienie skurczu prawej tętnicy wieńcowej

## Discussion

Variant angina is a form of ischaemic heart disease, presently classified as a stable coronary syndrome. It is found in 2-5% of patients with angina pectoris [1]. The spasm may occur in vessels with or without atherosclerotic lesions [2]. A significant stenosis of the coronary arteries in patients with Prinzmetal's angina is observed in 60% of cases [1]. Coronary artery spasm may be triggered by tobacco smoking, electrolyte imbalance (potassium, magnesium), hyperventilation, stress, low temperatures, using narcotics, and resistance to insulin. It may also occur in the course of an autoimmune disease [1, 2, 6]. It usually affects the right coronary artery [1], and may take a focal, multifocal or diffused form [2].

Potential mechanisms responsible for a vascular spasm include the increased cellular activity of Rho kinase, and abnormalities in the adenosine triphosphate-sensitive potassium channels and/or membrane sodium-hydrogen exchange [2].

Other factors contributing to vascular spasms include disturbed function of the autonomic nervous system, intracoronary elevation of the concentrations of vasoconstrictors, such as endothelin-1 [3], neuropeptide Y, or thromboxane B2, as well as hormonal changes [2, 6]. All these mechanisms result in increased intracellular calcium concentration and/or hypersensitivity of myosin to calcium [3].

Clinical symptoms typically occur at rest, usually between midnight and 6 am, while exercise tolerance remains unchanged, contrary to classic or microvascular angina [1, 2].

ECG may reveal two types of changes. ST elevation, if the vessel is completely closed by the spasm, which abates without permanent lesions, and ST lowering, when the distal vessel regions are affected, and only partially closed. To confirm the diagnosis and differentiate between other forms of ischaemic heart diseases, a coronarography is conducted. During the test, agents provoking vasoconstriction are administered intracoronally (ergonovine, acetylcholine) [1, 2, 6].

Prophylaxis involves fighting the risk factors for the development of atherosclerosis and its complications, as well as avoiding the situations that may provoke a spasm.

The basic medications for Prinzmetal's angina are calcium channel blockers, sometimes used at high doses (240-480 mg/d of verapamil, 120-360 mg/d of diltiazem, 40-60 mg/d of nifedipine) [1, 2, 5, 6]. If the effect of treatment with one calcium blocker is unsatisfactory, another blocker may be introduced, but from a different group [1]. In long-term treatment long-acting nitrates may be added, so that their time of action covers the occurrence of the symptoms. Such therapy allows the symptoms to be fought effectively in approximately 90% of patients [2]. In approx. 10% of cases, resistance to treatment may occur. In these patients alpha-adrenergic receptor blockers are additionally used (clonidine). To stop an episode of angina-related pain, short-acting nitrates are used. Contraindicated medications include beta-blockers, especially non-selective ones, as they may increase the spasms [2, 6].

Routine implantation of stents in the vessels without atherosclerotic lesions is not recommended, as it does not prevent vascular contractions in other locations [2]. The complications associated with Prinzmetal's angina include life-threatening disturbance of rhythm or conductivity, which may be indications for implantation of a stimulator of cardioverter-defibrillator [1, 2, 6]. In some cases, including in the presented patient, complications may include a myocardial infarction, or even a sudden cardiac death [2, 6].

Prognosis in variant angina is good, as 95% of patients survive 5 years [1]. The prognosis depends on a concurrence of ischaemic heart disease and its extent, frequency and duration of episodes of vascular spasm, the area of the cardiac muscle at risk of ischaemia during a spasm, and the rhythm and conductivity disorders observed during the ischaemia [2]. The prognosis is adversely affected by advanced atherosclerotic lesions combined with the vascular spasm, and the focal character of variant angina, which leads to a complete closure of the vessel [2].

## Conclusions

The presented case demonstrates complications rarely observed in the course of variant angina. Our patient experienced transmural myocardial infarction and conductivity disorders in the form of a complete atrio-ventricular block. A typical therapy in gradually increased doses was applied, and its effectiveness was confirmed by the absence of disease symptoms and the results of a follow-up coronography. Despite the common perception of the disease as a non-life-threatening one, in rare cases it may lead to a sudden cardiac death.

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# Difficulties in the treatment of pneumonia - a case report

## Trudności w leczeniu zapalenia płuc - opis przypadku

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**Abstract.** *Acinetobacter baumannii* is an opportunistic pathogen, causing serious hospital-acquired infections, especially of the respiratory tract. A case of nosocomial pneumonia with respiratory failure was presented. An infection was found in a 58-year-old female admitted to a hospital for *Streptococcus pneumoniae*. Despite appropriate therapy, the pneumonia progressed and massive structural changes in the lungs were observed and a multi-drug resistant strain *of Acinetobacter baumannii was* cultured from the sputum. After a targeted antibiotic was administered, the clinical status of the patient improved gradually, but there was no improvement in the radiological picture. However, in a chest X-ray performed 1.5 years later, only small fibrous changes in the left lung were observed. This demonstrates both the high and slow regenerative capacity of the pulmonary parenchyma.
**Key words:** *Acinetobacter baumannii,* antibiotherapy, lung regeneration, multi-drug resistance, nosocomial pneumonia

**Streszczenie.** *Acinetobacter baumannii* to oportunistyczny patogen powodujący groźne zakażenia szpitalne, najczęściej układu oddechowego. Przedstawiono przypadek ciężkiego, przebiegającego z niewydolnością oddechową, szpitalnego zapalenia płuc. Do zakażenia doszło u 58-letniej chorej leczonej z powodu pozaszpitalnego zapalenia płuc o etiologii *Streptococcus pneumoniae,* u której pomimo odpowiedniego leczenia pogorszył się obraz radiologiczny z pojawieniem się rozległych struktur jamistych, a z plwociny wyhodowano wielolekooporny szczep *Acinetobacter baumannii.*
Po zastosowaniu celowanej antybiotykoterapii stan kliniczny chorej stopniowo uległ poprawie, jednak nie obserwowano ustępowania zmian w obrazie radiologicznym. W wykonanym półtora roku później zdjęciu radiologicznym klatki piersiowej uwidoczniono jedynie niewielkie zmiany włókniste w płucu lewym. Świadczy to o dużej i zarazem powolnej zdolności regeneracyjnej miąższu płucnego.
**Słowa kluczowe:** *Acinetobacter baumanii*, wielolekooporność, antybiotykoterapia, szpitalne zapalenie płuc, regeneracja płuc

Delivered: 25/09/2017
Accepted for print: 13/12/2017
No conflict of interest was reported.
Mil. Phys., 2018; 96(1): 54-58


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## Introduction

Pneumonia is a disease characterised by inflammatory cells infiltrating the pulmonary parenchyma and effusions being present in the interalveolar space, which hinders gas exchange and leads to hypoxemia, with systemic consequences [1, 2]. Because of the different aetiologies and the resulting different diagnostic and therapeutic procedures, pneumonia was divided into non-nosocomial and nosocomial types [3]. The most common causes of non-nosocomial infections are: *Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Haemophilus influenzae* [3]. In the case of nosocomial infections, their aetiology depends on the length of the hospital stay. Over the first four days, they are usually caused by the same bacteria as in non-nosocomial pneumonia, with multidrug-resistant strains predominating from day five, most often aerobic gram negative bacilli, including *Acinetobacter spp.* [3]. Bacteria from the *Acinetobacter* genus are non-motile and non-fermentative bacilli, commonly found in soil and water bodies [4, 5]. *Acinetobacter baumannii* is the most common pathogen among all species of this genus [4, 5]. It can be a component of the physiologicmicroflora of the skin, pharynx and anus [4, 5]. It often colonises intensive care patients [6, 7]. This bacillus can occupy and stay for a long time on abiotic surfaces, is resistant to disinfectants and can easily acquire and transfer drug resistance mechanisms [8, 9]. Its multidrug-resistance is associated with the presence of various drug resistance

mechanisms, such as metallo-β-lactamases, changes in penicillin-binding proteins (PBPs) and reduced cell membrane permeability [8]. These characteristics predispose *A. baumannii* to triggering nosocomial epidemics [9]. Their source can be the medical personnel or patients transferred from one unit to another [4, 9]. Pneumonias constitute a major cause of death. In their course, mortality can vary significantly, and in Europe it can fluctuate from <1% to 48% [3].

This study describes the case of a female patient treated for non-nosocomial pneumonia caused by *Streptococcus pneumoniae,* who had nosocomial pneumonia caused by multidrug-resistant *Acinetobacter baumannii*, causing very extensive lesions in the lungs.

## Case report

A 58-year-old woman, a shop assistant in a general store, non-smoker for 3 years (previously 20 pack years of smoking), was admitted to the Department of Internal Diseases due to dyspnoea at rest, cough producing purulent sputum, headache, fever reaching 38.5 °C, diarrhoea and vomiting. She suffered from arterial hypertension, ischemic heart disease (a myocardial infarction 20 years ago), atherosclerosis of the lower extremities and type 2 diabetes. She took insulin, quinapril, acetylsalicylic acid, pentoxifylline and sulodexide. The patient was admitted in a generally severe condition. Hypoxemia ($SaO_2$ 77%), tachypnea (24/min), rales over the lungs, a blood pressure of 160/80 mm Hg, a heart rate of 100/min and oedemas in lower extremities were identified. Leukocytosis (19.51 thousand/µl) and an increased concentration of C-reactive protein (58 mg/dl) were found in laboratory tests. A chest X-ray revealed bilateral consolidations in both lungs and pleural effusions (Fig. 1). The patient was diagnosed with pneumonia, and amoxicillin combined with clavulanic acid was administered. *Streptococcus pneumoniae* susceptible to beta-lactam antibiotics were found in a sputum culture.

Despite treatment, the patient had a fever, reported dyspnoea at rest and was coughing up purulent sputum. Also respiratory failure persisted ($SaO_2$ 84% with oxygen therapy employed). Laboratory tests identified increased leucocytosis (25.36 thousand/µl) and a small decrease in CRP concentration (36.85 mg/dl). In an image of the chest performed after 4 days, an aggravation of the condition was observed. A CT angiogram performed at the time excluded pulmonary embolism and revealed bilateral inflammatory consolidations, much more pronounced on the left, with an effusion in pleural cavities and signs of pulmonary hypertension (Fig. 2.). As a result, clarithromycin and ceftriaxone were administered intravenously, and when *Citrobacter freundii* was found in a sputum culture, ciprofloxacin was also used in accordance with an antibiogram.

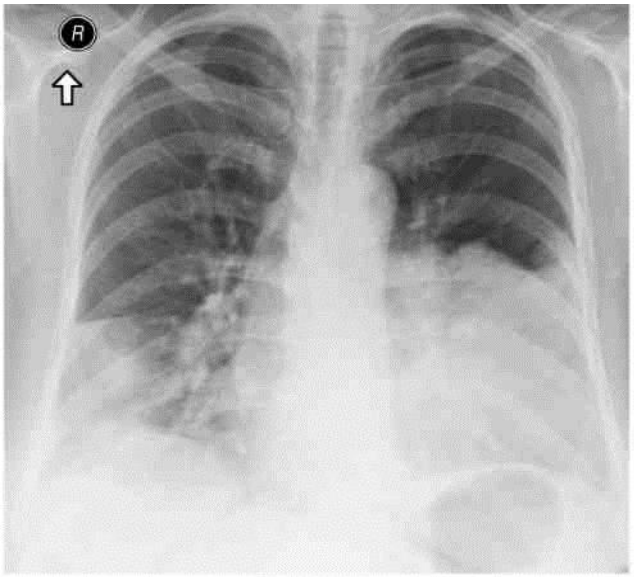


**Figure 1.** Posteroanterior chest X-ray (3/02/2013). Lower lobe consolidation and a right pleural effusion, reaching anterior part of rib IX. Middle and lower field opacities, consolidation and a left pleural effusion.
**Rycina 1.** Zdjęcie radiologiczne klatki piersiowej w projekcji tylnoprzedniej (3.02.2013). Płyn w prawej jamie opłucnowej sięgający do przedniego odcinka IX żebra, z ogniskiem zagęszczenia miąższu płucnego w polu dolnym. Zmniejszenie przejrzystości pola środkowego oraz dolnego płuca lewego, płyn oraz zagęszczenia miąższowe.

Low-grade fever was observed in the subsequent days, the dyspnoea subsided slightly, and $SaO_2$ increased to 93-96% with oxygen provided at a rate of 2 l/min. Leukocytosis and CRP concentration decreased (to 15.67 thousand/µl and 9.7 mg/dl respectively). In a check-up X-ray image no improvement was observed, and *Acinetobacter baumannii,* resistant to almost all antibiotics, with the exception of carbapenems, was grown in a sputum culture. In accordance with the result of the antibiogram, imipenem was applied. The condition of the patient improved gradually, she was no longer feverish, and $SaO_2$ increased to 94% without oxygen therapy. Leukocytosis was at 10.54 thousand/µl, and CRP concentration at 7.7 mg/dl. However, a check-up CT of the chest revealed progression of the lesions in the left lung, increased atelectasis and the consolidation of lesions in the parenchyma, with substantial air cavities appearing, a decreased volume of the left lung, rib retraction and decreased amount of liquid in the pleural cavities, as well as inflammatory lesions in the right lung (Fig. 3.). Bronchoscopy was performed, which revealed no hyperplastic lesions, but purulent discharge was observed in the bronchi, from which *Acinetobacter baumannii* was cultured. No acid-fast bacilli were found. As a result, the Imipenem therapy was continued.

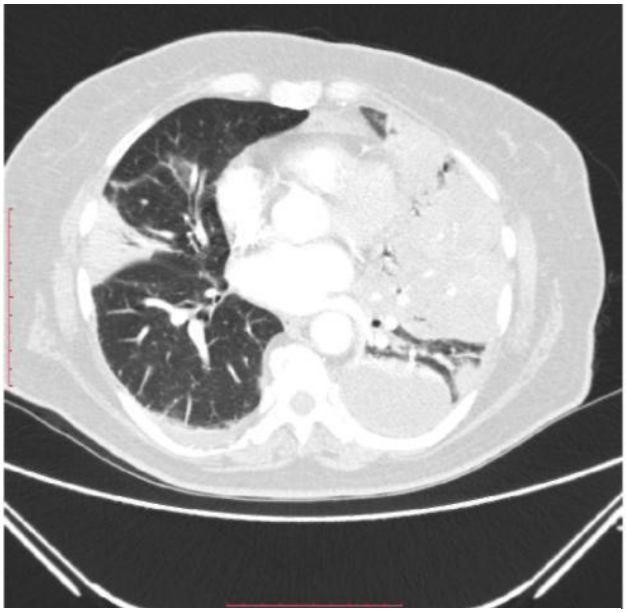

**Figure 2.** Chest computed tomography (8/02/2013). An effusion in the left pleural cavity, partially in adhesions. Massive left lung inflammatory consolidation. Inflammatory changes in seg. 4 of the right lung, mainly at the chest wall, a small amount of effusion in right pleural cavity.
**Rycina 2.** Badanie tomografii komputerowej klatki piersiowej (8.02.2013). W lewej jamie optucnowej częściowo otorbiony płyn. Masywne zagęszczenia zapalne w lewym płucu. W płucu prawym zmiany zapalne w segmencie 4., głównie przy ścianie klatki piersiowej, niewielka ilość płynu w jamie opłucnowej po stronie prawej.

Respiratory system ailments were accompanied by heart failure symptoms (dyspnoea, tachycardia and oedemas in lower extremities). A cardiac echo revealed left ventricular hypertrophy with heart chambers of the correct size, the ejection fraction at 65%, and NT-proBNP increased to 957 pg/ml. After diuretic treatment, the oedemas in the lower extremities were reduced and tachycardia subsided. Intensive insulin therapy was applied for the entire duration of the hospital stay, achieving blood glucose levels within 100-250 mg/dl. During the antibiotic treatment the patient had *Clostridium difficile* diarrhoea, treated with vancomycin to good effect.

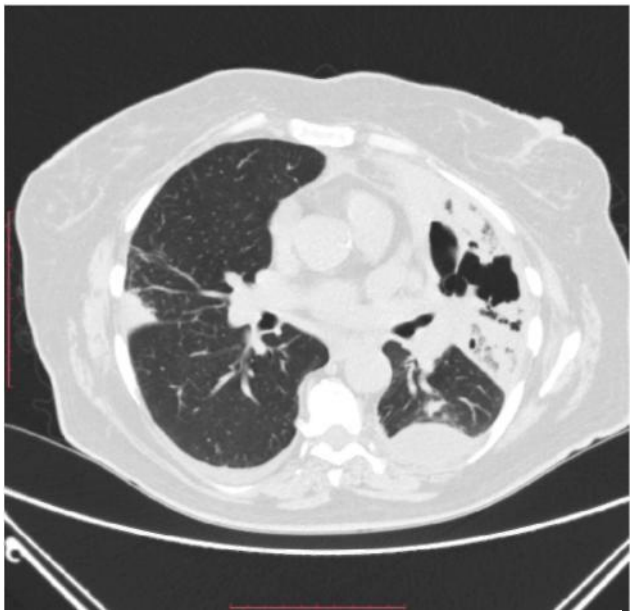

**Figure 3.** Chest computed tomography (7/03/2013). Signs of atelectasis, consolidation and irregular air spaces (cavities) in left lung. A smaller amount of encapsulated effusion. Consolidation and atelectasis in right lung, less than in previous CT.
**Rycina 3.** Tomografia komputerowa klatki piersiowej (7.03.2013). Cechy niedodmy i konsolidacji miąższu w płucu lewym z nieregularnymi przestrzeniami powietrznymi (jamy) w płucu lewym. Mniejsza ilość otorbionego płynu. Zagęszczenia miąższowe i obszary niedodmowe w płucu prawym mniejsze niż w poprzednim badaniu.

After concluding a 16-day imipenem treatment, the clinical condition of the patient gradually improved, as did the results of the laboratory tests. However, no relevant improvement was observed in the x-ray images of the lungs (Fig. 4). The patient was discharged after a 40-day stay with a correct body temperature, no dyspnoea, a slight cough, and respiratorily stable ($SaO_2$ 94% when breathing atmospheric air), with an x-ray check-up after one month and further treatment in a pulmonology clinic being recommended. One and a half years later, the patient was admitted to the hospital for pain in the lumbar spine. An x-ray of the chest performed at the time revealed only small fibrous changes in the left lung and a diaphragm retracted by adhesions (Fig. 5).

## Discussion

*Acinetobacter baumannii* is an opportunistic pathogen causing nosocomial infections [1]. These most often concern the respiratory system, with the lungs being a major point connected with mechanical ventilation [4, 6, 10]. Infections can also appear in other locations (e.g. surgical wounds, the urinary tract and the meninges) or in the form of sepsis [4, 6, 7, 10, 11]. They develop the most often in immunocompromised people and those treated in intensive care units [5]. Patients at the greatest risk are those who have recently had a surgery, are being mechanically ventilated, have an inserted tracheal tube or a central venous catheter, or are being treated with cephalosporin, fluoroquinolone or carbapenem antibiotics [5, 10].

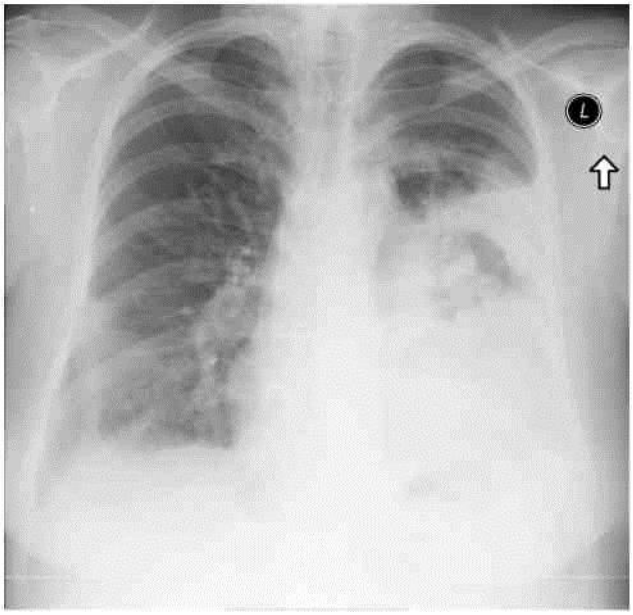

**Figure 4.** Posteroanterior chest X-ray (14/03/2013). Consolidation in the middle and lower left lung with cavity lesions in the central part and consolidation in right lung's lower lobe with effusion in both pleural cavities.
**Rycina 4.** Zdjęcie radiologiczne klatki piersiowej w projekcji tylno-przedniej (14.03.2013). Skonsolidowane zagęszczenia miąższowe w środkowo-dolnym polu lewego płuca z widocznym przejaśnieniem w części centralnej oraz zagęszczenia w dolnym płacie prawego płuca z płynem w obu jamach opłucnowych.

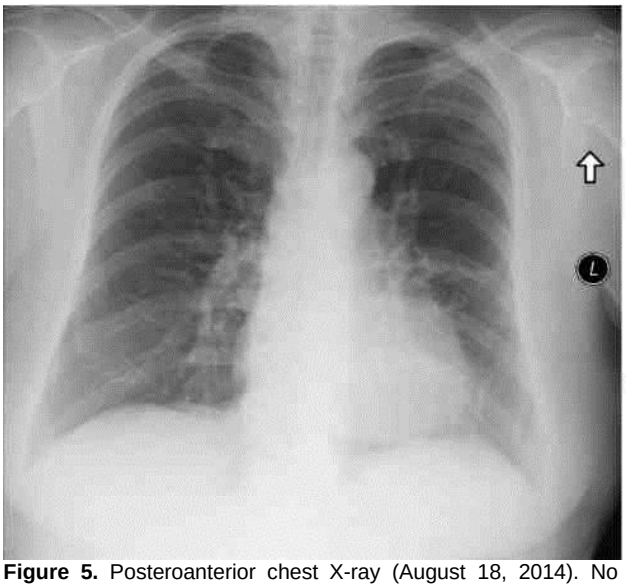

**Figure 5.** Posteroanterior chest X-ray (August 18, 2014). No consolidations in the lungs, small fibrous changes in the left lung, diaphragmatic retraction due to pleural adhesions.
**Rycina 5.** Zdjęcie radiologiczne klatki piersiowej w projekcji tylno-przedniej (18.08.2014). Miąższ płucny bez zmian naciekowych. Niewielkie zwłóknienia w płucu lewym. Przepona pozaciągana przez zrosty.

The patient in question was infected with *A. baumannii* during her stay at the hospital. Antibiotic treatment (amoxicillin combined with clavulanic acid, clarithromycin, ceftriaxone and ciprofloxacin) applied due to severe non-nosocomial pneumonia could have been a risk factor leading to the development of an infection with an opportunistic pathogen. While the administered antibiotics were part of empiric treatment, the sputum culture results confirmed the presence of bacteria *(S. pneumoniae* and *Citrobacter freundii)* susceptible to the medications used. Despite this, the progression of lesions illustrated by X-ray images was observed. Only after *A. baumannii* was cultured and imipenem administered in line with the antibiogram was a marked clinical improvement observed, with no improvement in the radiological image. Substantial atelectasic lesions with air cavities remained for a long time, suggesting irreversible lung damage. In pneumonia, the lesions seen in X-ray images last much longer than the clinical signs and the abnormal results of laboratory tests. For this reasons, it is recommended to produce a check-up X-ray image of the chest as late as 6 weeks after the treatment was discontinued, since it takes time for the effusion to be absorbed and inflammatory infiltrate to subside [12]. Sometimes, inflammatory lesions decrease without any trace being visible in X-ray images (e.g. the numerous abscesses in the course of staph infections). In other cases permanent fibrous or nodular changes or adhesions (e.g. after tuberculosis) are formed [13]. It remains unclear whether this is conditional on the extent of inflammatory lesions, virulence of the bacteria or the individually varied regenerative capacity. It is unknown to what extent the damaged lungs retain this capacity. The

presented case demonstrates that the regenerative capacity of this organ is significant. The regenerative capacity of the lungs has been investigated in a number of studies, which indicate the participation of endogenous stem cells in this process [14, 15]. This suggests that the impairment of this process can cause such chronic diseases as idiopathic pulmonary fibrosis and COPD [14]. Researching these issues can contribute to finding new medications, effective in their treatment [15].

In order to evaluate the efficacy of pneumonia treatment, attention is drawn to the clinical symptoms, abnormalities in laboratory tests and X-ray images. Difficulties occur when information from these sources is inconsistent and does not provide a clear answer as to whether the patient's condition is improving. The case in question indicates that lesions illustrated by X-ray images in the course of pneumonia can subside much later than the clinical symptoms and inflammatory markers. This is of great significance when it comes to making decisions on the duration of antibiotic treatment, which should depend primarily on the clinical response. Antibiotics kill bacteria but do not repair the structural changes which occur in the course of inflammation. It would be, therefore, wrong to use antibiotics until the lesions illustrated by X-ray images are no longer seen [16]. Time is needed for lesions to heal, and this time depends on many factors, such as their extent, the age and nutritional status of the patient, and the occurrence of comorbidities, etc.

Carbapenems are the preferred medications in treating infections of an *A. baumannii* aetiology [5]. However, because of the overuse of this group of antibiotics, resistant strains are appearing increasingly often [11]. A 9% to 40% increase in resistance to carbapenems was observed between 1995 and 2004 [17]. This constitutes a significant therapeutic problem. It is possible that soon there will be no antibiotics effective in treating such infections.

Without determining the aetiology of nosocomial pneumonia and the knowledge of drug susceptibility, it is difficult to effectively treat this disease, and cases of ineffective treatment are being reported increasingly often, especially when the administration of the correct antibiotic is delayed [18, 19]. According to Ye et al. [20], mortality in the course of *A. baumannii* infections varies between 8% and 23% for general nosocomial infections, and between 10% and 43% for ICU infections. The independent risk factors which increase the mortality of pneumonia caused by *A. baumannii* are: tracheostomy, diabetes, COPD and high concentrations of CRP and creatinine [11]. In the discussed case, the patient was diagnosed with diabetes and more than a 100 times higher concentration of CRP in the peripheral blood.

Despite severe nosocomial pneumonia with extensive pulmonary lesions and risk factors, it was possible to contain the inflammation caused by *A. baumannii.* What is noteworthy is the very slow reduction in the vast structural changes.

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# The red cross as a symbol

## Znak czerwonego krzyża

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**Abstract.** On 17 February 1863, Henry Dunant formed the International Committee for Relief to the Wounded with a group of activists. This committee was named the International Committee of the Red Cross. The Polish Red Cross (PCK) was established in 1919. It acts in accordance with the Act of 16 November 1964. The emblem of the Polish Red Cross is a Greek red cross on a white background - the Geneva Cross. The emblem and name of the Polish Red Cross are protected by law. It is prohibited to use the PCK emblem and name unlawfully.
**Key words:** symbol of the red cross

**Streszczenie.** 17 lutego 1863 roku Henry Dunant zawiązał wraz z grupą działaczy Międzynarodowy Komitet Pomocy Rannym Wojskowym. Komitet ten przyjął nazwę Międzynarodowy Komitet Czerwonego Krzyża. Polski Czerwony Krzyż został utworzony w 1919 roku. Funkcjonuje obecnie zgodnie z ustawą z 16 listopada 1964 roku. Godłem Polskiego Czerwonego Krzyża jest równoramienny czerwony krzyż na białym polu - Krzyż Genewski. Godło i nazwa Polskiego Czerwonego Krzyża pozostają pod ochroną prawną. Zakazuje się bezprawnego wykorzystywania godła i nazwy PCK.
**Słowa kluczowe:** znak czerwonego krzyża

Delivered: 10/05/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 59-63


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## Introduction

On 17 February 1863, Henry Dunant and a group of activists established the International Committee for the Relief to the Wounded. This committee was later named the International Committee of the Red Cross (ICRC). In 1964, the International Red Cross and Red Crescent Movement was created. The movement currently comprises the following entities: the International Committee of the Red Cross, the International Federation of the Red Cross and Red Crescent Societies (IFRCRCS), and national Red Cross and Red Crescent societies. The federation is important from the point of view of providing assistance, as it prevents human suffering, ensures respect for human dignity, especially during military conflicts, and offers help to the victims of epidemics, natural disasters and catastrophes [1]. The ICRC uses the symbol of the red cross. Both the federation, and the national societies, as well as military medical service may use the symbol during times of peace or war. This right is secured by a provision in the Geneva Convention from 1949. The red cross protects the victims of war, and any person providing help. It plays an informational role and indicates individuals providing medical help on the battlefield, people who cannot be attacked. It may be placed on medical units such as hospitals or medical aid stations, or on the medical means of land, air or water transportation. The symbol may be used by medical and religious personnel.

The principles of its use by the army are determined by appropriate provisions. The Minister of National Defence introduced in the Polish Army a unified presentation of the red cross symbol on the land medical facilities (Defence norm no. NO-02-A032. Geneva emblem. Camouflage of land-based medical facilities. Decision no. 302/MON of the Minister of National Defence of 17 December 2001 on the defence norms, Official Journal of the Ministry of National Defence of 2001, item 186 [updated in 2009]) The norm defines the principles of placing the red cross symbol on vehicles, planes, helicopters, trains and hospital tents.

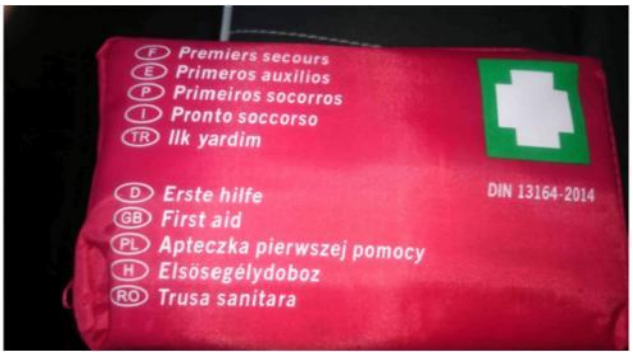


**Figure 1.** Lawful labelling of vehicle first aid kit
**Rycina 1.** Zgodne z prawem oznakowanie apteczki samochodowej


**Figure 3.** Lawful marking of a hospital emergency department
**Rycina 3.** Zgodne z prawem oznakowanie szpitalnego oddziału ratunkowego


**Figure 2.** Lawful labelling of a pharmacy
**Rycina 2.** Zgodne z prawem oznakowanie apteki


**Figure 4.** Unlawful use of the Red Cross emblem (bag for used personal hygiene products)
**Rycina 4.** Niezgodne z prawem wykorzystanie Znaku Czerwonego Krzyża (torebka na zużyte przedmioty higieny osobistej)

In Poland, a party to the Geneva conventions, the Polish Red Cross (PRC) functions, following the Act of 16 November 1964. The emblem of the PRC is a red cross on a white background – the Geneva cross. The emblem and name of the Polish Red Cross have legal protection, and their unlawful use is prohibited.

The PRC follows the same principles as the IRCRCM, i.e. humanitarianism, neutrality, independence, voluntariness, unity and universality.

A contemporary armed conflict taking place in towns and cities will activate both the military and the civil medical service systems. Identification of the medical services and medical assistance points should follow generally accepted rules, norms and customs, and be based on the law of the country in which the local or international armed conflict occurs [2-5]. Therefore, the red cross symbol should be used in a justified way by the entities operating in times of peace and war in Poland, as well as in certain European states [6, 7]. The fact of that abuses and the unlawful use of the red cross symbol occurs should be emphasised.

In common terminology the word "symbol" is used, expressed in the Geneva Convention of 12 August 1949 as the word "emblem". The Polish Red cross Act of 16 November 1964, and the Polish Red Cross Statute of 10 September 2011 use the terms "emblem" and "symbol" interchangeably.

## The emblem of the red cross

The red cross symbol may be used by the ICRC and PRC, as examples of national societies [6]. In Muslim countries, a red crescent symbol is used instead [1], but has the same protective functions, and is also protected by law as a consequence of the Geneva Conventions and their Additional Protocols [8-10]. In 2005, Additional Protocol no. 3 introduced another protective and indicative symbol: a red crystal on a white background. During times of peace, the protective symbol is used exclusively by the military medical services, whereas as the informative symbol is reserved for the national societies, such as the PRC [7].

Following the example of Italy, the Act of 30 June 1912 established the rules of use of the red cross symbol ("Protection of victims and the wounded during war and protection of the international symbols of neutrality" Official Journal of Laws of 17/07/1912, no. 168). The Italian Red Cross was created as the fifth national society, and it is closely associated with the tragic history of the battle of Solferino, described as "Un souvenir de Solferino".

The Italian Red Cross was established by a Milan-born Cesare Castiglioni. It was in his hometown that the first Italian committee, chaired by Castiglioni, was formed on 15 June 1864. The law in Italy (Act no. 740/1912) states that "anyone who uses the symbol of the red cross on the white background or the name "Red Cross" or "Geneva Cross" without the consent of the government, is subject to an administrative fine of five hundred thousand to three million lire, unless the fact constitutes an offence". A legal use of the red cross symbol requires permission from the government, and without it any use of the symbol will be considered an administrative infringement. Legislative decree no. 507 of 1999 "Depenalisation of minor offences and reforms of the sanction system, following art. 1 of the act of 25 June 1999 no. 205", in article 62 exchanged imprisonment (1-6 months) for a fine, which is an administrative sanction. In cases when the symbol is gravely abused, the penal law is applied and article 5 stating "a penalty of imprisonment for three to fifteen years for anyone who usurps, falsifies or uses unlawfully emblems, epaulettes and the name "Red Cross" and, generally, other symbols of neutrality". Moreover, article 6 also states that these offences are tried by a martial court, even if the offender is a civilian. This is due to the fact that the offence is "universal", i.e. it may be committed by anyone, and does not have to be committed exclusively by subjects with particular physical or legal features stipulated in legal provisions [12]. The same penalty applies also to those who falsify or distort the emblem or name "Red Cross" or "Geneva Cross", or use them in a confusing or misleading way. This refers both to an unlawful use of the emblem by individuals or groups of people who are not authorised to use it, and by individuals who normally are authorised to use it, but who do so for purposes other than intended. This includes falsification of the symbols, which could result in mistaking their form and colour for the real symbols. Moreover, articles 2 and 3 state: "Products introduced to the market with an unauthorised or falsified symbol of the red cross will be confiscated", and "The prohibition mentioned in article 1 also refers to brands, symbols and labels used at the moment the act becomes effective. The brands, symbols and labels in one, used for at least a year since the act is effective can be used until 1 January 1915" indicate that in Italy for over a century the symbol of the Red Cross could not be used for marketing purposes.

Presently, in Italy, following the Resolution of the Prime Minister of 6 May 2005 no. 97, published in the Official Journal of Laws of 08 June 2005, no. 131, a new Statute of the Italian Red Cross Organisation is in force. The statute, attached to the resolution, defines the principles referring to the emblem, and stipulates that the "Italian Red Cross uses the symbol of a red cross on the white background, under four Geneva Conventions of 1949, as amended. In the case of unlawful use of the name or symbol of the Red Cross, the sanctions defined by law, i.e. following Act no. 740/1912, will be applied."

In Poland, the use of the symbol of the Red Cross or the Red Crescent is considered against international law, as regulated by the Polish Criminal Code. Relevant information may be found in the Criminal Code (Official Journal of Laws of 1997, no. 88, item 553, Act of 6 June 1997. Principles of criminal sanction, published on: 02/08/1997, entered into effect on: 02/08/1997 - Chapter XVI. Crimes against peace, humanity and war crimes – article 126, para. 1 c.c.:§ 1. A person who during military activities uses the Red Cross or Red Crescent symbol against international law, is subject to the penalty of imprisonment of up to 3 years. This offence is considered

a misdemeanour, but not a crime. A misdemeanour is an offence of a slightly "lighter" character. Using the terminology of the International Humanitarian Law of Armed conflicts, it is a war crime other than a severe infringement of the Geneva Conventions or the Additional Protocol I). According to article 7, §1 of the Criminal Code, an offence is a crime or a misdemeanour. Following §2, crimes are criminal offences sanctioned by imprisonment of not less than 3 years, or by a more severe penalty. Summing up, a criminal offence under article 126 paragraph 1 of the Criminal Code, which is a misdemeanour, is subject to a penalty of imprisonment for up to 3 years (the sentence may be shorter, depending on the circumstances and the final decision of the court, but it cannot be less than 1 month). In addition, chapter three, article 15.1 of the Act on the Polish Red Cross of 16 November 1964 states: "He who uses the emblems, symbols or names mentioned in article 12, 13 and 14 against the provisions of law, is subject to a fine of up to PLN 4,500".

Since 1995, the Commission for Protection of the Symbol of the Red Cross has operated in Poland. It was established in March 1995 at the Main Board of the Polish Red Cross. Since 2013, the chair of the commission has been Magdalena Stefańska PhD. The aim of the commission is to ensure protection of the symbol, especially in the view of frequent abuse or improper use of the symbol by physical and legal entities, media or medical services. The principles of functioning of the Commission for the Protection of the symbol are based on the legal document: "Regulations regarding the Use of the Symbol of the Red Cross and Red Crescent", adopted by the Council of Delegates of the International Red Cross and Red Crescent Movement in November 1991 in Budapest, and on Resolution no. 54/2004 of the National Council of Representatives of 11/12/2004 regarding the protection of the symbol of the red cross and red crescent. The tasks of the Commission for Protection of the Symbol are listed on the PCR internet website [13].

In relation to the presented provisions of the Act of 1964, and in the view of article 126 of the Criminal Code, the relevant additional provisions regarding the symbol of the red cross should be mentioned.

**Common law**

- Geneva Conventions of 1949, especially articles 38, 44, 53 of the I GC (Official Journal of Laws of 1956, no. 38, item 179).
- Additional Protocols to the Geneva Conventions of 1977, articles 8 [c], 85 [2f] and articles 3 and 4 of the Annex 1 to the AP I (Official Journal. of Laws of 1992, no. 41, item 175).
- Act on the Polish Red Cross of 16 November 1964, especially articles 12 to 15 (Official Journal of Laws of 1964, no. 41, item 276).
- Roman Statute of the International Criminal Court, article 8, paragraph 2b, section vii (Official Journal of Laws of 2003, no. 78, item 708).
- Criminal Code, article 126 § 1 (Official Journal of Laws of 1997, no. 88, item 553).
- Additional Protocol to the Geneva Conventions of 2005 (Official Journal of Laws of 2010, no. 70, item 447).
- Statute of the Polish Red Cross of 20 September 2011, approved by the resolution of the Council of Ministers (Official Journal of Laws of 2011, no. 217, item 1284), especially paragraph 4.

**Military law**

- Resolution of the Minister of National Defence of 10 April 2008 (Official Journal of Laws of 2008, no. 105, item 675).
- Resolution of the Minister of National Defence of 16 April 2008 regarding identity cards and plates for professional soldiers and candidates for professional soldiers (Official Journal of Laws of 2008, no. 79, item 473).
- List of military symbols and abbreviations (part II) - symbol of the General Staff 1561/2004 Instruction APP6.
- Defence norm no. NO-02-A032: Geneva emblem. Camouflage of the land-based medical facilities. Decision no. 302/MON of the Minister of National Defence from 17 December 2001 on the defence norms, Official Journal of the Ministry of National Defence of 2001, item 186 (amended in 2009).

**Internal regulations on the Polish Red Cross and the Red Cross Movement**

- Regulations on the use of the emblem adopted by the Council of Delegates of the International Red Cross and Red Crescent Movement in November 1991 in Budapest (Resolution no. 5).
- Resolution of the National Council of Representatives of the Polish Red Cross no. 54/2004 regarding protection of the emblems of the red cross and red crescent.
- Resolution of the Main Board of the PRC no. 115/2011 regarding adopting the principles of proper use of the emblem of the Red Cross by all the organisational PRC units, including their application in collaboration with external entities.
- Resolution of the Main Board of the PRC no. 116/2011 regarding examining applications for permission to use the emblem of the Red Cross.
- Resolution of the Main Board of the PRC no. 117/2011 regarding Commission for the Protection of the Emblem of the Red Cross at the Main Board of the PCR.
- Resolution of the Main Board of the PRC no. 64/2012 regarding approval of the visual identity book [14].

## Conclusions

- The symbol of the red cross may be used according to the relevant provisions of law.
- Abuse and unjustified use of the emblem is punishable in the view of applicable law.

- There is a constant need for monitoring of the public space to detect unlawful use of the symbol of the red cross, and for reporting such cases to the PRC.

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# Hypertension - diagnostic and therapeutic differences in the elderly

## Odrębności diagnostyczne i lecznicze u chorych na nadciśnienie tętnicze w podeszłym wieku

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**Abstract.** Hypertension is the most common cause of excessive mortality in the global population. The incidence of hypertension is increasing with age, partly as a consequence of the processes taking place in the arteries, mainly their stiffness. A special form of hypertension, found particularly in the elderly, is isolated systolic hypertension. It increases the risk of death caused by cardiovascular diseases. Hypotensive therapy in the elderly gives them benefits, but it has to be conducted following particular principles, including individualization of the therapy and careful reduction of blood pressure.
**Keywords:** elderly, hypertension, hypotensive therapy

**Streszczenie.** Nadciśnienie tętnicze jest jedną z najczęstszych przyczyn nadmiernej umieralności w populacji ogólnej. Częstość występowania tej choroby zwiększa się wraz z wiekiem, co jest konsekwencją między innymi procesów zachodzących w naczyniach tętniczych, głównie zwiększającej się sztywności naczyń. Szczególną postacią nadciśnienia, występującą zwłaszcza u osób starszych, jest izolowane nadciśnienie skurczowe, które znacznie zwiększa ryzyko zgonów z przyczyn sercowo-naczyniowych. Leczenie hipotensyjne w osób w podeszłym wieku może dawać pacjentom wiele korzyści, ale musi być prowadzone z poszanowaniem określonych zasad, takich jak indywidualizacja terapii i ostrożne obniżanie ciśnienia tętniczego.
**Słowa kluczowe:** nadciśnienie tętnicze, podeszły wiek, terapia hipotensyjna

Delivered: 27/01/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 64-69


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## Introduction

Old age can be divided into early old age (60-74 years) and late old age (>74 years). People over 90 years old are referred to as long-lived. In the last decade, the mean life expectancy extended by nearly 5 years for men, and approximately 4.5 years for women. In 2014, over 8.5 million people in Poland were over 60 years old, and 17.9% of them were over 80 years old [1].

Globally, arterial hypertension is the most important risk factor for premature death. There is a linear relationship between blood pressure (BP) values and mortality, and the incidence of cardiovascular diseases (myocardial infarction, cerebral stroke, heart failure, peripheral vascular disease), as well as renal failure in all age and ethnic groups, both in men and women. In individuals over 50 years old, the cardiovascular risk is better described by the systolic blood pressure (SBP) value. Another indicator of increased risk is pulse pressure, i.e. the difference between SBP and diastolic blood pressure (DBP). In 2000, the number of patients with arterial hypertension around the world was 972 million, and it is estimated to increase to 1 560 million patients by 2025. The NHANES study conducted in the United States revealed arterial hypertension in 67% of the population over 60 years old. In Europe, the percentage of elderly patients with arterial hypertension is 60-80% [2].

The data from the past 20 years indicate an increased frequency of this disease in Poland. According to the NATPOL 2011 study, the incidence of arterial hypertension in the population aged 18-79 years old increased in 10 years from 30 to 32%, i.e. to approximately 9 million people. Added to this number should be the results of the POLSENIOR study, i.e. approximately one million individuals over 80 years old with arterial hypertension. If the tendency prevails, the number of patients with arterial hypertension will increase by 50% by 2035 [3]. Approximately 90% of patients are diagnosed with primary (spontaneous) hypertension. Only approx. 5-10% of all hypertensive patients have secondary hypertension [4].

The most common type of secondary hypertension is renal hypertension. One form is parenchymal renal hypertension, and it is found in 5-6% of all hypertensive patients. Renovascular hypertension is less common, being observed in 1% of all cases of hypertension. These are the most frequent types of secondary hypertension [5]. In patients with secondary hypertension the recovery, or at least improved control of BP, and a reduction in the cardiovascular risk are possible. As a result, it seems reasonable to perform a simple screening assessment for secondary forms of hypertension. The evaluation may be based on interviews, physical examinations and standard additional tests. Indicative of secondary hypertension may be a significant increase in BP values, sudden problems with blood pressure control or deterioration of BP control, limited response to hypotensive treatment, and exacerbation of organ complications disproportionate to the duration of the hypertension. If this basic assessment leads to a suspicion of secondary hypertension, specific diagnostic procedures, listed in Table 1, may be necessary [3].

The diagnostics of the secondary forms of hypertension, especially in those cases of suspected endocrine origin, should be conducted in specialist centres.

The basis for the diagnosis of arterial hypertension is a properly conducted indirect BP measurement in a doctor's office. To obtain a reliable BP value, a device that meets the criteria of measurement precision must be used, the patient needs to be prepared, and the measurement has to be made by a trained person. Measurements on the arm are recommended, and the list of certified devices is presented on the website of the Polish Society of Hypertension (PTNT). Arterial hypertension may be diagnosed if the mean BP values (from at least two measurements taken during two different appointments) are >140 mm Hg for SBP and/or 90 mm Hg for DBP. In patients with BP values <160/100 mm Hg the diagnosis should be confirmed by outpatient blood pressure monitoring (ABPM) and, if this is not possible, by BP measurements at home, using different norms for these results (Tab. 2). Patients with BP >180/>110 mm Hg may be diagnosed with arterial hypertension during the first visit, after other factors increasing BP values, e.g. fear, pain or alcohol consumption have been excluded.

Arterial hypertension may also be diagnosed on the basis of reliable interview data or patient records (BP values or using hypotensives). The 2015 PTNT guidelines maintained the classification of arterial hypertension, based on measurements in the doctor's office, into three degrees, with the isolated systolic hypertension (ISH) subtype (Tab. 3).

Age-related increases in arterial hypertension is associated with the processes taking place in the arteries due to ageing, and primarily with growing vascular stiffness. The stiffness is a result of the accumulation of calcium deposits, quantitative and qualitative changes in the collagen forming the vascular walls, proliferation of the smooth muscle cells in the middle layer of the arterial walls, impaired glomerular filtration, impaired vasodilation in response to the stimulation of ß-adrenergic receptors, increased activity of $\alpha_1$-receptors, and endothelial damage [6].

## Differences in diagnostics and therapy

Individual management of elderly patients with arterial hypertension begins at the diagnostics stage. Vascular stiffness is associated with what is termed pseudohypertension. Stiff arteries, resistant to the cuff's pressure, falsely elevate the readings so that they do not reflect the actual arterial pressure. A positive Osler's sign, where after inflating the blood pressure cuff the pulse in the radial artery is palpable, indicates increased vascular stiffness and can help diagnose pseudohypertension [7]. In such cases, the most reliable method of blood pressure measurement is the direct method. Compared to younger people, elderly patients are a heterogeneous group, so the assessment of the risks and benefits of the pharmacotherapy of arterial hypertension should be individualised, considering the patient's baseline status, comorbidities, and life expectancy.

**Table 1. Diagnosis of secondary causes of hypertension**
**Tabela 1. Diagnostyka wtórnych przyczyn nadciśnienia tętniczego**

| Cause of arterial hypertension | Medical history | Physical examination | Basic tests | Additional tests | First-choice examination | Tests to confirm diagnosis |
|---|---|---|---|---|---|---|
| Obstructive sleep apnoea | Characteristic symptoms – time of day and night symptom assessment using questionnaires | Abdominal obesity, neck circumference, craniofacial abnormalities | Increased glucose level, lipid disorders | Limited or absent reduction in BP at night in ABPM, increased morning BP values in ABPM, disturbed rhythm and/or conductivity in ECG Holter test | Type IV night test | Type I-III night test |
| Parenchymal renal disease | History of infection or abnormal structure of the urinary tract, haematuria, abusing analgesics, family history of renal diseases | Kidneys palpably enlarged (in the case of cystic renal degeneration) | Presence of protein, erythrocytes or leukocytes in urine, reduced GFR | Albuminuria and proteinuria of various intensity | Renal US | Detailed diagnostics of renal disease |
| Atherosclerotic renal artery stenosis (RAS) | Arterial hypertension with sudden onset, exacerbation or decreased control of hypertension, refractory or malignant hypertension, recurrent episodes of pulmonary oedema | Vascular murmur in the abdomen | Sudden deterioration of renal function (spontaneous or during therapy with RAA inhibitors) Hypokalaemia | Renal US: difference in kidney lengths >1.5 cm Small kidney | Doppler US of the renal arteries | Angio-CT angio-MR angiography |
| Cushing's syndrome | Fast weight gain, polyuria, polydipsia, mental disorders | Typical body build central obesity, moon face, buffalo hump), red striae, hirsutism, bruising | Hyperglycaemia | Accidental detection of adrenal lesion | Daily excretion of free cortisol in urine Inhibition test 1 mg of dexamethasone | Inhibition test with dexamethasone |
| Aortic coarctation | Temporary claudication, headaches, loss of consciousness, nasal bleeding | Audible murmurs in the precardiac or interscapular area, reduced pulse in femoral arteries and reduced pressure in the femoral artery compared to a simultaneous measurement on the upper limb, difference in the arterial pressure between the left and right upper limb | Image "3" in thoracic X-ray, erosions on inferior costal edges | Abnormalities in echocardiogram | Echocardiography | Angio-CT angio-MR |

**Table 2. Diagnosis of hypertension based on the results of measurements made in the surgery and beyond**
**Tabela 2. Rozpoznanie nadciśnienia tętniczego na podstawie wyniku pomiarów w gabinecie lekarskim i poza nim**

| Category | Systolic BP (mm Hg) diastolic BP (mm Hg) | | |
|---|---|---|---|
| BP in the office or clinic | ≥140 | and/or | >90 |
| Outpatient blood pressure monitoring (ABPM) | | | |
| During daytime (or while awake) | ≥135 | and/or | >85 |
| At night or during sleep) | ≥120 | and/or | >70 |
| Mean daily value | ≥130 | and/or | >80 |
| BP measured at home | ≥135 | and/or | >85 |

**Table 3. Classification of blood pressure in surgery measurements**
**Tabela 3. Klasyfikacja ciśnienia tętniczego w pomiarach gabinetowych**

| Category | Systolic BP (mm Hg) diastolic BP (mm Hg) | | |
|---|---|---|---|
| Optimal BP | <120 | | <80 |
| Normal BP | 120-129 | and/or | 80-84 |
| High normal BP | 130-139 | and/or | 85-89 |
| 1st degree arterial hypertension | 140-159 | and/or | 90-99 |
| 2nd degree arterial hypertension | 160-179 | and/or | 100-109 |
| 3rd degree arterial hypertension | >180 | and/or | >110 |
| Isolated systolic hypertension | ≥140 | | <90 |

As in other age groups, the first step in the diagnostic and therapeutic process is a reliable blood pressure measurement. If possible, peripheral renal and carotid arteries should be assessed for pathological murmurs associated with atherosclerosis. In elderly patients an orthostatic test should be performed, i.e. blood pressure taken while the patient is supine, and again after 1, 3 and 5 minutes, while the patient is standing. The test needs to be performed during the first visit, after treatment modification or if symptoms of hypotonia occur, as elderly patients are particularly susceptible to this condition [8]. In order to avoid hypotonia and peripheral hypoperfusion, the initial hypotensive treatment should consist in a monotherapy using doses lower than in younger patients. Reaching the target blood pressure values should be planned for 2-3 months. In the treatment of hypertensive elderly patients, polytherapy resulting from numerous comorbidities should be avoided, as it leads to many problems due to the number of medications and doses. Polypragmasy should also be avoided, as elderly patients may use many analgesics or anti-inflammatory products simultaneously (including OTC preparations), which reduce the effectiveness of hypotensive medicines, as well as exposing the patients to adverse reactions. Abnormal pharmacokinetic characteristics, i.e. impaired absorption, slower distribution of medication in the vascular system, reduced pool of circulating proteins, and reduced renal and hepatic clearance require more frequent controls of the renal parameters and ionogram during the hypotensive therapy. The blood potassium concentrations should be monitored particularly closely, as commonly used angiotensin convertase inhibitors, angiotensin-II mineralocorticoid receptor antagonists, or frequently overused non-steroid anti-inflammatory drugs result in hyperkalaemia. In elderly patients with renal insufficiency or other general systemic diseases, multidrug therapy, which often involve potassium supplementation, may lead to severe, life-threatening hyperkalaemia [9]. Moreover, the risk of hypokalaemia, hyponatraemia or hypomagnesia following the use of diuretics such as indapamide should be considered. The incidence of hyponatraemia increases with the age of patients, which is probably due to the concurrent renal, hepatic or cardiac diseases.

It should also be emphasised that with age, the impaired renal conservation of sodium syndrome may develop, which, especially when combined with a diuretic therapy, leads to the increased incidence of hyponatraemia.

Apart from basic laboratory tests, in elderly patients the diagnostics of hypertension and the assessment of the cardiovascular risk should always involve an electrocardiographic examination. More detailed diagnostics is required when secondary causes of hypertension are suspected (e.g. atherosclerotic stenosis of the renal artery). The causes of secondary arterial hypertension, especially in the elderly population, include thyroid disorders and use of non-steroid anti-inflammatory drugs [10].

## Current therapeutic management

Following the guidelines of the Polish Society of Hypertension and European Society of Cardiology / European Society of Hypertension (ESC/ESH), hypotensive therapy should be initiated following a careful analysis of the global cardiovascular risk. An age of over 55 years in men and over 65 years in women is an important cardiovascular risk factor. The principles of non-pharmacological management in the elderly do not differ from those recommended in younger patients [3]. In older age, non-pharmacological therapy, involving body weight reduction and limited sodium consumption, are associated with satisfactory hypotensive effects [11].

Increasing physical activity to the degree allowed by the patient's condition is also recommended. In this group of patients, isotonic exercises are particularly beneficial. It

should be emphasised that a combination of all the elements of non-pharmacological therapy is the most effective. The current guidelines regarding hypotensive treatment recommend continuation of the therapy in patients over 90 years old, if the treatment was previously well-tolerated and effective.

A breakthrough study for the assessment of benefits of hypotensive treatment in the elderly was the Hypertension in the Very Elderly Trial (HYVET), published in 2008. It involved 3,845 patients aged over 80 years, with persistent arterial hypertension (systolic blood pressure of 160-199 mm Hg, diastolic blood pressure of 90-110 mm Hg), but with SBP not lower than 140 mm Hg while standing. It eliminated potential ischaemic episodes due to orthostatic hypotonia. The exclusion criteria included creatinine concentration >1.7 mg/dl, concurrent terminal diseases, heart failure and dementia. Patients who could not take medications in the vertical position or who required nursing were not qualified for the study. The active group (n = 1,933) received 1.5 mg of prolonged-release indapamide, and the target blood pressure values were >150/80 mm Hg. If the values were not obtained during control visits, perindopril at a dose of 2 or 4 mg was additionally introduced. The mean age of patients was 83.5 years. 73% of the patients were 80-84 years old, 22% were 85-89 years old, and 5% were over 90 years old. Due to the positive outcomes, the study was finished earlier than planned. The target blood pressure values were obtained in 48% of patients receiving active treatment (indapamide or indapamide + perindopril), and in 19.9% of patients using a placebo. The significant reduction in overall mortality by 21% was achieved, mortality due to cardiovascular causes was reduced by 23%, the number of cerebral strokes was reduced by 30%, and the number of cerebral strokes resulting in death by 39%. In the active treatment group the number of new heart failure cases was reduced by 64% compared to the control group. The results of HYVET demonstrated the benefits of hypotensive treatment in the oldest population, as well as safety of the therapy (358 episodes of adverse reactions in the placebo group and 448 in the treatment group). The greatest treatment benefits were observed in patients with arterial hypertension without severe comorbidities, and in good physical condition. The HYVET results inspired modification of the recommendations regarding the treatment of elderly patients presented in the Polish Society of Hypertension guidelines. Such a therapy should be implemented in patients over 80 years old, without severe comorbidities, when blood pressure values are over 160 mm Hg SBP) or 90 m Hg (DBP). The target values are <150/80 mm Hg. A therapy with a long-lasting thiazide diuretic followed by angiotensin convertase inhibitor is recommended.

The safety and effectiveness of different hypotensive pharmacotherapy regimens, as well as their effect on the cardiovascular risk depending on patient's age were the subject of the Blood Pressure Lowering Treatment Trialists' Collaboration (BPLTTC) metaanalysis [12]. The frequency of the end-point (serious cardiovascular events) was assessed in the group of patients below and above this age limit. The metaanalysis demonstrated that reduction of arterial pressure by 5 mm Hg decreased the risk of serious cardiovascular events by 11.9% in the group of younger patients, and by 9.1% in the elderly patients. In both groups the effect of various hypotensive treatments on the reduction of cardiovascular events was similar. The metaanalysis demonstrated that the main benefit from hypotensive therapy for elderly patients is reduced blood pressure, and not using a particular product.

Long-lasting diuretics in low doses, dihydropyridine derivatives of calcium antagonists, and angiotensin convertase inhibitors are recommended in the hypotensive therapy of elderly patients. P-adrenolytic drugs are less effective, and are reserved for the high-risk groups, e.g. patients with ischaemic heart disease. Hypotensive therapy in elderly patients requires a careful assessment of associated benefits and risks.

## Conclusions

Large clinical studies and metaanalyses indicate that hypotensive treatment in patients over 65 years old significantly reduces the number of strokes, the risk of heart failure, and mortality due to cardiovascular causes. Therefore, hypotensive treatment is recommended in elderly patients with second degree arterial hypertension, including BP reduction to 140-150 mm Hg. However, for rational reasons, and due to the fact that in many clinical trials the percentage of patients aged over 65 years old was very high, hypotensive therapy should be considered with SBP >140 mm Hg, with target values of <140 mm Hg, if the patient has a good functional status and tolerates the treatment well.

In patients over 80 years old, based on the HYVET study, it is generally recommended to initiate the hypotensive treatment if SBP is over 160 mm Hg, and attempt to achieve SBP <150 mm Hg. However, due to the differences in general health status in this group of patients, the treatment decisions should be taken on an individual basis, and BP reduction should always be gradual and carefully monitored by the physician.

In elderly patients with comorbidities such as coronary disease, chronic renal disease or diabetes, the target BP values for these clinical situations apply. The benefits of hypotensive therapy in elderly patients are comparable to those achieved in younger patients. However, due to the reduced adaptation abilities of the cardiovascular system, and the risk of orthostatic hypotonia, the treatment should be more careful and the target BP values should be obtained more gradually.

While the basic principles of non-pharmacological treatment of arterial hypertension in elderly patients are the same as in the younger population, it is important to consider the limitations resulting from impaired physical fitness and exercise capacity that prevent regular physical exercise. Large clinical trials involving elderly patients examine hypotensive medications from all the basic groups, while a recent metaanalyses did not reveal differences in the effectiveness of hypotensive drugs

according to age. However, clinical experience dictates that when there are no special contraindications for the individualisation of therapy, thiazide / thiazide-like diuretics and dihydropyridine derivatives of calcium antagonists are used as first-line treatment (or a combination of the two). In studies on the most common form of arterial hypertension in elderly patients, ISH, only diuretics and calcium channel blockers were used, with the option to use an additional RAA blocker. In patients over 80 years old the study results (HYVET) indicate that therapies should be started with long-lasting thiazide-like diuretics (indapamide), with the option of ACE-I.

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# Rheumatoid arthritis treatment in patients with concomitant viral hepatitis B and C

## Leczenie reumatoidalnego zapalenia stawów u pacjentów ze współwystępującym wirusowym zapaleniem wątroby typu B i C

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**Abstract.** It is estimated that over 350 million people around the world are infected with hepatitis B, and 185 million with hepatitis C. Due to their prevalence both conditions are considered a global burden and require a multidisciplinary approach. The lack of clear and consistent guidelines for the treatment of rheumatoid arthritis with coexisting viral hepatitis remains a significant therapeutic problem, especially in regards to the risk of hepatitis reactivation and the potential hepatotoxicity of drugs. This paper focuses on rheumatoid arthritis as one of the most common inflammatory joint disorders. It provides a review of studies and guidelines related to arthritis treatment and the risk of adverse effects in patients with concomitant viral hepatitis.
**Key words:** rheumatoid arthritis, hepatitis B, hepatitis C

**Streszczenie.** Szacuje się, że na świecie jest około 350 milionów ludzi zakażonych wirusem zapalenia wątroby typu B oraz około 185 milionów zakażonych wirusem zapalenia wątroby typu C. Ich rozpowszechnienie sprawia, że choroby te są zagadnieniem globalnym i multidyscyplinarnym. W leczeniu reumatoidalnego zapalenia stawów ze współwystępującym wirusowym zapaleniem wątroby problem stanowi brak jednoznacznych wytycznych odnośnie do stosowania odpowiednich terapii. Szczególne znaczenie mają: ryzyko reaktywacji zakażenia wirusowego i hepatotoksyczność stosowanych leków. Artykuł skupia się na reumatoidalnym zapaleniu stawów - jednej z najczęstszych chorób zapalnych stawów, i stanowi przegląd badań oraz wytycznych dotyczących leczenia i ryzyka wystąpienia działań niepożądanych u chorych ze współwystępującym wirusowym zapaleniem wątroby.
**Słowa kluczowe:** reumatoidalne zapalenie stawów, wirusowe zapalenie wątroby typu B, wirusowe zapalenie wątroby typu C

Delivered: 03/04/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96 (1): 70-79


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## Introduction

Doctors often deal with cases where other comorbidities significantly affect both the course of and options for treatment of the primary disease. This also applies to patients with rheumatoid arthritis (RA) and a concomitant infection with the hepatitis B virus (HBV) or hepatitis C virus (HCV). In these cases the therapy has to be adjusted and the risks associated with the comorbidities taken into account. This is difficult, due to the number of factors that always need to be considered: the safety profile of the new medicine, its effectiveness and its potential interaction with other drugs.

This article presents a review and summary of studies and guidelines related to therapies for patients with RA and hepatitis B (Hep B) or C (Hep C).

## Rheumatoid arthritis (RA)

RA is an inflammatory autoimmune disease of unknown aetiology, manifested by progressive synovitis, articular damage and organ complications, leading to disability and increased mortality. RA is estimated to affect 0.5-1% of the global adult population [1].

The basic treatment includes disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids. DMARDs can be synthetic or biologic. Synthetic DMARDs include conventional types (methotrexate, sulfasalazine, leflunomide, hydroxychloroquine) and targeted types (Janus kinase inhibitors: tofacitinib and baricitinib). Biologic DMARDs are divided into original (TNF-α inhibitors, interleukin 6 inhibitors, and abatacept, rituximab) and biosimilar DMARDs. The current treatment for RA recommended by the European League Against Rheumatism (EULAR) [2] is presented in Figure 1. The

adverse effects associated with medications used in the treatment of RA are well-known. In the context of HBV and HCV infection, the most important ones include the increased risk of virus reactivation and hepatotoxicity [3].

## Viral hepatitis B (Hep B)

Hep B is a potentially life-threatening infectious disease caused by HBV, and it is associated with liver damage. The disease is typically transmitted through the blood, less often by sexual intercourse or during birth. It is estimated that 2 billion people across the world have had contact with HBV [4], and 350 million are infected [5]. In Poland over 1,500 new cases are observed annually, and the number of infected people is estimated to be 400-600 thousand [6]. The incidence of Hep B in RA patients is similar to that in the general population [1].

HBV may cause acute and chronic hepatitis. 70% of acute HBV infections are asymptomatic [7], and usually resolve spontaneously. However, they may lead to serious complications, such as fulminant hepatitis. 3-5% of patients infected with HBV develop chronic hepatitis, associated with increased risk of cirrhosis, hepatic failure and hepatocellular carcinoma.

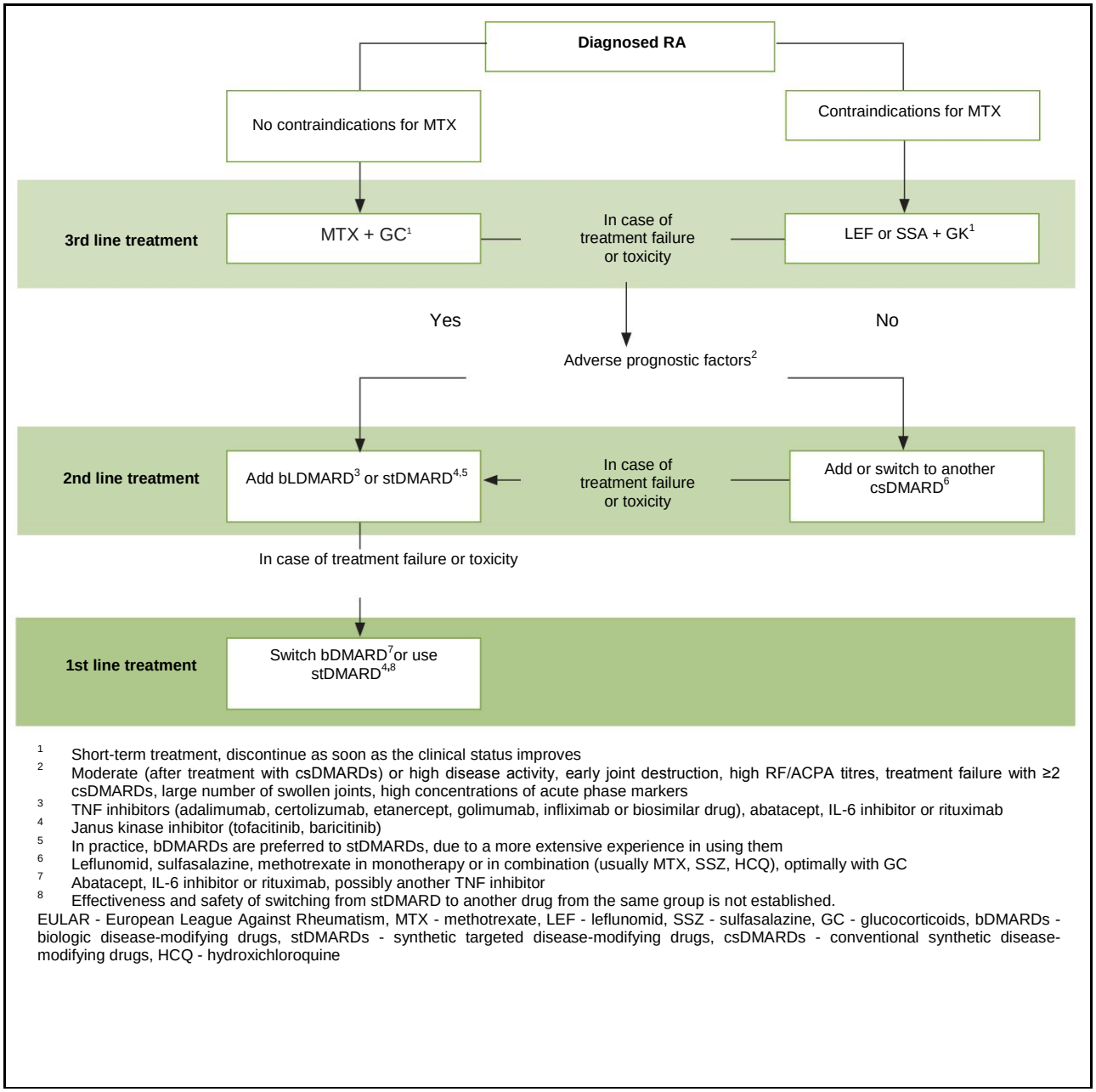
To implement an optimal therapy, it is necessary to determine the stage and phase of infection (Tab. 1). This can be achieved using tests assessing the blood concentration of the virus DNA, and detecting antigens and antibodies: surface antigen (HBsAg), antibodies against the surface antigen (anti-HBs), IgM and IgG antibodies against the core antigen (anti-HBc), and the secreted form of the core antigen and its antibodies (HbeAg and anti-HBe). In HBV infections we may encounter the following clinical situations:

- acute Hep B - HBsAg, IgM anti-HBc and high activity of alanine aminotransferase (ALT);
- chronic hepatitis B - HBsAg present for over 6 months, serum HBV DNA is >105 copies/ml, permanent or temporary increase in ALT and AST activity, while a hepatic biopsy reveals chronic necrotic and inflammatory lesions; two forms are distinguished: HBeAg-positive and HBeAg-negative with anti-HBe antibodies;
- HBsAg chronic carriage (inactive infection) - HBsAg present in the blood for over 6 months, HBeAg undetectable, anti-HBe antibodies present, number of virus DNA copies in the serum less than 104/ml, ALT values normal, liver biopsy may reveal minor signs of chronic inflammation;
- history of hepatitis B - in this case in a patient with confirmed acute or chronic hepatitis B, or with the presence of anti-HBc antibodies with or without anti-HBs HBsAg is not found, HBV DNA in the serum undetectable, and ALT and AST activity normal [8].

The phases and natural course of chronic hepatitis B is presented in Table 1. The main problems in the treatment of RA with concomitant HBV infection are drug hepatotoxicity and the risk of reactivation. Reactivation includes:

- exacerbation of chronic hepatitis B,
- recurrence of hepatitis B in a patient with a history of Hep B.

The principal criterion of reactivation is increased HBV replication (HBV DNA increased by 2 orders of magnitude compared to the baseline, or concentration of ≥100 IU/ml if it has not been observed before) in a patient with a stable level of virus DNA, or without the virus DNA in the blood [9]. De-novo HBV-related hepatitis [10] includes patients in whose blood HBsAg was found again (regardless of the HBV-DNA levels), or whose HBV-DNA concentration increased to $>10^5$ copies/ml after immunosuppressive therapy [10]. A number of factors increasing the risk of reactivation was identified, e.g. rituximab therapy, steroid therapy, certain chemotherapy regimens, discontinuation of antiviral prophylaxis, presence of HBsAg, presence of HBeAg, high HBV DNA concentrations, and absence of anti-HBs [11-14]. However, it should be emphasised that reactivation may also occur in patients without evident risk factors, also when anti-HBc antibodies are present in the blood, and the HBs antigen and virus DNA in the serum are undetectable [15], even when anti-HBs antibodies are present [16].




1 Short-term treatment, discontinue as soon as the clinical status improves
2 Moderate (after treatment with csDMARDs) or high disease activity, early joint destruction, high RF/ACPA titres, treatment failure with ≥2 csDMARDs, large number of swollen joints, high concentrations of acute phase markers
3 TNF inhibitors (adalimumab, certolizumab, etanercept, golimumab, infliximab or biosimilar drug), abatacept, IL-6 inhibitor or rituximab
4 Janus kinase inhibitor (tofacitinib, baricitinib)
5 In practice, bDMARDs are preferred to stDMARDs, due to a more extensive experience in using them
6 Leflunomid, sulfasalazine, methotrexate in monotherapy or in combination (usually MTX, SSZ, HCQ), optimally with GC
7 Abatacept, IL-6 inhibitor or rituximab, possibly another TNF inhibitor
8 Effectiveness and safety of switching from stDMARD to another drug from the same group is not established.

EULAR - European League Against Rheumatism, MTX - methotrexate, LEF - leflunomid, SSZ - sulfasalazine, GC - glucocorticoids, bDMARDs - biologic disease-modifying drugs, stDMARDs - synthetic targeted disease-modifying drugs, csDMARDs - conventional synthetic disease-modifying drugs, HCQ - hydroxichloroquine

**Fig.** Recommendations for RA treatment according to EULAR 2016
**Ryc.** Wytyczne leczenia RZS na podstawie EULAR 2016

Reactivation of HBV infection may take different courses: from asymptomatic and mild, to severe, resulting in damage to the hepatic cells, and in liver failure. The therapy may result in obtaining control of the reactivation and restoration of the baseline status, or recovery (disappearance of HBsAg). If the presence of HBsAg is persistent, or HBV DNA concentration increases, the disease is considered resistant to the treatment [9].

## Viral hepatitis C (Hep C)

Hepatitis C is an infectious disease caused by HCV, which can lead to liver damage. It is transmitted through the blood, sexual contact or during birth. According to QHO, approximately 185 million people are infected with HCV [17]. In Poland in 2013, 2705 new cases of hepatitis C were reported. The total number of patients with active HCV (detectable HCV-RNA) is estimated at 230 thousand, and approximately 320 thousand patients have

anti-HCV antibodies [18]. The incidence of hepatitis C in the studied populations and in patients with concomitant RA is similar [19, 20]. Similarly to HBV, HCV may also cause acute or chronic hepatitis. Acute infection is usually asymptomatic, but in 75-85% of patients it transforms into chronic hepatitis [21]. Chronically infected patients are at an increased risk of liver cirrhosis and hepatocellular carcinoma. In the diagnostics of hepatitis C, anti-HCV and HCV-RNA are determined (if the HCV RNA test is unavailable, then the HCV core antigen may be determined - a substitute marker of HCV replication). The virus genotype is also established. Similarly to hepatitis B, the main problems in the management of RA patients include: infection reactivation, the hepatotoxic effect of the medications used, and interactions between the therapies used in RA and hepatitis C.

## Treatment of RA in patients with hepatitis B

The American College of Rheumatology (ACR) guidelines from 2008 (including the update from 2012) regarding RA treatment in patients with hepatitis B contain recommendations regarding vaccinations, screening before implementation of therapy, and selecting the medication according to the Child-Pugh score [22, 23]. The score comprises three classes (A, B and C), and is based on the following parameters: total bilirubin and albumin concentration, prothrombin time, the presence of hepatic encephalopathy and ascites.

Following the 2008 ACR recommendation, patients with the hepatitis B risk factors who have not been vaccinated should receive vaccination against HBV prior to the DMARD therapy [23]. The updated ACR guidelines from 2012 recommend vaccination also in the patients previously treated with DMARDs, unless they were vaccinated before the treatment [22]. The European Association for the Study of the Liver (EASL) recommends vaccinations for HBV-seronegative patients who require immunosuppressive therapy. Importantly, in patients undergoing immunosuppressive therapy, the vaccine dose required to obtain immunity may be higher than the standard dose [24].

**Table 1. Natural history of chronic hepatitis B**
**Tabela 1. Przebieg naturalny przewlekłego WZW typu B**

| Phase | Characteristic features |
|---|---|
| Phase I: HBeAg-positive chronic HBV infection (previously known as the immunotolerance phase) | Active proliferation of the virus, HBV DNA >$10^6$ copies/ml, HBsAg +, HBeAg +, absent/minor necrotic inflammation and hepatic fibrosis, normal or slightly increased activity or aminotransferases |
| Phase II: HBeAg-positive chronic hepatitis B (previously known as the immunoreactive phase) | HBeAg+, HBV DNA +(high levels), increased ALT activity, moderate/severe necrotic inflammatory lesions, and rapid progression of hepatic fibrosis |
| Phase III: HBeAg-negative chronic HBV infection (previously known as the non-active HBV carriage phase) | Anti-HBe +, HBsAg + (may disappear and anti-HBs antibodies may occur), HV DNA <2000 IU/ml, ALT normal, monitoring for the development of liver cirrhosis, hepatocellular carcinoma and virus reactivation is required |
| Phase IV: HBeAg-negative chronic hepatitis B | HBeAg -, anti-HBe +, variations in HBV DNA and ALT levels, periods of exacerbations and remission, necrotic and inflammatory lesions and hepatic fibrosis, increased risk of liver cirrhosis, especially in the elderly |
| Phase V: HBsAg-negative (latent HBV infection) | HBsAg -, anti-HBc +, anti-HBs +/-, HBV DNA usually undetectable in the serum (may be revealed in hepatic biopsy), ALT normal, virus may reactivate |

(+) - present, (-) - absent, HBsAg - surface antigen, anti-HBs - antibodies against the surface antigen, anti-HBc - antibodies against the core antigen, HBeAg - secreted form of the core antigen, anti-HBe - antibodies against the secreted form of the core antigen, HBV DNA - DNA of hepatitis B virus, ALT - alanine aminotransferase

The 2009 guidelines of the American Association for the Study of Liver Diseases (AASLD) and the 2017 EASL guidelines recommend screening for HBV infection before implementing any immunosuppressive treatment. HBsAg, anti-HBs, anti-HBc and HBV DNA should be determined in patients for the presence of anti-HBc and absence of HBsAg [24]. The ACR recommends screening tests in patients from high-risk groups prior to therapy with leflunomide and methotrexate [22].

Interestingly, the ACR guidelines, contrary to those of EASL and AASLD, do not distinguish between the serological forms of HBV infection, and do not provide recommendations for antiviral prophylaxis.

In the EASL and AASLD guidelines the recommendations generally regard immunosuppressive treatment. The 2015 ACR guidelines and the previous ones from 2008 and 2012 are summarised in Table 2.

According to the 2017 EASL guidelines, patients with chronic hepatitis B using immunosuppressants should be treated with telbivudine, entecavir or tenofovir, similarly to immunocompetent patients. Follow-up and completion of treatment are the same as in immunocompetent patients.

The 2009 AASLD guidelines and 2017 EASL guidelines regarding prophylactic antiviral treatment in patients with chronic HBV infection (presence of HBsAg for > 6 months) recommend using telbivudine, entecavir or tenofovir (AASLD also mentions lamivudine, whereas

EASL approves it in patients without HBsAg, with anti-HBc and at high risk of reactivation) [24, 25] in patients who require immunosuppressive treatment [25]. According to the 2015AASLD guidelines and EASL guidelines, pegylated interferon alpha is contraindicated in patients with concomitant autoimmune disease, due to an increased risk of their exacerbation [26]. Lamivudine reduces the frequency of HBV reactivation, severity of exacerbations, and mortality. Similarly to telbivudine, it is recommended if the expected duration of immunosuppressive treatment is less than 12 months, and HBV DNA is not detected in the blood serum [25]. Entecavir and tenofovir are associated with a lower risk of resistance to therapy than lamivudine, and therefore they seem beneficial in patients at high risk of reactivation of infection, and when the expected duration of immunosuppressive therapy is longer than a year [24, 25]. There are certain discrepancies between AASLD and EASL guidelines regarding the duration of the antiviral therapy. AASLD recommends maintaining the prophylactic treatment for 6 months after completion of the immunosuppressive therapy in patients with a baseline HBV DNA of <2000 lU/ml, or until the end points of the hepatitis B therapy are achieved in patients with HBV DNA of >2000 lU/ml [25]. EASL from 2017 recommends maintaining the prophylactic treatment (regardless of the HBV DNA levels) for 12 months following the completion of the immunosuppressive therapy (18 months if rituximab is used). The prophylactic therapy may be discontinued when the primary disease is in remission [24]. During the prophylactic therapy the hepatic enzymes and HBV-DNA levels should be determined every 3-6 months, and for at least 12 months after the prophylaxis is discontinued [24].

The 2017 EASL guidelines also recommend antiviral treatment in patients without HBsAg with the presence of anti-HBc antibodies. When the risk of virus reactivation is high (>10%), prophylaxis should be continued for 18 months after the end of the immunosuppressive treatment, and patients should be monitored for 12 months after the end of the prophylactic treatment. If the risk of reactivation is moderate (<10%) or low (<1%), the guidelines recommend monitoring of HBsAg and/or HBV DNA every 1-3 months during and after immunosuppression. If HBsAg seroconversion occurs or HBV DNA is detected, telbivudin, entecavir or tenofovir should be introduced. In certain clinical situations, such as prolonged immunosuppression, limited monitoring options, or unknown risk of reactivation following the use of new medications, a prophylactic treatment should be introduced. Patients without HBsAg with the presence of anti-HBc antibodies and HBV DNA should receive the same therapy as patients with HBsAg [24]. The 2009 AASLD guidelines emphasise that in patients without HBsAg with the presence of anti-HBc the reactivation of infection may occur during the immunosuppressive treatment. However, this does not happen frequently, and the clinical data are too limited to justify routine antiviral prophylaxis in these patients. Therefore the recommendations suggest regular monitoring and introducing an antiviral treatment when HBV DNA becomes detectable. Due to an insufficient number of studies, the 2015 AASLD guidelines do not present new recommendations regarding the management of patients with HBV infection undergoing immunosuppressive therapy, and refer to previous guidelines [26, 27].

## Glucocorticoids (GC)

Glucocorticoids (GS) are anti-inflammatory medications used in rheumatology for the rapid control of disease symptoms. However, due to numerous adverse effects, whose risk increases with time, they should be used for only short periods of time [2]. The data regarding the risk of reactivation of HBV infection in RA patients treated with GC are inconsistent. Mo et al. did not find a relation between using low doses of GC and increased risk of reactivation of HBV infection in patients receiving DMARDs. However, the studied groups (especially the group of patients who did not use GC) were very small, at 36 patients in total [13]. Tan et al. observed that low doses of GC are a risk factor for reactivation in patients who do not receive an antiviral prophylactic therapy [14]. Vassilopoulos and Calabrese show that in patients with HBsAg the risk of reactivation of infection is considerable if high doses of GC are used, whereas with low doses the risk is small [12]. The authors also demonstrate that in patients with a history of HBV (presence of HBsAg and anti-HBc) the risk of reactivation of infection is insignificant [12], although individual cases of reactivation in patients with non-detectable HBsAg were reported (de-novo HBV-related hepatitis [10]) treated with GC in combination with DMARD [28, 29]. In summing up, the safety of GC therapy depends on the dose, concomitant use of DAMRDs, and the serological type of the HBV infection. In some cases regular monitoring of patients for the reactivation of infection is justified, or even antiviral prophylaxis should be considered.

**Table 2. Comparison of ACR 2008, 2012 and 2015 recommendations for DMARD use in patients with viral hepatitis [22, 23, 46]. The lack of contraindications does not indicate an affirmative recommendation for the use of the drug in a particular clinical circumstance.**
**Tabela 2. Porównanie zaleceń ACR 2008, 2012 i 2015 dotyczących stosowania LMPCh w wirusowym zapaleniu wątroby [22,23,46]. Brak przeciwwskazań nie stanowi jednoznacznego zalecenia do stosowania leku w konkretnej sytuacji klinicznej.**

| | | 2008 and 2012 ACR guidelines | | | | | | | 2015 ACR guidelines |
|---|---|---|---|---|---|---|---|---|---|
| **Diagnosis** | **Medications** | **MTX** | **LEF** | **SSZ** | **HCQ** | **TNFi** | **ABA** | **RTX** | |
| Infection | Acute hepatitis B | - | - | - | + | - | - | - | No recommendations |
| HBV | Chronic hepatitis B with antiviral therapy | - | - | **A, B** | + | **A** | **A** | **A** | Patients with active hepatitis B who are receiving/received effective antiviral treatment - RA therapy as in patients without hepatitis B |
| | Chronic hepatitis B without antiviral therapy | - | - | - | **A, B** | **A** | **A** | **A** | Patients with a history of hepatitis B** - RA treatment as in patients who were not exposed to hepatitis B, under the condition of regular monitoring of viraemia (every 6-12 months). In patients who do not receive antiviral treatment, it should be introduced before implementation of immunosuppressive therapy |
| HCV infection | Acute hepatitis C | – | – | – | + | – | – | – | No recommendations |
| | Chronic hepatitis C with antiviral therapy | - | - | **A** | + | **A*** | **A** | **A** | Patients with hepatitis C who are receiving/received effective antiviral treatment - RA treatment as in patients without hepatitis C (therapy in cooperation with hepatologist/gastrologist) - a conditional recommendation*** |
| | Chronic hepatitis C without antiviral therapy | - | - | **A** | **A, B** | **A*** | **A** | **A** | Patients with hepatitis C who are not receiving / do not require effective antiviral treatment - RA therapy selected on an individual basis, in co-operation with gastrologists/hepatologists. The preferred DMARDs are synthetic ones, although it is suggested to use synthetic medications other than LEF and MTX (e.g. SSZ, HQA) - a conditional recommendation*** |

hep B - hepatitis B, hep C - hepatitis C, ACR - American College of Rheumatology, MTX - methotrexate, LEF - leflunomide, SSZ - sulfasalazine, HCQ - hydroxychloroquine, TNF-α - tumour necrosis factor α inhibitors, ABA - abatacept, RTX - rituximab, (-) - contraindicated, (+) - medication acceptable in all Child-Pugh classes, (A) - medication acceptable in Child-Pugh class A, (B) - medication acceptable in Child-Pugh class B

* According to the updated ACR guidelines from 2012, in RA with concomitant hepatitis C, etanercept should be the preferred anti-TNF.

** Presence of anti-HBc and anti-HBs antibodies, absent HBs antigen, normal aminotransferase activity.

*** Recommendation is conditional due to very weak evidence.

## Synthetic disease-modifying anti-rheumatic drugs

In patients with chronic hepatitis, B DMARDs are rarely the cause of reactivation [12]. In the study by Laohapand et al. methotrexate, when used for long periods (even without antiviral therapy), did not cause virus reactivation [30]. On the other hand, there were also reports of virus reactivation after the discontinuation of methotrexate [31-32]. A study on leflunomide demonstrated an increased frequency of reactivation in patients with chronic HBV infection (presence of HBsAg and normal HBV-DNA levels). However, no increased risk of reactivation was observed in patients with a history of HBV infection (HBsAg and HBV DNA undetectable, the presence of anti-HBe and/or anti-HBc) and in patients without infection (presence of anti-HBs antibodies and absence of other indicators of HBV) [34]. SSZ is also associated with the risk of reactivation, as demonstrated by Petrov's study involving patients with chronic HBsAg [35].

It should be noted that the reactivation of infection may take place in patients with a history of infection (absence of HBsAg, presence of anti-HBc) treated with DMARDs. However, it is a rare phenomenon (5.3% [29]), and it appears to be associated primarily with the use of MTX [28, 36]. Generally, in this group of patients, therapy with DMARDs is considered relatively safe [12, 14].

Antiviral prophylaxis reduces the risk of reactivation of infection [12] but does not eliminate it completely, mostly due to virus mutation resulting in resistance to lamivudine [25, 37].

It is also worth noting that DMARDs have hepatotoxic properties, and can cause drug-induced hepatic damage [34, 38]. The risk of hepatotoxicity is significantly higher in patients with RA and concomitant chronic hepatitis. A study by Mok et al. demonstrated hepatotoxic effects of DMARDs in 55.2% of RA patients with concomitant chronic hepatitis, compared to 21% of RA patients without hepatitis [38].

### Inhibitors of tumour necrosis factor α (anti-TNF)

The risk of reactivation of HBV infection following anti-TNF treatment in patients with chronic hepatitis B is high, and in some studies it is even 62.5% [10]. Reactivation is much less frequent in non-active HBV carriers (0-16.7%) [37], and occasionally in patients with a history of hepatitis B (5.3%) [29].

Prophylactic treatment reduces the risk of reactivation of infection in patients treated with infliximab, adalimumab and etanercept [39-43]. The observed cases of reactivation are primarily due to virus mutation, which leads to resistance to lamivudine, estimated to occur in 15-30% of cases per year [37]. In these cases tenofovir should be introduced, whereas entecavir should be avoided, due to a potential cross-resistance with lamivudine [11].

It is worth emphasising that most data regarding the safety of anti-TNF therapy in patients with hepatitis B apply to infliximab, adalimumab and etanercept. The data regarding the safety of golimumab and certolizumab are scarce.

### Tocilizumab (TCZ) and rituximab (RTX)

The data regarding the use of TCZ in patients with chronic hepatitis B are very limited, and do not allow unambiguous conclusions to be drawn as to the safety of TCZ therapy in this group of patients. Nagashima and Minota described a case of a patient with chronic HBV infection, who used TCZ for seven years without concomitant antiviral prophylactic treatment, and no reactivation of infection was observed [44, 45].

The data on the safety of RTX in RA patients with HBV infection are also limited. The data from haematological registries indicate a high rate of reactivation of up to 27-80%. A prophylactic treatment (usually with lamivudine) reduces the incidence to 0-13% [12].

## Treatment of RA in patients with hepatitis C

The 2008 and 2012 ACR guidelines recommended screening tests for HCV before introducing therapies with MTX or LEF. They also offered recommendations regarding the use of individual DMARDs according to the Child-Pugh hepatic damage score (Tab. 2) [22, 23]. The 2015 ACR guidelines indicate that RA patients with concomitant hepatitis C who are receiving or received effective antiviral therapy should be treated according to the general recommendations for RA. In patients who do not receive antiviral treatment, the therapy should be developed on an individual basis, in co-operation with gastrologists/hepatologists. The preferred medications are synthetic therapies, especially SSZ and HCQ (Tab. 2). However, it should be noted that the 2015 ACR guidelines regarding the management of hepatitis C patients are conditional (due to very weak evidence). The 2015 guidelines do not provide recommendations regarding screening for HCV in RA patients before the introduction of DMARDs [46].

Until recently, the treatment of hepatitis C was based on pegylated interferon alpha and ribavirin. This therapy is not entirely effective (eradication effectiveness of 44-71% [12]), it is associated with severe adverse effects (2.6-10.9% [47]), and cases of induced RA were reported [50-52].

The WHO guidelines recommend therapies based on direct-acting antivirals (DAA) instead of previously used interferon alpha and ribavirin [47], due to the higher effectiveness of DAA in the eradication of the virus (94-98%), and lower risk of severe adverse effects (1-2%) [47]. DAA include: NS3/4A inhibitors (asunaprevir, paritaprevir, simeprevir), NS5A inhibitors (daclatasvir, ledipasvir, ombitasvir) and NS5B inhibitors (sofosbuvir, dasabuvir) [47]. These medications are used in polytherapies, due to the rapid resistance developed by the virus. The 2017 AASLD/IDSA guidelines and 2015 EASL guidelines regarding the management in hepatitis C do not refer directly to the problem of RA therapy or associated medications. However, they mention numerous drug interactions with DAA related to their metabolism in the liver, which may affect the blood concentrations of the medications [48, 49]. Following the

2016 EASL guidelines, if the therapy with a drug interacting with DAA cannot be suspended for the duration of the HCV treatment (usually 8-12 weeks), this drug should be switched to an alternative one, associated with a lower risk of interaction [49]. In order to verify that a given medication interacts with any DAA, updated databases with information about drug interactions may be used, such as www.hepdruginteractions.org/ [48, 49].

### Glucocorticoids (GC)

Low doses of GC (<5 mg/d of prednisone) are considered effective and relatively safe in joint inflammations associated with HCV infection [53]. On the other hand, GC may stimulate HCV replication and exacerbate the liver damage [54]. Therefore, special caution should be exercised during GC therapy in patients with chronic HCV infection.

### Synthetic disease-modifying anti-rheumatic drugs

It is generally acknowledged that immunosuppressive medications (and GC) may intensify HCV replication and hepatotoxicity [54]. However, it should be emphasised that study results are not consistent in that respect. For instance, Moka et al. observed that chronic hepatitis increases the risk of DMARD hepatotoxicity in RA patients [38]. It is noteworthy that most patients in this study had chronic hepatitis B (n=23), and only six patients had chronic hepatitis C. In a long-term prospective study, Lannone et al. demonstrated that MTX (and etanercept) in patients with active mild hepatitis C did not increase HCV replication or hepatotoxicity [55]. Also the study by Nissen et al. MTX did not show any adverse effects on the hepatic function or the course of HCV infection [56].

According to the 2015 ACR guidelines (as previously mentioned, the ACR recommendations regarding the management of hepatitis C, due to weak evidence, are conditional), patients with RA and concomitant hepatitis C who are receiving or received effective antiviral treatment should be treated following the general recommendations for RA. Simultaneously, it is recommended to consult a gastrologist/hepatologist about the treatment. The situation is more complicated in patients who, for various reasons, did not receive antiviral treatment. According to the 2015 ACR guidelines, it does not disqualify patients from DMARD treatment, but in this case decisions are made on an individual basis, in co-operation with hepatologists/gastrologists, and should be based not only on the activity and complications of hepatitis C, but also on comorbidities, contraindications for individual drugs, and the risk of deterioration of the liver function when a given DMARD is used (generally, in this group of patients conventional synthetic drugs are preferred, especially SSA and HCQ) (Tab. 2) [46]. SSZ may also demonstrate a hepatotoxic effect [54, 57], but it is assumed that the risk of liver damage is lower than with MTX [58].

According to some authors, cyclosporine A (CsA) could be an interesting therapeutic option for RA patients with concomitant hepatitis C. A few long-term studies demonstrated its safety and effectiveness in RA patients with concomitant chronic hepatitis C [59-61]. Some studies indicate that CsA shows an antiviral activity against HCV [57]. Certain authors suggest that a combined therapy with CsA and anti-TNF could be a beneficial therapeutic option [54, 61]. However, according to the current guidelines, CsA is not recommended in the therapy of RA.

### Inhibitors of tumour necrosis factor (anti-TNF)

The effectiveness of the anti-TNF in the treatment of RA patients with and without HCV is similar [62]. However, the potential risk of hepatotoxicity and exacerbation of the HCV infection raises concerns. Some studies demonstrated that TNF-α blocking may potentially inhibit apoptosis of the cells infected with HCV, and as a result increase virus replication and exacerbate chronic hepatitis [63]. However, this hypothesis is not confirmed by clinical observations. Numerous studies on anti-TNF medications (adalimumab, infliximab, etanercept, and golimumab) indicate that they are safe in patients with concomitant hepatitis C [44, 55, 60, 61, 64, 65]. One of the studies revealed a beneficial effect of etanercept in the treatment of HCV infection. Using this medication in addition to a standard therapy with ribavirin and IFN improved the effectiveness and tolerance of the antiviral treatment.

The 2015 ACR guidelines do not exclude the possibility of using anti-TNF, even in patients who do not receive antiviral treatment, but in this group synthetic DMARDs are preferred, especially SSZ and HCQ [46].

### Biologics other than anti-TNF

There are few reports on the use of tocilizumab and abatacept in patients with hepatitis C. In two patients with RA and concomitant hepatitis C treated with TCZ no increase in viraemia or worsening of the liver function were observed [67, 68]. Mahajan et al. described beneficial effects of a therapy with abatacept in 2 patients with RA and HCV infection; the drug was well-tolerated, although the activity of liver enzymes and viraemia changed during the observation [69].

Despite an apparently good safety profile of RTX in cryoglobulinaemia associated with HCV infection [64], in RA patients with concomitant hepatitis C there were cases of increased viraemia and reactivation of infection [64, 70]. Therefore, during RTX therapy in patients who do not receive antiviral treatment, monitoring of viraemia and hepatic enzymes should be considered [64].

## Conclusions

Hepatitis B and C are global and complex problems. Patients who suffer from hepatitis and concomitant rheumatic diseases, such as RA, require close monitoring and the application of special therapeutic strategies, as using GC and DMARDs is associated with a risk of exacerbation of hepatitis, reactivation of infection, and liver damage. It is generally assumed that patients who receive/are receiving effective antiviral therapy may be treated according to the general recommendations, and a

gastrologist/hepatologist should be consulted about the treatment. Significant limitations, however, apply to patients who did not receive antiviral treatment. In the case of chronic hepatitis B it is generally recommended to administer antiviral therapy before introducing immunosuppressants. In chronic hepatitis C, following the ACR guidelines, in certain cases DMARDs may be considered in patients who did not receive antiviral treatment. In these cases therapeutic decisions are made on an individual basis, in co-operation with a gastrologist/hepatologist, and considering the activity and advancement of the liver disease, concomitant diseases, contraindications for individual drugs and the risk associated with using DMARDs.

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# Air pollution and birth weight: past accomplishments and challenges for future research

## Zanieczyszczenia powietrza a masa urodzeniowa: dotychczasowe osiągnięcia i kierunki dalszych badań

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**Abstract.** This review provides concise and useful updates on the latest progress made in the area of research relating to the associations between prenatal air pollution exposure and the birth weight of neonates. It focuses on the critical windows of prenatal exposure, potentially sensitive populations such as male foetuses, and the foetuses of mothers who are multiparous, who smoke, or who are above 35 years of age. It also describes the methodological challenges for future research in the area of environmental epidemiology, such as monitoring of confounding, assessing individual exposure or the problem of simultaneous exposure to many pollutants.
**Key words:** air pollution, birth weight, critical windows, male foetus, multiparous

**Streszczenie.** Artykuł poglądowy przedstawia w zwięzły i precyzyjny sposób aktualny stan badań nad związkiem pomiędzy narażeniem na zanieczyszczenia powietrza w okresie prenatalnym a masą urodzeniowa noworodków. Koncentruje się przede wszystkim na okresach krytycznych w trakcie życia płodowego, a także na populacjach potencjalnie najbardziej wrażliwych na działanie zanieczyszczeń, takich jak płody męskie, płody matek wieloródek, matek palących tytoń i matek powyżej 35. roku życia. W artykule opisano ponadto wyzwania metodologiczne dotyczące badań w zakresie epidemiologii środowiskowej, takie jak kontrolowanie zjawiska zakłócania, oszacowanie wielkości indywidualnej ekspozycji czy problem jednoczesnego narażenia na wiele zanieczyszczeń atmosferycznych.
**Słowa kluczowe:** zanieczyszczenia powietrza, masa urodzeniowa, płody męskie, okresy krytyczne, wieloródki

Delivered: 17/11/2017.
Accepted for print: 13/12/2017
No conflict of interest was reported.
Mil. Phys., 2018; 96(1): 80-86


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## Introduction

Epidemiology and reproductive biology provide much evidence related to the increased sensitivity of foetuses and infants to environmental toxins in comparison to adults. The most sensitive periods in the life of a developing organism include the prenatal period, principally due to the speed of cellular proliferation and the changes in foetal metabolism [1]. Exposure to air pollution during this period can have very serious health consequences for the young body, such as the premature termination of pregnancy (before 37 weeks), intrauterine hypotrophy or low birth weight (<2500 g) [2]. Exposure to air pollution in the prenatal period can also lead to the development of many chronic diseases later in life. The theory of intrauterine programming describes the manner in which adverse environmental conditions in the foetal period affect the occurrence of irreversible structural and functional changes, the metabolism and function of the specific cells, and the tissues and organs of the foetus [3]. While these changes are of an adaptive nature – the condition for survival in an adverse environment, they can

lead to the development of diseases of affluence later in life, both during childhood and adulthood. For example, chronic intrauterine hypoxia, caused by placental function disorders resulting from the mother's exposure to carbon monoxide, mainly from tobacco smoke or pollution caused by transport, through impaired neuronal function and foetal brain development, can lead to the development of neurological diseases which manifest later in life [4]. Other consequences of prolonged intrauterine hypoxia can also include changes to the composition of the body, i.e. lower muscle mass compared to body fat, as well as the higher occurrence of the markers of insulin resistance in adults [5] or cardiovascular abnormalities [6].

When analysing the health consequences of exposure to air pollution measured at birth, most researchers focus on examining the birth weight of new-born infants, deeming it a good indicator of their early developmental condition. In many studies, birth weight is treated as a binary variable, with the cut-off point being the upper limit of what is termed as low birth weight - 2500 g. In the 1950s and 1960s, it was observed that low birth weight (LBW) could be caused both by premature delivery (before the 37th week of pregnancy) and intrauterine growth retardation (IUGR) [7]. The term birth with a low birth weight is treated as a marker in IUGR, provided that infants born at or after the 37th week of pregnancy and weighing less than 2500 g are characterised by growth restriction rather than a low birth weight solely due to a shortened pregnancy.

Although birth weight (treated both as a continuous variable and a binary variable - LBW) has many limitations, it is widely used in research, mainly due to its practicality. It can be easily and accurately measured, in contrast to gestational age or IUGR, which are much less accurate indicators, while ultrasound is still not widely available in all countries [8].

## Types of air pollutants vs. birth weight

Early research on the correlation between air pollution and neonatal condition at birth, conducted in China and the Czech Republic, most often underlined the harmfulness of suspended particles (measured as total suspended particles [TSP] and sulfur dioxide [$S0_2$]). The main source of pollution is stated to be the combustion of fossil fuels (mainly high-sulfur coal), primarily in the utilities and housing sectors, general industry and energy generation and transformation [9].

A recent meta-analysis [10] demonstrated that the majority of studies indicated a decreased birth weight or increased risk of LBW in relation to exposure to carbon oxide (CO), nitrogen dioxide ($N0_2$) and suspended particles smaller than 10 μm and 2.5 μm ($PM_{10}$ and $PM_{2.5}$). A stronger biological effect was observed when analysing the impact of the average pollution level throughout the entire pregnancy in comparison to the impact of pollution measured during various periods of pregnancy. The total effect in terms of lower birth weight was between 11.4 g (95% CI: 6.9-29.7) for each 1 ppm of CO exposure and 28.1 g (95% CI: 11.5-44.8) for each 20 ppb of $NO_2$ exposure. The total increase in LBW risk was between OR =1.05 (95% CI: 0.99-1.12) for each 10 μg/m$^3$ of $PM_{2.5}$ exposure and OR =1.10 (95% CI: 1.05-1.15) for each 20 ug/m$^3$ of $PM_{10}$ (the risk was estimated in relation to exposure throughout pregnancy). The results were less clear in respect to the exposure to ozone ($O_3$) and sulfur dioxide ($SO_2$).

## Critical windows during pregnancy

In the relatively short 9-month period of foetal life, one can distinguish critical windows in which exposure to environmental factors may significantly modulate the structure and functions of the individual systems and organs of the foetus. This is because cellular development changes depend on the stage of foetal life. The initial (embryonic) period is characterised primarily by the proliferation of cells, whereas between week 17 and 31 of pregnancy we can observe both the hyperplasia of foetal cells and their hypertrophy (increase in cellular volume), with the pace of cell division stabilising. After week 31 of pregnancy, foetal development consists mainly of cellular hypertrophy [11], which is why adverse environmental conditions that affect various stages of pregnancy have different consequences. Environmental stimuli restricting foetal growth, which impacts the early stage of pregnancy, are responsible for global growth retardation. Those affecting later stages influence growth restriction, but only of specific tissues, e.g. adipose and muscle tissues, with the key organs for survival such as the brain and heart remaining unaffected.

As regards exposure to air pollutants, however, the question arises as to at what stage the effects of environmental toxins cause the most severe health effects. In other words, whether it is possible to identify the moment in pregnancy when air pollutants have the greatest impact on the reduction in birth weight?

A review of the research indicates that exposure to air pollutants in the initial and terminal stages of pregnancy is of the greatest significance for foetal development. The mechanisms responsible for reducing birth weight as a result of exposure to toxins in the first trimester of pregnancy have yet to be researched well. It is, nevertheless, reckoned that air pollutants can impair the functions of the placenta, which is formed in the first trimester of pregnancy and conditions the provision of nutrients, oxygen and maternal antibodies to the foetus. Substances found in polluted air, such as dust, can cause oxidative stress and damage the DNA of placental and foetal cells, as well as induce inflammations in the placenta [12]. Inflammation mediators are capable of increasing blood viscosity, and, as a result, of impairing intrauterine blood flow. The substances which suspended particles contain can combine with receptors for placental growth factors, which constitute measures for the development of the placenta, impairing its normal growth [13]. When the placenta stops to function normally, foetal growth may be impaired.

This hypothesis is confirmed by research into the impact of particulate matter on the reduction of birth weight in the first trimester of pregnancy. In research involving infants in Brazil born at term it was demonstrated that increasing exposure to $PM_{10}$ by 10 $mg/m^3$ was associated with the birth weight being 14 g lower [14]. A similar interrelation was indicated in research involving infants in Kraków also born at term, i.e. increasing exposure to $PM_{10}$ by 30 $\mu m/m^3$ (interquartile range - IQR) in the first trimester of pregnancy was associated with a birth weight reduced by 14 g [15]. In research involving a Czech Republic population, increasing exposure to TSP by 50 $\mu g/m^3$ increased the risk of LBW by 15% [16].

In addition to particulate matter, also noted were the effects of other types of toxic substances in the air, whose increased concentrations in the first trimester affect the birth weight of infants. These include $SO_2$, which when the concentration in the air is increased by 50 $jug/m^3$ in the first trimester can be associated with the risk of 15% higher LBW [16].

Some research indicates that increased exposure to air pollutants in the last trimester causes decreased birth weight. It is believed that this may be connected with the highest growth rate of the foetus, which falls in the third trimester.

It was observed that increasing the exposure to $PM_{10}$ by 10 $\mu g/m^3$ in the third trimester can result in the birth weight of infants born at term being 11 g lower [17]. A similar effect was also observed in the case of a population of infants in California – increasing the exposure to $PM_{10}$ by 20 $\mu g/m^3$ was associated with the birth weight being 22 g lower [18]. Exposure to CO in the last trimester had a similar effect. Increasing exposure to CO by 1 ppm caused the risk of LBW to increase by 8% [19]. On the other hand, exposure to CO concentrations of >5.5 ppm (a 3-month average) during the last trimester increased the risk of LBW by 22% in the population of infants born at term [20].

## Sensitive populations

Studying populations which are sensitive to environmental factors constitutes a significant challenge in research into the impact of air pollution on the health of infants. There are hypotheses proposing that such sensitive populations may include male foetuses, and the foetuses of mothers who are multiparous, who are above 35 years of age or who smoke.

### Male foetuses

The results of some research indicate a higher mortality rate among male foetuses than among female ones [21], although the recently published study of a team from California [22] found that the opposite may also be true.

The higher sensitivity of male foetuses may be indicated by a faster rate of intrauterine growth. This leads to an increased demand for nutrients and oxygen, as well as by a faster rate of intrauterine blood flow [23], with slower development of the immune and respiratory systems, and an increased risk of intrauterine infections.

Does the sex of the foetus condition the impact of pollution on birth weight? Research involving infanta in Kraków indicates that, as a result of exposure to $PM_{2.5}$, the birth weight of male foetuses decreased more than that of female foetuses [24]. On the other hand, a paper analysing the impact of $PM_{10}$ on the birth mass depending on sex, also involving a population of infants in Kraków, did not reveal any variation of the effect of these pollutants on male and female infants [15]. One can infer from a systematic review that there is some evidence to indicate that in the case of mothers carrying male foetuses exposed to air pollutants, the risk of giving birth to a child with decreased birth weight is higher. This evidence is, however, limited and ambiguous [25].

### Multiparous mothers

Another population of infants which is more sensitive to environmental conditions consists of the children of multiparous mothers. A faster rate of umbilical blood flow was observed in these mothers in comparison to primiparas, thus facilitating maternal-foetal exchange [26]. It is assumed that explains why the birth weight of children of multiparous mothers is higher than that of mothers who give birth for the first time. Increased umbilical blood flow can translate into not only a more efficient supply of oxygen and nutrients to the foetus, but also of environmental toxins, especially when the mother lives in a heavily polluted environment. The streamlined uterine-placental circulation in multiparous mothers can, therefore, contribute to increased susceptibility of this group of women to the effect of the harmful foreign agents present in the air. This hypothesis was studied by a team of researchers from Kraków [27], analysing a representative group of more than 74,000 infants born at term. It was noted that, after standardising to confounders, the impact of CO on the risk of giving birth to a baby with a low birth weight was visible only in multiparous mothers (OR = 1.28, 95% CI: 1.06-1.54),

while in primiparous mothers no such effect was observed. The impact of carbon monoxide on the risk of giving birth to a child with LBW differed depending on the number of children, which was additionally confirmed by an interaction test.

A similar effect was also observed in a population of infants in California, in which the two times greater risk of giving birth to a child with LBW in multiparous mothers was associated with exposure to CO in the third trimester of pregnancy. This effect was caused by concentrations >95th percentile in comparison to exposure to concentrations below the median [20]. In the case of multiparous mothers, CO pollution can be the most significant since, unlike other pollutants, CO can easily cross the placental barrier, resulting in oxidative stress [28]. It presumed that the number of children can modify the effect for the dependence between air pollution and the risk of LBW; however, research is needed to corroborate this observation.

## Mothers who smoke and mothers above 35 years of age

Smoking can exacerbate the adverse effects of air pollution on foetal growth by increasing the inflammatory response and airway reactivity [29]. In pregnant women, exposure to CO resulting from tobacco smoke and road vehicle emissions decreases the availability of oxygen transported to the foetus. This compound easily crosses the placenta and combines with haemoglobin in the foetus more quickly than in the mother, and is removed slower from foetal blood than from maternal blood. Consequently, the concentration of carboxyhaemoglobin in foetal blood can be twice that in maternal blood [30].

Some studies also indicate that the infants of mothers above 35 years of age can be more sensitive to the adverse effects of prenatal tobacco exposure [31], and thus more susceptible to the adverse effects of air pollution. Three researchers attempted to verify these hypotheses [19]. They analysed a New Jersey population, by combining information on the place of residence of the mothers as recorded in the birth register with data from the air quality monitoring network. It was observed that the harmful impact of CO on the risk of LBW was higher for mothers who were smokers than for non-smokers. This effect was not statistically significant, although it was found that exposure to CO in the third trimester had a significant impact on decreased birth weight and increased risk of LBW only in mothers above 35 years of age, while a similar effect among women under 19 years of age was not statistically significant.

Further research is required to confirm whether the effect of pollution in the subgroups of mothers above 35 years of age and smokers differs significantly from that observed among the general population of pregnant women.

## Methodological challenges

Most studies focusing on evaluating the relationship between air pollution and the health of infants are based on data collected from air monitoring stations and birth registers. The application of this method of data collection has several methodological challenges. These include confounding control, estimating the values of individual exposure and the problem of simultaneous exposure to many air pollutants.

### Confounding

Difficulties in confounding control is one of the methodological problems faced in environmental epidemiological studies.

Birth mass can be influenced by a number of factors, such as the mother's nutritional habits before and during pregnancy, weight gain during pregnancy, mother's height, socio-economic status and exposure to passive and active smoking, which can have even a greater impact on foetal and infant health than pollution alone. Due to the applied methodology, which uses previously collected register data, these factors are often not taken into account. This results in errors associated with confounding, whose value is difficult to estimate. For example, mothers who had an opportunity to choose a place of residence characterised by better air quality, were more well-off individuals. At the same time, they probably invested more in their offspring than families with lower incomes. Therefore, mothers less exposed to air pollution are, at the same time, in a better financial situation, and thus benefit from better prenatal care and lead healthier lifestyles than mothers who are less-exposed but in a poorer financial state. If factors such as social status, income or prenatal care are not taken into account in the analyses, this might lead to an incorrect estimation of the effect of pollution (overestimation of its impact). On the other hand, the effect of exposure can be underestimated due to the fact that people living in urban areas are both more exposed to pollution (negative effect) and often better educated, with better access to medical care (positive effect) than individuals who live in rural areas. Because of this, the effect of confounding is very difficult to estimate in studies on the interrelations between the mothers' exposure to air pollution and infant health.

Studies based on register data also have clear advantages. One of these is the use of analyses on extensive research groups, which reduces the uncertainty connected with the random effect, often observed in studies involving small samples. Registry data on births are often also characterised by significant completeness and often include information about important risk factors, such as mother's age, duration of pregnancy, number of children, type of professional activity, and the mother's education. The two last factors can provide some information on the socio-economic status when more precise data cannot be obtained.

## Errors in estimating exposure

The results of measurements of the air pollutant concentration recorded in monitoring station registers are characterised by their high completeness and provision of high quality data over time. However, they do not facilitate the reliable evaluation of the spread of pollutants in a given area [32].

Land-use regression (LUR) makes it possible to more precisely estimate individual exposure to air pollution. This method takes into account spatial data, such as traffic flows, total road length, the characteristics of heavy good vehicle traffic, population density and land area. One advantage of this method is the possibility to model emissions in a given area, which provides an opportunity for increasing the study group. Recently, there has been dynamic development in the spatial methods used for estimating exposure to pollution, as a way of supplementing the standard methods. The use of satellite imagery or biomonitoring, i.e. the observation of the state of the environment using specific species of plants or animals, such as moss, lichen, leaf composition, animal fur and cobweb, is also worth mentioning.

Another methodological solution facilitating a more precise measurement of exposure to air pollution entails the use of personal pollution sensors, which allow the continuous monitoring of personal exposure both during the day and night. This type of recording exposure was used in the study involving women in Kraków, for example [24]. Due to their high costs and time-consuming nature, studies utilising personal measurement devices often cover small samples, which limits their representativeness. Comparative studies demonstrate that, despite the temporal and spatial variability of air pollutant levels, measurements made with the use of portable personal sensors and air monitoring stations correlate well [33]. Therefore, extrapolating pollution measurements from monitoring stations to individual exposure can be justified in this case.

In a study investigating the impact of air pollution on the health of infants, errors in estimating individual exposure can also appear if it is assumed that pregnant women do not move and are constantly exposed to the pollution levels recorded by the monitoring station closest to their place of residence. Other problems with estimating the exposure can appear when the data on the precise place of residence of the studied individuals are unavailable. In such a case, the only solution appears to be to average out the concentrations recorded by all monitoring stations within a given location. This does not rule out exposure estimation errors, as this type of error will be of a random rather than of a systemic nature, and its most serious consequence will be a weakening of the studied effect [34].

## The multi-pollutant approach

The impact of air pollution on the health of infants has been the subject of intensive research for more than 30 years; however, most researchers focus on studying a single type of pollution. There are still few papers concerning simultaneous research into the impact of many environmental toxins on birth weight [35].

The multi-pollutant approach, recently postulated by some researchers and recommended by the U.S. National Research Council, assumes that since we breathe air that is a mixture of different substances, including many different air pollutants to which we are exposed at the same time, health impact analyses should employ modelling which takes into account such simultaneous effects of many toxic substances on the body.

There are several ways of modelling the complex ways in which the mix of air pollutants impact the body. One of these is replacing the studies based on the effect of a single pollutant with the effect from exposure to various sources of pollutant emissions [36]. The simplest way is to use a single pollutant as a marker for a specific type of emission source. For example, $NO_2$ as the indicator of transport pollution [37]. The concentrations of $NO_2$ in the atmosphere correlate with traffic indicators and the concentrations of other substances emitted in relation to the impact of road transport, which is why they can be regarded as a good marker for this source of emission. Some markers, however, are not that specific. For example, carbon dioxide, which is the product of incomplete combustion of fuels containing carbon, and is emitted to the atmosphere both as a result of road transport and technological processes, e.g. as a side product of steel production.

Another approach proposed for researching the impact of the mix of pollutants on health entails the application of statistical methods, such as principal component analysis (PCA) for the identification of sources of emissions. It facilitates the determination of the number of sources and their estimate percentage share in total emissions. This makes it possible to isolate the factors of the most intercorrelated pollutants, and then classify the individual factors and assign them to a specific emission source (e.g. power generation, transport or the metal-mining industry) [38]. One of the advantages of such an analysis is the determination of mutually orthogonal factors, i.e. those independent of each other. In subsequent stages of analysis, it is possible to study the simultaneous impact of numerous sources of pollution on the selected health effect, avoiding the problem of colinearity that appears when many intercorrelated predictors are introduced into a model. This is the case when the effects of many highly correlated pollutants (e.g. $PM_{10}$ and $SO_2$) are analysed at the same time.

The interrelations between individual pollutants are complicated and yet to be fully determined, and their biological impact on infant health and the physiology of the female reproductive system has still to be comprehensively explained. This suggests that analyses taking the multi-pollutant approach into account are an interesting direction for further research into environmental epidemiology.

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# Hospital No. 2 and the Chief Surgeon during the Defence of Lviv on 1-22 November 1918

## Szpital nr 2 i jego naczelny chirurg podczas Obrony Lwowa 1-22 listopada 1918 roku

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**Abstract.** The Defence of Lviv is one of the most important episodes in Polish history. On 1-22 November 1918, some of the civilian population of the city (mostly secondary school and university students) fought against the Ukrainian soldiers of Dmytro Vitovsky, who made a coup d'etat. On 1 November 1918, the Lviv Defence Headquarters (LDH) was organized, headed by Capt. Czesław Mączyński (1881-1935). The health service was based on two main hospitals: Hospital No. 1 ("Technika") and Hospital No. 2 ("Dwójka"). Colonel Henryk Jan Glaser was the commandant of Hospital No. 2. Ludwik Rydygier (1850-1920), a world famous Polish physician, who was the first man to perform a resection of the stomach for a peptic ulcer, served as chief surgeon at "Dwójka". He was also the second doctor in history to undertake a pyloric resection for gastric cancer. For many years he was the head of the Surgery Clinical Hospitals of the Jagiellonian University in Krakow and the University of Lviv. During World War I he was the commander of the Austrian Military Hospital in Brno in Moravia. He was famous for his love of the uniform and the military lifestyle. At that time Poland was partitioned by Russia, Austro-Hungary and Prussia. Rydygier was a great Polish patriot and, despite his age, he joined the Polish Army in 1918 to fight for the independence of his homeland. During the Defence of Lviv, Rydygier was the chief surgeon of Military Hospital No. 2, where he assisted the wounded soldiers of both sides of the conflict. He died on 26 June 1920 in Lviv, and was buried in his general's uniform in the Cemetery of the Defenders of Lviv.
**Key words:** Defence of Lviv, Military Hospital No. 2, Rydygier, Glaser, Cemetery of the Defenders of Lviv

**Streszczenie.** Obrona Lwowa to jeden z najważniejszych epizodów w historii Polski. W dniach 1-22 listopada 1918 roku mieszkańcy miasta (głównie gimnazjaliści i studenci) walczyli z ukraińskimi żołnierzami Dmytra Wytowskiego, który przeprowadził zamach stanu. 1 listopada 1918 roku zorganizowano Naczelną Komendę Obrony Lwowa, którą dowodził kpt. Czesław Mączyński (1881-1935). Służba zdrowia działała w oparciu o dwa główne szpitale: Szpital nr 1 „Technika" i Szpital nr 2 „Dwójka". Komendantem Szpitala nr 2 był płk Henryk Jan Glaser. W „Dwójce" funkcję naczelnego chirurga pełnił Ludwik Rydygier (1850-1920), światowej sławy polski lekarz, który jako pierwszy na świecie wykonał zabieg resekcji żołądka w przebiegu choroby wrzodowej. Był drugim lekarzem w historii, który wykonał wycięcie odźwiernika żołądka z powodu raka. Przez wiele lat prowadził Klinikę Chirurgiczną Uniwersytetu Jagiellońskiego w Krakowie i Uniwersytetu Lwowskiego we Lwowie. W czasie I wojny światowej był komendantem austriackiego Szpitala Wojskowego w Brnie na Morawach. Słynął z zamiłowania do munduru i wojskowego stylu życia. W tym czasie Polska była okupowana przez Rosję, Austro-Węgry i Prusy. Rydygier był wielkim polskim patriotą i mimo podeszłego wieku wstąpił w 1918 roku do Wojska Polskiego, by walczyć o niepodległość ojczyzny. W czasie obrony Lwowa był naczelnym chirurgiem Szpitala nr 2, gdzie udzielał pomocy rannym żołnierzom obu stron konfliktu. Zmarł 26 czerwca 1920 roku we Lwowie, został pochowany w mundurze generała na Cmentarzu Obrońców Lwowa.
**Słowa kluczowe:** Obrona Lwowa, Szpital Wojskowy nr 2, Rydygier, Glaser, Cmentarz Obrońców Lwowa

Delivered: 5/10/2017
Accepted for print: 13/12/2017
No conflicts of interest were declared.
Mil. Phys., 2018; 96(1): 87-96


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## Introduction

In 2018 we will be celebrating the centenary anniversary of the Defence of Lviv (1-22 November 1918), one of the fundamental events relating to regaining independence by the Polish state after 123 years of imprisonment. Its significance for the nation is proved most distinctly by the fact that after 1945 an attempt was made to expunge this city and its extraordinary history from the Polish historiography, aptly fearing this may become an inspiration for later generations of Poles, for whom independence and sovereignty are key values. From the tomb of the unknown soldier in Warsaw, where one of the defenders of Lviv rests, a plate with the word "Lviv" on it disappeared, and in all publications information was censored about the heroic fight of the inhabitants of Lviv for their city. The authorities of the Polish People's Republic were fully aware that a generation of "columbuses" was brought up on the epic of the Lviv Eaglets, who in following their example took up arms on 1 August 1944 to defend the Polish capital. It requires much bad will or ignorance not to notice that the Warsaw teenagers, while attacking German tanks with a bottle of petrol or breaking through channels to deliver post or a dispatch, followed the example of Antoś Petrykiewicz (1905-1919, the youngest in history Knight of the Military Order of Virtuti Militari), Jurek Bitschan (1904-1918) or Jaś Kukawski (1908-1918) - all boys killed during the Defence of Lviv. Despite many years of preventive censorship, they failed to expunge the truth about the city whose motto is "Semper Fidelis". Throughout the centuries it was the most faithful borderland redoubt, defending Poland's rights to these territories, which was expressed beautifully by Kornel Makuszyński in: "...This city is gruesome, this Republic of Poland's faithful and vigilant dog...". On the centenary anniversary of the heroic Defence of Lviv, it is certainly worth remembering also the efforts by Polish doctors during this great and noble undertaking, including one of the then most eminent of world surgeons, Gen. Prof. Ludwik Rydygier [1, 2].

## Genesis of the Conflict

Since the end of the 18th century, Poland was under occupation of the three neighbouring superpowers: Russia, Austria-Hungary and Prussia. Lviv was a part of the territory annexed by Austria. Polish patriots pinned their hopes on regaining independence upon an armed conflict between the invaders. Their dream came true with the outbreak of World War I in 1914, resulting in clashes between the armies of Russia, Prussia and Austria-Hungary. The end of this terrible cataclysm was extremely fortunate for Poles, as all the invaders suffered some form of defeat. The empire of Franz Joseph collapsed, the bloody Bolshevik Revolution was raging across the by then former Russian Empire, Prussian soldiers were defeated on all fronts, and over most German cities was sweeping a revolutionary wave. Although Poland regained independence following 123 years of imprisonment, it had to fight for its borders until 1921. On 1 November 1918 the Ukrainian ataman colonel, Dmytro Vitovsky, staged an armed putsch in Lviv, in an attempt to take over the city for Ukrainians (Rusyns).

It needs to be remembered that the city of Lviv had always been a part of the Polish state and a great centre of research, culture and art. Here Polish mathematicians creating the worldwide famous Lviv School of Mathematics, making many discoveries: Stefan Banach (1892-1945), Hugo Steinhaus (1887-1972), and Stanisław Ulam (1909-1984). One cannot ignore the discoveries by other Polish intellectuals linked with Lviv who exerted considerable influence on the development of their fields, such as Benedykt Dybowski (1833-1930), Kazimierz Bartel (1882-1941), Ignacy Mościcki (1867-1946), and Tadeusz Kotarbiński (1886-1981). Lviv and its doctors made a significant contribution to the development of Polish and world medical science, it is enough to quote a few names to clearly understand the significance of the city and its higher education institutions for Polish medical thought: Ludwik Rydygier (1850-1920), Rudolf Weigl (1883-1957), Adam Gruca (1893-1983), Bolesław Jałowy (1906-1943), Antoni Gluziński (1856-1935), Antoni Cieszyński (1882-1941), Aleksander Domaszewicz (1887-1948), Emanuel Machek (1852-1930), Tadeusz Ostrowski (1881-1941), Hilary Schramm (1864-1941), and many, many others. It is impossible to name all the great artists who were inspired by this very beautiful city on the Poltva River when creating works in different branches of art. This circle included: Maria Konopnicka (1842-1910), Gabriela Zapolska (1857-1921), Tadeusz Boy-Żeleński (1874-1941), Artur Grottger (1837-1867), Henryk Siemi-radzki (1843-1902), Kornel Makuszyński (1884-1953), Stanisław Lem (1921-2006), Wojciech Kilar (1932-2013), Jerzy Janicki (1928-2007), and Adam Hanuszkiewicz (1924-2011).

At the beginning of the 20th century, even after more than 100 years under Austrian occupation, the largest group of residents in Lviv were Poles, at 51%, with Jews accounting for 28%, and the smallest of Ukrainians (Rusyns) at 18%. Because of that it was difficult to imagine that the Poles would agree for this great centre of Polish identity to be outside the borders of their rising-from-ruins country. It is particularly worth emphasising that the Ukrainians (Rusyns) did not come to Lviv until the second half of the 19th century; they took advantage of the extremely tolerant and friendly atmosphere created there over the years by the local Poles. The Ukrainian (Rusyn) strangers, like the Jews, Germans, Russians and Armenians, were allowed to set up their social organizations, political parties, schools and run publishing activities without any hindrance, which was unthinkable in the territories under the jurisdiction of tsarist Russia. The Polish leaders did not expect that their guests from the East would speedily break this territory owners' rights to own it and would make an armed attempt to grab the

Polish city, despite only being the third largest national group in it. Here arises the unwitting reflection about the sense and purpose of bringing enormous numbers of economic immigrants from Ukraine to contemporary Poland, the generation brought up to worship the perpetrators of the genocide of the Polish nation (Stepan Bandera, Roman Szuchewycz) and an exceptionally expansive one who may at some point acknowledge themselves the owners of a part of the Polish territories like their predecessors did in 1918 (Ukrainian nationalist environments are openly bringing territorial claims to the south-eastern provinces of contemporary Poland). *Historia magistra vitae?* [3-5]

On 1 November 1918 at 3.30 a.m., when the units of ataman Dmytro Vitovsky began occupying major strategic buildings in Lviv, there were no regular Polish units at that time that could mount any resistance. This was a result of the Austrio-Hungarian authorities' conscious policy which, fearing independence tendencies amidst Polish society, dispatched Imperial-Royal army units in which the Poles served to distant front lines (e.g. Italy), and the only left units in which the Ukrainians (Rusyns) served, the result of which was a huge disproportion in strength to the benefit of the latter.

It must be particularly strongly emphasised that the entire Polish society joined the battle to defend their city, to a large extent secondary school and university students, later famous as the Lviv Eaglets. In charge of the defence was the Lviv Defence Headquarters (LDH), which were probably constituted as early as during the evening hours of 1 November 1918. As its commander was chosen Czesław Mączyński (1881-1935), with the duties of Head of Health Services by Lieutenant Doctor Lesław Węgrzynowski (1885-1956). The fighting within the city was extremely fierce and bloody. One needs to respect the defenders' absolute determination and dedication that, although they were not a regular army, they managed to gradually drive the Ukrainian units from the city. Unfortunately many people died or were injured due to the actions of both conflicting sides during the military activities [6, 7].

## Organization of Hospital No. 2 during the Defence of Lviv

By the Head of Health Services of LDH's decision, the health services were based on the two major hospitals: Hospital No. 1 ("Technika"), in the Technical University building on Sapiehy Street (with branches in the Piramowicz boarding house at 6 Listopada Street, in a women's prison and in the Russian secondary school in Sapiehy Street), and Hospital No. 2, located in the House for the War-Disabled on Kleparowska Street, and the so-called Air Health Service Patrols (AHSP). The other field hospitals were established mainly in the barracks located across from the War-Disabled Person House's main building. Its headquarters were taken over by a forty-six-year-old officer doctor of the Imperial-Royal Army, Colonel Doctor Henryk Jan Glaser (Fig. 1.). The facility had three main wards: surgical, isolation and skin-venereal. The function of the chief surgeon was performed by Prof. Ludwik Rydygier and was supported by medical students working as medics in a medical corps. A fifth-year student of the University of Lviv, Zdzisław Zygmunt Górecki (1895-1944), later head of the 1st Internal Disease Research Hospital of the Józef Piłsudski University of Warsaw, who died under the rubble of his own hospital during the Warsaw Uprising, gained his experience in the fields of diagnostics and treating infectious diseases here. Patients of the skin-venereal ward were attended by Second Lieutenant Orderly Michał vel Mejlech Parille, also a student of the University of Lviv, a dermatologist at the National General Hospital in Lviv during the interwar period. At this facility was also Second Lieutenant Orderly Leon Koziołkowski, who however never finished his studies and became a sub-doctor in reserve.

**Figure 1.** Colonel Henryk Jan Glaser (1872-1942), commandant of Military Hospital No. 2 ("Dwójka") during the Defence of Lviv in November 1918 (Wawrzkowicz E, Klink J, eds. Obrona Lwowa 1-22 listopad 1918. Organizacja listopadowej Obrony Lwowa. Ewidencja uczestników walk. The list of the casualties. [The Lwów Defence on 1-22 November 1918. Organization of the November Lwów Defence. Register of engaged in the struggle] vol. III. Volumen Publishing House, Warsaw 1994)
**Rycina 1.** Pułkownik Henryk Jan Glaser (1872-1942), komendant Wojskowego Szpitala nr 2 „Dwójki" podczas Obrony Lwowa w listopadzie 1918 roku (Wawrzkowicz E, Klink J, eds. Obrona Lwowa 1-22 listopad 1918. Organizacja listopadowej Obrony Lwowa. Ewidencja uczestników walk. Lista strat. Tom III. Oficyna Wydawnicza Volumen, Warszawa 1994)

The head of the hospital's administration and management department was Second Lieutenant Bolesław Dąbrowski, while the function of provisions supplier was performed by Julian Lachcik. The ministry activities were performed by Rev. Alfred Dobiecki (1880-1951), a chaplain of the Lviv health service during the defence of the city in 1918, and later in World War II he performed a similar function at the Malta Hospital in Warsaw - a facility whose activity became famous in the resistance movement and during the Warsaw Uprising. It is difficult to accurately estimate the number of female orderlies, medics and stretcher men serving at the facility in November 1918, especially as the changes occurring at that time were highly dynamic in nature, and the AHSP members were filling in for personnel shortages at the field hospitals if need be.

At this point it is worth explaining in more detail about the Air Health Service Patrols, whose commandant was twenty-two-year-old Second Lieutenant Orderly Tytus Nowak, who ran a popular dental practice in Lviv in the interwar period. The tasks of this the most dynamic group of the military health facilities and located the nearest to the front line were as follows: providing medical help and evacuating the injured to hospitals. Individual patrols consisted of people having a very diversified degree of medical training, from medical students having the function of medics, through nurses and female orderlies, to the youngest defenders trained in first aid ad lib. It was really dangerous duty, involving a direct life hazard when attending the injured on the front line. In a notorious war crime that the Ukrainians (Rusyns) committed on 29 December 1918 in Dawidów, the soldiers of the Polish health services were killed, together with the Head of Health Services of LDH's adjutant, Second Lieutenant Lech Gluziński, and three AHSP orderlies: fifteen-year-old Franciszek Walerian Manowarda de Jana, seventeen-year-old Adam Nestarowski and his peer Jerzy Henryk Adamowicz-Leliwa [8-11].

It is also worth mentioning the Polish Health Services (PHS), organized by Prof. Antoni Cieszyński (1882-1941), later head of the Dental Department and Research Hospital of the Jan Kazimierz University in Lviv, murdered in the Wuleckie Hills in 1941. PHS' central task was to provide medical help within the city occupied by the Ukrainians (Rusyns). For this reason a dozen or so dressing stations were created where 10 doctors, 10 medical students, 3 female medical students, 53 female orderlies and couriers, 4 male orderlies, and 5 altar boys worked. From the surviving archival materials, it is clear that it was attended not only by Poles, but also Ukrainians (Rusyns), Hungarians and Jews. It was still a time when the conflicting sides respected the health service and medical centres, and their personnel were not an object of attack, although the Ukrainian (Rusyn) side repeatedly broke these fine rules at a later time (e.g. the crime in Dawidów and the shooting at Polish hospitals) [12].

## General Professor Ludwik Rydygier

Undoubtedly the most important figure at Hospital No. 2 was its chief surgeon, Prof. Ludwik Rydygier (Fig. 2). This world-famous doctor was born on 21 August 1850 in Dusocin near Grudziąc (within the territory of Poland, occupied at that time by Prussia). His parents were Karol and Elżbieta née Koenig, middle-class landowners. He received his initial education at Collegium Marianum in Pelplin, then at the secondary school in Chojnice and the Royal Catholic Secondary School in Chełmno, where he passed his secondary school-leaving examination in 1869 [13, 14]. He studied medicine at the universities in Greifswald, then Berlin and Strasbourg, where the teachers of the profession were eminent lecturers: Karl Heuter, Paul Friedrich Vogt, August Martin, and Karl Ernst Schweigger. On 8 December 1873 he received a doctor's diploma in Greifswald. While there he defended his PhD thesis entitled "Experimentelle Beitrage zur Lehre von der Wirkung der Carbolsaure" ["A Contribution to Research into the Action of Carbolic Acid"] under Karl Heuter's supervision in March 1874. In 1878 he was habilitated under the thesis entitled "Eine neue Methode zur Behandlung von Pseudoarthrosen" ["A New Way of Treating Nonunions"] at the University of Jena. Soon afterwards he set up a private surgical clinic in Chełmno, where he performed his pioneering surgery that made him famous throughout the medical world. On 16 November 1880 he was the second in the world to perform an operation for the removal of a stomach pylorus due to cancer (the first was Jules Pean [1830-1898]). The next year he was the first in the world to perform an operation for the resection of the stomach due to a peptic ulcer. He developed many new methods of surgical treatments for various diseases, e.g. hernia incarcerata, ovarian tumours, breast cancer, and many others. He was one of the most eminent surgeons in the world at the turn of the 19th and 20th centuries. He published more than 200 academic works in Polish, German, French, and Italian. His articles featured in many eminent scientific journals, e.g. "Przegląd Lekarski", "Gazeta Lekarska", "Wiener klinische Wochenschrift", "Berliner klinische Wochenschrift", "Zentralblatt fur Chirurgie", and "Revue de Chirurgie". He was an initiator and organizer of the first ever Convention of the Polish Surgeons in 1889 in Kraków [13, 15, 16]. As an already very experienced and well-known doctor, he had been awarded the title of full professor and assumed the function of the head of the Surgical Research Hospital of the Jagiellonian University in Kraków in 1887. After ten years he moved to Lviv, where he was the head of the Surgical Research Hospital of the University of Lviv from 1897 to 1920 (since 1919 the Jan Kazimierz University). He died 26 June 1920 in Lviv and was buried in the Cemetery of the Defenders of Lviv on Łyczakowska Street [15].

**Rycina 2.** General Profesor Ludwik Rydygier (1850-1920) (źródło: Sokół S. Ludwik Rydygier 1850-1920. In: Skarżyński B, ed. Sześćsetlecie medycyny krakowskiej. Życiorysy. Akademia Medyczna w Krakowie, Kraków 1963: 245)
**Figure 2.** General Prof. Ludwik Rydygier (1850-1920) (Sokół S. Ludwik Rydygier 1850-1920. In: Skarżyński B, ed. Sześćsetlecie medycyny krakowskiej. Życiorysy [Six Hundredth Anniversary of Cracow Medicine. Biographies]. Medical University of Kraków, Kraków 1963: 245)

## The Military and War in the Great Surgeon's Life

Rydygier was famous for liking military uniforms and harsh military discipline a lot. He even organized the work at his clinic partly following the example of solutions universally adopted in the army. He attached considerable weight to punctuality and a sense of responsibility, which he demanded of all his assistants. Appreciating his extraordinary practical and teaching skills, the War Ministry of Austria-Hungary dispatched military doctors there to develop their professional skills under the tutelage of this outstanding specialist. In 1914, at the outbreak of World War I, large numbers of civilian doctors, especially surgeons, were called up to serve in the army. Ludwik Rydygier assumed the position of commander of the Military Hospital in Brno (K.u.K. Kriegsspital Brunn) in Moravia, where he worked alongside many doctors from Lviv. There essential help was provided to injured soldiers from the Austrian army from the Italian front. Appreciating the great surgeon's services and all his achievements, Austro-Hungarian Emperor Franz Joseph decorated him with the 3rd Class Order of the Iron Crown, whereas the Pope with the Commander's Cross of the Order of St. Gregory. In June 1916 Rydygier returned to Lviv and shortly after began working once more at the largely war-destroyed clinic [4, 14, 15].

During the Defence of Lviv in November 1918, there were many dead and injured from both sides of the conflict as a result of fierce fighting. The situation was particularly hard amidst civilians. On 3 November 1918 the emergency ambulance service stopped operating in the city because most doctors were called up for military service. Rydygier, who was a great Polish patriot, immediately yielded to LDH and joined the Polish military health service's activities. He became the chief surgeon of Military Hospital No. 2, commonly known as "Dwójka", which was organized in the premises of the House of the War-Disabled at 35 Kleparowska Street (Fig. 3). The doctors of Lviv provided essential surgical help to 1090 injured people badly in need of surgery, including 790 Poles and 300 Ukrainians (Rusyns). Despite Rydygier's enormous effort, 269 people were not saved. It must be particularly strongly emphasised that the Polish surgeon was providing help to injured soldiers of both sides of the conflict, and paid no attention to the nationality of a person in need of help, by which he referred to the Polish military health service's noblest traditions. During the Defence of Lviv the workings of the military health service were supported by a son of the great surgeon, Antoni Rydygier, also a doctor and an officer of the Polish Army. During the major escalation in military activities, the professor only left the operating table to snatch meals and a few hours' sleep [2].

The workings of the military health service during the Defence of Lviv entailed great risk, with 439 members of the medical service dying, including 8 doctors. Such great losses were a result of the absolute commitment and dedication of the medical personnel, who tried to save injured people without the slightest delay heedless of the fact that they themselves were in danger [4]. At that time Rydygier also suffered a great personal tragedy, a stray bullet hit his wife Maria, née Borkowska, who was buried on 28 November 1918 in the Lychakiv Cemetery in Lviv. He did not break down, but decided to be even more active in the fight for independence. He became the chief surgeon of the "Wschód" Army, in command of which was General Tadeusz Rozwadowski (1866-1928). He gave his commander 4 thousand crowns as a donation for his country. That is how the outstanding surgeon and Polish patriot gave an example of sacrificing his own fortune for his country, for as the Polish national hero Tadeusz Kościuszko (1746-1817) said in 1794: "Sacrifice a part of your fortune for your country. Do not flinch from giving credit to your own Homeland, which will repay you as a token of its appreciation".

**Figure 3.** Former Military Hospital No. 2 ("Dwójka"), where Rydygier was the chief surgeon during the Defence of Lviv in November 1918, today the Lviv State University of Life Safety, Lviv, 2006 (photograph by Z. Kopociński)
**Rycina 3.** Dawny Szpital Wojskowy nr 2 „Dwójka", w którym profesor Rydygier byt naczelnym chirurgiem podczas Obrony Lwowa w listopadzie 1918 roku, obecnie Lwowski Państwowy Uniwersytet Bezpieczeństwa Życia, Lwów, 2006 (fot. Z. Kopociński)

The fighting for Lviv ended in victory for the Polish side, on 22 November 1918, when LDH-subordinate forces took control of the city. However, it was fully united with the homeland not until the spring of 1919 [2]. At that time Poland's struggle with Bolshevik Russia was continuing, with its apogee taking place next year. The now seventy-year-old Rydygier, having the rank of second lieutenant general, became the Head of the Health Services for the General Headquarters of the "Pomorze" General District, and once again fought for his country [15]. At that time he also put out an excellent textbook entitled "Krótki zarys chirurgii wojennej dla lekarzy Wojska Polskiego, czynnych w pierwszych linjach sanitarnych" ["A Brief Outline of Military Surgery for Doctors of the Polish Army, Performing their Duty on First Health Service Lines "] for the military health service, in which he shared his experiences with younger trainee doctors [17]. During the military action he visited Lviv in June 1920 for a short time, to settle his matters related to his fortune and property. He died suddenly on 26 June 1920 around 04.30 p.m. at the Loewenherz and Apen-Zeller law office from a heart attack [18, 19].

An excellent illustration of Rydygier's affection for the army was his funeral on 1 July 1920. For his funerary rites the world-famous surgeon was attired in a Polish Army general's uniform, and the funeral service at the Boim Chapel was held by Archbishop Józef Bilczewski (1860-1923). From there the funeral procession passed to the Lychakiv Cemetery. The procession was headed by a cavalry troop and a guard of honour of the Polish Army, while amidst thousands of citizens of Lviv saying goodbye to the brilliant doctor there were also outstanding representatives of the army and of the academic world. The funeral was attended by General Robert Lamezan-Salins, General Juliusz Albinowski and Professor Maksymilian Thullie [20], General Professor Ludwik Rydygier was buried in the Cemetery of the Defenders of Lviv (section 1, grave 15), which has a special place in every Pole's heart. His fanciful gravestone, pyramid-shaped, was topped with a statue of an eagle and the following engraving: "Defender of Lviv", beneath the engraving was the inscription "Doctor Ludwik Rydygier, Professor of the Jan Kazimierz University General of the Polish Army, 1850-1920".

In 1945, after the end of World War II, the Soviet Union annexed the eastern part of Poland together with Lviv. The Cemetery of the Defenders of Lviv was profaned, with Rydygier's grave being destroyed completely. After 1989 a gradual restoration of the Lviv necropolis began, with the graves of distinguished Polish heroes being restored, regrettably partly in a considerably changed form. Now Rydygier's gravestone is in the shape of a classic cross (Fig. 4), deprived of the beautiful statue of the eagle and of the engraving informing that this world-famous surgeon was a defender of Lviv [2],


**Figure 4.** Gravestone of General Prof. Ludwik Rydygier, Cemetery of Defenders of Lviv, Lviv, 2006 (photograph by Z. Kopociński)
**Rycina 4.** Nagrobek generała profesora Ludwika Rydygiera, Cmentarz Obrońców Lwowa, Lwów, 2006 (fot. Z. Kopociński)

## Conclusions

On the centenary of the achievements in the Defence of Lviv by the Polish health service, which chalked up one of the most commendable pages in the history of the military health service during the fighting in November 1918, must be recounted. Heedless of the mortal danger, doctors and nurses, orderlies and stretcher men provided the injured of both sides of the conflict with help, thereby proving that they had the highest moral and humane values. Prof. Ludwik Rydygier became famous in the medical world as a precursor of many new surgical techniques, in particular concerning the abdominal cavity. He went down in history as being the first in the world to perform the stomach resection related to an ulcer. Regrettably not many people know that this world-famous surgeon was also a general in the Polish Army, who fought in the Defence of Lviv in 1918 and in the war with Russia in 1920, contributing to Poland regaining its independence.

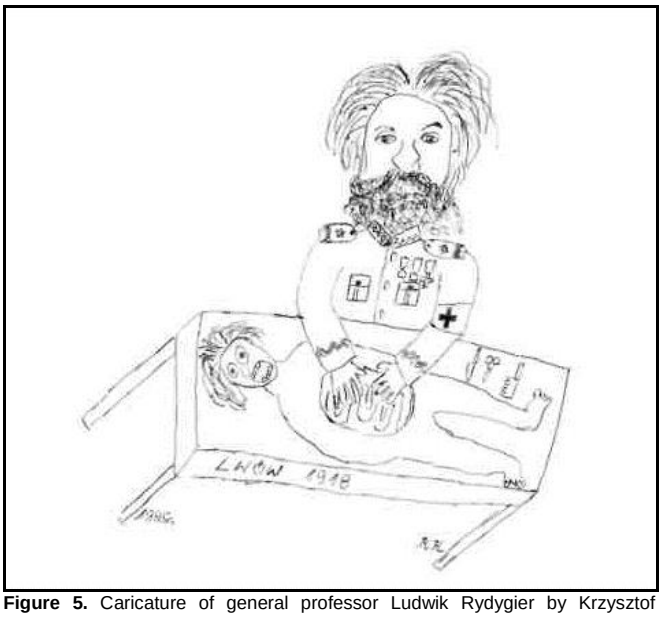


**Figure 5.** Caricature of general professor Ludwik Rydygier by Krzysztof Kopociński, 1995
**Rycina 5.** Karykatura generała profesora Ludwika Rydygiera autorstwa Krzysztofa Kopocińskiego, 1995 r.

It is worth introducing this lesser-known, yet indeed praiseworthy activities of this great surgeon and Polish patriot.

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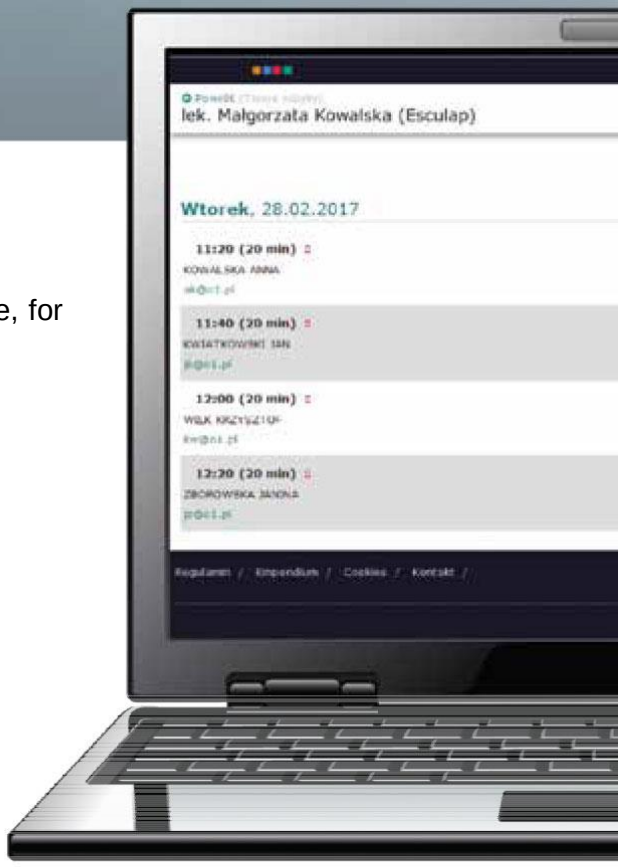


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