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**MEDICAL  
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SCIENCES**

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REVIEW / PRACA POGLADOWA

Agnieszka Łukomska, Iwona Nowakowska, Justyna Szrajda

**CREATING THE IMAGE OF A HEALTH CARE CENTRE**

**KSZTAŁTOWANIE WIZERUNKU ZAKŁADU OPIEKI ZDROWOTNEJ**

Z Zakładu Polityki Zdrowotnej i Zabezpieczenia Społecznego UMK w Toruniu, Collegium Medicum w Bydgoszczy  
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**S u m m a r y**

The purpose of the article is to show the importance of actions in the field of public relations in health care. Communication with environment has a strong influence on creating a positive image of a medical health center and ensuring a stable position in the competitive market of medical services.

The article divides organization's environment into segments and points at groups of people, owing to whom it is possible to influence public opinion. The article discusses forms of public relations actions, underlying the continuous, systematic character and planning strategy of these actions to make them effective and bringing about expected results.

**S t r e s z c z e n i e**

Celem artykułu jest przedstawienie znaczenia działań z zakresu public relations w ochronie zdrowia. Działania komunikacyjne z otoczeniem mają duży wpływ na kształtowanie pozytywnego wizerunku zakładu opieki zdrowotnej, jak i zapewnienie stabilnej pozycji na konkurencyjnym rynku usług medycznych.

W artykule dokonano segmentacji otoczenia organizacji i wskazano grupy osób, dzięki którym można skutecznie oddziaływać na kształt opinii publicznej. Omówiono formy działań public relations, wskazując na ich ciągły i systematyczny charakter oraz strategię planowania tych działań, aby były one efektywne i przyniosły oczekiwane rezultaty.

**Key words:** public relations, image, publicity, health care

**Słowa kluczowe:** public relations, wizerunek, polityka rozgłosu, ochrona zdrowia

The introduction of market mechanisms into the health sector has contributed to the increase of competitiveness between healthcare centres. Therefore, establishing a good reputation and a positive image seems to be justified. The image is a strategic device of an organization and one of the crucial elements of success. When it is well promoted, it makes a healthcare centre more attractive, credible and trustworthy for the receivers of medical services. The value of the image has two dimensions – economical and social [1, 2, 3]. The economical dimension is added in the process of achieving targets and during the market activity of a healthcare centre. The social dimension is acquired on the basis of the reactions of the public and the way a healthcare centre is perceived by an environment. The positive image of an organization contributes to devel-

oping trust and better assessment of a healthcare centre.

The creation of a desired image, i.e the one that an organization wishes to be associated with, is possible through communication with an environment. All the communication actions designed to create and sustain a coherent image in an environment are termed public relations.

The concept of public relations was coined in 1832 by J. Eaten while delivering a lecture at the Tale Law School (USA). Eaten remarked that „public relations refer to the development of relations for the sake of common welfare” [4].

The term of public relations does not have a generally accepted Polish equivalent. “The promotion of reputation has been suggested in order to convey the

factual meaning of the term and coin the same abbreviation - PR [5].

Public relations involve conscious, planned and long-lasting care for relations between an economical or a non-economical institution and its immediate and distant environment called the public, considering an environment's attitudes, opinions and interest in an organization. The activity is designed for the development, predominantly by means of an information system and feedback, of an environment's trust and positive attitudes towards an organization.

PR activity thus not only creates the image of an institution but also provides the public with information on an organization's activity, provides justification for an institution's decisions and balances social relations inside and outside of an organization.

PR activity brings advantages not only to an organization but also to an environment, it contributes to the creation of bonds and an environment's approval and friendly attitude towards an organization's actions.

When communicating with an environment, one needs to consider the fact that an environment is extremely broad. Therefore, public relations should address not the whole environment but clear and specific publics, often called groups, which currently or potentially can contribute to the achievement of an organization's targets [6, 7, 8]. In the field of public relations, it is thus necessary to classify a market into groups and to target groups of importance which form the strategic environment of an organization. While classifying an environment, the following groups are taken into consideration:

- groups which have an immediate and direct impact on the achievement of an organization's targets,
- groups which influence the general functioning, including the creation of conditions for the optimum development of an organisation (public opinion institution, the mass media),
- short-term groups, which form the unstable environment of an organization, request services or criticize an organization.

By defining specific receivers, it is possible to identify the so-called doormen who facilitate the access to the groups [9]. It is a common mistake to mainly consider public and private media (the press, radio, television) to be responsible for the creation of the public opinion. Most often, it is not anonymous newspaper journalists but respected authorities who are able to influence the public opinion. It is their opinions which

are repeated by information media and have a significant impact on our opinions, attitudes and beliefs [10].

Several groups which successfully influence the public opinion can be identified:

- individuals who hold formal power, e.g. politicians, high government officials, religious hierarchy,
- individuals who do not hold formal power but enjoy social prestige,
- individuals who have a vast knowledge and experience,
- individuals who particularly endear themselves to others.

The more one identifies with their views, the stronger the impact and ability to modify attitudes they have.

An organization which intends to influence the public opinion needs to incorporate PR methods in an invariable, continuous and systematic manner [11]. The incorporation needs to be systematic because of the nature of targets to be achieved by the exertion of influence on an environment. It is impossible to form a desired opinion and a positive attitude in a short time through a single contact with an environment. Typical mistakes, which should be avoided, committed by organizations are the following:

- functional shortsightedness – unwillingness to fully acknowledge a significant impact of public relations on successful management,
- philosophy of a water tap – “we will turn the public opinion on when we need it”,
- local anaesthetic – “we will handle this at a local level”,
- neurosis of good news – “we believe in accurate and complete information presented to the community at large as long as it is positive and shows our good side”,
- illusion of shadow – philosophy of hiding and moving into the shadows, a belief that a company can be invisible whenever it wants to [9].

Systematic activity and contacts with an environment can vary in their forms. The most important factor behind the creation of the image of an organisation is cooperation with the mass media. The media make it possible to deliver information to various receivers without the necessity of paying high costs. Another feature of cooperation with the mass media is reliability of information provided through this channel and a stronger impact, either positive or negative, of the information. Cooperation with journalists should be a carefully planned activity, it should not be coincidental

or occasional. An institution which frequently endears itself to journalists can rely on their help in times of trouble (e.g. in a situation of crisis). It is essential to provide journalists with information on a regular basis. Common examples are: the opening of a new ward, the buying of a piece of advanced diagnostic equipment, the introduction of innovative technologies and solutions, promotional actions.

Often underestimated, press conferences and interviews are also popular means of communication with the media. Press conferences are organized usually when a highly important piece of information needs to be presented to the public or a complex problem needs to be discussed in detail. Then, an interview offers a good opportunity to present an institution in public. Hence, it serves for the creation of a positive self-image and helps to quash vicious rumours and resolve doubts about the functioning of an institution.

Apart from developing and cultivating good relations with the media, other PR methods can be employed. One can communicate with an environment by:

- organizing open lectures, readings, talks, actions,
- organizing seminars or conferences to promote scientific achievements,
- preparing and publishing informational materials, accounts, reports,
- supporting various social, cultural and sports actions,
- attending events held by various social organizations.

In order to be effective and bring desired results, PR actions need to be carefully planned. The starting point for the process of planning is a detailed analysis of available information and data, making it possible to create the shared image of an institution for patients, local authorities, the media, the competition and the workers of a healthcare centre. A detailed analysis points to advantages and disadvantages, opportunities and threats, which can be used in the process of designing the action plan to strengthen positive aspects of an image and change disadvantageous elements. It is highly important to adapt the plan to an external (economic and political conditions, moods of the public opinion) and internal situation (moods experienced by the staff, internal conflicts or financial possibilities). The use of adequate methods and means, adapted to the group of receivers facilitates the realisation of a plan. Unsuccessful selection of methods, formulation of a message and provision of information can make the efforts come to nothing. Therefore, it is essential to

evaluate the actions. Yet, the issue of evaluating the efficiency of public relations raises a number of various doubts [5]. The doubts arise, for instance, from the fact that the result of these actions cannot be measured in a quantitative manner, but only in a qualitative one, which is reflected in changes in the attitudes of the public opinion. It is also difficult to determine exactly whether an image owes its improvement only to PR actions or to other means of communication with an environment. It is important to consider the fact that the outcome of PR actions becomes visible only after a certain time and that it is modified by various internal and external factors.

Institutions which consciously intend to follow the policy of public relations and minimize the risk of uncertainty accompanying these actions, can employ not only their own PR units but can also use services provided by specialist agencies [12]. It is also possible to use indirect solutions and conduct these actions on one's own, relying on the advice of an experienced external consultant. Each of these opportunities brings certain advantages along with some disadvantages. Numerous factors are responsible for the choice of a solution. The size of an organization, past experiences and personal preferences of managers are usually taken into consideration.

The support provided by these individuals, their specialist knowledge and skills can certainly win the recognition of an environment and establish a secure position on the competitive market of medical services.

## BIBLIOGRAPHY

1. Maćkowska R. Efektywność działań PR w budowie społecznego zaufania do organizacji. W: Knechta Z (red). *Public relations na tle problemów zarządzania*. Wyd. Wyższej Szkoły Zarządzania, Wrocław 2001.
2. Gruning J., Gruning L. Conceptual differences in public relations and marketing : the case of health care organizations. *Public Relations Review*, 17 (3) 1991.
3. Hart N. Is there a new role for public relations in marketing? *International Public Relations Review*, 1/1995.
4. Wójcik K. *Public relations od A do Z*. Agencja Wydawnicza Placet. Warszawa 2001.
5. Rozwadowska B. *Public relations – teoria, praktyka, perspektywy*. Wydawnictwo Studio EMKA. Warszawa 2002.
6. Kotler P. *Marketing*. Wyd. Felberg SJA. Warszawa 1999.
7. White J. *How to understand and manage public relations*. Business Books Limited, 1991.
8. Goban – Klas. *Teoria komunikowania jako fundament public relations*. *Marketing i Rynek*, 4/1997.

9. Black S. Public relations. Dom Wydawniczy ABC. Warszawa 1998.
10. Lasota M., Rychlicka A., Ryś A., Stępień W. Public relations w ochronie zdrowia. Uniwersyteckie Wydawnictwo Medyczne „Vesalius”. Kraków 2000.
11. Pycio I. PR po polsku – spontanicznie i bez planu. Manager 1/2001.
12. Szymańska A. Aspekty organizacyjne działań public relations. W: Knechta Z (red). Public relations na tle problemów zarządzania. Wyd. Wyższej Szkoły Zarządzania. Wrocław 2001.

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**THE IMPACT OF BAROQUE DANCE FORMS ON THE PSYCHOLOGICAL CONDITION OF PATIENTS AFTER PROCEDURES ON THEIR LOWER LIMB VEINS**

**WPŁYW FORM TANECZNYCH EPOKI BAROKU NA STAN PSYCHICZNY PACJENTÓW PO ZABIEGACH ŻYLNÝCH KOŃCZYN DOLNYCH**

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**S u m m a r y**

Music therapy is closely linked with psychiatry, psychology and medicine and is defined as: „a form of psychotherapy that uses music and its elements as means of stimulation, emotional expression and verbal communication in the process of diagnosis, treatment and the development of per-

son's personality". The world of sounds calms down, reduces fear and provides energy, which is why music can be included in the rehabilitation of patients who have undergone procedures on their lower limb veins.

**S t r e s z c z e n i e**

Muzykoterapia ściśle wiąże się z psychiatrią, psychologią oraz medycyną i jest definiowana jako „forma psychoterapii, która wykorzystuje muzykę i jej elementy jako środki stymulacji oraz ekspresji emocjonalnej i komunikacji werbalnej w procesie diagnozy, leczenia i rozwoju osobowości czło-

wieka". Świat dźwięków uspokaja, łagodzi strach, dodaje energii. Muzykoterapię można więc włączyć do rehabilitacji pacjentów po operacjach przeprowadzonych na kończynach dolnych.

**Key words:** music, music therapy, relaxation music

**Słowa kluczowe:** muzyka, muzykoterapia, relaksacja muzyką

*Music expresses that which cannot be said  
and on which it is impossible to be silent.*

V. Hugo

The healing qualities of music have been known for a long time, the first record dating back to three thousand years ago. However, the rise of music as a form of therapy occurred in the twentieth century as a result of extensive studies in the field of experimental psychology and psychiatry, which have shown great value of music therapy. [4]

Music therapy is closely linked with psychiatry, psychology and medicine and is defined as: „a form of psychotherapy that uses music and its elements as means of stimulation, emotional expression and verbal communication in the process of diagnosis, treatment and the development of person's personality". Excess of music can affect us adversely, which is why it is worth to mention the so-called "healthy music" which

does not interfere with the natural physiological and emotional processes. Such music should be characterized by a rhythm slower than the heart beat per minute, balanced tones and cannot be louder than 70 decibels.

Researchers at the **Chair and Department** of Music Therapy at Collegium Medicum in Bydgoszcz have begun studying the influence of Baroque dance forms on the psychological condition of patients who have undergone venous procedures conducted on their lower limb veins.

Baroque is the longest period in the history of music (1560-1740). Proper Baroque was a period of full bloom of forms and types of music which were developed in the early stages of this epoch. It was in that period when new ideas from Italy were transformed to France and Germany. J.S. Bach is considered as the most eminent representative of that era.

The period is marked by the spectacular rise of the Venetian school of opera (Monteverdi) and beginnings of the French national opera (Lully). The proper Baroque has attained many new stylistic possibilities for new forms. The forms themselves are not fixed but are created on a free basis. This period is also notable for the rise of instruments which have been subordinated to the idea of general bass and various related harmonic forms. The most important characteristics of Baroque style depend on the achievements of its greatest artists including among others Frescobaldi, Carissimi, Schutz and Lully, who determined an individual understanding of the opportunities of the new style and early Baroque heritage. What we experience is detachment from vocal forms and emancipation of the instrumental style. Works are divided into parts like in the typical for that period Italian trio Sonata. The various parts occur in either equally or in terce or quint related keys. The introduction of dance into instrumental music made it livelier. Baroque is noted for an incredible development of instrumental music resulting in creating a particular instrumental style based on new forms.

The world of sounds calms down, reduces fear and provides energy, which is why music can be included in the rehabilitation of patients who have undergone procedures on their lower limb veins.

The consequence of chronic vein failure are varicose veins, i.e. persistent widening of surface veins of the leg, as a result of vein lever failure and weakened elasticity of surface vein walls. It is a progressive and irreversible disease.

Varicose veins treatment should be applied at the right time in order to avoid such complications as ulcers, surface veins clot inflammation.

In the initial period, preservative treatment is applied, which is to reduce the pressure in the venous system of the limb and eliminate edema and inflammation. Preservative treatment includes: treatment with pressure (compression therapy), exercise (physical therapy), medication and sclerotherapy. Sclerotherapy involves injecting solution in the vein, which makes the veins gradually close.

The preservative treatment can also involve dance as a form of rehabilitation. With the help of music, patients overcome pain, do not think about the disease and regular physical activity helps them keep in shape.

Should preservative treatment of varicose veins prove to be ineffective, or vein changes are advanced, operative treatment should be applied.

Another symptom associated with ischemic disease of lower extremities is called pausing lameness (claudicatio intermittens). This term denotes painful cramps or muscle fatigue, occurring in only one or both legs, occurring at the time of exercise (walking) and passes after rest. This condition results from the fact that the demand for oxygen is much smaller and even considerably narrowed arteries deliver sufficient quantity of blood. Most commonly, patients experience pain in their calves, rarely in their feet, buttocks, hips or thighs area. Pausing lameness is more common among men than women, usually after the age of 50 [8].

This condition also requires regular physical activity. Movement stimulates the formation of new, small blood vessels in the subcutaneous tissue and muscle, which may substitute larger clogged arteries.

When faced with a disease, each patient's psychological condition is far from desirable, hence it is important to generate mutual relationship between the patient and the therapist. It is music that can play the role of the mediator while it can not only allow us to release from illness-related stress but can also reduce pain. An example of Baroque music that heals is, for example, "Piano Concerto in A major, part. II, " by J. S. Bach.

As once said by Schopenhauer "Music is a bath for the soul, cleansing it of all its impurity."

## REFERENCES

1. „Dzieje muzyki” - B. Schaeffer
2. „Muzykoterapia aktywna” - M. Janiszewski

3. „Czym jest muzykoterapia” - P. Horn, Magazyn Medycyny naturalnej i niekonwencjonalnej
4. „O muzyce i muzykoterapii” - H. Cesarz; Muzykoterapia Polska nr 1 , 2003
5. „Formy oddziaływań niewerbalnych. Muzykoterapia” - E. Borowicz-Czuchryta
6. [www.erodzina.com](http://www.erodzina.com)
7. [www.psycholog.alleluja.pl](http://www.psycholog.alleluja.pl)
8. [www.emedica.pl](http://www.emedica.pl)
9. [www.jestem.pl](http://www.jestem.pl)
10. [www.nadzieja.pl/lekarz/nowe/muzykoterapia.html](http://www.nadzieja.pl/lekarz/nowe/muzykoterapia.html)

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**MUSIC THERAPY AS AN IMPORTANT ELEMENT OF RELAXATION AND TREATMENT**

**MUZYKOTERAPIA JAKO WAŻNY ELEMENT RELAKSACJI I WSPÓŁLECZENIA**

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**S u m m a r y**

Ever since the 1960s, music therapy has been regarded as a well acknowledged and developing field, its impact noticeably growing in various moments of human life [1]. The knowledge concerning music's therapeutic capabilities is being constantly broadened. Cooperation between musical

therapists, psychologists, psychiatrists, psychical therapists and physical therapists creates even better possibilities of preventing diseases of modern civilization and their treatment [1]. Music's influence on the emotional sphere is an undeniable fact, confirmed by physiologists' research.

**S t r e s z c z e n i e**

Muzykoterapia już od lat sześćdziesiątych XX wieku jest uznaną i rozwijającą się dziedziną wiedzy. Jej udział w różnych momentach życia stale rośnie [1]. Możliwości wykorzystywania muzyki są coraz szersze. Współdziałanie muzykoterapeutów, psychologów, psychiatrów, psychotera-

peutów i fizjoterapeutów pozwala na przeciwdziałanie chorobom współczesnej cywilizacji i wspomaga ich leczenie [1]. Wpływ muzyki na sferę emocjonalną jest faktem niezaprzeczalnym, potwierdzonym w badaniach fizjologów.

**Key words:** music, music therapy, relaxation music

**Słowa kluczowe:** muzyka, muzykoterapia, relaksacja muzyką

For centuries, music has played a vital role in people's everyday lives. It has a calming, relaxing as well as stimulating effect, bringing back memories, encouraging reflection. Not surprisingly, music has become an integral part of therapy in cases of many diseases. Ever since the 1960s, music therapy has been a well acknowledged and developing field, its impact noticeably growing in various moments of human life [1]. Music as part of therapy has its application especially in treating various psychosomatic disturbances, diseases affecting hearing organs or minimizing mental discomfort caused by stress, overstrain and the rapid pace of everyday life. The knowledge concerning music's therapeutic capabilities is being constantly broadened. Cooperation between musical therapists, psy-

chologists, psychiatrists, psychical therapists and physical therapists create even better possibilities of preventing and treatment of diseases of modern civilization [1].

Music's influence on the emotional sphere is an undeniable fact, confirmed by physiological research. For instance, music can cause activation and release of emotional processes, stimulate emotional activity, especially the one concerning conflicting and pathogenic experiences [4]. The above mentioned thesis can be proved by research conducted by the Chair and Department of Music Therapy at the Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, headed by Assoc. Prof. Wojciech Pospiech. His engagement in numerous research projects as well as in

promoting music therapy all around the country are the main contributors to the success of the department. Looking at the research paper topics currently being worked on by Professor Pospiech and his team, one may observe in how many fields music therapy contributes to the treatment. Research is being conducted among others in many clinics, health centers, rehabilitation clinics, residential and nursing homes. The most extensive research works are being currently conducted at the Arka Medical Spa in Kołobrzeg. Their aim is to present the influence of different kinds of music (using works by W.A. Mozart, Baroque music, popular and classical music) on the relaxation of patients and Spa clients during various procedures. As mentioned before, music plays a vital role also in medicine, which has been confirmed by research conducted in such areas of medicine as gynecology or midwifery, where it has been observed that Baroque music of Heandel and Mozart has a relaxing effect on pregnant women and during family labor. Another field, substantially influenced by music therapy is geriatrics, where through different types of music one can relax, motivate to more active way of life and decrease the level of stress. What is more, research is also being conducted in the Chair and Clinic of Cardiology and in a pediatric ward. It is worth mentioning that Professor Pospiech is conducting research in a very broad range, covering various aspects from entertainment through every day life burdens to serious illnesses. Professor's love to music and people is visible in his research programs for children with cerebral palsy, long suffering patients, the lonely, the elderly, the ill and the physically as well as mentally disabled. One needs to remember that the disease does not affect only particular organs, but also affects the psyche and soul, to which music can bring great peace. All data quoted in the research papers have been obtained not only in Bydgoszcz but in other Polish cities as well such as Torun, Poznan, Szczecin, Słupsk, Warsaw and many other, which proves music therapy to be growing and expanding its horizons. In order to attain the best results, different types of music are used depending on the age, health condition and personal preferences of the patient. The calming influence of music on the human psyche has been known from the beginnings of human kind history, first theories regarding music's aesthetic and educational values dating back to antiquity. Studies on the influence of music on human body have been regarded as an interesting research field not only for many Polish but also international scientists. Studies on Mozart's music influence on one's health began in France in the late 50's when Dr Alfred Tomatis started his experiments in the area of hearing stimulation in children with speech and communication

disorders. By 1990, hundreds of therapeutic centers were established all around the world, where Mozart's music, especially his concerts and violin symphonies, were used in the treatment. In the 90's experiments were conducted at the University of California in Irvine and in 2001 scholars in England began studying the influence of Mozart's music on patients with epilepsy [5].

#### ABSTRACT

Many years of research on the influence of music on human body and various areas of life have proven that the world of sounds allows us to calm down, but also activates, teaches sensitivity and broadens our horizons. Therefore, by including music in relaxation or treatment we can influence many senses and obtain very good therapeutic results. Both in Poland, as well as all around the world observations of negative influence of music therapy have not been noted.

#### REFERENCES

1. Boryna M. „SONGIA – leczenie dźwiękiem” Wykład przeprowadzony podczas targów medycyny naturalnej EXPO – MEDIA, Aula UMK Marzec 2003
2. Galijka E. „Diagnostyczne i terapeutyczne aspekty stosowania muzyki w lecznictwie”, Polski Tygodnik Lekarski nr 26, 1981
3. Kasprzak W. Mańkowska A. „Fizykoterapia, medycyna uzdrowskowa i SPA”
4. PZWL Warszawa 2008
5. Konturek S. „Neurofizjologia” W. „Fizjologia człowieka” Tom 4, wydawnictwo
6. Uniwersytet Jagielloński, Kraków 1990
7. Kwolek A. „Rehabilitacja medyczna”, Tom 2, Urban & Partner, Wrocław 2003
8. Natanson T. „Programowanie muzyki terapeutycznej,” Akademia Muzyczna im. K. Lipińskiego we Wrocławiu, Wrocław 1992
9. Szulc Wita „Muzykoterapia jako przedmiot badań i edukacji” Lublin 2005
10. <http://www.psycholog.alleluja.pl/tekst.php?numer=149>
11. <http://www.sciaga.pl/tekst/28338-29-muzykoterapia>

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**EFFECT OF MUSIC THERAPY (MUSIC PROGRAMS) ON INVASIVE CARDIAC SURGERY, CARDIOLOGISTS' RELAXATION AFTER THE SURGERY, PATIENTS' RELAXATION AFTER THE SURGERY**

**WPLYW MUZYKOTERAPII (PROGRAMÓW MUZYCZNYCH) NA PRZEBIEG KARDIOLOGICZNEGO ZABIEGU INWAZYJNEGO, RELAKSACJI KARDIOLOGÓW PO ZABIEGU, RELAKSACJI PACJENTÓW PO ZABIEGU**

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**S u m m a r y**

Stress is an integral part of cardiology. It is an external factor originating from the human environment, burdening the psyche and disturbing body's internal homeostasis. It is important that both cardiologists and patients have an opportunity to relax. Music therapy can be used in order to pursue psychophysical well being, reduce stress and eliminate pain.

Presented examples, as well as knowledge on the overall impact of music on the human body, show that further research on the effects of music therapy programs on the invasive cardiac surgery, as well as cardiologists and patients' relaxation after the surgery is highly justified and needed.

**S t r e s z c z e n i e**

Stres w kardiologii dotyczy zarówno lekarzy, jak i pacjentów. Jest czynnikiem obciążającym dla psychiki człowieka, zaburza wewnętrzną homeostazę organizmu. Ważne jest, aby kardiologowie, a także pacjenci przebywający na oddziałach kardiologicznych, poddawani byli zabiegom relaksacyjnym. Terapia muzyką może być wykorzystywana w celu: dążenia do dobrostanu psychofizycznego, redukcji

stresu, eliminacji bólu. Przeprowadzane badania oraz wiedza o wpływie muzyki na organizm człowieka świadczą o tym, jak bardzo uzasadnione i potrzebne są dalsze badania nad wpływem programów muzycznych na przebieg inwazyjnego zabiegu kardiologicznego, relaksacji kardiologów po zabiegu, relaksacji pacjentów po zabiegu.

**Key words:** music, music therapy, relaxation music

**Słowa kluczowe:** muzyka, muzykoterapia, relaksacja muzyką

Heart has long been considered as a particularly peculiar organ. As an example might be regarded Spanish wall paintings dating back to the Stone Age. The beginnings of modern cardiology date back to the year 1628, when an English physician, William Harvey, published his findings regarding the discovery of circulatory system. Today, cardiology is one of the leading

branches of medicine. Cardiology deals with the treatment of congenital and acquired heart diseases, as well as with the entire circulatory system. Cardiac surgeries are procedures requiring the utmost precision and control. Invasive cardiac surgeries result in patients' stress. Not much is spoken about the "masters of the ceremony" themselves i.e. the cardiologists. They work

under constant stress and tension, when even the smallest mistake can decide about not only health but sometimes even human life. Stress is an integral element of cardiology. It is an external factor, a stimulus or a set of stimuli that originate in the human environment, are harmful, irritating, aggravating the psyche. Stress is a factor disturbing body's internal homeostasis. In order to fight the stress, human body activates defense mechanisms. As a result, a reaction is initiated by the circulatory and hormonal systems, during which nervous system and adrenals are stimulated. It is important that both cardiologists and patients have an opportunity to relax. In the recent years, music therapy as a form of relaxation has been gaining popularity. According to the definition by the American Music Therapy Association music therapy is an area of health protection that uses music to meet the social, mental and emotional needs of people of all ages. Music therapy can be used in order to pursue psychophysical well being, reduce stress, eliminate pain, express feelings, eliminate bad memories, and improve communication between the patient and the therapist. What is more, researches indicate that music can display emotions that are able to reveal the depths of every human being. Music is a form of non-verbal communication. It can also divert attention from, nor even sometimes eliminate the physical pain. Music is initially received through the hearing organ but the melody also reaches the sensory-nervous structures, which are subject to human thinking. Along with the idea that music may improve our mood, an idea known from the ancient times, music therapy has been used in modern medicine since 1950 and is now incorporated as an element of conventional medicine. It is a form of therapy to use music, its qualities and elements as means of stimulation, emotional expression and non-verbal communication in the process of relaxation. It can be used during cardiac surgeries. In the process of cardiac relaxation, one must apply relaxation music which calms down. According to Galinski (1981), it is characterized by short duration from 3 to 10 minutes, slow to moderate pace, no disharmony, transparency, flow, lack of vocals and compositions with vivid content. Appropriate here would be the music of Beethoven, Vivaldi or Strauss as studies show that their music harmonizes and integrates the rhythm at which heart and brain work. Studies on the effects of music on the invasive cardiac surgery were conducted in Hong Kong from September 2004 to May 2005. The purpose of the study was to determine the effect of music on patients

being given a C-ray after a PCI (Percutaneous coronary intervention). This procedure involves pain and physical discomfort, which can lead to stress. The study included 43 patients (20 as a study group - listening to music during the procedure and 23 as a control group). Patients were listening to slow, rhythmic tracks excluding ballads. It was observed in the group listening to music that their pain was reduced, their blood pressure decreased, along with the number of breaths and heart beats. This study has shown that music therapy is a simple, safe and inexpensive method to minimize the physical and psychological symptoms occurring in the form of pain during the surgery. A study conducted in the United States has shown that listening to music during and after the surgery (CABG) is beneficial for the patients. Music is a patient-friendly way to unwind after the surgery, influencing not only the physiological but also psychological parameters. (7).

Another study evaluating the impact of music on patients undergoing cardiac procedures was conducted in the United States among patients undergoing Heart Bypass Surgery (coronary artery bypass graft - CABG). It is known that fear is an integral part of cardiac procedures. Researchers examined 60 people over 65 years old selected at random for the study group and control group. Study group members listened to music during and after the cardiac procedure whereas people included in the control group underwent the traditional procedure. Among patients taking part in the music therapy program, less fear along with a shorter outside procedure intubation have been observed (7).

Presented examples, as well as knowledge on the overall impact of music on the human body, show that further research on the effects of music therapy programs on invasive cardiac surgery, as well as cardiologists' and patients' relaxation after the surgery is highly justified and needed. Before proceeding with the relaxation, patients should be provided with accurate information on the effects of music therapy. Patients should also know what attitude to take before proceeding with the program, what to focus their thoughts and attention on in order to experience full relaxation.

## REFERENCES

1. E. Galińska: Muzykoterapia jako jedna z form terapii przez sztukę. *Zeszyty Naukowe Akademii Muzycznej we Wrocławiu*, nr 48, 1989, str. 73-84.



2. Rymaszewska J, Kiejna A, Hadyrs T. (2004). Depression and anxiety in coronary artery bypass grafting patients. *European Psychiatry*; 18.
3. Biley F.C. (2000) The effect on patient well-being of music listening as a nursing intervention: a review of the literature. *Journal of Clinical Nursing* 9.
4. Davis W.B. & Thaut M.H. (1989) The influences of preferred relaxing music on measures of state anxiety, relaxation and physiological responses. *Journal of Music Therapy* 26.
5. Fscher M. (1990) Music as therapy. *Nursing Times* 86, 39-41.
6. Moon Fai Chan; Oi Chi Wong; Hoi Lam Chan; Mei Chu Fong; Suet Yan Lai; Ching Wah Lo; Siu Mei Ho; Suk Ying Ng; Suk Kit Leung Effects of *music* on patients undergoing a C-clamp procedure after percutaneous coronary interventions. *Journal of Advanced Nursing*, Mar2006, Vol. 53 Issue 6, p669-679.
7. Elizabeth Twiss, Jean Seaver and Ruth McCaffrey The effect of music listening on older adults undergoing cardiovascular surgery 2006 *British Association of Critical Care Nurses, Nursing in Critical Care* 2006 • Vol 11 No 5.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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**COGNITIVE FUNCTIONS AND INCREASE IN ANXIETY, DEPRESSION AND SYMPTOMS OF POSTTRAUMATIC STRESS DISORDER AMONG VICTIMS OF DOMESTIC VIOLENCE**

**FUNKCJE POZNAWCZE A NASILENIE LĘKU, DEPRESJI I OBJAWÓW ZESPOŁU STRESU POURAZOWEGO U OFIAR PRZEMOCY DOMOWEJ**

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**S u m m a r y**

**B a c k g r o u n d .** The study evaluated a selection of cognitive functions in victims of domestic violence in relation to presence and degree of anxiety, depression, or post-traumatic stress disorder (PTSD). 78 women aged 17-64 with a history of domestic violence trauma were chosen for the study.

**M e t h o d s .** I. Neuropsychological evaluation: TMT A&B (Trail Making Test A & B), Stroop's Test (Colour-Word Perseveration Test), Verbal Fluency Test II. Psychometric scales: Mississippi PTSD Scale- Civilian, Beck Depression Inventory, Hamilton Anxiety Scale.

**R e s u l t s .** PTSD was diagnosed in nearly 18% of the victims, psychic and somatic anxiety symptoms were found in more than 17%, whereas half of the women had symptoms of at least moderate depression. The results did not reveal significant differences in selected neuropsychological tests'

performance between the group with at least moderate depression and anxiety. PTSD positive subjects performed worse on the RCnb part of the Stroop's Test. Longer completion time of TMT B correlated with elevation of PTSD symptoms, while longer completion time of TMT A&B correlated with elevation of depression and anxiety. PTSD symptoms elevation correlated with a reduction of words produced in the Verbal Fluency Test. Older persons performed some neuropsychological tests longer than the younger ones.

**C o n c l u s i o n s .** PTSD patients demonstrated a deterioration of some cognitive functions. Elevated symptoms of lowered mood and anxiety as well as the post-traumatic stress disorder and older age correlates with deterioration of some cognitive functions.

**S t r e s z c z e n i e**

**C e l e m** pracy była ocena wybranych funkcji poznawczych u ofiar przemocy domowej w zależności od obecności i nasilenia lęku, depresji i PTSD. Grupę badaną stanowiło 78 kobiet w wieku od 17 do 64 lat, które miały traumatyczne przeżycia związane z przemocą ze strony bliskiej osoby, partnera życiowego.

**M e t o d y .** I. Ocena neuropsychologiczna: Test Łączenia Punktów Reitana A i B - TMT A i B (Trail Making Test A & B), Test Stroopa (Color-Word Intererence Test), Test Fluencji Słownej II. Skale psychometryczne: Mississippi PTSD Scale-Civilian, Beck Depression Inventory, Hamilton Anxiety Scale.

**W y n i k i .** W badanej grupie ofiar przemocy u prawie 18% stwierdzono objawy PTSD, u ponad 17% objawy psychiczne i somatyczne lęku o umiarkowanym nasileniu, a ponad połowa badanych kobiet obserwowała u siebie objawy depresji o umiarkowanym nasileniu. Wyniki badania nie

wykazały istotnych różnic w zakresie sprawności w wykonywaniu wybranych testów neuropsychologicznych między grupą z przynajmniej umiarkowanym nasileniem depresji i lęku. Osoby z diagnozą PTSD gorzej wykonywały część RCnb testu Stroopa. Wydłużenie czasu wykonywania części B testu TMT korelowało z nasileniem objawów PTSD, a wydłużenie czasu wykonywania obu części A i B testu TMT korelowało z nasileniem depresji i lęku. Nasilenie objawów PTSD korelowało ze zmniejszeniem liczby wypowiedzianych słów w Teście Fluencji Słownej. Osoby w starszym wieku dłużej wykonywały niektóre testy neuropsychologiczne.

**W n i o s k i .** U osób z diagnozą PTSD zanotowano osłabienie niektórych funkcji poznawczych. Większe nasilenie objawów obniżonego nastroju, lęku, zespołu stresu pourazowego oraz starszy wiek koreluje z osłabieniem niektórych funkcji poznawczych.

**Key words:** violence, cognitive functions, posttraumatic stress disorder (PTSD), depression, anxiety

**Słowa kluczowe:** przemoc, funkcje poznawcze, zaburzenie stresowe pourazowe, depresja, lęk

## INTRODUCTION

Domestic violence is a pattern of behaviour that is used by one of the family members against the rest of the family to gain or maintain control or power, infringing their rights, personal goods, or lives or health (both physical and mental) in particular, and resulting in damage or suffering. Domestic violence is not a marginal problem but rather a major issue affecting many families, with mostly female victims. Domestic violence causes health deterioration of various type: physical, including disability or life loss, and mental, such as disorders and mental diseases or psychological changes in victims. Psychiatric consequences of domestic violence trauma can be no different from consequences of any other major stress [1]. Victims of domestic violence may experience trauma that meets the criteria of a post-traumatic stress syndrome and are highly at risk of revealing posttraumatic stress disorder (PTSD) symptoms [2]. Painful experiences are one of depression risk factors [3]. Anxiety and other psychopathological symptoms are often identified in victims of violence [4]. Mood and emotional disorders have a great effect on neuropsychological functions [5]. Cognitive functions allow for acquiring an event according to individual interpretation [6]. Structural abnormalities revealed in MRI study of patients with PTSD are found in regions traditionally related to memory. In subjects with a history of trauma, such as war, physical violence, or sexual abuse, shrinkage of the right hippocampus could be observed in MRI of the brain. The loss was proportionate to the degree of short-term memory disorders [7, 8]. The study evaluated a selection of cognitive functions in victims of domestic violence in relation to the presence and degree of anxiety, depression, or PTSD.

## INVESTIGATED GROUP

The group comprised 78 women aged 17-64 (mean age 38.5 yrs, SD=11 yrs). All women had histories of domestic violence traumas. When examined, most females had been separated from perpetrators anywhere between a few months and several years. Prevailing majority inhabited hostels for victims of domestic violence or single mothers' homes. Perpetrators used physical violence and psychological abuse lasting from a few months up to over 20 years. All subjects experienced psychophysical integrity- or health- or life-threatening situations.

Various forms of abuse comprised: threatening to kill, strangulation, beating with a heavy tool, threatening with a knife, threatening with an axe, pushing and knocking over, or forcing sexual activity. Most of the subjects stressed that acute psychological abuse was particularly painful: humiliation, verbal abuse, ridicule, circumscription of personal freedom, facing money use restrictions, wangling money, and indebting. Besides, the women endured particularly harsh psychological tortures when compelled to witness child abuse. All subjects fitted intellectual norms. Majority of women had vocational training. A few subjects worked irrespectively of their children's age. All females but two youngest ones were mothers of 1 to 4 children.

## METHODS

### I. Neuropsychological evaluation

#### 1. Trail Making Test A& B

Trail Making Test comprises two parts. Part A evaluates psychomotor speed, and part B examines visuospatial working memory and set-shifting ability after learning one rule of conduct. Good visuomotor coordination is an advantage. Test's result is provided in seconds (tasks are timed).

#### 2. Stroop's Test (Colour-Word Perseveration Test)

It is used to assess verbal working memory and attention span [9] and comprises two parts: RCnb (reading colour names in black) and NCWd (naming colour of word-different). The test assesses perseveration and during its second part a previously studied criterion needs to be neglected and substituted with a novel criterion, with the former one still present. Test's results are based on completion times of parts one and two, and on the number of perseveration mistakes made during part two.

#### 3. Verbal Fluency Test (FAS)

The test measures the creation and fluency of verbal expression according to a provided criterion. The letter version of the FAS was used (formal reaction criterion). Abnormalities of performance such as few words, perseveration, or intrusions indicate a dysfunction of the frontal and temporal lobe of the cerebral cortex. Verbal fluency abnormalities partly result from online activities related to working memory, which impair the speed of activating words based on a designed criteria.

## II. Evaluation of PTSD presence and elevation

39-point Mississippi PTSD Scale – Civilian (PTSD-C) emerged from the Mississippi scale for combat-related PTSD [10]. PTSD symptoms going back as far as one month are rated on a 5-point Likert scale. The civil version is suited to evaluate mental effects of any type of trauma e.g. PTSD. The scale comprises six sub-scales: 1. Intrusive memories and depressive symptoms, 2. Social adjustment problems, 3. Lability of affect and memory, 4. Ruminative features of PTSD, 5. Other interpersonal problems, 6. Sleep disorders. The Polish version was presented by Lis-Turlejska and Luszczynska-Cieslak (2001) [11]. 107 points and above on a 39-point scale was considered as PTSD elevation in the present study.

## III. Evaluation of depression

A) BDI-Beck Depression Inventory. It is a self-report instrument used for the evaluation of depression. The study investigated a one-month period. Anything above 12 points was recognised as depression (12-18 mild-moderate, 19-26 moderate-severe, 27-49 severe, and 50-63 extremely severe depression) [12, 13].

## IV. Evaluation of anxiety

### Hamilton Anxiety Scale (HAS)

It is a 14-item instrument for measuring anxiety, and its psychic (tension, psychological distress) and somatic (physical complaints resultant from anxiety) aspects [14]. The interviewer rates patients on a 5-point scale (0-4 points) for each of the 14 items ( 0 points – no anxiety symptoms, 1 point – mild, 2 points – moderate, 3 points – severe, and 4 points – paralyzing anxiety)[14]. Seven items address psychic anxiety, whereas the remaining seven address somatic anxiety. Scores of both parts range from 0 to 28. Mild anxiety was determined in patients scoring between 18-24, moderate 25-30, and severe >30 points on HAS [14, 15]. The scale was applied to anxiety evaluation in patients with a history of trauma e.g. war victims [16].

## STATISTICAL ANALYSIS

Results of examinations were statistically analysed. A set of statistical tests SPSS for Windows, ver.13.0 was applied. T-test and Pearson's Correlation Test were used for independent variables.

## RESULTS

PTSD was diagnosed in 17.9% of the examined domestic violence victims (>107 points on the Mississippi PTSD Scale). Mean score on the Mississippi PTSD Scale was 99 points (SD=25.9). At least moderate depression was found in 51.4% of the women (19 points and above on the Beck Depression Inventory). Mean depression in the group was 19.9 points (SD=13.8). Moderate and severe (>25 points) anxiety measured on the Hamilton Anxiety Scale was determined in 17.5% of the subjects. Mean anxiety value in the group was 16.5 points (SD=14.7). Mean TMT A score in the group was 38.4 seconds (SD=21.2), whereas in TMT B it was 94.9 seconds (SD=65.2). Mean result of RCnb of the Stroop's Test was 26.3 seconds (SD=7.7), and of NCWd it was 59.8 seconds (SD=18.4). Average number of words produced in the Verbal Fluency Test was 34.4 words (SD=11.6).

Subjects with pronounced PTSD symptoms (>107 points on the Mississippi PTSD Scale) performed worse on the RCnb part of the Stroop's Test than individuals with no definite PTSD diagnosis (tab. I).

Table I. *Differences in neuropsychological tests' means in victims with and without PTSD*

Cognitive Functions	PTSD (+) Group		PTSD (-) Group		Difference		
	Mean	SD	Mean	SD	t	df	p
TMT A / sec	41.9	26.9	35.6	14.9	1.14	56	0.258
TMT B / sec	114.6	82.5	83.4	50.9	1.75	54	0.085
Stroop RCnb / sec	29.6	8.4	24.0	6.7	2.54	47	0.014
Stroop NCWd /sec	65.5	18.0	58.4	18.1	1.36	47	0.180
FAS / words no.	30.6	12.0	37.0	12.0	-1.92	52	0.060

t-test for equality of means

No difference in the performance of neuropsychological tests was recorded between the group with at least moderate degree of depression (>19 points in BDI) and subjects with mild or without depressive symptoms (tab. II).

Table II. *Differences in neuropsychological tests' means in victims with and without depression*

Cognitive Functions	Depression (+) Group		Depression (-) Group		Difference		
	Mean	SD	Mean	SD	t	df	p
TMT A / sec	42.3	25.1	35.2	12.9	1.60	53	0.115
TMT B / sec	109.7	79.2	78.8	45.5	1.68	51	0.097
Stroop RCnb / sec	26.9	7.2	25.4	8.7	0.67	47	0.506
Stroop NCWd /sec	64.8	17.5	55.9	19.1	1.70	47	0.096
FAS / words no.	32.5	11.6	38.1	10.9	-1.73	49	0.089

t-test for equality of means

Likewise, there were no differences in the performance of neuropsychological tests between subjects with at least moderate symptoms of anxiety (>25 points on HAS), and women with mild or without anxiety symptoms (tab. III).

Table III. *Differences in neuropsychological tests' means in victims with and without symptoms of anxiety*

Cognitive Functions	Anxiety (+) Group		Anxiety (-) Group		Difference		
	Mean	SD	Mean	SD	t	df	p
TMT A / sec	52.9	37.2	36.2	18.0	1.37	10	0.200
TMT B / sec	123.7	103.7	95.1	61.0	0.83	10	0.422
Stroop RCnb / sec	30.0	10.5	25.8	6.2	1.07	8	0.312
Stroop NCWd /sec	65.2	22.5	60.7	19.2	0.58	41	0.562
FAS / words no.	32.6	10.6	36.6	11.9	-0.97	47	0.338

t-test for equality of means

Elevation of PTSD symptoms correlated with longer completion time of TMT B and reduction of words produced in the Verbal Fluency Test (tab. IV). Elevation of depressive symptoms correlated with prolonged completion times of both TMT parts (tab. IV). Similarly, higher level of anxiety correlated with prolonged completion times of TMT A&B (tab. IV).

Table IV. *Correlation between neuropsychological tests results and elevation of PTSD, depression and anxiety*

Cognitive Functions	PTSD		Depression		Anxiety	
	r	p	r	p	r	p
TMT A / sec	0.206	0.121	0.268*	0.048	0.404**	0.003
TMT B / sec	0.271*	0.043	0.271*	0.05	0.340*	0.017
Stroop RCnb / sec	0.220	0.128	0.151	0.302	0.124	0.427
Stroop NCWd /sec	0.114	0.437	0.187	0.199	0.130	0.405
FAS / words no.	-0.332*	0.014	-0.227	0.109	-0.161	0.269

Pearson's correlation; r - correlation coefficient. \* - significance 0.05, \*\* - significance 0.01

An evident correlation was noted between older age and time needed to complete part A of TMT test ( $r=0.249$  with 0.05 significance level) as well as part B of the test ( $r=0.357$  with 0.01 significance level) (in Pearson's correlation test). There was no correlation between age and performance in all other parts of neuropsychological tests.

There was no significant difference between the average scores in neuropsychological tests between the groups of women with only primary or vocational education in comparison with women who obtained

Secondary School Leaving Certificate or third level degree.

## DISCUSSION

18% of violence victims included in the study were diagnosed with PTSD, 17% showed psychic and somatic symptoms of at least a moderate anxiety level, and more than half of the women had symptoms of at least a moderate depression. To find out whether a history of trauma itself lowers cognitive functions whether it is trauma-instilled mental condition that has detrimental effects on cognitive functions, neuropsychological tests results between the group with pronounced PTSD, anxiety, and depressive symptoms, and subjects with no symptoms present were compared. Our findings showed no significant differences in neuropsychological tests performances between the group with at least a moderate level of depression and anxiety. PTSD diagnosed subjects performed worse on RCnb part of the Stroop's Test, which assessed functioning of frontal lobes, verbal operational memory, and attention processes. Longer completion time of TMT B correlated with elevation of PTSD symptoms, whereas longer completion times of TMT A&B correlated with elevation of depression and anxiety. Elevation of PTSD symptoms correlated with reduction of words produced in the Verbal Fluency Test. Nearly 18% of the investigated women showed PTSD symptoms, which indicates that domestic violence is a source of serious trauma, comparable to other traumatic experiences resulting in PTSD. Older persons performed some neuropsychological tests longer than younger. Post-traumatic stress disorder comprises several memory distortions e.g. intrusive memories (hypermnnesia) or temporary loss of memories (amnesia) [17]. Some researchers have proved that symptomatic PTSD is accompanied by other disorders, 47.9% accounting for depression [18]. More than half of the investigated group showed at least a moderate depression, despite the fact that victims were separated from perpetrators at the time of examination. Other studies demonstrated that among individuals with histories of abuse severe depression assessed on BDI is detected significantly more frequently [19]. Symptoms of anxiety in 17.5% of the subjects were at least moderate. Increased anxiety level was probably caused by long-lasting threatening situations. Presence of depressive or anxiety symptoms as psychic and somatic syndromes alone did not differentiate the assessed cognitive func-

tions. Nevertheless, elevation of anxiety and depression correlated with worse performance on TMT A&B. PTSD diagnosed subjects performed worse on RCnb part of Stroop's test, while elevation of PTSD symptoms correlated with longer completion time of TMT B and reduction of words produced in FAS. Domestic violence deteriorates victims' mental health for long. Subjects had not been treated prior to our examination, even though a large part of victims were diagnosed with PTSD and demonstrated symptoms of anxiety and low mood. Therefore, there is a need for designing a special psychiatric aid programme for victims of domestic violence.

## CONCLUSIONS

PTSD patients demonstrated a deterioration of some cognitive functions. The elevated symptoms of lowered mood and anxiety as well as post-traumatic stress disorder and older age correlate with deterioration of some cognitive functions.

## REFERENCES

- Muenzenmaier K, Meyer L, Struening E, Ferber J: Childhood abuse and neglect among women outpatients with chronic mental illness. *Hosp Community Psychiatry* 1993; 44:666-70.
- Jones L, Hughes M, Unterstaller U: Post-Traumatic Stress Disorder (PTSD) in victims of domestic violence. A review of the research. *Trauma Violence Abuse* 2001; 2(2):99-119.
- Tanskanen A, Hintikka J, Honkalampi K, Haatainen K, Koivumaa-Honkanen H, Viinamaki H: Impact of multiple traumatic experiences on the persistence of depressive symptoms--a population-based study. *Nord J Psychiatry* 2004;58(6):459-64.
- Avdibegović E, Sinanović O: Consequences of domestic violence on women's mental health in Bosnia and Herzegovina. *Croat Med J* 2006;47(5):730-41.
- Jaracz J: Zaburzenia funkcji poznawczych w depresji: badania neuropsychologiczne i neuroobrazowe; In Zaburzenia funkcji poznawczych w chorobach psychicznych, A. Borkowska (eds): Biblioteka Psychiatrii Polskiej, Kraków 2005:31-44.
- Granvold DK: Cognitive treatment. In L. Beebe, N. A. Winchester, F. Pflieger, & S. Lowman (eds.): *Encyclopedia of social work* Washington, DC: NASW Press 1995, pp 525-538.
- Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB: MRI based measurement of hippocampal volume in patients with combat related posttraumatic stress disorder. *Am J Psychiatry* 1995;152:973-981.
- Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure CM, Capelli S, McCarthy G, Innis RB, Charney DS: MRI-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse: A preliminary report. *Biol Psychiatry* 1997;41:23-32.
- Stroop JR: Studies of interference in serial verbal reactions. *J Exp Psychol* 1935;18: 643-661.
- Keane TM, Caddell JM., Taylor KL: *The Mississippi scale*. Boston, MA:VA Medical Centre, 1986.
- Turlejska M, Łuszczynska-Cieślak A: Adaptacja cywilnej wersji Kwestionariusza Zespołu Stresu Pourazowego: Mississippi PTSD Scale. *Czasopismo Psychologiczne* 2001;7:165-173.
- Beck AT, Ward CH, Mendelson M, Mock J, Erbaug J: An inventory for measuring depression. *Arch Gen Psychiatry* 1961;4:561-571.
- Parnowski T, Jernajczyk In: Inwentarz Depresji Becka w ocenie nastroju osób zdrowych i chorych na choroby afektywne(ocena pilotażowa). *Psychiatr Pol* 1977;11:417-425.
- Hamilton A: Diagnosis and rating of anxiety. *Brit J Psychiat* (special publication) 1969;3:76-79.
- Beneke M: Methodological investigations of the Hamilton Anxiety Scale. *Pharmacopsychiatry* 1987;20(6):249-55.
- Hashemian F, Khoshnood K, Desai MM, Falahati F, Kasl S, Southwick S: Anxiety, depression, and posttraumatic stress in Iranian survivors of chemical warfare. *J Am Med Assn* 2006;296:560-566.
- Van der Kolk BA, Fisler R: Dissociation and fragmentary nature of traumatic memories. Overview and exploratory study. *J Trauma Stress* 1995;4:505-525.
- Breslau N, Davis GC, Peterson EL, Schultz L: Psychiatric sequelae of posttraumatic stress disorder in women. *Arch Gen Psychiatry* 1997;54:81-7.
- Csoboth CT, Birkás E, Purebl G: Living in fear of experiencing physical and sexual abuse is associated with severe depressive symptomatology among young women. *J Womens Health (Larchmt)* 2005;14(5):441-8.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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**JUSTIFICATIONS OF THE CHOICE OF PHYSIOTHERAPY PROFESSION  
AND SELECTED ENVIRONMENTAL CONDITIONS OF YOUTH  
FROM POST-SECONDARY SCHOOLS IN THE MAZOVIAN REGION**

**MOTYWACJE WYBORU ZAWODU FIZJOTERAPEUTY A WYBRANE UWARUNKOWANIA  
ŚRODOWISKOWE MŁODZIEŻY SZKÓŁ POLICEALNYCH REGIONU MAZOWIECKIEGO**

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**S u m m a r y**

Physiotherapist's work should be treated as social service, in the realization of which incessant improvement of the professional skills is indispensable and also the formation of fully developed personality in a person executing the profession. The choice of the profession should result from appropriately steered justifications. The aim of the present study is to define both.

It was taken for granted that young people learning at secondary schools in cities differing in the urbanizational level and coming from different social environments differ in the regard of choosing the physiotherapist occupation.

By the method of diagnostic opinion poll two groups of youth were examined: students from Medyczne Szkoły Policealne (post-secondary medical schools) in Sokołów Podlaski - assembling young people from small cities and villages and students from Warsaw - who, in majority, are youth from large cities.

Both examined groups gave similar opinion of the relationship between the physiotherapy profession and chosen

areas of professional activity. The desire to carry help was identified as the basic justification of choosing the career. Essential differences were observed in the preferences of the place of the realization of the future employment, the expected age of patients and the fields of physiotherapy applied in the future work - the youth from a small city prefer the work with children and using characteristic standards for physical therapy, youth learning in a large city declare greater willingness to work with adults and the superiority of professional actions from the range of kinesitherapy.

Results of the investigations and the necessity of further education declared by the youth from both small and large cities, allowed for formulating conclusions relating to the legitimacies of considering differences in the educational programmes of higher education resulting from the justification of the choice of physiotherapist profession and his or her place of future work - and also the need of monitoring this problem among the academic youth.

**S t r e s z c z e n i e**

Pracę fizjoterapeuty należy traktować jako służbę społeczną, w realizacji której niezbędne jest nieustanne doskonalenie warsztatu zawodowego oraz kształtowanie wszechstronnie rozwiniętej osobowości osób ją wykonujących. Na wybór tej pracy powinny wpływać odpowiednio ukierunkowane motywacje – próbę ich określenia uczyniono celem niniejszej pracy.

Założono, że osoby podejmujące naukę na poziomie średnim w ośrodkach znacznie różniących się poziomem urbanizacyjnym i tym samym pochodzące z innych środowisk społecznych kierują się innymi względami w wyborze zawodu fizjoterapeuty.

Metodą sondażu diagnostycznego zbadano uczniów dwóch medycznych szkół policealnych: w Sokołowie Podlaskim – gdzie uczy się młodzież z małych miast i wsi i w Warszawie – do której przeważnie uczęszcza młodzież z ośrodków wielkomiejskich.

Wśród młodzieży obu badanych szkół stwierdzono podobną ocenę związku zawodu fizjoterapeuty z wybranymi obszarami działalności zawodowej, a chęć niesienia pomocy zidentyfikowano jako podstawową motywację jej wyboru. Istotne różnice zaobserwowano w preferencjach miejsca realizacji przyszłego zatrudnienia, oczekiwanego wieku pacjentów oraz dziedzin fizjoterapii wykorzystywanych

w przyszłej pracy – młodzież z małego ośrodka preferowała pracę z dziećmi oraz wykorzystywanie standardów znamienych dla fizykoterapii, a podejmująca naukę w dużym mieście deklarowała większą chęć pracy z osobami dorosłymi i przewagą czynności zawodowych z zakresu kinezyterapii.

Wyniki badań i deklaracja potrzeby dalszego kształcenia młodzieży obu ośrodków, pozwoliły na sformułowanie wnio-

sków dotyczących zasadności uwzględnienia różnic wynikających z motywacji wyboru zawodu fizjoterapeuty i miejsca jego przyszłej pracy w programach edukacyjnych studiów wyższych – także potrzeby monitorowania tego problemu wśród młodzieży akademickiej.

**Key words:** post-secondary schools, large city, small city, the area of the professional activity, workplace, the field of physiotherapy, patient's age

**Słowa kluczowe:** szkoły policealne, duże miasto, małe miasto, obszar działalności zawodowej, miejsce pracy, dziedzina fizjoterapii, wiek pacjenta

## INTRODUCTION

The physiotherapist's work should be treated as the social service. The necessity of possessing individual features to achieve the aims, and which patients often attribute to the representatives of this profession, creates the need of the incessant improvement of professional skills and formation of fully developed personality.

The present-day physiotherapy studies are realized by universities with various profiles of education, different modes and educational levels - recently often on the medium level. It is possible that candidates to this profession differ in the opinions about its social part and their own suitability to the chosen occupation. The fact of one's identification with the difficult job which is the focus of the public opinion and which requires strong and grounded justifications supported by the possession of special individual qualities was made the aim of the present study.

Among many works relating to this occupation and the physiotherapist individual qualities [1, 2, 3, 4, 5, 6] we do not find the full answer to the question pertaining the causes of choosing by young persons this and not other profession and preparing to its realization [7]. Earlier own investigations show, that the frequent motivation for choosing the physiotherapy profession is the desire to give help, and that the calling is indispensable to realize it successfully [8, 9]. We also find similar statements in different authors' works, and the problem of the justification of the choice of the occupation and studying physiotherapy remains the interest of many academic centres, also that of Poznań and Warsaw [1, 3].

It was presumed in the present study, that persons who undertake studying physiotherapy on the medium level in the cities considerably differing in the urbanizational level and coming from different social environments were prompted by different causes in the comparatively early choice of the future occupation.

## THE MATERIAL AND METHOD

The material used in the investigations was gathered in the spring 2006 among the listeners of post-secondary medical schools in Sokołów Podlaski and Warsaw. These centres differed as to the facilities and didactic-scientific potential, which is not, however, a decisive factor as far as the skills and the students' level of knowledge is concerned. It may impinge on the studied aspects, though.

The method of diagnostic investigation supported by the questionnaire technique was applied in the work [10]. The questionnaire contained questions which a respondent answered choosing from among the suggested points, what in his or her opinion was the most important. Partly these answers allowed to gather information about the person questioned, and six questions concerned the justification of the choice of the occupation and the basic preferences of the conditions of the future work.

157 filled out forms were received: 94 from the students in Sokołów Podlaski and 63 from the students in Warsaw. In both those centres male students made up approximately 30% of all the respondents and due to the fact there was no need to differentiate the material with the regard to sex. The attention was focused rather on the place of studying, and hence the size of the living environment.

After obtaining the quantitative data, the basic methods of descriptive statistics were applied, counting the proportional parts of the given categories from the questionnaire, and to describe the differences of the proportions of individual profiles, the Fisher-Snedecor test was applied [11].

## THE RESULTS OF THE INVESTIGATIONS

As it was mentioned already, the percentage structure of the sex of the youth from both centres did not differ significantly. A similar situation was observed as for the level of education and the age of the investigated persons; 96% of persons had secondary education, and were under the age of 25.

The size of the place of residence was the factor differentiating these groups. Those who studied in Sokołów Podlaski came from small cities (54%) and villages (35%). Their colleagues studying in Warsaw lived mostly in large cities (62%) and small cities (25%), the percentage from villages being almost three times smaller - fig. 1.

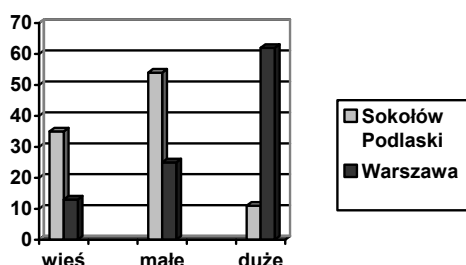


Fig. 1. The graphic profile of the size of the youth's place of residence

Fig. 1. Graficzna charakterystyka wielkości miejsca zamieszkania młodzieży badanych zespołów

The ideas of the inquired students about connections between the physiotherapist's profession and some chosen areas of that professional activity and expectations pertaining to the future work have been presented by means of numerical profiles in Tab. I.

Tab. I. The numerical profile of the relationship of the physiotherapist's occupation with the chosen areas of professional activity in the opinion of the youth

Tab. I. Liczbowa charakterystyka związku zawodu fizjoterapeuty z wybranymi obszarami działalności zawodowej w ocenie młodzieży badanych ośrodków

Category of expected professional activity	Sokołów Podlaski	Warszawa	n	Test probability of the proportion differences essentiality
Medical care	54   57.45%	33   52.38%	87	0.5371
Preventive treatment	12   12.77%	11   17.46%	23	0.4867
Care	8   8.51%	3   4.76%	11	0.4645
Physical exercise	3   3.19%	4   6.35%	7	0.3585
Posture correction	29   30.85%	26   41.27%	55	0.1988
Physical activity	14   14.89%	11   17.46%	25	0.7362
Sport	7   7.45%	9   14.28%	16	0.4309
Personal hygiene	1   1.06%	1   1.59%	2	0.6010
Wholesome education	4   4.25%	2   3.17%	7	0.7645

As one may deduce from the results, basic justifications of the choice of the occupation connected with the character of the physiotherapist work did not differ much between the youth of the studied groups - the greatest share was allotted to medical care and the correction of posture, the smallest to personal hygiene, and wholesome and physical education.

The preferred fields of the future occupation are presented in the Tab. II. The youth from Sokołów Podlaski in their future work would like most to actualise the standards characteristic for physical therapy, the youth studying in Warsaw - actions constituting kinesitherapy. The differences of the percentages of these preferences were statistically significant - however, they were not affirmed that much in the indications of the massage as basis of the future professional work.

Tab. II. The numerical profile of the fields of physiotherapy preferred in the future work of the youth

Tab. II. Liczbowa charakterystyka dziedzin fizjoterapii preferowanych w przyszłej pracy młodzieży badanych ośrodków

Category of physiotherapy area	Sokołów Podlaski	Warszawa	n	Test probability of the proportion differences essentiality
Physiotherapy	52   55.32%	2   3.17%	54	0.0000*
Kinesitherapy	28   29.79%	49   77.78%	77	0.0000*
Massage	14   14.89%	12   19.05%	26	0.5101

Tab. III shows the justification of the choice of the occupation of the youth from the two centres. The most commonly mentioned reason for undertaking physiotherapy studies was the desire to carry help - the pro-social attitude prevailing among the students from Sokołów Podlaski. Significant differences were observed only in the justification connected with the financial protection which this occupation ensures - Warsaw students ignored this point entirely.

The profile of the awaited place of work for the studied groups is presented in the Tab. IV. Differences in the accepted categories of the future work place were frequent and in most cases statistically significant. They were not affirmed only in the cases of students who intended to undertake work in a hospice, a hospital and in the case of planning to start a kind of private business which was indicated most often by youth from both groups. A considerable percentage of the youth pursuing studies in Sokołów Podlaski declare the intention of undertaking employment in the lounge

of the biological refit in a sanatorium and in an outpatient clinic. These justifications are different in the compared groups, and similarly in those relating to the undertaking the work at school, a day care centre and as a representative of a rehabilitation firm. Among the youth studying in Warsaw these factors had the smallest percentage.

Tab. III. *The numerical profile of the justification of the choice of the physiotherapist occupation*

Tab. III. *Liczbowa charakterystyka motywacji wyboru zawodu fizjoterapeuty młodzieży badanych ośrodków*

Justified category of a chosen profession	Sokołów Podlaski		Warszawa		n	Test probability of the proportion differences essentiality
Willingness to carry help	62	65.96%	45	71.43%	107	0.3592
Social prestige of a given job	10	10.63%	3	4.76%	13	0.0711
Easiness to find a job	12	12.77%	10	15.87%	22	0.4788
Financial reasons	6	6.38%	0	0%	6	0.0349*
Other motivations	4	4.26%	5	7.94%	9	0.2918

Tab. IV. *The numerical profile of the expected place of employment*

Tab. IV. *Liczbowa charakterystyka oczekiwanego miejsca zatrudnienia młodzieży badanych ośrodków*

Category of expected work place	Sokołów Podlaski		Warszawa		n	Test probability of the proportion differences essentiality
Hospital	28	29.79%	18	28.57%	46	1.0000
Out-patient clinic	32	34.04%	7	11.11%	39	0.0013*
Hospice	13	13.83%	4	6.35%	17	0.1148
Sanatorium	54	57.45%	11	17.46%	65	0.0000*
Day care centre	15	15.96%	2	3.17%	17	0.0109*
School	10	10.64%	1	1.59%	11	0.0361*
Lounge of biological refit	55	58.51%	14	22.22%	69	0.0000*
Own activity	50	53.19%	27	42.86%	77	0.2201
Sales representative of rehabilitation company	12	12.77%	2	3.17%	14	0.0332*

Table V presents the general profile of age of patients awaited by the studied groups. Students from Sokołów would most willingly undertake the work with children, and their colleagues from Warsaw with adults - expectations in this area, in the light of the statistical opinion, differed indeed. The work with aging patients was awaited by the similar and also the smallest percentage of the youth of both groups.

Tab. V. *The numerical profile of the developmental age of the patients as expected by the youth in their future professional work*

Tab. V. *Liczbowa charakterystyka wieku rozwojowego pacjentów oczekiwanych przez młodzież badanych ośrodków w przyszłej pracy zawodowej*

Patients' age category	Sokołów Podlaski		Warszawa		n	Test probability of the proportion differences essentiality
Children	52	55.32%	23	36.51%	75	0.0280*
Youth	20	21.28%	11	17.46%	31	0.5345
Adults	18	19.15%	27	42.86%	45	0.0013*
Elderly	4	4.25%	2	3.17%	7	0.5405

## DISCUSSION

Justifications have emotional nature and they stimulate the purposefulness of activity [12]. From the point of view of cognitive psychology, of great importance is the knowledge of aims which furthers motivation in taking up activities - „the most exact observation will not let understand behaviour, if we do not know its aim” [13]. The cognitive models of justification take for granted that we choose the aim of our activity consciously. In our investigations, despite the similar opinion about the connection between the physiotherapist occupation and the chosen different fields of the professional activity, there were essential differences in choosing the future workplace and the subject of the therapeutic working of the groups of the youth undertaking the study in the centres differing in size. It should be noted here that the youth studying in the small city shows greater motivation to undertake employment even in places fundamentally different. One may suppose that the fact is associated with their real opinion of the possibility of finding work in smaller centres. These observations allow us to deduce that such differences of aims and the justification of their realization will also appear among the academic youth studying physiotherapy from other similarly differing centres. This is backed up by the fact of insignificant differences occurring in the profile and the education programmes in this occupation.

Human nature possesses positive drive to develop and to satisfy needs. They differed among the studied groups in the matter of satisfying the financial needs connected with the future occupation. Greater importance of this factor among the youth coming from smaller cities is due to their greater determination to acquire higher social status and probably the worse financial situation of their families.

According to the A.H. Maslow's conception of the hierarchy of motivations, one's internal need to help others pertains to the level called self-realization and is fulfilled through the accomplishments of deeds for those in need [14]. As distinct from earlier discussed justifications of the choice of occupation of the studied youth groups which can be counted as the shortage group, the desire to carry help may be regarded as higher in the hierarchy group of the needs of existence; this was listed most often by the future physiotherapists [15].

This investigation confirmed considerably the common opinion about the desire of carrying help as the basic factor motivating young people to choose the physiotherapy profession [6, 7, 8], and also different observations of other authors and the results of our own earlier investigations relating to the physiotherapist occupation - they also allowed to formulate these basic observations and conclusions.

## CONCLUSIONS

1. The desire to carry help is young persons' basic motivation in the choice of the physiotherapist occupation. It is a symptom of possession a set of direction features and hence, taking into account the necessity of identification of their occurrence with the candidates to these type of studies seems well-founded.
2. Differences in expectations relating to the place of work, the subject of exerting one's professional interaction and the hierarchy of satisfying financial needs by the future occupation among persons coming from areas with various degree of urbanization and undertaking the study in the centres of the various size testify about the influence of environmental factors on the motivation in choosing the physiotherapist occupation.

## BIBLIOGRAPHY

1. Kabsch A.: Dobór na studia, kształcenie osobowości i praca specjalistów rehabilitacji ruchowej, [w:] H. Grabowski (red.) Społeczno – pedagogiczne problemy kształcenia w uczelniach, AWF Kraków 1993.
2. Herberger J.: Psychospołeczne uwarunkowania przydatności magistrów rehabilitacji ruchowej do pracy zawodowej. Zeszyty Naukowe AWF, Wrocław, 1990, 51, 152-182.
3. Biniakiewicz B.: Kształcenie, zawód i praca magistrów rehabilitacji ruchowej, AWF Poznań 1994.
4. Zembaty A.: Rola fizjoterapeuty w rehabilitacji leczniczej. w: Kultura Fizyczna, 1981, 5, 57-112.
5. Srokosz W., Szczygieł A.: Wzorzec magistra rehabilitacji w świadomości studentów kierunku rehabilitacji ruchowej w Akademii Wychowania Fizycznego w Krakowie. Rocznik Naukowy, 1988, 23, 183-203.
6. Marosz A. Lewandowski A.: Model zawodowy fizjoterapeuty w opinii pacjentów na przykładzie badań miejskich przychodni rehabilitacyjnych regionu kujawsko-pomorskiego. Medical and Biological Science, 2005, 19/3, 69-75.
7. Biniakiewicz B.: Opinie magistrów rehabilitacji ruchowej o ich przygotowaniu do pracy zawodowej, AWF Poznań 1994.
8. Lewandowski A., Sobolewski M.: Opinie studentek fizjoterapii z Bydgoszczy na temat znaczenia cech indywidualnych w realizacji przyszłego zawodu. w: Medical and Biological Sciences, 2007, 21/1, 55-62.
9. Lewandowski A., Isbrandt K., Smeja B.J.: Praca i zawód fizjoterapeuty w opinii nauczycieli akademickich. w: Medical and Biological Sciences, 2007, 21/4.
10. Babbie E.: Badania społeczne w praktyce. PWN, Warszawa 2004.
11. Barańska Z.: Podstawy metod statystycznych dla psychologów. Wyd. UG, Gdańsk 1999.
12. Skinner B. F.: The Technology of Teaching. New York, Appleton-Century-Croft, 1968.
13. Wieczorkowska-Nejtardt G.: Inteligencja motywacyjna, mądre strategie wyboru celu i sposobu działania. Wydawnictwa Instytutu Studiów Społecznych, Warszawa 1998.
14. Maslowa. H.: Toward a Psychology of Being. wyd. II, New York 1968.
15. Galloway Ch.: Psychologia uczenia i nauczania. t.II, PWN, Warszawa 1988.

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**THE ESTIMATION OF SPERM TRANSFECTION EFFICIENCY  
USING ELECTROPORATION METHOD**

**OCENA SKUTECZNOŚCI TRANSFEKCJI PLEMNIKÓW METODĄ ELEKTROPORACJI**

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**S u m m a r y**

The aim of this study was to examine the efficiency of the gene transfer into the sperm using electroporation method. The first step of the experiment was to determine the survivability of sperm depending on the used parameters: isoosmolar or hypoosmolar solution; voltage 500 V or 800 V; pulse length of 50  $\mu$ s, 100  $\mu$ s or 150  $\mu$ s and pulse number of 1 or 3 pulses. The second step of this study involved transfection of sperms green fluorescent protein GFP in two selected conditions: ensuring the largest and the smallest survivability of sperm in hypoosmolar solution, and further, determination of the efficiency of transfection. The highest survivability of sperm was confirmed in hypoosmolar solution when a single

pulse of length 50  $\mu$ s was applied at the voltage of 500 V (79.9%) and 800 V (39.8%), the lowest survivability (0% alive sperm) was confirmed in isoosmolar solution when a single pulse of length 150  $\mu$ s was applied at voltage 800 V. The chosen conditions of electroporation ensured the expression of GFP gene and fluorescence continued throughout the observation time. The efficiency of transfection in hypoosmolar solution when applying a single pulse of length 50  $\mu$ s and voltage 500 V was 57.3%. However, when a single pulse of length 150  $\mu$ s was applied at voltage 800 V, it was 23.6%. This study indicates that electroporation is an effective method of the gene transfer into the sperm.

**S t r e s z c z e n i e**

Badanie miało na celu ocenę skuteczności transfekcji plemników metodą elektroporacji. W pierwszym etapie eksperymentu została zbadana przeżywalność plemników w zależności od zastosowanych parametrów, takich jak: bufor izoosmolarny lub hypoosmolarny; napięcie 500 V lub 800 V; długość impulsu 50  $\mu$ s, 100  $\mu$ s lub 150  $\mu$ s oraz liczba impulsów: 1 lub 3 impulsy. W drugim etapie badań przeprowadzono transfekcję plemników z użyciem genu kodującego białko zielono fluoryzujące GFP dla dwóch wybranych kombinacji parametrów: zapewniających największą i najmniejszą przeżywalność plemników w buforze hypoosmolarnym oraz określenie jej skuteczności. Najwyższą przeżywalność plemników stwierdzono w buforze hypoosmolarnym przy

zastosowaniu pojedynczego impulsu o czasie trwania 50  $\mu$ s przy napięciu 500 V (79,9%) i 800 V (39,8%), a najniższą przeżywalność – 0% żywych plemników, w buforze izoosmolarnym przy zastosowaniu pojedynczego impulsu o czasie trwania 150  $\mu$ s i napięciu 800 V. Wybrane kombinacje parametrów zapewniły ekspresję genu GFP, a fluorescencja utrzymywała się przez cały czas prowadzenia obserwacji. Skuteczność transfekcji w buforze hypoosmolarnym wynosiła przy zastosowaniu jednego impulsu o długości 50  $\mu$ s i napięciu 500 V – 57,3%, a w buforze hypoosmolarnym przy zastosowaniu jednego impulsu o długości 150  $\mu$ s i napięciu 800 V – 23,6%. Badanie dowiodło, że elektroporacja jest skuteczną metodą wprowadzania egzogennej DNA do plemników.

**Key words:** electroporation, SMGT (sperm-mediated gene transfer), sperm, transfection

**Słowa kluczowe:** elektroporacja, SMGT (sperm-mediated gene transfer), plemniki, transfekcja.

**1. INTRODUCTION**

The interest in the possibility of utilization of spermatozoa as natural vectors to introduce exogenous

DNA (SMGT - sperm-mediated gene transfer) has been increasing during last years. In this way Lavitrano

et al. [1] obtained transgenic mice which after achieving sexual maturity were able to transfer to the offspring the introduced gene, which gives evidence of transformation of germ line cells. Many researchers have carried out research on the possibility of using spermatozoa as vectors. The greatest advantage of the use of spermatozoa as carriers exogenous DNA is simplicity and efficiency of the method and the fact that it is useful for every species reproducing with their participation. The idea, to use spermatozoa as vectors appeared, when Brackett et al. [2] showed that exogenous DNA could penetrate spermatozoa. After incubation of devoid of the plasma rabbit spermatozoa with radioactively labeled DNA they found the presence of foreign DNA inside the spermatozoa head. However, the incubation of spermatozoa of different species of animals with exogenous DNA, gave different results. Horan et al. [3] obtained 30% transformed spermatozoa of pig, whereas Wang et al. [4], working over rabbit spermatozoa, obtained as many as 66% transformations. Accordingly, various techniques raising the efficiency of transfection of spermatozoa and consequently the efficiency of transformation with their use as vectors have been formulated. Among the methods of introducing DNA to spermatozoa, apart from incubation with exogenous DNA, most of techniques of introducing foreign genetic material to cells, the electroporation method is used.

The principle of introducing DNA to cells by electroporation method is based on increasing the capacity of cell membranes under the influence of changeable electric field. Transfection efficiency by electroporation method is dependent on applied voltage (V), length of single electric pulse, electric (F) capacity and the geometry of chambers in which the electroporation is carried out (from the volume and distance between electrodes in the chamber), conductivity of solution in the chamber (phosphate solution, PBS or culture medium), type of introduced DNA and the kind of cells. The introduction of DNA to cells by electroporation method is commonly used in view of a possibility of a quick introduction of DNA both to primary germ cells and to differential somatic cells. However, one ought to underline that this method is rather expensive due to the necessity to purchase expensive equipment and disposable chambers in which electroporation is carried out. The efficiency of transfection by this method increases linearly together with the voltage rise, however the mortality of transfected cells grows then from 30% to 80% of the aggregate of cells, therefore

for various sensitivity of cells to the electric current, it is necessary to determine the optimal electroporation conditions for treated cells and to determine their mortality curve. Symonds et al. [5] working on the salmon sperm achieved the expression on level 85%. Kang et al. [6], according to the used osmotic conditions, obtained 8, 20 and 66% efficiency of the transfection of fish spermatozoa. Tsai et al. [7] obtained 50% transformed spermatozoa of loach *Misgurnus anguillicandatus*, while Müller et al. [8] reached the expression of foreign gene at 2,6-4,2% of transformed carps, catfishes and tilapia. Sin et al. [9] obtained 40% of transformed spermatozoa of mollusc *Haliotis iris*, while Hu et al. [10], working on the transfection of the sperm of mollusc *Pinctada maxima*, obtained, according to the amount of DNA used, the efficiency of the transcription on the level 5,6, 20 and 50%. Working on transformation of the pig sperm Horan et al. [11] obtained the expression of the gene within 70-75% of spermatozoa.

The transfection with the utilization of spermatozoa as vectors might become a method not only able to greatly simplify the production of transgenic animals. For its simplicity, efficiency and „naturalness” might rival the popularity of microinjection in the future, while in cases in which manipulation of oocytes or zygotes is difficult, may turn out to be a method of choice. In consideration of the universality of insemination of domestic animals, the use of spermatozoa as vectors not only of own, but also of exogenous DNA may appear optimum. This method might also be efficient and inexpensive, as well as might contribute to the development of new methods used in research and biotechnology.

The usefulness of electroporation method to introduce foreign genetic material to spermatozoa was assessed in this study. Survivability of bovine spermatozoa after electroporation depending on the used electroporation solution, voltage, length and number of pulses and expression of pEGFP-C1 gene was determined.

## 2. MATERIALS AND METHODS

Research on efficiency of transfection of cattle spermatozoa with pEGFP-C1 plasmid (Clonotech, № 6084-1; 4.7 kb) by an electroporation method was carried out in two steps. The first step of the experiment was to determine the survivability of sperm depending on the used parameters: solutions: hypoosmo-



lar ( $90 \pm 10$  mOsmol/kg; Eppendorf, № 4308 070.501), isoosmolar ( $285 \pm 15$  mOsmol/kg; Eppendorf, № 4308 070.510); voltage: 500 V, 800 V; pulse length: 50  $\mu$ s, 100  $\mu$ s, 150  $\mu$ s and pulse number: 1 puls or 3 pulses.

The second step of this study involved transfection of sperms and evaluation of its effectiveness on the basis of green fluorescence protein GFP in two selected conditions of electroporation.

### I. Assessment of sperm survivability after electroporation

Bovine sperm freeze in the liquid nitrogen situated in 0,25 ml straws (available at points of animal insemination) were used for this study. The sperm was thawed through submerge of the straw in the water bath at 38°C for 5 minutes. Then the sperm was transferred from the straw to the tube (25 ml) and centrifuged in a centrifuge with a tangent-balance rotor (2000 rpm/20 min - rotational speed 2000 rpm, the time of the rotation: 20 min). After removal of seminal plasma (about 200  $\mu$ l), the sediment of spermatozoa was resuspended in 2 ml PBS (pipetting 3x) (Gibco, Invitrogen Corporation) and sperm suspension was centrifuged (2000 rpm/20 min). The wash was repeated three times. After a second centrifuging and removal of seminal plasma, the sperm sediment was resuspended first in 1 ml of PBS then 100  $\mu$ l of sperm suspension was received for staining with 1% eosin and estimation of sperm count, then 1 ml of PBS was added to sperm suspension destined to centrifuge. After third centrifuging and removal of seminal plasma, the sperm sediment was resuspended in 300  $\mu$ l of selected electroporation solution, and electroporated (electroporator „Multiporator”, Eppendorf Scientific, № 940000807; the eukaryotic module- pulse voltage: 20-1.200 V, pulse form: exponentially diminishing, electronically controlled; time constant: 15-500  $\mu$ s, in increments 5  $\mu$ s; plastic cuvettes, Amaxa, № VCA-1003). Immediately after experiment sperm suspension was transferred to another tube (Eppendorf 1.5 ml) and centrifuged (2000 rpm/20 min). After centrifuging supernatant was removed, the sperm sediment was resuspended in 1 ml of PBS and 100  $\mu$ l of sperm suspension was received for staining and estimations of sperm count with the aim of determination of the survivability of sperm after electroporation.

### II. Transfection of spermatozoa and estimation of transfection efficiency

The culture medium Opti-MEM (Gibco, Invitrogen Corporation) was set to equilibrations (saturation CO<sub>2</sub>) for 12 hours before planned transfection. The sperm sediment was prepared analogically as in the point I (i.e. thawed, the seminal plasma was removed and three times centrifuged along with estimation of spermatozoa count). After third centrifuging spermatozoa was resuspended in 70  $\mu$ l compound of selected electroporation solution and the circular form of the pEGFP-C1 plasmid against 1  $\mu$ g plasmid on 10.29  $\mu$ l suspension of spermatozoa (6,8  $\mu$ g plasmid on 70  $\mu$ l compound), and electroporated. Immediately after electroporation the sperm suspension was transferred to another tube (Eppendorf 1.5 ml) and centrifuged (2000 rpm/20 min). After removal of seminal plasma (compound of solution and the possibly remaining plasmid) sperm sediment was resuspended in 0.5 ml of the prepared earlier culture medium. The sperm suspension was transferred to the culture well, and plate placed in the incubator (CO<sub>2</sub> the Incubator, Heal Force) (temperature 37°C, 5% CO<sub>2</sub>). The gene expression was examined 40 hours after the transfection every 4 hours until the 72nd hour of the incubation. The observation was performed using the inverted fluorescent microscope (Axiovert 40, Carl Zeiss Lichtmikroskopie) with the phase contrast, and PlasDIC (contrast which allows the use of plastic dishes for microscopic examinations) with utilization of reflected light fluorescence (fluorescence of pEGFP-C1 - excitation maximum: 488 nm; emission maximum: 507 nm).

The total sperm count and count of spermatozoa with expression of pEGFP-C1 (the visible fluorescence) were determined using MultiScan program on the basis of microscopic picture photographs (40x magnification) performed in particular hours of incubation. The efficiency of transfection (%), i.e. the part of all spermatozoa with expression of green fluorescent protein GFP, was calculated according to the equation:

$$E = (S_E / S_T) * 100\%$$

where: E - efficiency of the transfection (%);

S<sub>E</sub> – count of spermatozoa with expression of the gene;

S<sub>T</sub> - total count of spermatozoa.

### III. Staining procedure and estimation of the sperm count after electroporation

Determination of the sperm count in semen was performed by counting the spermatozoa in the Bürker chamber before and after electroporation. Both staining and estimation of the sperm count were performed

immediately after collecting 100 ml of suspension of cells. Spermatozoa were stained in the eosin solution (0.5 ml of 1% eosin, 19.5 ml of 3% NaCl solution, 100 ml of sperm suspension, time of staining - 5 min). Spermatozoa were counted in all 100 squares of the chamber. The sum and mean of the sperm count counted from three countings with differentiation to stained and non-stained head (live spermatozoa, sperm motility was not taken into consideration). Survivability of spermatozoa (%), i.e. the part of alive spermatozoa before electroporation that remained alive after the use electroporation, was calculated according to the equation:

$$S = (S_{\text{after}}/S_{\text{before}}) * 100\%$$

where: S - Survivability of spermatozoa after electroporation (%);

$S_{\text{after}}$  - alive (no-stained) spermatozoa after electroporation;

$S_{\text{before}}$  - alive (no-stained) spermatozoa before electroporation.

#### IV. Statistical calculations

Obtained results of survivability of spermatozoa were compared by analysis of variance for independent three-factor system (multifactorial ANOVA, Multiplicative methods), the significance was verified by Student's t-test at the level of significance of  $P \leq 0.01$  and  $P \leq 0.05$  [11].

### 3. RESULTS

Survivability of spermatozoa after electroporation in the hypoosmolar solution was higher than in the isoosmolar solution, as well as in the case of applied voltage 500 V in comparison with voltage 800 V (Fig. 1).

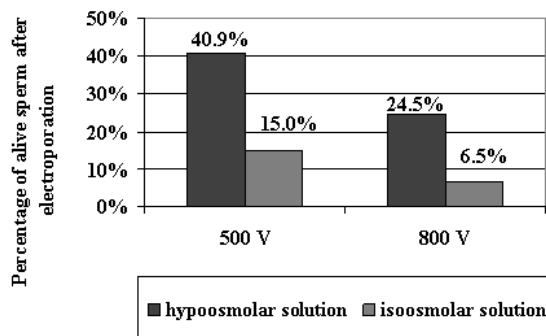


Fig. 1. The impact of solution and voltage on sperm survivability after electroporation

The highest survivability after electroporation was found after application of the hypoosmolar solution

and the single pulse at voltage 500 V and length 50  $\mu\text{s}$  and it was 79.9%, while the highest mortality (0% alive spermatozoa) was found after application of the isoosmolar solution and the single pulse at voltage 800 V and length 150  $\mu\text{s}$ . The effect of the pulse length on survivability ( $P \leq 0.01$ ) was also statistically significant, however no significant differences between applied of pulse lengths were found. Statistically significant differences ( $P \leq 0.01$ ) in survivability of spermatozoa after electroporation were noticed between mean values for: hypoosmolar solution (32.7%) and isoosmolar solution (10.8%); the pulse length 50  $\mu\text{s}$  (38.9%) and 100  $\mu\text{s}$  (14.2%); the pulse length 50  $\mu\text{s}$  (38.9%) and 150  $\mu\text{s}$  (12.2%) (Fig. 2).

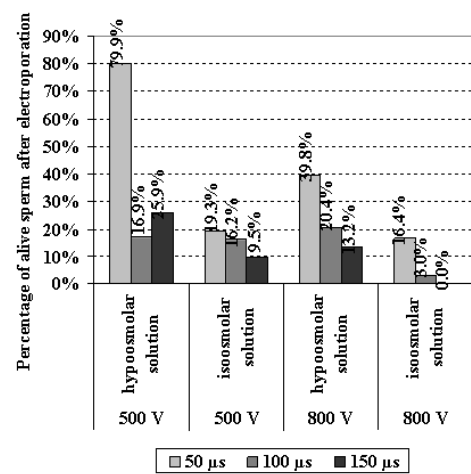


Fig. 2. The impact of solution, voltage and single pulse length on sperm survivability after electroporation

#### a) Hypoosmolar solution

Statistically significant differences ( $P \leq 0.01$ ) between mean survivability of spermatozoa were shown for the pulse length 50  $\mu\text{s}$  and 100  $\mu\text{s}$  and the pulse length 50  $\mu\text{s}$  and 150  $\mu\text{s}$  at application of the single pulse (Table I).

Table I. Percentage of alive sperm after electroporation in hypoosmolar and isoosmolar solution

HYPOOSMOLAR SOLUTION		
1 PULSE	Voltage	
Length of pulse	500 V	800 V
50 $\mu\text{s}$	79.9 <sup>a</sup> <sub>A</sub>	39.8 <sup>a</sup> <sub>B</sub>
100 $\mu\text{s}$	16.9 <sup>b</sup> <sub>A</sub>	20.4 <sup>a,b</sup> <sub>A</sub>
150 $\mu\text{s}$	5.9 <sup>b</sup> <sub>A</sub>	3.2 <sup>b</sup> <sub>A</sub>
ISOOSMOLAR SOLUTION		
1 PULSE	Voltage	
Length of pulse	500 V	800 V
50 $\mu\text{s}$	19.3 <sup>a</sup> <sub>A</sub>	16.4 <sup>a</sup> <sub>A</sub>
100 $\mu\text{s}$	16.2 <sup>a</sup> <sub>A</sub>	3.0 <sup>a,b</sup> <sub>A</sub>
150 $\mu\text{s}$	9.5 <sup>a</sup> <sub>A</sub>	0.0 <sup>b</sup> <sub>A</sub>

<sup>a,b</sup> - significant differences ( $P \leq 0.05$ ); the comparison of means for different times within the same value of voltage.

<sub>A,B</sub> - significant differences ( $P \leq 0.05$ ); the comparison of means for different voltage within the same value of length of pulse.

Differences between mean survivability of spermatozoa after electroporation after application of three pulses of the length 50  $\mu\text{s}$  for voltages 500 V and 800 V were not statistically significant (Fig. 3).

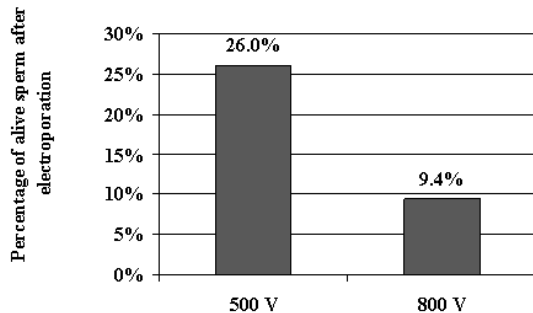


Fig. 3. The impact of voltage on sperm survivability after electroporation in hypoosmolar solution using 3 pulses of length of 50  $\mu\text{s}$

#### b) Isoosmolar solution

After applying the isoosmolar solution significant differences ( $P \leq 0.05$ ) between means for voltages 500 V and 800 V and between means for lengths of pulse 50  $\mu\text{s}$  and 150  $\mu\text{s}$  ( $P \leq 0.01$ ) were shown (Table I).

It should be emphasized that significant difference ( $P \leq 0.01$ ) occurred for both the hypoosmolar and isoosmolar solution were observed just between mean survivability concerning the pulse length 50  $\mu\text{s}$  and 150  $\mu\text{s}$ .

#### c) Transfection

The transfection was carried out for optimal parameters enabling the highest survivability of spermatozoa after electroporation: the hypoosmolar solution, voltage 500 V and the pulse length 50  $\mu\text{s}$ . Because of higher survivability of spermatozoa after electroporation in the hypoosmolar solution than in the isoosmolar solution, for the comparison, the transfection was carried out in the hypoosmolar solution at voltage 800 V and the pulse length 150  $\mu\text{s}$ . In both groups expression of introduced green fluorescent protein GFP was found and observed in the form of sperm fluorescence in the UV light which was continued throughout the observation time. The higher transfection efficiency was observed in the case of application of voltage 500 V and the pulse length 50  $\mu\text{s}$  – 57,3%. After application of voltage 800 V and the pulse length 150  $\mu\text{s}$ , transfection efficiency was about 2.5 times lower and it was 23.6% (Fig. 4). However, these results can be erroneous as a consequence of determining transfection efficiency on the basis of microscopic picture photographs.

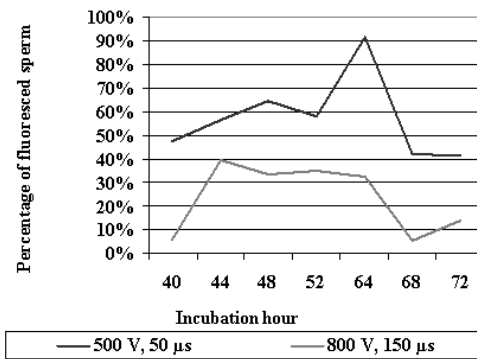


Fig. 4. Sperm transfection efficiency in hypoosmolar solution

## 4. DISCUSSION

Transgenic animals create unique research possibilities and find many current and potential applications. Despite many techniques of introduction of exogenous DNA, most of them do not find practical application in domestic animals reproduction or encounter considerable difficulties in their usage (microinjection of DNA into a pronuclei of fertilized ova, the transgenesis by genetic modification of embryonic stem cell) [13].

Therefore, the effects of Lavitrano et al. [1] research in which they obtained transgenic mice as result of using spermatozoa as vectors of exogenous DNA became a beginning of series of experiments. The large number of researchers have analyzed the mechanism of introduction of exogenous DNA to spermatozoa. They have found, the presence of both, factors permissive to the exogenous DNA uptake, and inhibitors of this process [14-20].

Although the mechanisms which determine when the conducive factors affect introduction of foreign DNA and when the inhibitors of this process, are still unknown, the repeatability of research with spermatozoa as vectors is really various.

Survivability of spermatozoa after electroporation substantially determines the success and efficiency of transfection. Research of West [21] demonstrated relativeness of electroporation efficiency die to used voltage. He ascertained that electroporation efficiency increased linearly with the voltage rise, increasing at the same time the mortality of transfected cells. Symonds et al. [5] and Sin et al. [9] researching the sperm motility marked that it decreased with increasing field strength (V/cm), pulse length and number of pulse. After application of 1000 V/cm with two pulses of the length 27.4 msec, the sperm motility decreased below

5%, and at five pulses of the length 18,6 msec and four of the length 27.4 msec decreased to 0%. Tsai et al. [7] noticed that sperm motility significantly decreased at application of voltage 8 kV, while at 10 kV totally stopped.

This research also examined the effect of applying voltage and the pulse length on survivability of spermatozoa. Significant differences between the influence of voltages 500 V and 800 V during length of pulse 50  $\mu$ s for both solutions and for all length of pulse in the isoosmolar solution was shown. Statistically significant differences in survivability of spermatozoa after electroporation between lengths of pulse 50  $\mu$ s and 100  $\mu$ s as and 50  $\mu$ s and 150  $\mu$ s in both solutions were also observed. The significant effect of applied voltage value and the pulse length on survivability was also found (Table I, Fig. 2.).

This research aimed to determine optimal electroporation conditions of bovine spermatozoa in consideration of survivability of spermatozoa after electroporation. The highest survivability (79.9%) was obtained after application the hypoosmolar solution, voltage 500 V and single pulse about the length 50  $\mu$ s. The highest mortality (0% alive spermatozoa) was observed after electroporation in isoosmolar solution at application of voltage 800 V and single pulse of the length 150  $\mu$ s. Greater survivability of spermatozoa was also observed after application of single pulse, than three pulses (Fig. 2, 3).

Kang et al. [6] examined the influence of the osmotic pressure of medium on efficiency of the transformation of spermatozoa by electroporation. They suggest that transfection efficiency might be enlarged through electroporation during rehydration of earlier dehydrated spermatozoa. As result of dehydration of spermatozoa by their location in hypoosmolar solution and after rehydration in hypoosmolar solution containing DNA, they obtained efficiency of transfection on the level of 66%. It was 8% in isoosmolar conditions, while in the hypoosmolar solution it reached 20%. Furthermore we found that spermatozoa placed in hypoosmolar solution become motile, but rapidly lose their fertility.

This study examined the effect of osmolarity of electroporation solutions on survivability of spermatozoa. These solutions were added to electroporator „Multiporator”: the hypoosmolar and isoosmolar solutions. Significant differences in survivability of spermatozoa, respectively 32.7% and 10,8% between them, were shown. In the hypoosmolar solution the highest

survivability (79.9%) was observed, while in the isoosmolar one – the lowest value was stated (0%). The obtained results suggest that hypoosmolar solution is the better electroporation medium of spermatozoa than the isoosmolar one (Table I, Fig.1).

Spermatozoa were transfected in the hypoosmolar solution at application of single pulse using following combinations of parameters: 500 V, 50  $\mu$ s, and 800 V, 150  $\mu$ s. In both groups the expression of introduced green fluorescent protein GFP was found and it continued throughout the whole observation time. The obtained efficiency of transfection after voltage application of 500 V and the pulse length 50  $\mu$ s was 57.3%, and at voltage 800 V and the pulse length 150  $\mu$ s – 23.6%.

## 5. CONCLUSIONS

The electroporation appeared an effective method of the gene transfer into bovine spermatozoa preserved in the liquid nitrogen. The highest survivability of spermatozoa and the greatest efficiency of transfection were obtained after electroporation in the hypoosmolar solution after application of single pulse of the length of 50  $\mu$ s, at voltage 500 V. However, enlarging of the pulse length, the voltage and number of pulses and also using the isoosmolar solution, caused lower survivability of spermatozoa after electroporation and lower efficiency of transfection.

## REFERENCES

1. Lavitrano M., Camaioni A., Fazio V. M., Sperm cells as vectors for introducing foreign DNA into eggs: genetic transformation of mice., *Cell*, (1989), 2;57(5):717-23.
2. Brackett B. G., Baranska W., Sawicki W., Uptake of Heterologous Genome by Mammalian Spermatozoa and Its Transfer to Ova through Fertilization., *Proc. Nat. Acad. Sci. USA*, (1971), 68(2):353-357.
3. Horan R., Powell R., McQuaid S., Gannon F., Houghton J. A., Association of foreign DNA with porcine spermatozoa., *Arch. Androl.*, (1991), 26:83-92.
4. Wang H. J., Lin A. X., Chen Y. F., Association of Rabbit Sperm Cells with Exogenous DNA., *Anim. Biotechnol.*, (2003), 14(2)155.
5. Symonds J. E., Walker S. P., Sin F. Y. T., Development of a mass gene transfer method in chinook salmon: optimization of gene transfer by electroporated sperm., *Mol. Mar. Biol. Biotechnol.*, (1994), 3(2) 104-111.
6. Kang J. H., Yoshizaki G., Homma O., Effect of an osmotic differential on the efficiency of gene transfer by electroporation of fish spermatozoa., *Aquaculture*, (1999), 173(1-4):297-307.

7. Tsai H. J., Tseng F. S., Liao I. C., Electroporation of sperm to introduce foreign DNA into genome of loach (*Misgurnus anguillicaudatus*), *Can. J. Fish. Aquat. Sci.*, (1995), 52:776-787.
8. Müller F., Iviec Z., Erdélyi F., Introducing foreign genes into fish eggs with electroporated sperm as a carrier., *Mol. Mar. Biol. Biotechnol.*, (1992), 1(4/5):276-281.
9. Sin F. Y. T., Mukherjee U. K., McKenzie J. C., Electroporation of abalone sperm enhances sperm-DNA association., *J. Fish Biol.*, (1995), 47(A):20-28.
10. Hu W., Yu D. H., Wang YP., Electroporation of sperm to introduce foreign DNA into the genome of *Pinctada maxima* (Jameson), *Sheng Wu Gong Cheng Xue Bao.*, (2000), 16(2):165-8.
11. Horan R., Powell R., Bird J. M., Effects of Electroporation on the Association of Foreign DNA with Pig Sperm., *Arch. Androl.*, (1992), 28:105-114.
12. Ruszczyc Z., *Metodyka doświadczeń zootechnicznych.*, Państwowe wydawnictwo rolnicze i leśne., Warszawa, (1981).
13. Niemann H., Kues W., Carnwath J. W., Transgenic farm animals: present and future., *Rev Sci Tech.*, 2005, 24(1):285-98.
14. Carballada R., Esponda P. Regulation of Foreign DNA by Mouse Spermatozoa., *Experimental Cell Research*, (2001). 262:104-113.
15. Gandolfi F. Sperm-mediated transgenesis., *Theriogenology*, (2000). 53:127-137.
16. Lavitrano M., French D., Zani M., The interaction between exogenous DNA and sperm cells., *Mol. Reprod. Dev.*, (1992). 31(3):161-9.
17. Lavitrano M., Maione B., Forte E., The interaction of sperm cells with exogenous DNA: a role of CD4 and major histocompatibility complex class II molecules., *Exp. Cell Res.*, (1997). 25:233(1):56-62.
18. Magnano A. R., Giordano R., Moscufo N., Sperm/DNA interaction: Integration of foreign DNA sequences in the mouse sperm genome., *Jurnal of Reproductive Immunology.*, (1998). 41: 187-196.
19. Szczygiel M. A., Moisyadi S., Ward W. S., Expression of Foreign DNA Is Associated with Paternal Chromosome Degradation in Intracytoplasmic Sperm Injection-Mediated Transgenesis in the Mouse., *Biology of Reproduction.*, (2003). 68:1903-1910.
20. Zani M., Lavitrano M., French D., The Mechanism of Binding of Exogenous DNA to Sperm Cells: Factors Controlling the DNA Uptake., *Experimental Cell Research.*, (1995)., 217:57-64.
21. West C. M. L., Int. J., A Potential Pitfall in the Use of Electroporation: Cellular Radiosensitization by Pulsed High-voltage Electric Fields., *Radiat. Biol.*, (1992), 61, 329-334.

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**FUNGI AND FUNGUS-LIKE ORGANISMS FOUND IN CHOSEN BATHING SITES  
OF THE NAREW RIVER WITHIN THE NAREW NATIONAL PARK**

**GRZYBY WODNE I ORGANIZMY GRZYBOPODOBNE WYSTĘPUJĄCE W WYBRANYCH  
KĄPIELISKACH NARWI NA TERENIE NARWIAŃSKIEGO PARKU NARODOWEGO**

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**S u m m a r y**

**I n t r o d u c t i o n .** Mycological investigations are not included in the assessment of bathing sites. However, long-term observations of many authors seem to indicate that they are important from the sanitary-epidemiological point of view, since certain fungi found in water can be potentially pathogenic to humans.

**T h e a i m** of the present study was to establish the diversity of micromycetes found in four bathing sites of the river Narew, to determine or exclude potential etiological factors of mycotic infections and to demonstrate the effect of physical and chemical factors on the growth of fungi in the spring, autumn and winter 2006/2007.

**M a t e r i a l a n d m e t h o d s .** The baiting method was used to isolate the respective fungi and fungus-like

organisms, including the material of plant origin (seeds of clover, hemp and onion skin) and of animal origin (grass snake skin and crustacean).

**R e s u l t s .** Fifty-three species were identified, with the predominance of saprotrophic fungi, among which four species (*Alternaria alternata*, *Aspergillus flavus*, *Candida albicans*, *Mucor hiemalis*) could be potentially pathogenic to man. The greatest diversity was noted in the spring and autumn months at the bathing sites with lower burden of biogenic compounds.

**C o n c l u s i o n .** Species diversity of the fungal biota at the investigated bathing sites depended on a number of abiotic and biotic factors and season of the year.

**S t r e s z c z e n i e**

**W s t ę p .** W ocenie wód kąpielisk naturalnych nie uwzględnia się badań mykologicznych. Jednak, wieloletnie obserwacje licznych autorów wskazują, że są one ważne z punktu widzenia sanitarno-epidemiologicznego, ponieważ niektóre grzyby występujące w wodzie mogą być potencjalnie chorobotwórcze dla człowieka.

**C e l e m b a d a ń** było ustalenie występowania grzybów i organizmów grzybobodobnych, w tym potencjalnie chorobotwórczych, w czterech kąpieliskach rzeki Narwi na terenie Narwiańskiego Parku Narodowego oraz wpływu na ich rozwój czynników fizycznych i chemicznych wiosną, jesienią i zimą w latach 2006/2007.

**M a t e r i a ł i m e t o d y .** Do izolowania poszczególnych grzybów wodnych i organizmów grzybobodobnych

zastosowano metodę przynęt, w badaniach tych wykorzystano materiał pochodzenia roślinnego (nasiona gryki, koniczyny, konopi i łuski cebuli) oraz zwierzęcego (wylinka zaskrońca i skorupiaka).

**W y n i k i .** Obserwowano rozwój 53 gatunków, wśród których dominowały saprotrofy. Największą różnorodność bioty grzybów zaobserwowano wiosną i jesienią w kąpieliskach obciążonych w mniejszym stopniu związkami biogennymi. Cztery gatunki (*Mucor hiemalis*, *Candida albicans*, *Aspergillus flavus* i *Alternaria alternata*) mogą być potencjalnie patogenne dla człowieka.

**W n i o s k i .** Różnorodność gatunkowa grzybów w badanych kąpieliskach zależy od czynników biotycznych i abiotycznych oraz pory roku.

**Key words:** fungi, fungus-like organisms, Narew National Park

**Słowa kluczowe:** grzyby, organizmy grzybobodobne, Narwiański Park Narodowy

## INTRODUCTION

Water at natural bathing sites should meet the requirements stated in the Ordinance of the Minister of Health [1], which was elaborated based on the EU Directive [2].

According to this Ordinance, water purity at natural bathing sites should be assessed through the physicochemical and microbiological analyses. Microbiological analysis involves determination of the number of bacteria coli and faecal streptococci, and detection of the presence or absence of *Salmonella bacilli*. Mycological investigations are not included in the assessment of waters. However, long-term observations of many authors seem to indicate that they are important from the sanitary-epidemiological point of view, since certain fungi found in water, e.g. *Candida albicans*, can be potentially pathogenic to humans [3].

In Poland, no norms exist for *Candida albicans* to be used as a water quality index, although in 1983 an American norm –ASTM D4249-83 (2005) Standard Test Method for Enumeration of *Candida albicans* in Water was implemented [4].

Fungi and fungus-like organisms are a major component of the microbial loop. The microbial loop is an integral part of the trophic network of each aquatic ecosystem and constitutes a complex set of processes due to which the originally unavailable dissolved organic matter (DOM) becomes available to higher trophic organisms. Bacteria, fungi and fungus-like organisms are responsible for the transfer of organic matter to higher levels of the trophic chain. There are many literature reports referring to the role of bacteria in the functioning of the microbial loop [5], but not of fungi and fungus-like organisms. Only few researchers have described their involvement in the functioning of the microbial loop [6, 7].

The mechanisms and ecological factors contributing to species diversity of fungi and fungus-like organisms in the microbial loop have not been fully known. Similarly, the significance of this diversity within the microbial loop for the microbiological processes associated with flow of the organic matter and with biogenic regeneration has not been investigated.

Taking the above into consideration, the mycological assessment of the water from four bathing sites of the river Narew within the borders of the Narew National Park appears justified. Until now, the bathing sites of the Narew have never been the object of mycological investigations, contrary to the non-recreational sites [8-10].

The aim of presented study was to establish the diversity of micromycetes found in four bathing sites of the river Narew, to determine or exclude potential etiological factors of mycotic infections and to demonstrate the effect of physical and chemical factors on the growth of fungi in the spring, autumn and winter 2006/2007.

## MATERIAL AND METHODS

The Narew river is the right-side tributary of the Vistula, its length on the Polish territory being 455 km (total length 484 km). The river has its source on the swampy area of the Białowieża Forest in Belarus. The upper Narew basin district is relatively poorly industrialized, typically agricultural and with large forest area. The river Narew flows through the Narew National Park and due to its natural sights is called „the Polish Amazon”. It belongs to the unique anastomosing river system in Europe. Aquatic conditions in its valley are the result of natural factors and man’s actions within the Park and in the whole Narew river basin [11].

Water for the experiment was collected in spring and autumn 2006 and in winter 2007 from four bathing sites situated in the upper part of the Narew in Suraż, Uhowo, Łapy and Bokiny. Water samples (2.0 dm<sup>3</sup>) for physicochemical analyses were obtained from the respective sites at a depth of about 0.20 m. Hydrochemical analysis of 19 parameters (Table 1) was performed according to Standard Methods [12].

The baiting method was used to isolate the respective fungi and fungus-like organisms including the material of plant origin (seeds of clover, hemp seeds and onion skin) and of animal origin (grass snake skin and crustacean) [13]. The samples were stored in a room in thermal and light conditions resembling those of the natural environment. Sterilized baits were placed into one-litre containers and poured with water from the respective reservoirs, and then stored for approximately a month. The fungi that appeared were successively observed under an optic microscope (100x and 400x) every few days, starting from the first day of the culture. Next, several microscopic preparations were prepared from each sample.

At the same time, the developmental stages of the respective fungi were determined using a micrometric ocular. The fungi were identified based on the vegeta-



tive organs (shape and size of the hypha), organs of asexual reproduction (shape of the sporangium, sporangiospores, conidiospores) and generative organs (oogonia with oospores and antheridia) [14, 15]. The systematic of straminipilous organisms was defined according to Dick [15].

Table 1. *Physical and chemical properties of water in particular water sites on the River Narew (mean from 3 samples)*

Tabela 1. *Fizyczne i chemiczne parametry wody na poszczególnych stanowiskach rzeki Narwi*

Parametry Specification	Suraż	Uhowo	Łapy	Bokiny
Temperatura Temperature (°C)	8	7	9	9
pH	7.60	7.75	7.10	7.85
O <sub>2</sub> (mg l <sup>-1</sup> )	13.68	15.44	12.67	14.68
BOD <sub>5</sub> (mg l <sup>-1</sup> )	7.81	7.21	9.22	7.22
COD (mg l <sup>-1</sup> )	9.02	7.00	9.05	14.64
CO <sub>2</sub> (mg l <sup>-1</sup> )	11.0	13.2	11.0	8.8
Zasadowość Alkalinity in CaCO <sub>3</sub> (mval l <sup>-1</sup> )	4.3	4.0	4.5	4.2
N-NH <sub>3</sub> (mg l <sup>-1</sup> )	0.770	1.070	0.970	0.54
N-NO <sub>2</sub> (mg l <sup>-1</sup> )	0.0236	0.0262	0.0236	0.022
N-NO <sub>3</sub> (mg l <sup>-1</sup> )	0.220	0.460	0.220	0.160
P-PO <sub>4</sub> (mg l <sup>-1</sup> )	0.520	0.760	0.560	0.400
Fosforany Sulphates (mg l <sup>-1</sup> )	26.32	19.33	20.15	38.26
Chlorki Chlorides (mg l <sup>-1</sup> )	65	58	54	53
Twardość Total hardness (mg Ca l <sup>-1</sup> )	72.00	72.00	69.84	69.12
Twardość Total hardness (mg Mg l <sup>-1</sup> )	3.44	9.03	10.32	10.74
Fe (mg l <sup>-1</sup> )	1.90	2.40	1.90	2.40
Sucha pozostałość Dry residue (mg l <sup>-1</sup> )	294	256	256	283
Subst. rozpuszczone Dissolved solids (mg l <sup>-1</sup> )	281	247	240	271
Zawiesina Suspended solids (mg l <sup>-1</sup> )	13	9	16	12

## RESULTS

Chemical analysis of such water parameters as oxidability, all the three forms of nitrogen and phosphates has revealed that the water of the river Narew at the studied bathing sites satisfies the requirements stated in the Ordinance of the Minister of Health (Table 1). Water samples showed similar temperature, pH and oxygen content. BOD<sub>5</sub> index was the highest in Łapy, whereas COD showed the highest value in Bokiny, where the level of CO<sub>2</sub> was the lowest. Alkalinity as CaCO<sub>3</sub> was similar in all the water samples. The high-

est concentration of ammonium nitrogen (N-NH<sub>4</sub>) was noted in Suraż, the lowest in Bokiny. The amounts of nitrite nitrogen (N-NO<sub>2</sub>) were similar at all the sites, whereas the level of nitrate nitrogen

(N-NO<sub>3</sub>) was the highest in Uhowo. The highest level of phosphates was observed in Uhowo, the lowest in Bokiny. The content of sulphates varied, being the highest in Bokiny, where the level of chlorides was the lowest. Calcium concentration ranged from 69.12 to 72 mg/L<sup>-1</sup>, whereas the fewest magnesium cations were found in Suraż. The levels of iron were the same at bathing sites in Suraż, Łapy, Uhowo and Bokiny. The highest values of dry residue and dissolved substances were noted in Suraż, whereas suspension was the most abundant in the water in Łapy.

Fifty-three species of micromycetes were found to grow in the water of the investigated sites. They represented Chromista (39), Fungi (11) and Anomorphous Fungi (3) (Table 2). Most taxons were detected in the water in Bokiny (47), the fewest in Łapy (34). At all the bathing sites, fungi and straminipilous organisms showed greater species diversity in the spring and autumn periods as compared to winter (Fig. 1).

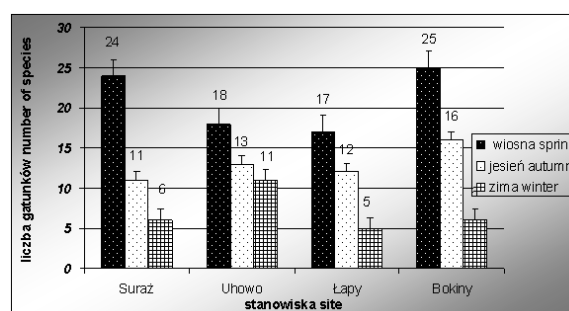


Fig. 1. *Number of fungal biota found in different seasons at the investigated sites of the River Narew*

Ryc. 1. *Liczba bioty grzybów w poszczególnych sezonach w badanych stanowiskach rzeki Narwi*

Worthy of note is the finding of such pathogenic fungi as *Mucor hiemalis* (in Suraż), *Candida albicans* (Uhowo) and *Aspergillus flavus* (Uhowo and Łapy).

The most attractive baits included seeds of clover (26 species) and crustaceans (22 species), whereas the least colonized were cellophane (2) and hemp seeds (*Cannabis sativa*) (4) (Table 3).

Table 2. *Fungi and fungus-like organisms found in water from the respective bathing sites of the River Narew*  
 Tabela 2. *Grzyby i organizmy grzybobodobne stwierdzone w wodzie badanych kąpielisk rzeki Narwi*

Królestwo, klasa, rząd i gatunek Kingdom, class, order and species	Kąpieliska w rzece Narwi. Bodies on the river Narew											
	Suraz			Uhowo			Lapy			Bokiny		
	wiosna spring 2006	jesień autumn 2006	zima winter 2007	wiosna spring 2006	jesień autumn 2006	zima winter 2007	wiosna spring 2006	jesień autumn 2006	zima winter 2007	wiosna spring 2006	jesień autumn 2006	zima winter 2007
<b>FUNGI</b>												
<b>Ascomycetes</b>												
<b>Pleosporales</b>												
1. <i>Alternaria alternata</i> (Fr.) Keissl	x											
2. <i>Aspergillus flavus</i> Link				x	x			x				
<b>Mucorales</b>												
3. <i>Mucor hiemalis</i> Wehmer	x											
<b>Chytridiomycetes</b>												
<b>Blastocladales</b>												
4. <i>Achlyogeton entophytum</i> Schenk										x		
5. <i>Catenaria anguillulae</i> Sorokin							x			x		
6. <i>Blastocladiopsis parva</i> (Whiffen) Sparrow						x						
7. <i>Catenophlyctis variabilis</i> (Karling) Karling				x						x	x	
8. <i>Micromycopsis cristata</i> Scherffel							x					
<b>Chytridiales</b>												
9. <i>Karlingia rosea</i> (de Bary et Woronin) Johanson		x			x			x			x	
10. <i>Nowakowskiella elegans</i> (Nowakowski) Schröter	x			x			x					
11. <i>Polyphagus euglenae</i> Nowakowski				x						x		
<b>Monoblepharidiales</b>												
12. <i>Monoblepharis hypogyna</i> Perrot						x					x	
<b>Saccharomycetes</b>												
<b>Saccharomycetales</b>												
13. <i>Candida albicans</i> . (Robin) Berkhout					x							
<b>Zoopagales</b>												
14. <i>Zoopagus insidians</i> Sammerstorff				x			x				x	
<b>STROMINIPILA (CHROMISTA)</b>												
<b>Peronosporomycetes</b>												
<b>Lagenidiales</b>												
15. <i>Olpidiopsis saprolegniae</i> (Braun) Cornu	x						x			x		
<b>Leptomitales</b>												
16. <i>Rhipidium interruptum</i> Cornu				x					x			
<b>Pythiales</b>												
17. <i>Pythium acanthicum</i> Drechsler		x					x				x	
18. <i>Py. afertile</i> Kanouse et Humphrey										x		
19. <i>Py. aquatile</i> Höhnk						x				x		
20. <i>Py. debaryanum</i> Hesse				x			x			x		
21. <i>Py. catenulatum</i> Matthews											x	
22. <i>Py. complectens</i> Braun										x		
23. <i>Py. oedochilum</i> Drechsler	x									x	x	
24. <i>Py. rostratum</i> Butler		x			x	x		x			x	x
25. <i>Py. torulosum</i> Coker et Patterson						x				x		x
26. <i>Py. undulatum</i> Petersen	x	x						x	x	x		
<b>Saprolegniales</b>												
27. <i>Achlya americana</i> Humphrey	x			x			x			x		x
28. <i>Ac. apiculata</i> de Bary	x							x		x		
29. <i>Ac. caroliniana</i> Coker				x		x	x					
30. <i>Ac. debaryana</i> Humphrey	x											
31. <i>Ac. dubia</i> Coker	x									x		
32. <i>Ac. flagellata</i> Coker	x											
33. <i>Ac. oligocantha</i> de Bary	x				x							
34. <i>Ac. orion</i> Coker et Couch	x		x		x	x	x					
35. <i>Ac. polyandra</i> Hildebrandt	x	x	x	x			x		x	x	x	
36. <i>Ac. prolifera</i> Nees	x	x		x						x		x
37. <i>Ac. rodrigueziana</i> . Wolf	x			x			x			x	x	
38. <i>Ac. ireleaseana</i> (Humphrey) Kauffman	x	x			x							
39. <i>Aphanomyces irregularis</i> Scott		x	x	x	x	x		x	x	x	x	x
40. <i>Ap. laevis</i> de Bary							x	x		x		
41. <i>Aplanes androgynus</i> (Archer) Huphrey							x				x	
42. <i>Dictyochus sterilis</i> Leitgeb	x	x		x								
43. <i>Isoachlya unisporea</i> (de Bary) Coker	x			x			x				x	
44. <i>Pythiopsis cymosa</i> de Bary	x			x		x	x					
45. <i>Saprolegnia anisosporea</i> de Bary							x			x		
46. <i>S. dielina</i> Coker	x		x				x	x				
47. <i>S. ferax</i> (Gruih) Nees		x	x		x	x		x			x	
48. <i>S. latvica</i> Apinis	x			x	x	x				x	x	
49. <i>S. mixta</i> de Bary					x			x				
50. <i>S. monoica</i> Pringsheim					x			x				
51. <i>S. paradoxa</i> Maurizio	x			x								
52. <i>S. parasitica</i> Coker		x			x			x		x	x	x
53. <i>S. subterranea</i> Disssmann	x		x							x		
Ogólna liczba w sezonie Total number in seasons	24	11	6	18	13	11	17	12	5	25	16	6
Ogólna liczba Total number		41			42			34		47		

Table 3. Occurrence of aquatic fungi and fungus-like organisms on the investigated baits

Tabela 3. Występowanie grzybów i organizmów grzybobodobnych na badanych przynętach

Rodzaj przynęty Type of bait	Grzyby i organizmy grzybobodobne (patrz Tabela 2) Fungi and fungus-like organisms (see Table 2)	Ogólna liczba gatunków Total number of species
skorupiak crustacean <i>Gammarus pulex</i> L.	1, 6, 7, 10, 16, 17, 19, 20, 21, 23, 24, 25, 26, 27, 28, 30, 32, 33, 34, 36, 38, 49	22
wyłinka zaskrońca grass snake skin <i>Natrix natrix</i> L.	5, 11, 14, 20, 22, 25, 33, 38, 41, 42, 43, 44, 45, 49	14
nasiona koniczyny clover seeds <i>Triforium pratense</i> L.	1, 4, 5, 6, 7, 10, 11, 12, 13, 15, 16, 17, 20, 21, 22, 24, 25, 26, 27, 29, 30, 32, 33, 34, 38, 39	26
nasiona konopi hemp seeds <i>Cannabis sativa</i> L.	18, 24, 25, 27	4
luszka cebuli onion skin <i>Allium cepa</i> L.	3, 4, 10, 12, 25, 45, 46, 51	8
celofan cellophane	33, 43	2

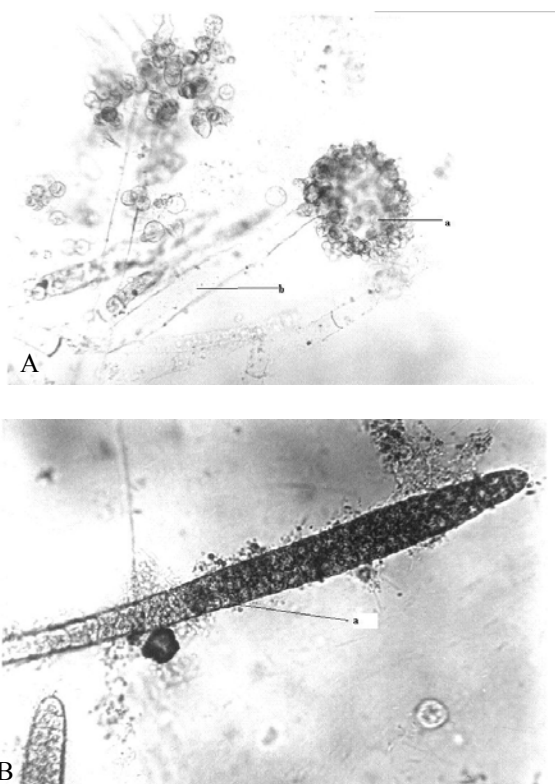


Fig. 2. Some species of fungus-like organisms in the chosen bathing sites of the River Narew: A - *Achlya oligacantha* - a) spores, b) discharge sporangium; B - *Dictyuchus sterilis* - a) sporangium with zoospores

Ryc. 2. Niektóre gatunki organizmów grzybobodobnych stwierdzone w kąpieliskach rzeki Narwi: A - *Achlya oligacantha* - a) zarodniki b) opróżniająca się zarodnia; B - *Dictyuchus sterilis* - a) zarodnia z zarodnikami

## DISCUSSION

The ecological status of surface waters is taken into consideration in the assessment of their purity and in

classification [2]. Therefore, the role of biological analysis in river monitoring has gained importance in recent years.

The results of mycological and physicochemical analyses of water collected at four bathing sites of the Narew river have confirmed their good ecological status.

The bathing site in Suraz contains relatively pure water. In the whole commune of Suraz there are no enterprises that could pollute surface waters. The only problem is insufficient sewage system in comparison with the produced amounts of sewage and waste disposal. Mycological analysis showed the presence of 41 species of fungal biota. Most taxons were detected in the spring period (26), the fewest in winter (6). The results have been confirmed by other authors. A considerable decrease in the number of micromycetes in summer and their intensified growth in spring and autumn have been observed in rivers by Czezuga et al. [9, 16]. Similar seasonal changes have been reported by Nejadsttari in Teheran [17]. In our study, phytosaprophytes were found to predominate, with the species of the genus *Achlya* being the most numerous (11). Worthy of note is *Achlya flagellata*, found to colonize hemp seeds. This species, previously detected in the river Narew [10], can also affect fish eggs [18].

The genus *Pythium*, frequently encountered in the river Nile and in soil in Egypt was represented by 4 species [18].

The genus *Saprolegnia* was represented by 6 species. In spring, *S. latvica* was detected. This species, which is rather characteristic of low pH waters [19,20], was reported earlier from the river Narew water of pH above 7 [10].

Only at this bathing site, *Alternaria alternata* and *Mucor hiemalis*, both belonging to Anamorphic fungi, were found. They may evoke allergic reactions in humans [21].

The bathing site in Uhowo – municipal waste disposal contributes extremely to water pollution there. The norms for phosphates recommended by the Ordinance of the Minister of Health (1) have been found to be exceeded. Of 40 species identified at this bathing site most worthy of note is *Candida albicans*, which can be an aetiological factor of 80% of mycotic infections in humans. Dynowska [22] associates the presence of this species with water pollution by municipal sewage. Water samples collected from bathing sites in Uhowo contained *Aspergillus flavus*, a pathogen secreting aflatoxins [23]. In spring months, *Pythium*

*debaryanum* was detected in the water examined. This phytopathogen affects roots and seeds of young plants [3]. This species has also been identified by Czczuga [8] in the river Narew.

The bathing site in Łapy – there are three industrial plants in Łapy, considered to cause environmental pollution of the river Narew: Sugar Plant "Łapy", ZNTK (Rolling Stock Repair Factory) and Milk Plant. Mycological analysis of the bathing site in this town showed the lowest mycobiota species diversity (33 species) among the sites studied. Pollutants, especially municipal and industrial sewage, suppress the growth of many organisms, including fungi. Czczuga et al. [9] have demonstrated that pure water shows a greater species diversity that decreases with the pollution increase. The presence of *Aspergillus flavus* was also detected and *Zoophagus insidians*, a predacious fungus, was identified in Łapy. It had been previously noted in the river Narew [8, 9].

The bathing site in Bokiny. Bokiny is a small agricultural place. Water there showed the lowest content of biogenic compounds. Within the distance of Łapy-Bokiny, the Narew river maintains its primeval character, with a network of old river beds. Its abundant aquatic vegetation acts as a biological filter. According to some authors, the presence of plants and their fragments significantly affects the growth of fungi [24]. Of the four bathing sites examined, most fungus species were found in Bokiny (47). The ecological status of water at this site had a positive effect on the species diversity of fungi, their growth being promoted by flowing waters [25].

The application of various baits allowed us to culture different groups of physiological fungi. Certain species were found to colonize most of the baits, showing features of eurybionts (*Achlya treleaseana*, *Aphanomyces irregularis* and *Aphanomyces androgynus*). Also stenobiont species were found (*Alternaria alternata* and *Karlingia rosea*). A similar phenomenon has been observed by Czczuga et al. [9, 10].

Water chemism in these rivers undergoes fluctuations; the amount of dissolved substances and their chemical composition in fresh waters change according to the site, season of the year, and is associated with local geological condition, precipitation rate and man's activity.

Until now, studies concerning the effect of water chemism on fungi and fungus-like organisms have been dispersed, each referring to different mycobiota, their different aspects and employing different research

methods [19, 20, 25]. This does not allow a synthetic treatment of the issue.

The current study shows that physicochemical properties of water affect significantly the diversity of mycobiota as components of the microbial loop.

In the river Narew, the optimum conditions for mycobiota were observed at the study sites in Suraż and Bokiny.

The similar contents of three forms of nitrogen (ammonia, nitrate and nitrite), phosphates, sulphates and high indices of dry residues and dissolved substances observed in water samples from Suraż and Bokiny were found to have a stimulatory effect on mycobiota growth; hence, species diversity of fungi and fungus-like organisms included in the microbial loop. A beneficial effect of sulphates on microorganisms has also been observed in the River Nile [26]. Sulphate ions are very stable in water and do not easily undergo chemical and biochemical transformations. Their high concentrations at the study sites are probably due to an increased inflow from the outside. Water investigations in Bokiny conducted in the year 1981 seem to confirm the beneficial effect of the level of phosphates on fungal species diversity [9].

However, lower species diversity of fungi and fungus-like organisms was observed at bathing sites in Uhowo and Łapy, which might have been caused by too high biogenic levels. The bathing site in Uhowo had the highest phosphate content of all the study sites. Fungi and fungus-like organisms, similarly to heterotrophic bacteria, take up energy, indispensable to life and growth, from the decomposition processes. An inflow of substantial amounts of pollutants causes imbalance between production and degradation, thus leading to reduced species diversity. Sewage flowing to the river from a purification plant in Łapy may have disturbed natural biological transformations in the microbial loop. Such parameters as temperature, pH and dissolved oxygen underwent slight fluctuations and were the optimum for mycobiota growth.

Water temperature ranged from 7 to 9 °C at all the study sites. However, in warm climatic zones, the optimum temperature for mycobiota growth is substantially higher [27].

Lack of a direct effect of pH on mycobiota growth observed in the current study is consistent with the findings reported by other researchers [28].

A study on the water in ponds in India conducted by Misra [27] has revealed that the growth of fungi and fungus-like organisms is limited by temperature, dis-

solved oxygen and calcium. However, Rattan et al. [29] during their study on some ecological factors affecting seasonal occurrence of aquatic fungi claim that dissolved oxygen has no direct effect on the growth of these microorganisms. This has also been confirmed by our investigations in the river Narew.

The concentrations of calcium were similar at the bathing sites involved in the current study. Misra [27] demonstrates a beneficial effect of this element on the growth of fungi. However, the level of magnesium showed a broad range and only slightly affected diversity of these microorganisms. Similar results have been reported from the River Nile [26].

The concentration of chlorides was the highest in Suraz and it was the site with the largest number of mycobiotic species. A study conducted in the river Narew in the year 1999 found the concentration of these ions to be an environmental factor determining the growth of microorganisms [10].

The remaining chemical indices of water in the river Narew had no significant effect on species composition of fungi and fungus-like organisms as the microbial loop components.

According to a study conducted by the Provincial Institute of Environmental Protection in Białystok in 2001, the River Narew in Uhowo was classified into the purity class II, whereas in Bokiny into class III [30].

Ecological stability and functioning of the aquatic ecosystems depend, among others, on biodiversity of groups of microorganisms included in the microbial loop [5, 6, 7]. Apart from viruses, phytoplankton, protozoa and small crustaceans, also fungi and fungus-like organisms play a role in the transformation rate and exploitation of organic and mineral substrates in water.

The microbial loop components are linked by means of trophic and paratrophic interactions, which determines definite biodiversity of respective groups of these microorganisms. This also refers to fungi and fungus-like organisms, which incorporate biogenic elements and transfer them to higher trophic nets, serving as food for certain Protista, small crustaceans and nematodes.

According to the recommendations of the EU Water Framework Directive [2], ecological evaluation of waters is based on the sets of organisms called biological quality components, such as phytoplankton, macrophytes, macrozoobenthos and fish. Aquatic fungi or fungus-like organisms are not included. However, as shown by the current mycological study, these organ-

isms significantly affect the ecological and sanitary conditions of the bathing sites studied. Thus, supplementation of the list of biological quality components with aquatic fungi and fungus-like organisms seems to be fully justified.

## CONCLUSIONS

Species diversity of the fungal biota at the investigated bathing sites depended on a number of abiotic and biotic factors and season of the year.

The physico-chemical status of the river Narew has been undergoing changes over the years and is one of the mechanisms conditioning species diversity of fungi and fungus-like organisms as the microbial loop components.

## REFERENCES

1. Dziennik Ustaw [The Official Journal of Laws]. The Ordinance of the Minister of Health of 16 October 2002, D. U. No 183, item 1530. (In Polish)
2. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. (In Polish)
3. Kiziewicz B., Kozłowska M., Godlewska A. et al.: Water fungi occurrence in the River Supraśl-bath Jurówce near Białystok. *Wiad. Parazytol.* 2004, 50, 143-150. (In Polish)
4. <http://www.astm.org/Standards/D4249.htm>.
5. Bouvy M., Pagano M.M., Boup M. et al.: Functional structure of microbial food web in the Senegal River Estuary (West Africa): impact of metazooplankton. *J. Plankton Res.* 2006, 28, 195-207.
6. Meyer J.L.: The microbial loop in flowing waters. *Microb. Ecol.* 1994, 28, 195-199.
7. Newell S.Y.: Established and potential impacts of eukaryotic mycelial decomposers in marine/terrestrial ecotones. *J. Exp. Mar. Biol. Ecol.* 1996, 200, 187-206.
8. Czeczuga B.: Aquatic fungi of the Narew River and its tributaries in the stretch from Siemianówka to Doktorce. *Ann. Acad. Med. Bialostociensis.* 1996, 41, 245-257.
9. Czeczuga B., Próba D.: Studies of aquatic fungi. VII. Mycoflora of the part of the River Narew and its tributaries in a differentiated environment. *Nova Hedwigia.* 1987, 44, 151-161.
10. Czeczuga B., Kiziewicz B., Godlewska A. et al.: Further studies on aquatic fungi in the river Narew within the Narew National Park. *Ann. Acad. Med. Bialostociensis.* 2002, 47, 58-79.
11. Göbel P., Keller C., Steinbach G. et al.: *Paradise National Parks in Europa.* Gütersloh/München, Verlagshaus Stuttgart im Bertelsmann Lexicon Verlag 2000.
12. Greenberg A.E., Clesceri L.S., Eaton A.D.: *Standard Methods for the Examination of Water and Wastewater.*

- American Public Health Association, Washington, DC 1995.
13. Seymour R.L., Fuller M.S.: Collection and isolation of water molds (Saprolegniaceae) from water and soil.- In: M. S.Fuller & A. Jaworski [eds]. Zoosporic fungi in teaching and research. Southeastern Publishing, Athens. 1987.
  14. Batko A.: [Hydromycology—an overview] PWN, Warszawa. 1975. (In Polish)
  15. Dick M.W.: Straminipilous fungi, systematics of the Peronosporomycetes including accounts of the marine straminipilous protists, the plasmodiophorids and similar organisms. Kluwer, Dordrecht, NL. 2001.
  16. Czeżuga B.: Mycoflora of the Supraśl river and its tributaries. Acta Mycol. 1996, 31, 13-32.
  17. Nejadstari T.: Occurrence and distribution of aquatic Saprolegniaceae in northwest and south of Teheran. Iran. Internat. J. Sci. 2000, 1, 1-5.
  18. El-Hissy F.T., Khalil A.M., Abdel-Raheem A.A.: Occurrence and distribution of zoosporic fungi and aquatic Hyphomycetes in Upper Egypt. Bull. Fac. Sci. Assuit Univ. 1992, 21, 45-64.
  19. Stpiczyńska E.: 1963. *Saprolegnia latvica* Apinis in Central Europe. Monogr. Bot. 1963, 15, 423-425. (In Polish)
  20. Zaborowska D.: Aquatic fungi from the peat-bog Bocian. Acta Mycol. 1965, 1, 31-52. (In Polish)
  21. Lugauskas A., Krikstaponis A.: Filamentous fungi isolated in hospitals and some medical institutions in Lithuania. Indoor and Built Environment. 2004, 13, 101-108.
  22. Dynowska M.: 1995. Yeast and yeast-like fungi as a pathogenic factors and a water ecosystems indicators. Studia i materiały WSP 77. Olsztyn 1995. (In Polish)
  23. Hedayati M.T., Pasqualotto A.C., Warn P.A. et al.: *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiol. 2007, 153, 1677-1692.
  24. El-Hissy F.T., Khalil A.M., El-Nagdy M.A.: Fungi associated with some aquatic plants collected from freshwater areas at Assiut (upper Egypt). J. Islam. Acad. Sci. 1990, 3, 298-304.
  25. Marano A.V., Steciow M.M.: Frequency and abundance of zoosporic fungi in some lotic environments of Buenos Aires Province (Argentina). J. Agric. Tech. 2006, 2, 17-28.
  26. El-Hissy F.T., Nassar M.S.M., Khallil A.M. et al.: Aquatic fungi recovered from water and submerged mud polluted with industrial effluents. J Biol. Sci. 2001, 1, 854-858.
  27. Misra J.K.: Occurrence, distribution and seasonality of aquatic fungi as affected by chemical factors in six alkaline ponds of India. Hydrobiologia. 1982, 97, 185-191.
  28. Ali E.H., Nasser L.: Incidence of mycobiota (zoosporic and terrestrial fungi) in accumulated rainfall water and submerged mud from three common valleys in the south-eastern region. Saudi Arabia. Bull. Fac. Sci. Assiut Univ. 2001, 30, 195-208.
  29. Rattan S.S., Muhsin T.M., Ismail A.L.S.: Notes on the occurrence and seasonal periodicity of Saprolegniaceae in Shatt Al-Arab (Iraq). Kavaka 1980, 8, 41-46.
  30. Kędzierzawski M.: The environmental conditions of Podlasie Province in 2000-2001. Wydawnictwo i Drukarnia, Białystok. 2002. (In Polish)

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**C-REACTIVE PROTEIN AND PROCALCITONIN CONCENTRATIONS  
AT CANCER DIAGNOSIS IN CHILDREN**

**WARTOŚCI BIAŁKA C-REAKTYWNEGO I PROKALCYTONINY  
PRZY ROZPOZNANIU CHOROBY NOWOTWOROWEJ U DZIECI**

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**Summary**

**Background.** C-reactive protein (CRP) is an acute phase protein produced in the liver. Procalcitonin (PCT) is a precursor of calcitonin, hormone produced in thyroid C cells. Both of them are markers of inflammation and their level increases in patients with cancer.

**The aim of the study** is to examine levels of C-reactive protein and procalcitonin observed during the diagnosis of cancer in children.

**Patients and methods.** The study group consisted of 359 patients with cancer. Concentration of serum CRP and PCT were analyzed.

**Results.** Increased levels of CRP and PCT were observed in 49,6% patients with cancer. The group with the most increased parameters were patients with acute myeloblastic leukaemia (73,9%). The lowest values of CRP were observed in patients with chronic myeloblastic leukaemia. Increased level of PCT was observed in 19/62 (30,6%) patients.

**Conclusions.** In patients with cancer increased level of CRP was observed more often than of PCT. There is no correlation between CRP and PCT.

**Streszczenie**

**Wstęp.** Białko C-reaktywne (C-reactive protein, CRP) jest białkiem ostrej fazy produkowanym w wątrobie. Prokalcytonina (PCT), jako prekursor hormonu kalcytoniny, wytwarzana jest w komórkach C tarczycy. Obydwa białka są markerami stanu zapalnego, których poziom wzrasta u pacjentów z chorobą nowotworową.

**Celem pracy** jest ocena wartości białka C-reaktywnego i prokalcytoniny przy rozpoznaniu choroby nowotworowej.

**Pacjenci i metody.** Badania przeprowadzono w grupie 359 pacjentów z chorobą nowotworową. Analizie poddano wyniki oznaczeń stężenia CRP i PCT w surowicy.

**Wyniki.** Podwyższone stężenie CRP przy rozpoznaniu stwierdzono u 49,6% pacjentów z chorobą nowotworową. Grupą, w której zaobserwowano najczęściej podwyższonych wyników CRP byli chorzy z ostrą białaczką szpikową (73,9%). Najniższe wartości CRP wystąpiły u pacjentów z przewlekłą białaczką szpikową. Podwyższone stężenie prokalcytoniny przy rozpoznaniu stwierdzono u 19/62 (30,6%) pacjentów.

**Wnioski.** U pacjentów z chorobą nowotworową częściej stwierdzano podwyższone stężenie CRP niż PCT. Nie zaobserwowano korelacji między CRP a PCT.

**Key words:** C-reactive protein, CRP, procalcitonin, PCT, children, cancer

**Słowa kluczowe:** białko C-reaktywne, CRP, prokalcytonina, PCT, dzieci, choroba nowotworowa

## INTRODUCTION

C-reactive protein (CRP) is an acute phase protein produced in the liver [1]. The synthesis of this protein is stimulated by pro-inflammatory cytokines, especially interleukin-6 (IL-6), interleukin-1 (IL-1) i TNF $\alpha$  [1,2,3,4]. CRP is sensitive and reliable but non-specific marker of inflammatory state [1,5,6]. Increased level of this protein is regarded as an important risk factor of atherosclerosis, myocardial infarction, peripheral blood vessels disease and ischaemic stroke [3, 5]. There is also a coincidence between increased CRP level and weight loss, anorexia – cachexia syndrome, advancement and recurrence of cancer. It plays a role as a prognostic factor in such diseases as multiple myeloma, melanoma, lymphoma, as well as ovarian, kidney, pancreatic and gastrointestinal tract tumors [3, 4]. There is a positive correlation between CRP level and the size of the tumor, the size of metastases and spread into lymphatic vessels in patients with esophageal cancer and non-small cell lung carcinoma [7, 8]. Elevated CRP concentration is related to bad prognosis in patients with non-small cell lung cancer [9]. The inflammatory state is an important component of carcinogenesis but it is not evident whether the inflammatory markers may be useful in predicting the risk of developing cancer [10].

Procalcitonin (PCT) is a precursor of calcitonin, hormone produced in thyroid C cells. Normally, PCT does not diffuse into the circulatory system and its level is below detectable limit (<0,1 ng/ml). The level of PCT increases in inflammatory states, especially caused by bacteria, and stays low in viral infections, organ transplant rejection and autoimmune diseases [11, 12, 13, 14, 15]. Presence of procalcitonin in blood is related to the presence of bacterial endotoxins, but can also be caused by other unknown factors. Clinical tests show that PCT is a sensitive and specific marker of sepsis in critically ill patients, in patients with hematologic malignancies and febrile neutropenia [13]. An increased level of PCT in patients with gastric cancer may be a useful marker in diagnosis of infection and staging [14].

Since some tumors, such as gastric cancer, as well as planoepithelial throat and laryngeal cancer, may cause an increase of PCT and CRP levels, diagnostic process of infection in patients with malignancies may be difficult [14, 16].

The aim of the study is to examine concentrations of C-reactive protein and procalcitonin during the diagnosis of cancer in children.

## MATERIALS AND METHODS

The study group consisted of 359 patients with cancer: from 1 month to 33 years of age (including 23 patients of more than 19 years of age). The characteristics of the examined patients are presented in Table 1. Concentration of serum CRP and PCT (in 62 cases) were analyzed at cancer diagnosis.

Table 1. *Patients' characteristics according to diagnosis and CRP concentration*

Tabela 1. *Charakterystyka pacjentów według rozpoznań i stężenia CRP*

Diagnosis	Number of patients	Number of patients with normal CRP level n (%)	Number of patients with increased CRP level [mg/l] and %				Total
			5-10	10-50	50-100	>100	
ALL	94	37 (39.4%)	15 (16.0%)	29 (31.0%)	10 (10.6%)	3 (3.3%)	57 (60.6%)
AML	23	6 (26.1%)	4 (17.4%)	9 (39.1%)	3 (13.0%)	1 (4.3%)	17 (73.9%)
Solid tumors	163	94 (57.7%)	14 (8.6%)	32 (19.6%)	11 (6.7%)	12 (7.36%)	69 (42.3%)
HD	37	16 (43.2%)	4 (10.8%)	7 (18.9%)	6 (16.2%)	4 (10.8%)	21 (56.8%)
LCH	9	8 (88.9%)	0	0	1 (11.1%)	0	1 (11.1%)
NHL	27	16 (59.3%)	4 (14.8%)	3 (11.1%)	3 (11.1%)	1 (3.7%)	11 (40.7%)
CML	6	4 (66.7%)	2 (33.3%)	0	0	0	2 (33.3%)
Total	359	181 (50.4%)	43 (11.9%)	80 (22.3%)	34 (9.5%)	21 (5.8%)	178 (49.6%)

Abbreviations used in the Table 1:

ALL – acute lymphoblastic leukaemia

AML – acute myeloblastic leukaemia

HD – Hodgkin disease

LCH – Langerhans cell histiocytosis

NHL – non-Hodgkin lymphoma

CML – chronic myeloblastic leukaemia

Serum CRP was measured with immunonephelometric method using monoclonal antibodies specific to human CRP molecule on Dade Behring nefelometer. PCT level was measured with immunoluminometric method using Lumitest PCT set (Brahms Diagnostica GmbH Berlin). CRP level was considered increased when above 5 mg/l; and PCT was considered increased when above 0.5 ng/ml. Patients were divided into four groups according to CRP level (CRP 5-10 mg/l, 10-50 mg/l, 50-100 mg/l, and >100 mg/l).

Our analysis included determination of the percentage of patients with elevated CRP level relative to the diagnosis. For patients with determined PCT concentration, a correlation with CRP concentration was also analyzed.



## RESULTS

Increased levels of CRP and PCT were observed in 49.6% patients with cancer (Table 1). The group with the highest concentrations of these parameters, included patients with acute myeloblastic leukaemia (73.9%), while the group with the lowest ratio of increased parameters were patients with Langerhans cell histiocytosis (1/9, i.e. 11.1%). Concentration of CRP above 100 mg/l was most commonly observed in children with Hodgkin's disease (10.8%). The lowest values of CRP were observed in patients with chronic myeloblastic leukaemia (the lowest value was 5.7 mg/l). In the whole group of patients most values (22.3%) ranged from 10 to 50 mg/l. The highest level of CRP was observed in the group of patients with solid tumors, and it was 329 mg/l.

Increased concentration of PCT at cancer diagnosis was observed in 19/62 (30.6%) patients. In 17/19 patients with increased PCT concentration, an increased CRP level was also observed. In 23/55 patients with CRP above 50 mg/l, the PCT concentration was also measured, and it was increased in 11 cases (the highest value was 8,30 ng/ml), including 7 patients with solid tumors.

## DISCUSSION

CRP may be an unspecific biochemical marker related to the growth of the malignancy in children and adults. In the analysis of Bień and Balcerska, an increased CRP concentration (value >15mg/l) was observed in 50,8% patients with diagnosed malignancies (acute leukaemias, Hodgkin's disease, non-Hodgkin lymphomas, hepatoblastoma, neuroblastoma, nephroblastoma, osteosarcoma and Ewing's sarcoma) [17]. Good clinical response to anti-cancer treatment was related to decrease of the CRP level. However, this parameter did not return to its normal concentration even after treatment completion and *actual remission* [17]. The clinical usefulness of CRP in the follow-up with patients with solid tumors was also observed by Wood et al. [18]. Wieland et al. reported that increased CRP level was observed in 64% of patients during the diagnosis of Hodgkin's disease. It was correlated with stage and was higher in patients with B symptoms. Higher CRP levels were associated with an increased risk of relapse [1]. Child et al. reported that increased serum CRP level (value >20mg/l) was observed in 31% of patients with Hodgkin's disease with stage I and II, while in 53% patients with stage III and IV. In

patients with non-Hodgkin lymphomas, increased CRP concentrations were observed in 23% of patients with stage I and II and in 40% with stage III and IV [19]. Our study showed an increased CRP concentration in 56.8% children with Hodgkin's disease and in 40,7% with non-Hodgkin lymphomas.

Ilhan et al. reported that the concentration of serum CRP and PCT in patients with gastric cancer was significantly higher than in the control group (the mean value of PCT was 0,59 ng/ml, the mean value of CRP was 78.21 mg/l) [14]. In our study, when both parameters were measured during the diagnosis, the mean value of CRP and PCT was 51.7 mg/l and 0.70 ng/ml, respectively (Table 2). The highest mean concentration of PCT was observed in the group of patients with solid tumors, and it was 1,01 ng/ml. The highest mean value of CRP was observed in patients with acute myeloblastic leukaemia, and it was 68.5 mg/l. Our study showed no correlation between CRP and PCT concentrations (Fig. 1)

Table 2. Values of CRP and PCT in the group of patients in which both parameters have been determined

Tabela 2. Wartość CRP i PCT w grupie pacjentów, u których oznaczono obydwa markery przy rozpoznaniu

Diagnosis	Number of patients	Mean CRP value [mg/l]	Range of CRP values [mg/l]	Mean PCT value [ng/ml]	Range of PCT values [ng/ml]
Acute lymphoblastic leukaemia	20	40.2	5-124	0.48	<0.10-1.97
Acute myeloblastic leukaemia	5	68.5	5-248	0.54	<0.10-1.43
Solid tumors	27	60.1	5-245	1.01	<0.10-9.48
Hodgkin disease	4	43.2	5-64	0.32	<0.10-0.52
Non-Hodgkin lymphomas	5	46.9	5-108	0.49	1.12-1.45
Langerhans cell histiocytosis	1	5	-	0.1	-
Total	62	51.7	5-248	0.70	<0.10-9.48

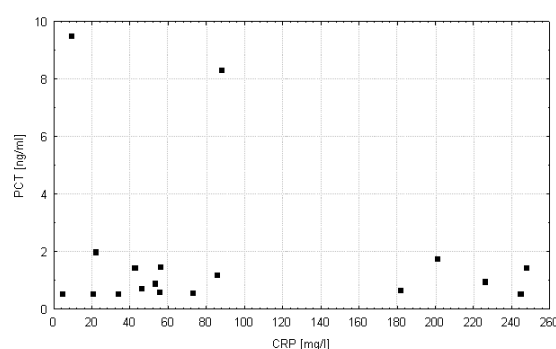


Fig. 1 Chart of relationship between CRP and PCT in the group of patients with increased PCT level

Ryc. 1 Wykres zależności między CRP i PCT w grupie pacjentów z podwyższoną PCT

## CONCLUSIONS

1. Increased concentrations of CRP and PCT were observed in 49,6% children with cancer at diagno-

sis. Abnormal CRP concentrations were most commonly observed in patients with acute myeloblastic leukaemia, and rarely in patients with Langerhans cell histiocytosis.

2. Increased serum PCT concentration was observed in 30.6% of patients.
3. There was no correlation between CRP and PCT concentrations.

## REFERENCES

1. Wieland A, Kerbl R, Berghold A, Schwinger W, Mann G, Urban C. C-reactive protein (CRP) as tumor marker in pediatric and adolescent patients with Hodgkin disease. *Med Pediatr Oncol* 2003; 41: 21-25.
2. Legouffe E, Rodriguez C, Picot MC, Richard B, Klein B, Rossi JF, Commes T. C-reactive protein serum level is a valuable and simple prognostic marker in non Hodgkin's lymphoma. *Leuk Lymphoma* 1998; 31: 351-357.
3. Mahmoud FA, Rivera NI. The role of C-reactive protein as a prognostic indicator in advanced cancer. *Curr Oncol Rep* 2002; 4: 250-255.
4. Chung YC, Chang YF. Serum C-reactive protein correlates with survival in colorectal cancer patients but is not an independent prognostic indicator. *Eur J Gastroenterol Hepatol* 2003; 15: 369-373.
5. Adamowicz A, Stróżecki P, Włodarczyk Z, Brzezińska B, Dymek G, Manitus J, Junik R. Ocena zależności pomiędzy białkiem C-reaktywnym oznaczanym metodą wysokiej czułości (hs-CRP) a czynnikami ryzyka sercowo – naczyniowego u chorych po przeszczepieniu nerki. *Med Biol Sci* 2008; 22: 27-33.
6. Min CK, Kim SY, Eom KS, Kim YJ, Kim HJ, Lee S, Kim DW, Lee JW, Min WS, Kim CC. Patterns of C-reactive protein release following allogeneic stem cell transplantation are correlated with leukemic relapse. *Bone Marrow Transplant* 2006; 37: 493-498.
7. Nozoe T, Saeki H, Sugimachi K. Significance of preoperative elevation of serum C-reactive protein as an indicator of prognosis in esophageal carcinoma. *Am J Surg* 2001; 182: 197-201.
8. Lee JG, Cho BC, Bae MK, Lee CY, Park IK, Kim DJ, Ahn SV, Chung KY. Preoperative C-reactive protein levels are associated with tumor size and lymphovascular invasion in resected non-small cell lung cancer. *Lung Cancer* 2008 May 29. [Epub ahead of print].
9. Hara M, Matsuzaki Y, Shimuzu T, Tomita M, Ayabe T, Enomoto Y, Onitsuka T. Preoperative serum C-reactive protein level in non-small cell lung cancer. *Anticancer Res* 2007; 27: 3001-3004.
10. Trichopoulos D, Psaltopoulou T, Orfanos P, Trichopoulou A, Boffetta P. Plasma C-Reactive Protein and Risk of Cancer: A Prospective Study from Greece. *Cancer Epidemiol Biomarkers Prev* 2006; 15: 381-384.
11. Cuesta MP, Llorente JL, Gómez JR, López F, Suárez C. [Procalcitonin plasma levels do not alter after uncomplicated skull base surgery.] *Acta Otorrinolaringol Esp.* 2008; 59: 341-344.
12. Gendrel D, Raymond J, Coste J, Moulin F, Lorrot M, Guérin S, Ravilly S, Lefèvre H, Royer C, Lacombe C, Palmer P, Bohuon C. Comparison of procalcitonin with C-reactive protein, interleukin 6 and interferon-alpha for differentiation of bacterial vs. viral infections. *Pediatr Infect Dis J* 1999; 18: 875-881.
13. Jimeno A, García-Velasco A, del Val O, González-Billalabeitia E, Hernando S, Hernández R, Sánchez-Muñoz A, López-Martín A, Durán I, Robles L, Cortés-Funes H, Paz-Ares L. Assessment of procalcitonin as a diagnostic and prognostic marker in patients with solid tumors and febrile neutropenia. *Cancer* 2004; 100: 1462-1469.
14. İlhan N, İlhan N, İlhan Y, Akbulut H, Kucuk M. C-reactive protein, procalcitonin, interleukin-6, vascular endothelial growth factor and oxidative metabolites in diagnosis of infection and staging in patients with gastric cancer. *World J Gastroenterol* 2004; 10: 1115-1120.
15. Karzai W, Oberhoffer M, Meier-Hellmann A, Reinhart K. Procalcitonin - a new indicator of the systemic response to severe infections. *Infection* 1997; 25: 329-334.
16. Stasik Z, Tarapacz J, Migas B, Skołyśzewski J. Białka ostrej fazy u chorych na nowotwory głowy i szyi. *Współcz Onkol* 2006; 6: 268-273.
17. Bień A, Balcerska A. Kliniczne znaczenie oznaczenia OB, CRP i LDH w diagnostyce, rokowaniu i monitorowaniu leczenia choroby nowotworowej u dzieci. *Med. Wieku Rozw.* 2004; 8: 1081-1089.
18. Woods WG. The use and significance of biologic markers in the evaluation and staging of a child with cancer. *Cancer* 1986; 58: 442-448.
19. Child JA, Spati B, Illingworth S, Barnard D, Corbett S, Simmons AV, Stone J, Worthy TS, Cooper EH. Serum beta 2 microglobulin and C-reactive protein in the monitoring of lymphomas: findings in a multicenter study and experience in selected patients. *Cancer* 1980; 45: 318-326.

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**NEPHROTOXICITY DURING ANTI-CANCER CHEMOTHERAPY**

**NEFROTOKSYCZNOŚĆ W PRZEBIEGU CHEMIOTERAPII PRZECIWNOWOTWOROWEJ**

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**S u m m a r y**

**B a c k g r o u n d.** Nephrotoxicity is an inherent adverse effect of certain anticancer drugs and a common problem in oncology. Drugs which cause nephrotoxicity are: cisplatin, methotrexate (especially in high doses), ifosfamide, cyclophosphamide, streptozocin, bleomycin, while gemcitabine, dacarbazine, mercaptopurine and carboplatin cause kidney failure less often.

**O b j e c t i v e.** Analysis of nephrotoxicity grade in children treated for neoplastic diseases with chemotherapy.

**P a t i e n t s a n d m e t h o d s.** The study group consisted of 333 patients with cancer. Concentration of serum creatinine was analyzed. For grading the nephrotoxicity common toxicity criteria for adverse events CTCAE v3.0 were used.

**R e s u l t s.** Biochemical symptoms of nephrotoxicity were observed in 8,4% of children with cancer. III grade of toxicity was observed in 0,9% of patients, there were no cases of grade IV of nephrotoxicity. Nephrotoxicity was most often observed in children treated for acute lymphoblastic leukaemia and solid tumors, while it was not observed in patients with Hodgkin's disease, and acute or chronic myeloblastic leukaemia.

**C o n c l u s i o n s.** Nephrotoxicity is not a frequent adverse effect of chemotherapy in children. However, because of the risk of chronic renal failure, the proper supportive therapy is required during chemotherapy.

**S t r e s z c z e n i e**

**W s t ę p.** Nefrotoksyczność jest nieodłącznym skutkiem ubocznym stosowania niektórych leków przeciwnowotworowych. Leki najczęściej powodujące objawy niepożądane ze strony nerek to: cisplatyna, metotreksat (zwłaszcza w dużych dawkach), ifosfamid, cyklofosfamid, streptozocyna, bleomycyna, natomiast gemcytabina, dakarbazyna, merkaptopuryna i karboplatyna rzadziej powodują uszkodzenie funkcji nerek.

**C e l p r a c y.** Ocena stopnia nefrotoksyczności u dzieci poddawanych chemioterapii z powodu chorób nowotworowych.

**P a c j e n c i i m e t o d y.** Badania przeprowadzono w grupie 333 pacjentów z chorobą nowotworową. Analizie poddano wyniki oznaczeń stężenia kreatyniny. Do oceny stopnia uszkodzenia nerek stosowano skalę CTCAE (Common Terminology Criteria for Adverse Events v3.0).

**W y n i k i.** Biochemiczne wykładniki nefrotoksyczności chemioterapii stwierdzono u 8,4% dzieci z chorobami nowotworowymi, przy czym tylko u 0,9% była to toksyczność III stopnia, IV stopień nie wystąpił u żadnego z pacjentów. Nefrotoksyczność występowała najczęściej u dzieci leczonych z powodu ostrej białaczki limfoblastycznej i guzów litych. Nie obserwowano neurotoksyczności u pacjentów z chorobą Hodgkina, ostrą białaczką mieloblastyczną oraz w przewlekłej fazie białaczki szpikowej.

**W n i o s k i.** Nefrotoksyczność nie jest częstym objawem niepożądanym chemioterapii chorób nowotworowych u dzieci, ale ze względu na ryzyko rozwoju przewlekłej niewydolności nerek, wymaga prowadzenia właściwej terapii wspomagającej.

**Key words:** : nephrotoxicity, children, chemotherapy, creatinine, acute lymphoblastic leukaemia

**Słowa kluczowe:** : nefrotoksyczność, dzieci, chemioterapia, kreatynina, ostra białaczka limfoblastyczna

## INTRODUCTION

Nephrotoxicity is an inherent adverse effect of certain anticancer drugs and a common problem in oncology [1, 2]. Intrinsic renal damage may result from prolonged hypoperfusion, exposure to exogenous or endogenous nephrotoxins, renotubular precipitation of xenobiotics or endogenous compounds, renovascular obstruction, glomerular disease, renal microvascular damage or disease, and tubulointerstitial damage or disease. Clinical signs of nephrotoxicity and methods used to assess renal function are still discussed. Chemotherapy-induced renal failure may result from damage to vasculature or structures of kidneys, haemolytic uraemic syndrome and prerenal perfusion deficits, presenting as prerenal uraemia, intrinsic damage or postrenal uraemia [1]. Furthermore, patients with cancer are frequently at risk of renal damage secondary to disease-related or iatrogenic causes [1, 2].

Some of anti-cancer drugs are potentially nephrotoxic (Table 1). Methotrexate may cause increased serum creatinine levels, uraemia and haematuria; high dose methotrexate may result in acute or chronic renal failure [1, 2]. Dose- and age-related proximal tubular damage, glucosuria, aminoaciduria and Fanconi syndrome may be adverse effects of ifosfamide [2, 5, 6]. Cisplatin and carboplatin cause dose-related renal dysfunction presenting with: increased serum creatinine levels or uraemia, and electrolyte abnormalities such as hypokalaemia and hypomagnesaemia [1, 2, 7-10]. A rare adverse effect of gemcitabine is haemolytic uraemia [1]. The risk factors of renal failure are previous or coexisting kidney diseases and exposure to potential risk of renal damage, especially with simultaneous chemotherapy (e.g. nephrotoxic antibiotics like aminoglycosides, vancomycin, amphotericin or antibiotics eliminated by kidneys like penicillins, cephalosporins, carbapenems, monobactams, tetracyclines, chinolones, cotrimoxazole) [6, 11, 12].

Table 1. *Drugs with high risk of nephrotoxicity [3-4]*Tabela 1. *Leki o dużym i umiarkowanym ryzyku nefrotoksyczności [3-4]*

Drugs with high-risk nephrotoxicity	Drugs with intermediate-risk nephrotoxicity
Cisplatin Methotrexate (high doses) Ifosfamide, cyclophosphamide Streptozocine Bleomycin	Gemcitabine Dacarbazine Merkaptopurine Methotrexate (medium doses) Carboplatin

The aim of this study was to analyze the grade of nephrotoxicity in children treated for neoplastic diseases with chemotherapy.

## MATERIALS AND METHODS

The study group consisted of 333 patients with cancer, who were hospitalized on 3040 occasions during 27 consecutive months. Patients' characteristics with respect to diagnosis are presented in Table 2. Concentration of serum creatinine was analyzed. Common toxicity criteria for adverse events (CTCAE) v3.0 were used for grading nephrotoxicity (<http://ctep.cancer.gov/forms/CTCAEv3.pdf>) (Table 3).

Table 2. *Patients' characteristics according to diagnosis*Tabela 2. *Charakterystyka pacjentów wg rozpoznania*

Diagnosis	Number of patients
Solid tumors	148 (44.5%)
Acute lymphoblastic leukaemia	95 (28.5%)
Hodgkin's disease	38 (11.4%)
Non-Hodgkin lymphomas	27 (8.1%)
Acute myeloblastic leukaemia	18 (5.4%)
Chronic myeloblastic leukaemia	7 (2.1%)
Total	333 (100%)

Table 3. *Common toxicity criteria in CTCAE scale*Tabela 3. *Skala toksyczności CTCAE*

	Grade 1	Grade 2	Grade 3	Grade 4
Creatinine concentration	>ULN – 1.5×ULN	>1.5×ULN – 3×ULN	>3×ULN – 6×ULN	>6×ULN

ULN – upper limit of normal value

In the analysis, the frequency of each nephrotoxicity grade was determined, as well as the percentage of results specific for each grade. Each patient was assigned the highest observed nephrotoxicity grade. Complications related to the tumor lysis syndrome in the first period of chemotherapy were excluded from the research.

## RESULTS

Biochemical symptoms of nephrotoxicity were observed in 8.4% of children with cancer and in 1.3% of all hospitalizations (Table 4). Grade III nephrotoxicity was observed in 0.9% of all patients. There was no case of grade IV nephrotoxicity. Grade III nephrotoxicity was observed only in one patient with acute lymphoblastic leukaemia and in two patients with solid

tumors (with nephroblastoma). Grade II nephrotoxicity was mostly observed in patients with acute lymphoblastic leukaemia and solid tumors (Table 5). Symptoms of nephrotoxicity were not observed in patients with Hodgkin's disease, neither in acute nor chronic myelogenous leukaemia.

Table 4. *Nephrotoxicity according to number of hospitalizations and patients*

Tabela 4. *Nefrotoksyczność wg liczby hospitalizacji i liczby pacjentów*

Grade	0	1	2	3	4	Total
Patients	305 (91.6%)	14 (4.2%)	11 (3.3%)	3 (0.9%)	0	333
Hospitalizations	2999 (98.7%)	20 (0.6%)	15 (0.5%)	6 (0.2%)	0	3040

Table 5. *Nephrotoxicity according to diagnosis*

Tabela 5. *Nefrotoksyczność wg rozpoznań*

Diagnosis	n	Nephrotoxicity grade				
		0	1	2	3	4
Solid tumors	148	135 (89.9%)	8 (5.4%)	5 (3.4%)	2 (1.3%)	0
Acute lymphoblastic leukaemia	95	85 (89.5%)	5 (5.3%)	4 (4.2%)	1 (1.0%)	0
Non-Hodgkin lymphomas	27	24 (88.9%)	1 (3.7%)	2 (7.4%)	0	0

## DISCUSSION

Chemotherapy-induced nephrotoxicity often demonstrates as an acute and potentially reversible renal failure, but may also cause chronic and irreversible kidney damage, decreasing the quality of life, affecting the short and long time survival, and leading to an increase in the mortality of patients with malignancies [13]. Nephrotoxicity is observed in 30% of patients treated with chemotherapy schedules based on cisplatin analogues [14]. As Langer et al. reported, 1% suffered from tubulopathy, 1% had a reduced creatinine clearance, 5% had glucosuria and 12% had proteinuria. High grade nephrotoxicity was observed in 7-9% of patients [15]. Skinner reported that ifosfamide may cause chronic renal failure in 30-60% of patients [13]. In our study, nephrotoxicity was observed in 8.4% of children.

Potential risk factors for ifosfamide nephrotoxicity are: higher cumulative dose, treatment at younger age (<3 years of age), previous or concurrent administration of cisplatin, previous unilateral nephrectomy, pre-existing renal impairment or tumor invasion, and inter-individual variability in ifosfamide pharmacokinetics or ifosfamide administration method [13].

Urinary alkalization, hydration and folic acid supplementation confer protection against methotrexate-induced renal dysfunction [1, 2]. It has been reported recently that carboxypeptidase G2 (Glucarpidase) may be useful in patients with delayed methotrexate elimination [11]. Appropriate strategy to circumvent platinum-induced toxicity are intensive hydration, forced diuresis, mannitol, magnesium supplementation, amifostine, and BNP 7787 (disodium 2,2-dithio-bis-ethanesulfonate), which is currently in clinical trial. However, there are no clear standards for such prophylaxis based on multicenter trials carried out in homogenous groups of patients [7,8,9]. The possible nephroprotective role of mesna has not been addressed specifically in clinical studies and, therefore, remains unproven [13]. In patients with malignancies, in which before or during multidrug chemotherapy renal failure was observed, amifostine (Ethyol) may be used as a nephroprotective agent [14].

## CONCLUSIONS

1. Biochemical symptoms of nephrotoxicity were observed in 8.4% of children with cancer. Grade III toxicity was observed in 0.9% of patients, while there were no cases of grade IV nephrotoxicity.
2. Nephrotoxicity was most often observed in children treated for acute lymphoblastic leukemia and solid tumors.
3. Nephrotoxicity was not observed in patients with Hodgkin's disease, neither in acute nor chronic myeloblastic leukaemia.

## REFERENCES

1. Kintzel PE. Anticancer drug-induced kidney disorders. *Drug Safety* 2001; 24: 19-38.
2. Miyazaki J, Kawai K. Prevention and management of nephrotoxicity from anti-cancer agents. *Nippon Rinsho* 2003; 61: 973-977.
3. Krzakowski M. Metody zmniejszania ryzyka narządowych powikłań leczenia systemowego. W: *Onkologia Kliniczna*. Red: Krzakowski M, Borgis, Warszawa 2006; 609-610.
4. Renal and electrolyte abnormalities due to chemotherapy. W: *The Chemotherapy Source Book Second Edition*. Red: Perry MC, Williams & Wilkins, Baltimore USA, 1996; 709-720.
5. Ho PT, Zimmerman K, Wexler LH, Blaney S, Jarosinski P, Weaver-McClure L, Izraeli S, Balis FM. A prospective evaluation of ifosfamide-related nephrotoxicity in children and young adults. *Cancer* 1995; 76: 2557-7564.
6. Lee BS, Lee JH, Kang HG, Hahn H, Lee JH, Shin HY, Ha IS, Cheong HI, Ahn HS, Choi Y. Ifosfamide nephrotoxicity in pediatric cancer patients. *Pediatr Nephrol* 2001; 16: 796-799.

7. Cornelison TL, Reed E. Nephrotoxicity and hydration management for cisplatin, carboplatin, and Ormaplatin. *Gynecol Oncol* 1993; 50: 147-158.
8. Szczylik C, Wcisło G, Bodnar L, Miedzińska-Maciejewska M. Leczenie nefroprotecyjne w trakcie chemioterapii analogami platyny u chorych nowotworowych. *Współcz Onkol* 2003; 7: 702-709.
9. Lehane D, Winston A, Gray R, Daska IY. The effect of diuretic pre-treatment on clinical morphological and ultrastructural cis-platinum-induced nephrotoxicity. *Int J Radiat Oncol Biol Phys* 1979; 5: 1393-1399.
10. Ries F, Klastersky J. Nephrotoxicity induced by cancer chemotherapy with special emphasis on cisplatin toxicity. *Am J Kidney Dis* 1986; 8: 368-379.
11. Schwartz S, Borner K, Müller K, Martus P, Fischer L, Korfel A, Auton T, Thiel E. Glucarpidase (Carboxypeptidase G2) intervention in adult and elderly cancer patients with renal dysfunction and delayed methotrexate elimination after high-dose methotrexate therapy. *Oncologist* 2007; 12: 1299-1308.
12. Dworacka M. Współczesna terapia chorób układu oddechowego. Antybiotykoterapia w leczeniu zapaleń płuc. *Przew Lek* 2008; 1: 50-52.
13. Skinner R. Chronic ifosfamide nephrotoxicity in children. *Med Pediatr Oncol*. 2003; 41: 190-197.
14. Drożyńska E, Stefanowicz J, Połczyńska K, Balcerska A, Czauderna P. Zastosowanie Etyolu u pacjentów z upośledzoną funkcją nerek poddawanych chemioterapii. Opis 3 przypadków. *Med. Wieku Rozw.* 2001; 5 (3) supl. 1:7-13.
15. Langer T, Stöhr W, Bielack S, Paulussen M, Treuner J, Beck JD. Late effects surveillance system for sarcoma patients. *Pediatr Blood Cancer* 2004; 42: 73-79.

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**PROFESSIONAL PRESTIGE OF NURSES IN OPINION OF OTHER MEMBERS  
OF THE THERAPEUTIC TEAM**

**OCENA PRESTIŻU ZAWODOWEGO PIEŁĘGNIARKI/PIEŁĘGNIARZA  
W OPINII POZOSTAŁYCH CZŁONKÓW ZESPOŁU TERAPEUTYCZNEGO**

Z Katedry i Zakładu Pedagogiki i Dydaktyki Pielęgniarskiej

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**S u m m a r y**

The aim of the study was to assess the prestige of a nursing profession based on the opinions of other members of therapeutic team and answer the following questions: is a nurse viewed as a partner by the team members? Will academic education increase the importance of the nursing profession? Is a nurse an important team member who influences goal accomplishment? The study was also to identify measures necessary to improve the prestige of the nursing profession.

The study was conducted in four hospitals in Bydgoszcz and included 139 professionals (doctors, nutritionists, medical laboratory specialists and physiotherapists). A specially designed questionnaire was used in the study. Quantitative and qualitative analyses of the results were performed.

The study shows that according to other members of therapeutic team, the prestige of nursing profession is average, despite the fact that nurses' work is highly rated by other specialists. A nurse is considered to be an important member of the team who is essential for the process of patient's treatment. The respondents expect nurses to gain more competence. The respondents believe that academic education will enhance the prestige of the profession (however nurses with university degree do not command much greater respect than other nurses – about 50% of the respondents notice greater respect for nurses with academic degree). According to the respondents, factors which might enhance the prestige of nursing profession are: constant education and training and higher salary.

**S t r e s z c z e n i e**

Celem pracy było określenie, jak wysokim prestiżem cieszy się pielęgniarka w zespole: czy jest postrzegana jako partner zawodowy, czy wprowadzenie kształcenia zawodowego na poziomie akademickim podniesie rangę zawodu, czy jest istotnym elementem zespołu, mającym wpływ na osiągnięcie celu misji oraz określenie działań, jakie należy podjąć, żeby podnieść prestiż zawodu według innych profesjonalistów.

Badania były przeprowadzone w czterech szpitalach bydgoskich, a udział w nich wzięło 139 osób wykonujących zawód lekarza, dietetyka, analityka medycznego i rehabili-

tanta. Wykorzystano do nich specjalnie skonstruowany kwestionariusz ankiety. Uzyskane wyniki poddano analizie ilościowej i jakościowej.

Wyniki badań wykazały, że pielęgniarki cieszą się przeciętnym prestiżem zawodowym w zespole, mimo że ich praca jest oceniana wysoko przez innych specjalistów. Pielęgniarka jest również postrzegana jako pełnoprawny partner zawodowy, bez którego nie można przywrócić zdrowia choremu. Respondenci oczekują rozszerzenia kompetencji pielęgniarki. Stwierdzają również, że wyższe wykształcenie podniesie rangę zawodu, jednak wzrost uznania wobec pielę-

gniarek z wyższym wykształceniem dostrzega tylko około połowa. Wśród czynników mających podnieść prestiż zawo-

du badani najchętniej wymieniali: stałe podnoszenie kwalifikacji oraz podniesienie wynagrodzeń za pracę.

**Key words:** professional prestige, nurse, therapeutic team.

**Słowa kluczowe:** : prestiż zawodowy, pielęgniarstwo, zespół terapeutyczny

## INTRODUCTION

Frequent contacts between specialists from different areas of medicine and nurses during patient treatment lead to the creation of an image of a nurse as a person and a professional in terms of caretaking.

The work done by nurses is an indicator of their social-professional position, e.i. their position in the class structure. This position is defined by their position within the profession, their income, the level of their independence in making decisions, education, external symbols of social position, such as the clothes they wear, lifestyle, professional and social identification [1]. Their job defines their position in the social hierarchy and the scope of duties, as well as enables them to use certain material goods and privileges, and defines their lifestyle.

The main aim of this work was to assess the professional prestige of a nurse among the other members of the medical team. In order to achieve this goal, the prestige of a nurse was evaluated on the scale of “very high – very low” and research was conducted to discover what factors influence the prestige. A minor aim was also set – to attempt to determine if the current model of nurse education influences their professional prestige, and what is the evaluation of its usefulness for taking care of patients, as well as what steps need to be taken, according to other specialists, in order to improve the professional prestige of a nurse.

## MATERIALS AND METHODS

Approximately 150 questionnaires were distributed among the Bydgoszcz hospital staff, out of which 139 were completed and returned. The respondents represented the following professions: nutritionists, rehabilitants, doctors, medical analysts. The most numerous group were rehabilitants – 49 respondents and medical analysts – 39. The other professional groups were represented by 28 nutritionists and 23 doctors. Among the population, 5 people possessed the degree of Doctor of Medicine (4 physicians and 1 medical analyst). Most respondents were women (90.6%).

Among the respondents, 79 (56.8%) had a degree and 60 (43.2%) graduated from secondary schools. Most respondents were aged between 31 and 40 (48 people). The second largest age group were employees aged between 41 and 50 (38 people). There were 26 and 25 respondents aged between 20 and 30, and between 51 and 60, respectively.

The research used the method of diagnostic questionnaire. For this purpose an anonymous questionnaire was designed consisting of 4 basic questions about the respondents (age, sex, profession and education) together with 16 principle questions. The principle questions referred to the issue of professional prestige both in general and in detail. They were both closed and open questions with wide variety of answers.

The research was conducted in 4 Bydgoszcz hospitals: the Dr A. Jurasz University Hospital, the J. Brudzinski Provincial Children’s Hospital, Dr J. Biziel Provincial Hospital and the 10th Military Teaching Hospital with a Polyclinic in randomly chosen wards, between November 2007 and April 2008.

The statistical analysis was done with the use of the STATISTICA PL 7.1 software by StatSoft. The analysis of co-dependence of responses to particular questions with the demographic factors was conducted with the use of the  $\chi^2$  test, which describes the interrelationships of the variables based on the normal scale. The level of significance of  $p \leq 0,05$  was assumed as statistically significant. The answer to the questions asked were presented in the form of percentage which facilitated capturing of the relations between the answers and particular groups of respondents. The percentage achieved in this way does not always total 100%, since the results in the “no answer category” were not taken into account, and some questions gave the opportunity to provide several answers.

## THE RESULTS

According to 51.09% respondents, the professional prestige of a nurse is average. Such answer was most often provided by medical analysts (71.79%). 52 respondents (38%) assessed the professional prestige of a



nurse as high (such evaluation was especially high among nutritionists and doctors, among whom 64.29% and 52.17% respectively provided such an answer), including 8 (15.2%) who said it was very high. Only 15 respondents (10.9%) assessed the professional prestige of a nurse as low, including 3 who believed it was very low. This group was dominated by people aged 41-50 (18.42%) and rehabilitants (23.40%). The differences in answers concerning the professional prestige of a nurse were statistically significant ( $p=0.0002$ ).

According to the respondents, in order to improve the professional prestige of a nurse, the following steps need to be taken: continuously improve qualifications – 83.4%, raise salaries 61.1%, choose nursing course candidates more carefully – 35.9%, encourage more men to become nurses 34.5%, put more emphasis on foreign languages during nursing courses – 18.7%. 80.4% of the respondents would not encourage any of their relatives to choose this profession ( $p=0.0073$ ). This option was most often chosen by rehabilitants (91.8%) and medical analysts (81.5%), and least often chosen by doctors (65.2%). These answers were supported by the following arguments: low salaries (39.6%), high level of work inconvenience (32.4%) and lack of appreciation (7.2%). Almost 39% respondents could not justify their answers.

Financial benefits is one of the factors influencing the level of professional prestige of a profession. 74.1% of the respondents decided that nurses were underpaid. Doctors were unanimous in this view, while 75% rehabilitants and 69.2% nutritionists believed so too. The data were statistically significant ( $p=0.0073$ ).

Higher education is one of the factors positively correlating with an increase in the professional and personal prestige of nurses which was represented in the survey. 66.7% of the respondents agreed that introduction of academic education for nurses would increase the prestige of the profession. Such response was most frequently given by rehabilitants (76.60%) and medical analysts (70.27%) and young people – aged between 20 and 30 (76.92%). Generally, such an answer was given by respondents with higher education (73.33%). On the other hand, respondents with secondary education and aged 51-60 were of the opposite view (41.67% and 48% respectively). The results were statistically insignificant ( $p=0.1318$ ).

Despite the positive relation between education and professional prestige, 68 respondents (50.7%) believed that nurses with higher education did not enjoy higher esteem within their teams, which was also statistically

insignificant ( $p=0.1915$ ). These respondents were mostly rehabilitants (57.78%) and medical analysts (56.41%). 66 respondents (49.3%) declared that nurses with higher education enjoyed higher esteem within their teams. Most of the respondents who shared this view were nutritionists (66.67%). Also, people aged 20-30 (56%) having secondary education (54.24%) were of the same view.

The respondents agreed that nurses deserved esteem for the following reasons: skills – 87.7%, being hard-working – 72.6%, reliability – 71.9%, knowledge – 71.9%, kindness – 4.3%, empathy and friendliness – 2.8%, attitude to patients – 2.1%, being communicative – 1.4%, and experience – 0.7%.

Vast majority (88.4%) perceived nurses as partners in the process of treatment ( $p=0.0201$ ). Such an answer was given by 100% of doctors and 96.43% of all nutritionists. The least supportive of this view were medical analysts (76.93%).

Similar percentage of respondents – 89.2% – declared that they appreciated and considered nurses' opinions concerning patient care and treatment. 100% of doctors and nutritionists shared this view. On the contrary, 23.08% of medical analysts were of the opposite opinion. The differences in answers were statistically significant ( $p=0.0062$ ).

91.3% of all respondents confirmed the usefulness of nurses' knowledge and declared that a medical team would not be able to exist without them. Again, doctors and nutritionists were unanimous in this opinion (100%). The most critical group were rehabilitants (18.37%) and medical analysts (7.69%), who declared that a team could function properly without a nurse's knowledge. The results were statistically significant ( $p=0.0132$ ).

Moreover, only 70.6% of the respondents believed that it was not possible to fully cure patients without nurses' participation. The largest percentage of respondents with this view could be found among nutritionists (92.8%). Within the remaining professions, a similar percentage of respondents believed that nurses' participation was necessary (between 63.3 and 66.7%). The opposite view was shared by 40 out of all the respondents (29.4%). The results were statistically significant ( $p=0.0361$ ).

Most of the respondents were in favour of extending the scope of activities during which nurses could make independent decisions ( $p=0.1837$ ). This opinion was mainly expressed by people with higher education (61.54%), nutritionists (76.92%) and doctors (69.5%).

Despite this, a similar proportion of respondents (65.15%) wanted nurses to be dependent on other medical

team members in terms of basic activities conducted within their duties. Such an answer was chosen most frequently by doctors (75%) and medical analysts (70.27%). Only 46 people (34.8%) declared nurses' full independence of other members of the interdisciplinary team. The results were statistically insignificant ( $p=0.5085$ ). It is worth noticing that dependence on other team members was most frequently expected by respondents aged 51 to 60 (80%) and 41 to 50 (77.8%), while the opposite opinion was expressed by mainly young respondents (aged 20-40). These results were statistically significant. ( $p=0.0228$ ).

The respondents agreed that nurses within their teams did not conduct any activities from beyond their scope of competence. 72 respondents were of this opinion (55.8%). Such answers were most common among doctors (86.9%) and rehabilitants (70.2%). The results were statistically significant. ( $p=0.0001$ ). Even more respondents believed that by conducting activities for which they are not qualified, and which are other peoples' responsibilities, nurses will not improve their professional prestige or gain them more esteem (108 of the surveyed staff, i.e. 80.6 % of all respondents). Such answer was most often given by rehabilitants (93.8%). The results were statistically significant. ( $p= 0.0256$ ).

Professionals highly valued the work of nurses in their teams. Such assessment was given by 68 of the questioned staff (50%), including 12 (17.6%) who described it as "very high". This very high evaluation of nurses' work was provided by 91.3% of doctors and 71.4% of nutritionists. The value of work of nurses within their teams was defined as low by 9 respondents (6.6%), 4 of whom actually gave a "very low" rating. 14.58% of low ratings were provided by rehabilitants. The results were statistically significant. ( $p=0.0001$ ).

## DISCUSSION OF RESULTS

After analysis of the research material and literature it seems that the social-professional situation of a nurse, in the opinion of the interdisciplinary team they work with, is not as bad as it is sometimes pictured by researchers and nurses themselves. As for some factors of prestige (salary, independence) low, depreciating opinions of nurses' profession can be noticed, which correlates with other available literature. In terms of other factors (such as importance and relevance of work, social usefulness) the situation is better than previous works may suggest.

74.1% of respondents agreed that nurses were underpaid in view of the effort made at their work. Most

such answers came from doctors and rehabilitants – professional groups with relatively high income. It is worth pointing out that these two groups were also characterized by highest percentage of staff with higher education (100% and 57.1% of doctors and rehabilitants respectively). Higher education is a factor influencing employee's higher expectations in terms of pay and work itself. These two groups, whose interpersonal contacts with nurses are the most frequent, also notice that nurses' work is challenging and involves many different duties. Nurses themselves are exposed to many harmful factors, e.g. health hazards or difficult situations during treatment and nursing, such as conflicts, violence, etc. However, it is probably low pay (51.09%) that influences the average prestige of nurses within a team, which seems to be confirmed by research conducted by Kądalska et al., in which more than 50% of respondents decided that low pay was the factor responsible for low or average prestige of nurse's profession. [2]. More than 60% of professionally-active nurses thought of changing their job for a more profitable one, since statistics show that they only earn approximately 74% of the national average (gross national average in April 2008 was PLN 2786), while the assistant staff's salaries were only approximately 70% of the national average [3]. Due to the increased demand for caretaking and nursing personnel many European countries have been making efforts to attract Polish nurses, who are known for their reliability and excellent professional skills. Foreign employers tempt Polish nurses with high salaries, professional development opportunities, and potential promotion in the future. The highest demand for such personnel can be noticed in Norway, Italy, the United Kingdom, Ireland, Sweden, the Netherlands and the USA, despite the fact that the number of nurses per every 10 thousand citizens in those countries is sometimes even 3 times higher than in Poland (in 2006 the ratio in Poland was 47 nurses per every 10 thousand citizens) [4]. Those countries offer monthly pay of approximately 1500 euro (2000 euro in Germany), and American employers offer 28-35\$ per hour (approx. 3500 \$ per month). Moreover, the level of nursing in those countries is high, not only for the quality of services provided but also for the financial and social offer, and social recognition. All this means that Polish nurses are willing to leave the country and improve their standard of living. Polish employers are not attractive enough to them, not only for financial reasons, but also because of non-material reasons, such as recognition and esteem. In

West European countries Polish nurses are well-paid and enjoy high esteem within the society. Such a high social position encourages young people to train for this job which means that there is better choice of candidates when recruiting nursing staff which leads to the possibility of choosing the candidates that are best-suited for this job. In the research conducted by Binkowska - Bury et al. [5] among students of nursing, 34.7% of them declared they had chosen the profession because they had not been accepted to other courses. However, in most cases the reasons behind the decision to study nursing were idealistic and highly ethical. The analysis conducted by Kulik et al. [6], Tęcza [7] and Kądalska [8] shows that the most common reason for choosing the course was the need to help others and interest in medicine. Only few respondents based their decision on the prestige of the profession, and even fewer made the decision for financial reasons. The respondents, as well as nurses, are aware that in order to improve the professional prestige of a nurse, it is necessary to introduce various changes on different levels, both dependent on and independent of nurses themselves. [9].

One of the factors defining the professional prestige of a job is its social relevance, which is represented in individuals' reasons for choosing a job. In today's society, the least often chosen jobs are those, which require high physical effort [10]. 80.4% of respondents underlined that they would not encourage their relatives or acquaintances to become a nurse. They argued that this was due to: low salary and hard work. Such opinion was most common among medical analysts and rehabilitants. The most willing to encourage others to become a nurse were young people (aged 20-30 lat), which probably results from the change in the perception of the job due to changes in nursing education and nurses' legal situation. 43% of nursing students declare that having experienced the nature of the job during their practical training, they would not choose the same course again. [5]. Another issue is the nature of the job. Most nursing students surveyed by Gotlib et al. [11] declared that it was a "feminised" and "unmanly" profession of low prestige. These are the main reasons for men not to choose the profession. Therefore, in order to encourage men to choose the job, many countries promote the profession in media, underlining the importance of men in modern nursing and raising the nurses' pay. It needs to take many years, however, before social stereotypes are brought down and men

will be free to choose nursing courses without the risk of being suspected of effemination.

CBOS (Public Opinion Research Centre) survey on honesty and professional reliability conducted in the year 2000 showed that nurses occupied a high position in the hierarchy of professions in terms of honesty and reliability. Among the 23 evaluated jobs, the profession of a nurse came second, directly after scientists, with 9% very high and 48% rather high ratings [1]. In own previous research, members of the medical team, when asked "What influences the respect shown to nurses", most often answered "skills" (87.7%) and "being hard-working" (72.6%). Some other answers were reliability (71.9%) and "honesty" (61.1%). This shows that, in terms of character traits and ethical standards, nurses are highly situated within the society, whose members believe that, in order to do this job, one needs to have a special calling, because it is difficult to imagine that someone would study for a job that is badly recognised, underpaid, and often done in hazardous conditions.

Nurses complain that their contribution to the patient care is underestimated and they do not have a say in the decision-making about the treatment [12]. However, as it has been shown in this work, it is contrary to the opinions of the other members of the team. 88.4% of respondents declared that they perceived nurses as professional partners, and 89.2% considered their opinion on treatment and nursing. 70.6% of respondents believed that without nurses' participation it was not possible to accomplish the medical team's mission, i.e. patient's recovery. From all the professional groups involved in the process of treatment, the highest percentage of medical analysts did not treat nurses as professional partners and did not consider their opinion on patient care and treatment. At the same time they were mostly young people (aged 20-30). It probably results from the fact that medical analysts do not cooperate with nurses directly and their contacts are infrequent.

When addressing nurses' independence, it is necessary to point out that this independence is of alleged nature and, in the view of the society, illusive. Why? There are several reasons. Although, by law, a nurse is an independent team member, whose work does not require supervision or orders to do the activities included in the scope of nurses' duties, it is their superiors who limit their professional independence to manual work, and nurses themselves assume that their job is only to follow doctors' recommendations. [13, 14]. According to Jankowska et al. [15], only 37.5% of

nurses believe they possess good or very good qualifications to perform the function of health-promotion, the function of rehabilitant (30%), the function of educator (25%). Nurses themselves want to gain additional qualifications in terms of prevention (85%), rehabilitation (66.3%), therapy (61.3%). Nurses are least willing to improve their qualifications in health promotion (17.5%) and nursing research (6.3%). Large percentage of nurses are unwilling to self-educate. What is more, trainings are not accessible enough, as they are expensive and there are not enough of them.

More than half of the respondents believe that nurses conduct activities from beyond their scope of duties. According to Kapala and Sawicka[16], 82% nurses admit that they conduct activities beyond their competence. Among these nurses, 14% do it often and 64% sometimes.

The job of a nurse is hard, done in difficult conditions and underpaid. It requires dedication and many sacrifices. This effort is recognised by other professionals who highly esteem nurses' contribution to the complex process of patient care.

## CONCLUSIONS

1. Professional prestige of a nurse is average, according to the other professional groups in the medical team. This results from, among other things, low salary and high level of work inconvenience.
2. Nurses' work in a team is highly esteemed by other team members.
3. Academic education of nurses is one of the factors improving professional prestige. However, the correlation between higher education and increase in professional prestige is only understood by half of the respondents.
4. Members of the medical team support the idea of increasing nurses' professional independence.
5. In order to improve nurses' professional prestige, nurses should continuously improve their qualifications.

## BIBLIOGRAPHY

1. Kucharz M.: Zawód pielęgniarki, [www.wizard.ae.krakow.pl](http://www.wizard.ae.krakow.pl), wizyta na stronie 15.04.2008.
2. Kądalska E., Fronczyk K., Wrońska I.: Postrzeżenie zawodu pielęgniarki przez studentów studiów licencjackich. *Pielęgniarstwo XXI wieku*. Wyd. Czelej, Lublin, 2006, 1/2, s. 115-119.
3. Domagała H.: Problemy płacowe i emigracja zarobkowa polskich lekarzy i pielęgniarek. *Zeszyty Naukowe Ochrony Zdrowia. Zdrowie Publiczne i Zarządzanie*. Instytut Zdrowia Publicznego, WOZ CM UJ, 2004, 2, s. 55-65.
4. Biuletyn statystyczny Ministra Zdrowia 2007, [www.csioz.gov.pl](http://www.csioz.gov.pl), wizyta na stronie 15.07.2008r.
5. Binkowska-Bury M., Wrońska I., Iwanowicz-Palus G.: Orientacja życiowa przyszłych pielęgniarek a motywy wyboru zawodu. *Pielęgniarstwo XXI wieku*. Wyd. Czelej, Lublin, 2004, 2, s. 37-40.
6. Kulik K., Eszyk J., Ordys D. i wsp.: Motywy i okoliczności wyboru zawodu pielęgniarki. *Problemy Pielęgniarstwa*. Wyd. Auxilium, Warszawa, 2004, 1-2, s. 76-79.
7. Tęcza B.: Motywacja wyboru zawodu pielęgniarki w aspekcie powołania i altruizmu. *Pielęgniarstwo Polskie*. Wydawnictwo Akademii Medycznej im. K. Marcinkowskiego w Poznaniu, 2003, 2, s. 170-174.
8. Kądalska E., Fronczyk K.: Motywy wyboru studiów licencjackich na kierunku pielęgniarstwo w Polsce. *Pielęgniarstwo XXI wieku*. Wyd. Czelej, Lublin, 2006, 1-2, s. 111-114.
9. Nowak J.: Z problematyki prestiżu zawodowego pielęgniarek w Polsce. *Zdrowie Publiczne*. Centrum Organizacji i Ekonomiki Ochrony Zdrowia, Warszawa, 1997, 8, s. 193-196.
10. Tobiasz-Adamczyk B., Bajka J., Marmon G.: Wybrane elementy socjologii zawodów medycznych. *Collegium Medicum UJ, Kraków*, 1996, s.79.
11. Gotlib J., Łabęda W., Rebandel H.: Do mężczyzny „siostrzy”?- postrzeżenie zawodu pielęgniarki w opinii studentów Akademii Medycznej w Warszawie- doniesienie wstępne. *Pielęgniarstwo XXI wieku*. Wyd. Czelej, Lublin, 2008, 1, s. 19-24.
12. Halota I.: Sytuacja zawodowa i społeczna pielęgniarek w Polsce. *Polityka Społeczna, Instytut Pracy i Spraw Socjalnych*, Warszawa, 2004, 10, s. 4-7.
13. Cegła B., Faleńczyk K.: Samodzielność pracy pielęgniarek w ich własnej ocenie. *Valetudinaria - Postępy Medycyny Klinicznej i Wojskowej*, 10 Wojskowy Szpital Kliniczny z Polikliniką w Bydgoszczy, 2002, 3, s. 90-95.
14. Niebrój L., Kosińska M.: Autonomia pielęgniarstwa: teoria i praktyka. *Health Care: Professionalism and Responsibility*. Wyd. ŚAM, Katowice, 2005, s. 47-51.
15. Jankowska B., Krajewska-Kułak K., Bartoszevska B. i wsp.: Przygotowanie pielęgniarek do wykonywania funkcji zawodowych. *Pielęgniarstwo XXI wieku*, Wyd. Czelej, Lublin, 2003, 5, s. 43-46.
16. Kapala W., Sawicka M.: *Pielęgniarka orkiestra*, [www.termedia.pl](http://www.termedia.pl), wizyta na stronie 13.04.2008r.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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**RELATIONSHIP BETWEEN WALL MOTION SCORE INDEX AND LEFT VENTRICULAR EJECTION FRACTION AND THE EXTENT AND THE ADVANCEMENT OF CHANGES IN CORONARY ARTERIES IN PATIENTS WITH NON-ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION**

**ZALEŻNOŚĆ POMIĘDZY WSKAŹNIKIEM KURCZLIWOŚCI I FRAKCJĄ WYRZUTOWĄ LEWEJ KOMORY A ROZLEGŁOŚCIĄ I ZAAWANSOWANIEM ZMIAN W TĘNICACH WIĘNCOWYCH U CHORYCH Z ZAWAŁEM SERCA BEZ UNIESIENIA ODCINKA ST**

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**S u m m a r y**

**I n t r o d u c t i o n .** Patients with non-ST-segment elevation myocardial infarction (NSTEMI) compose a non-homogeneous group while considering the extent of atherosclerosis and the degree of narrowing in coronary arteries. It is suggested that estimation of wall motion score index (WMSI) and left ventricular ejection fraction (LVEF) enhance morbidity and mortality risk stratification in patients with NSTEMI. Could echocardiography performed before a NSTEMI treatment decision is made be helpful in the actual decision making process?

**T h e a i m** of this study was to examine the correlation between WMSI, LVEF and markers of myocardial necrosis and the extent and the degree of narrowing in coronary arteries in patients with NSTEMI.

**M a t e r i a l a n d m e t h o d s .** A retrospective analysis based on medical records of 191 patients with NSTEMI was performed. Demographic, biochemical, angiographic and echocardiographic parameters were assessed. Patients were divided into groups using the following criteria: the number

of significantly narrowed coronary arteries, TIMI flow grade in the infarct-related artery before angioplasty and history of previous myocardial infarction.

**R e s u l t s .** LVEF was significantly higher and WMSI was significantly lower in patients without significant narrowings in coronary arteries and in patients with 1-vessel disease than in patients with multivessel disease (LVEF 53.3% vs 48.3% vs 43.6%,  $p=0.0058$ ; WMSI 1.35 vs 1.39 vs 1.51,  $p=0.0099$ ). Significant differences in LVEF and WMSI were observed between groups of patients with patent (TIMI 2-3) and non-patent (TIMI 0-1) infarct-related artery (LVEF 50 vs 45%,  $p=0.000045$ ; WMSI 1.32 vs 1.47,  $p=0.002475$ ) and in groups of patients without and with history of previous myocardial infarction (LVEF 48.3% vs 40.2%,  $p=0.000001$ ; WMSI 1.38 vs 1.63,  $p=0.0000001$ ).

**C o n c l u s i o n s .** Decrease in LVEF and increase in WMSI may indicate the extent and the advancement of changes in coronary arteries.

**S t r e s z c z e n i e**

**W s t ę p .** Chorzy z zawałem serca bez uniesienia odcinka ST (NSTEMI) stanowią niejednorodną grupę pod względem rozległości miażdżycy, jak i stopnia zwężenia w tętnicach wieńcowych. Sugeruje się, że ocena wskaźnika kurczliwości (WMSI) i frakcji wyrzutowej lewej komory (LVEF) wzbogając ocenę ryzyka chorobowości i śmiertelności

u chorych z NSTEMI. Czy badanie echokardiograficzne wykonane przed decyzją o rodzaju leczenia NSTEMI może być pomocne w podejmowaniu decyzji?

**C e l e m** pracy była ocena zależności pomiędzy WMSI, LVEF i poziomem markerów martwicy mięśnia

sercowego a rozległością zmian i stopniem zwężenia w tętnicach wieńcowych u chorych z NSTEMI.

**Materiał i metody.** Retrospektywnej analizie poddano historie chorób 191 pacjentów z rozpoznaniem NSTEMI. Oceniano parametry demograficzne, biochemiczne, koronarograficzne i echokardiograficzne. Chorych podzielono na grupy w zależności od liczby zwężonych istotnie tętnic wieńcowych, przepływu TIMI w tętnicy odpowiedzialnej za zawał przed interwencją wieńcową oraz w zależności od wcześniej przebytego zawału serca.

**Wyniki.** LVEF była znamienne wyższa, a WMSI znamienne niższy u chorych bez istotnych zwężeń w tętnicach wieńcowych i u chorych z chorobą 1-naczyniową

w porównaniu do chorych z chorobą wielonaczyniową (LVEF 53,3% vs 48,3% vs 43,6%,  $p=0,0058$ ; WMSI 1,35 vs 1,39 vs 1,51,  $p=0,0099$ ). Znamienne różnice w zakresie LVEF i WMSI obserwowano pomiędzy grupami chorych z drożną (TIMI 2-3) i niedrożną (TIMI 0-1) tętnicą odpowiedzialną za zawał (LVEF 50 vs 45%,  $p=0,000045$ ; WMSI 1,32 vs 1,47,  $p=0,002475$ ) oraz w grupach bez zawału i z wcześniej przebyłym zawałem serca (LVEF 48,3% vs 40,2%,  $p=0,000001$ ; WMSI 1,38 vs 1,63,  $p=0,0000001$ ).

**Wnioski.** Obniżenie LVEF i wzrost WMSI u pacjentów z NSTEMI może przemawiać za rozległością i zaawansowaniem zmian w tętnicach wieńcowych.

**Key words:** non-ST-segment elevation myocardial infarction, wall motion score index, left ventricular ejection fraction

**Słowa kluczowe:** zawał serca bez uniesienia odcinka ST, wskaźnik kurczliwości lewej komory, frakcja wyrzutowa lewej komory

## INTRODUCTION

Patients with non-ST-segment elevation myocardial infarction (NSTEMI) compose a non-homogeneous group while considering the extent of atherosclerosis and the degree of narrowing in coronary arteries [1,2]. Compared to patients with ST-segment elevation myocardial infarction (STEMI), they more often have multivessel disease. It is often difficult to unambiguously specify which lesion is responsible for myocardial infarction. Short-time prognosis in patients with NSTEMI is better than in patients with STEMI, but long-term prognosis is worse [3]. Factors determining prognosis include the value of left ventricular ejection fraction, the extent of changes in coronary arteries and the presence of potentially life-threatening cardiac arrhythmias. Several risk scores for morbidity and mortality risk stratification in this group of patients are available [4,5,6]. It is suggested that estimation of wall motion score index (WMSI) along with left ventricular ejection fraction (LVEF) enhance risk stratification [7,8]. Some of the currently used risk scores (TIMI, PURSUIT, Gensini score) are useful in the evaluation of the extent of changes narrowing coronary arteries [7,9,10,11]. However, to date, the relationship between wall motion score index (WMSI) alone or in association with left ventricular ejection fraction and markers of myocardial necrosis and the extent of changes and the degree of narrowing in coronary arteries has not been assessed. Could echocardiography performed before a NSTEMI treatment decision is made be helpful in the actual decision making process?

The aim of this study was to examine the association of WMSI, LVEF and markers of myocardial necrosis with the extent and the degree of narrowing in coronary arteries in patients with NSTEMI.

## MATERIAL AND METHODS

The retrospective analysis based on medical records of 191 patients with NSTEMI hospitalised in the Cardiology Clinic in 2005 was performed. Myocardial infarction was defined as level of TnI  $\geq 0,3$ ng/ml (recognized as cut-off point) registered in patients with typical chest pain lasting at least 20 minutes or with other clinical symptoms indicating myocardial necrosis. Demographic (age, sex, presence of classical risk factors), biochemical (level of creatinine, troponin I, CPK, CK-MB) angiographic (number of significantly narrowed coronary arteries, TIMI flow grade in the infarct-related artery) and echocardiographic parameters (contractility of individual left ventricular segments, left ventricular ejection fraction and wall motion score index) were assessed. For the echocardiographic assessment of wall motion abnormalities, standardized 17-segment model was applied [12]. Contractility was assessed as normokinesis, hipokinesis, akinesis and dyskinesis, assigning them a score of 1 to 4 accordingly. Average score constitutes WMSI assessed. Artery lumen reduction  $>50\%$  was considered angiographically significant. Artery that underwent pPCI was considered as infarct-related artery. Decision regarding coronary intervention was made by the interventional cardiologist who performed cardiac catheterization. Patients were divided into groups taking into account the number of significantly narrowed coronary

arteries (patients with 1-vessel disease, patients with 2- and 3-vessel disease and patients without significant narrowings in coronary arteries). Furthermore, patients were divided on the basis of the patency of the infarct-related artery during PCI (division was based on TIMI score- patent artery was described by the TIMI flow grade 2 or 3, non-patent artery TIMI 0 or 1) and the history of previous myocardial infarction. In all groups value of left ventricular ejection fraction and wall motion score index, serum concentration and activity of myocardial necrosis markers and blood leukocyte count was compared.

Statistical analysis was performed using the StatSoft's STATISTICA software. Parameters with normal distribution were expressed as the arithmetic mean and standard deviation. Variables with distribution other than normal were expressed as medians and quartiles. Significance of the differences between groups was assessed by the t-test, the Mann-Whitney U test and the ANOVA rang Kruskal-Wallis test. Estimation of the relationship between qualitative variables was tested by the Chi-square test. The p value  $\leq 0.05$  was deemed significant.

## RESULTS

All of 191 patients, whose medical records were analysed, underwent coronary angiography. Primary coronary angioplasty was performed in 141 patients and the remaining 50 patients either received conservative treatment or were scheduled for coronary artery bypass graft surgery. The study group characteristic is shown in table I and II. Women accounted for 32.9% and they were older than men by an average of 3 years ( $p=0.07$ ). 73.8% of patients had hypertension, 53.4% had hypercholesterolemia, 38.7% were current smokers and 28.7% had diabetes mellitus. The mean time from the onset of chest pain to hospitalization was 4 hours and 15 minutes. 37.2% of patients from the study group had history of previous myocardial infarction (Table I).

Patients with 1-vessel disease accounted for 27.2% (52 patients) of the study group, with multivessel disease (2- or 3-vessel disease)- 64.9% (124 patients) and patients without significant narrowings in coronary arteries- 7.9% (15 patients).

47.6% of patients had patent infarct-related artery (TIMI 2-3).

62.8% of patients did not have history of previous myocardial infarction.

Table I. *Study group characteristics part 1*

Tabela I. *Charakterystyka badanej grupy część 1*

Parameter	n	%	Mean
Females/ <i>Kobiety</i>	63	32.9	—
Males/ <i>Mężczyźni</i>	128	67.0	—
Females age/ <i>Wiek Kobiet</i>	63	—	68.72
Males age/ <i>Wiek Mężczyzn</i>	128	—	65.29
Diabetes mellitus/ <i>Cukrzyca</i>	55	28.70	—
Hypertension/ <i>Nadciśnienie tętnicze</i>	141	73.8	—
Hypercholesterolemia/ <i>Hipercholesterolemia</i>	102	53.4	—
Smoking/ <i>Palenie tytoniu</i>	74	38.7	—
Mean chest pain duration (h)/ <i>Średni czas trwania bólu (h)</i>	4.25	—	—
Previous myocardial infarction/ <i>Przebyty zawał serca</i>	71	37.2	—
Troponin I/ <i>Troponina I</i>	168	—	10.12
CPK	117	—	897.99
CK-MB	180	—	119.04
Cholesterol/ <i>Cholesterol</i>	175	—	198.79
LDL	169	—	124.51
HDL	174	—	52.66
WBC	191	—	9.83
Creatinine/ <i>Kreatynina</i>	191	—	1.38
LVEF (%)	169	—	45.47
WMSI	175	—	1.46

In 22% of patients with NSTEMI the infarct-related artery and the artery that underwent angioplasty was left anterior descending artery, in 22.5% it was right coronary artery, in 14.2% left circumflex artery and in 11.8% of patients obtuse marginal branch (Table II). In 91.5% of all percutaneous interventions stent was implanted.

Table II. *Study group characteristics part 2*

Tabela II. *Charakterystyka badanej grupy część 2*

Parameter	n	%	Entire group
Number of coronary arteries with stenosis >50% <i>Liczba naczyń wieńcowych ze stenozą &gt; 50%</i>	0	15	7.85
	1	52	27.23
	2	68	35.60
	3	56	29.32
Patency of coronary arteries/ <i>Drożność tętnic wieńcowych</i>			
Patent artery/ <i>Tętnica drożna</i>	91	47.64	n=191
Non-patent artery/ <i>Tętnica niedrożna</i>	100	52.34	
Dilated artery/ <i>Tętnica poszerzana</i>			n=204
LAD	45	22.05	
LCx	29	14.21	
RCA	46	22.54	
IM	3	1.47	
OM 1-3	24	11.76	
D 1-3	7	3.43	
Number of stents implanted/ <i>Ilość wszczepionych stentów</i>			n=191
0	50	26.18	
1	129	67.42	
2	11	7.70	
3	1	0.52	

Patients with 1-vessel disease, multivessel disease and without significant narrowings in coronary arteries.

In comparison with patients with 1-vesel disease (27.2%) and patients without significant narrowings in coronary arteries (7.9%), patients with multivessel disease were significantly more often diabetic. Diabetic patients also significantly more often than other patients had history of previous myocardial infarction. No differences could be found in concentration of myocardial necrosis markers between the group of patients with 1-vessel disease and the group of patients with multivessel disease. In the group of patients in whom myocardial infarction developed in the absence of significant narrowings in coronary arteries serum levels of necrosis markers were significantly lower than in patients from the other groups (Troponin I 1.07 vs 4.06,  $p=0.0301$ ; CPK 94 vs 419,  $p=0.0023$ ; CK-MB 36.5 vs 60.5,  $p=0.0067$ ).

Left ventricular ejection fraction (LVEF) value was considerably higher and wall motion score index (WMSI) was substantially lower in patients without significant narrowings in coronary arteries (53.3% and 1.35) and in patients with 1-vessel disease (48.3% and 1.39) than in patients with multivessel disease (43.6% and 1.51) (Table III, Figure 1-2).

Table III. LVEF and WMSI in patients without significant narrowings in coronary arteries, with 1-vessel disease and multivessel disease

Tabela III. LVEF i WMSI u chorych bez istotnych zwężeń w tętnicach wieńcowych, z chorobą 1- naczyniową i wielonaczyniową

LVEF/WMSI		N	Mean	SD	p	p
<b>LVEF (%) in patients/ u chorych:</b>						
without significant narrowings in coronary arteries <i>bez istotnych zwężeń w tętnicach wieńcowych</i>		14	53.29	17.13040		<b>0.0058</b>
with 1-vessel disease <i>z chorobą 1-naczyniową</i>		39	48.28	8.52843	<b>0.00949</b>	
with multivessel disease <i>z chorobą wielonaczyniową</i>		116	43.58	9.38653		
<b>WMSI in patients/ u chorych:</b>						
without significant narrowings in coronary arteries <i>bez istotnych zwężeń w tętnicach wieńcowych</i>		15	1.35	0.303926		<b>0.0099</b>
with 1-vessel disease <i>z chorobą 1-naczyniową</i>		49	1.39	0.289520	<b>0.01810</b>	
with multivessel disease <i>z chorobą wielonaczyniową</i>		111	1.51	0.316923		

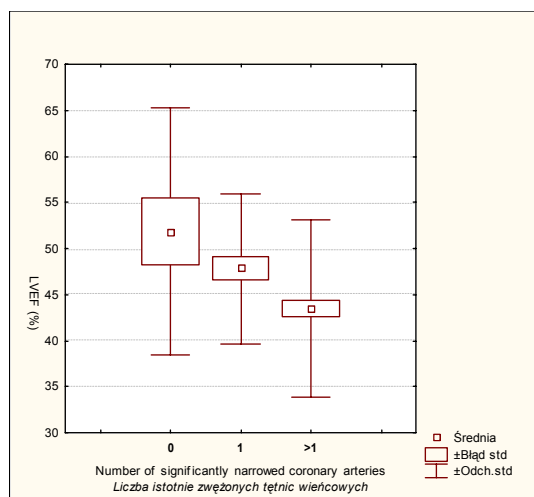


Fig. 1. LVEF in patients without significant narrowings in coronary arteries, with 1-vessel disease and multivessel disease

Ryc. 1. LVEF u chorych bez istotnych zwężeń w tętnicach wieńcowych, z chorobą 1-naczyniową i wielonaczyniową

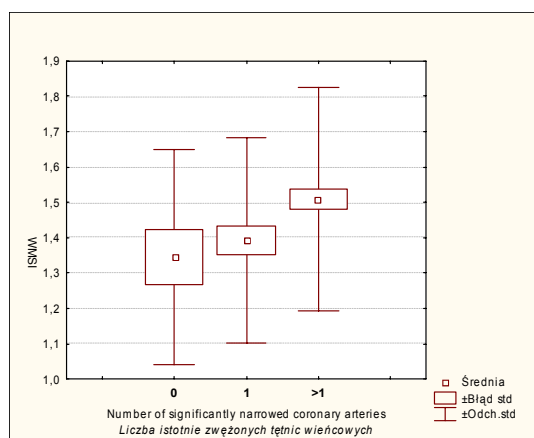


Fig. 2. WMSI in patients without significant narrowings in coronary arteries, with 1-vessel disease and multivessel disease

Ryc. 2. WMSI u chorych bez istotnych zwężeń w tętnicach wieńcowych, z chorobą 1-naczyniową i wielonaczyniową

In the group of patients with LVEF in the range of 20-40%, percentage of patients with multivessel disease was 82.1, and only 14.3% had 1-vessel disease and 3.6% of patients did not have significant narrowings in coronary arteries. In the group of patients with the highest WMSI 1.6-2.6 prevailed patients with multivessel disease- 77.3%, patients with 1-vessel disease comprised 18.2% and 4.5% were patients without significant narrowings in coronary arteries.



Patients with patent and non-patent infarct-related artery. Groups of patients divided in this way (patent artery 47.6%, non-patent artery 52.4%) differed significantly while considering concentration of myocardial necrosis markers, blood leukocyte count, LVEF (50 vs 45%,  $p=0.000045$ ) and WMSI (1.32 vs 1.47,  $p=0.002475$ ) and it was irrespective of the number of narrowed coronary arteries (Figure 3-4).

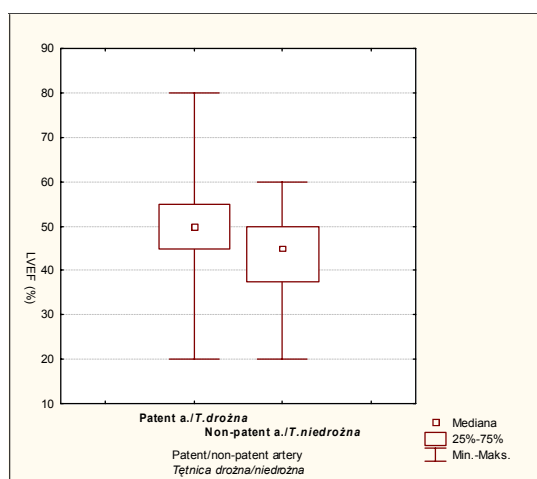


Fig. 3. LVEF in patients with patent and non-patent infarct-related artery

Ryc. 3. LVEF u chorych z tętnicą odpowiedzialną za zawał drożną i niedrożną

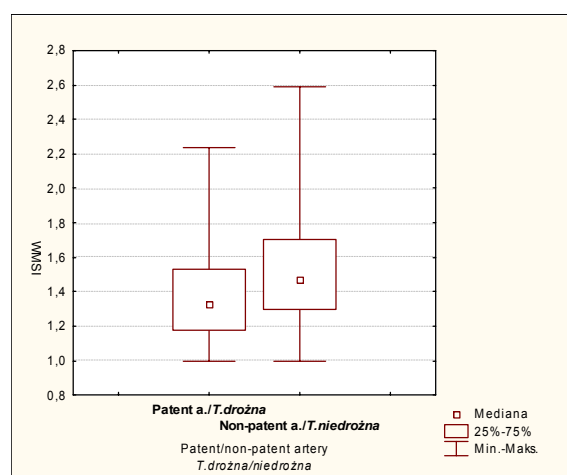


Fig. 4. WMSI in patients with patent and non-patent infarct-related artery

Ryc. 4. WMSI u chorych z tętnicą odpowiedzialną za zawał drożną i niedrożną

Patients with and without history of previous myocardial infarction. No significant differences could be found in concentration of myocardial necrosis markers

between these two groups. However, significant differences were observed in LVEF (48.3% vs 40.2%,  $p=0.000001$ ), WMSI (1.38 vs 1.63,  $p=0.0000001$ ) and in leukocyte count (9.27 vs 8.6,  $p=0.0177$ ) (Figure 5-6).

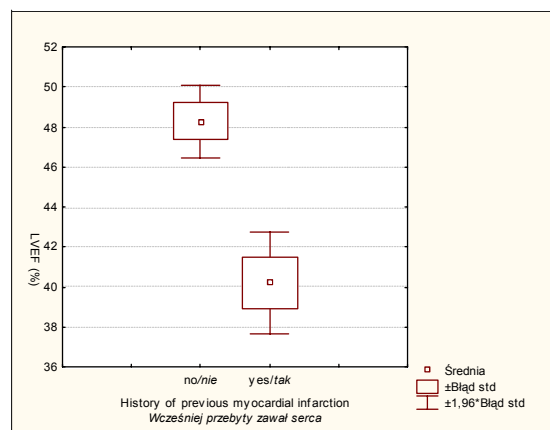


Fig. 5. LVEF in patients without and with history of previous myocardial infarction

Ryc. 5. LVEF u chorych bez i z wcześniej przeżytym zawałem serca

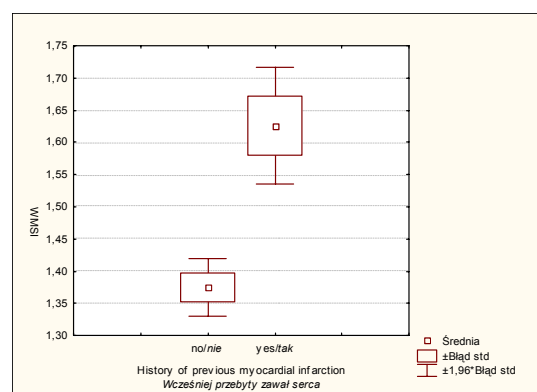


Fig. 6. WMSI in patients without and with history of previous myocardial infarction

Ryc. 6. WMSI u chorych bez i z wcześniej przeżytym zawałem serca

No significant differences could be found in all of biochemical, echocardiographic and angiographic parameters analysed between group of women and men.

## DISCUSSION

It is well recognised that patients presenting with NSTEMI are patients with more advanced coronary disease and worse long-term prognosis than patients

with STEMI. It is a result of the fact that multivessel disease is more often present in these patients and thus it is more difficult to identify infarct-related artery [13,14,15]. Time of coronary intervention is delayed while compared with patients with STEMI due to lack of typical for myocardial infarction electrocardiographic changes and also due to the usually prolonged waiting time for Troponin I assay needed to allow identification of myocardial infarction, which is at least 6 hours. In our study we sought to characterise a group of patients hospitalised with a diagnosis of non-ST-segment elevation myocardial infarction. In 2005, 1667 patients diagnosed with myocardial infarction were hospitalised in the Cardiology Clinic. In this group 20.9% were patients with NSTEMI. Mean age of this group was 67 years. Women accounted for 30% of all patients and they were not significantly older than men (by an average of 3 years). Regarding the percentage of women with myocardial infarction, our finding is in accordance with other studies [16, 17, 18] although women are usually significantly older than men. Different data from our study may be associated with a relatively small-sized group of patients considered.

The rate of classical risk factors in the study group reflects world's statistics [16, 17, 18].

In our group, patients with NSTEMI and with 2- or 3-vessel disease comprised the great majority- 64.9% (124 patients). Patients with 1-vessel disease comprised 27.2% and in 7.9% of patients myocardial infarction developed in the absence of significant narrowings in coronary arteries. It is known that in patients with multivessel disease, myocardial infarction often develops in the presence of narrowed but patent coronary arteries [19]. In our group, among patients with multivessel disease 32% (40 patients) had patent infarct-related artery (TIMI 2-3). In this case, when all coronary arteries remain patent, the artery chosen for coronary intervention is the most tightly narrowed one- the one which may correspond with ST depression observed in ECG, or the one with picture of atherosclerotic plaque rupture or with visible thrombus. Therefore, in the case of our patients the decision regarding dilated artery was made by interventional cardiologist on the grounds of angiographic picture. Echocardiographic parameters assessed in the study- LVEF and WMSI showed statistically significant dependence on the patency of the infarct related artery, the extent of coronary artery disease and on the fact whether patient

had previous myocardial infarction. These facts are obvious and no comment is required. It is also obvious that levels of myocardial necrosis markers were significantly higher in the group of patients with non-patent infarct-related artery in relation to patients with patent artery. No difference was observed in levels of myocardial necrosis markers between patients with 1-vessel and multivessel disease but such a difference existed in relation to LVEF and WMSI. Levels of myocardial necrosis markers (TnI and CK-MB) as well as LVEF, differing in sensitivity, determine indirectly the magnitude of myocardial necrosis [20]. Our result confirms that echocardiographic examination is a more sensitive method than biochemical analysis in the assessment of the extent of changes in coronary arteries but it is less sensitive in the assessment of the magnitude of myocardial necrosis area. The reason may be due to the fact that both necrosis as well as segmental hibernation of myocardium resulting from multivessel coronary disease contribute to the decrease in LVEF and the increase in WMSI. However, levels of myocardial necrosis markers as well as LVEF and WMSI differed significantly between the group of patients in whom myocardial infarction developed in the absence of significantly narrowed coronary arteries and the other group of patients. In the former patients hibernating areas of myocardium have no justification, which in fact can be used to support the previously mentioned situation. Echocardiographic parameters also showed significant difference between the group of patients without or with history of previous myocardial infarction, both groups lacking differences statistically significant with respect to markers of myocardial necrosis.

Despite the fact that many studies showed women with NSTEMI have coronary artery disease less advanced than men [19], our study remains in disagreement over this issue.

WMSI is regarded by some researchers to be a better indicator assessing left ventricular contractility than LVEF because in some patients with extensive wall motion abnormalities LVEF is relatively preserved due to compensatory regional hyperkinesis, and the WMSI estimation eliminates this kind of mistake [8]. In our study we did not prove superiority of this parameter over LVEF. All of statistically significant differences were related to both of parameters.

Currently, decision regarding diagnostics and invasive treatment is made on the grounds of biochemical markers and clinical presentation. If echocardiographic examination does not delay the decision, the results of our study indicate that echocardiographic examination performed before coronary intervention can be useful in a decision making process regarding invasive treatment.

## CONCLUSIONS

1. The extent of changes in coronary arteries and the patency of the infarct-related artery before vessel intervention (PCI) have significant influence on LVEF and WMSI in patients with NSTEMI.
2. In patients with NSTEMI equally often infarct-related artery is RCA, LAD and LCx.

## REFERENCES

1. Shishehbor M.H., Lauer M.S., Singh I.M., et al.: In Unstable Angina or Non-ST-Segment Acute Coronary Syndrome, Should Patients With Multivessel Coronary Artery Disease Undergo Multivessel or Culprit-Only Stenting? *J Am Coll Cardiol* 2007; 49: 849–54.
2. Hirsch A., Windhausen F., Tijssen J.G.P., et al.: Invasive versus Conservative Treatment in Unstable coronary Syndromes (ICTUS) investigators. Long-term outcome after an early invasive versus selective invasive treatment strategy in patients with non-ST-elevation acute coronary syndrome and elevated cardiac troponin T. (the ICTUS trial): a follow-up study. *Lancet* 2007; 369: 827–35.
3. Eng Wei Tang, Cheuk-Kit Wong, Herbison P.: Global Registry of Acute Coronary Events (GRACE) hospital discharge risk score accurately predicts long-term mortality post acute coronary syndrome. *Am Heart J* 2007; 153: 29–35.
4. Antman E.M., Cohen M., Bernink P.J., et al.: The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making. *JAMA* 2000; 284: 835–42.
5. Boersma E., Pieper K.S., Steyerberg E.W., et al.: Predictors of outcome in patients with acute coronary syndromes without persistent ST-segment elevation: results from an international trial of 9,461 patients. The PURSUIT Investigators. *Circulation* 2000; 101: 2557–67.
6. Armstrong P.W., Fu Y., Chang W-C., et al.: Acute coronary syndromes in the GUSTO-IIb trial: prognostic insights and impact of recurrent ischemia. The GUSTO-IIb Investigators. *Circulation* 1998; 98: 1860–8.
7. Bosch X., The'roux P.: Left ventricular ejection fraction to predict early mortality in patients with non-ST-segment elevation acute coronary syndromes. *Am Heart J* 2005; 150: 215–20.
8. Moller J.E., Hillis G.S., Oh J.K., et al.: Wall motion score index and ejection fraction for risk stratification after acute myocardial infarction. *Am Heart J* 2006; 151: 419–25.
9. Moise A., Clement B., Saltiel J.: Clinical and angiographic correlates and prognostic significance of the coronary extent score. *Am J Cardiol* 1988; 61: 1255–1259.
10. Brilakis E.S., Wright R.S., Kopecky S.L., et al.: Association of the PURSUIT risk score with predischARGE ejection fraction, angiographic severity of coronary artery disease, and mortality in a nonselected, community-based population with non-ST-elevation acute myocardial infarction. *Am Heart J* 2003; 146: 811–8.
11. Garcia S., Canoniero M., Peter A., et al.: Correlation of TIMI Risk Score With Angiographic Severity and Extent of Coronary Artery Disease in Patients With Non-ST-Elevation Acute Coronary Syndromes. *Am J Cardiol* 2004;93: 813–816.
12. Cerqueira M.D., Weissman N.J., Dilsizian V., et al.: Standardized Myocardial Segmentation and Nomenclature for Tomographic Imaging of the Heart A Statement for Healthcare Professionals From the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. *Circulation* 2002; 105: 539–542.
13. Fuster V., Moreno P.R., Fayad Z.A., et al.: Atherothrombosis and high-risk plaque part I: evolving concepts. *J Am Coll Cardiol* 2005; 46: 937–54.
14. Bhatt D.L. Diffuse coronary disease and atherothrombosis: a rationale for long-term therapy to prevent recurrent ischemic events. *J Invasive Cardiol* 2003; 15 Suppl B: 3B–9B; discussion 9B–10B.
15. Topol E.J., Nissen S.E. Our preoccupation with coronary luminology. The dissociation between clinical and angiographic findings in ischemic heart disease. *Circulation* 1995; 92: 2333–42.
16. Heer T., Gitt A.K., Juenger C., et al.: Gender Differences in Acute Non-ST-Segment Elevation Myocardial Infarction. *Am J Cardiol* 2006; 98: 160–166.
17. Zairis M.N., Lyras A.G., Bibis G.P., et al.: Association of inflammatory biomarkers and cardiac troponin I with multifocal activation of coronary artery tree in the setting of non-ST-elevation acute myocardial infarction. *Atherosclerosis* 2005; 182: 161–167.
18. Patel M.R., Chen A.Y., Peterson E.D., et al.: Prevalence, predictors, and outcomes of patients with non-ST-segment elevation myocardial infarction and insignificant coronary artery disease: Results from the Can Rapid risk stratification of Unstable angina patients Suppress Adverse outcomes with Early implementation of the ACC/AHA Guidelines (CRUSADE) initiative. *Am Heart J* 2006; 152: 641–647.
19. A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction): ACC/AHA 2007 Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocar-

- dial Infarction- Executive Summary. JACC, 2007; Vol. 50, No. 7: 652-726.
20. Apple F.S, Sharkey S.W., Falahati A., et al.: Assessment of left ventricular function using serum cardiac troponin I measurements following myocardial infarction. Clinica Chimica Acta 1998; 272: 59-67.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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**A COMPARISON OF THE EFFECTIVENESS OF CLASSICAL MASSAGE  
AND MANUAL TRACTION IN THE TREATMENT OF CERVICAL DISC HERNIATION**

**PORÓWNANIE SKUTECZNOŚCI MASAŻU KLASYCZNEGO I WYCIĄGÓW RĘCZNYCH  
W LECZENIU DYSKOPATII KRĘGOSŁUPA SZYJNEGO**

Chair and Department of Manual Therapy, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz

head: Maciej Dzierżanowski MD, PhD

**S u m m a r y**

**I n t r o d u c t i o n .** Spinal disc herniation – a disease which results from the wear out of the intervertebral disc in which the gelatinous nucleus bulges out through a cracked or torn fibrous ring and can exert pressure on the spinal cord or root.

**T h e a i m** of the study is to compare the effectiveness of massage and traction in the therapy of cervical spine herniation and to evaluate the mobility of the cervical spine.

**M a t e r i a l a n d m e t h o d s .** The study was carried out among 30 patients with symptoms of cervical disc herniation. Half of the patients were treated using classical mas-

sage only and the second half using traction. The mobility of the cervical spine was measured before and after the treatment. VAS pain scale was also measured.

**C o n c l u s i o n .** Both types of treatment have a positive therapeutic effect but it is faster in the case of traction. The pain relief effect is also faster in the case of traction. The therapy lessens excess muscular tension which in consequence increases the mobility of the cervical spine. Downward head compression was observed only in several patients.

**S t r e s z c z e n i e**

**W s t ę p .** Dyskopatia to choroba uwarunkowana zużyciem krążka międzykręgowego, w którym jądro galaretowate przedziera się przez pęknięty lub rozerwany pierścień włóknisty i może uciskać korzeń lub rdzeń kręgowy.

**C e l e m b a d a ń** było porównanie skuteczności masażu z trakcjami w leczeniu dyskopatii kręgosłupa szyjnego oraz sprawdzenie zakresu ruchomości odcinka szyjnego

**M a t e r i a ł i m e t o d y .** Badaniem objęto 30 osób z objawami dyskopatii szyjnej. Połowa z nich poddana została tylko i wyłącznie zabiegowi masażu klasycznego, a druga

połowa trakcjom. Zbadano zakres ruchomości odcinka szyjnego przed terapią oraz po jej zakończeniu. Oceniono także skalę bólu VAS.

**W n i o s k i .** Oba zabiegi przynoszą pozytywny efekt terapeutyczny, jednak szybszy jest on w przypadku trakcji. Efekt przeciwbólowy jest szybszy również w przypadku trakcji. W wyniku terapii zmniejsza się nadmierne napięcie mięśniowe, a z tym wiąże się zwiększenie zakresu ruchomości odcinka szyjnego. Objaw szczytowy był obecny u niewielu pacjentów.

**Key words:** manual traction, cervical spine, classical massage, cervical discopathy

**Słowa kluczowe:** wyciąg ręczny (trakcja), kręgosłup szyjny, masaż klasyczny, dyskopatia szyjna

**INTRODUCTION**

The cervical part of the spine is very mobile and it is prone to destructive environmental and external factors that can influence the whole organism, sometimes lowering psychophysical capabilities. Most people's current lifestyle, in a great part sedentary, full of

physical and mental strain, improper way of spending free time, the lengthening of human lifespan which leads to the muscular system insufficiency and the aging of tissues along with an improper diet, all lead to the destruction of the cervical spine [10, 11, 12].

Spinal disc herniation – a disease which is conditioned by the wear out of the intervertebral disc in which the gelatinous nucleus bulges out through a cracked or torn fibrous ring and can exert pressure on the spinal cord or root.

It is a multistage process. It usually starts with a pain between the shoulder blades during the bend forward to anteflexion, which later changes into a nape and head pain, often causing buzzing in the ears. The mobility of the cervical spine is limited.

The pain can radiate to the upper extremity. Pain ailments mostly originate in the lower dermatomes: C5-C6, C6-C7, C7-Th1. Traction, along with massage, is a method of relieving the symptoms of spinal disc herniation. Traction is also an element of the control of the joints. It causes a better irrigation of the disc and the phenomenon of the repositioning of the nucleus pulposus.

Unlike massage it consists of stretching the soft tissues and widening the joint gap. The aim of the study is to compare the efficiency of massage and traction in the treatment of spinal disc herniation and to evaluate the mobility of the cervical spine.

## MATERIAL AND METHODS

The study was carried out among a group of 30, aged from 22 to 60 years, patients of the Reh-Med Rehabilitation and Rheumatology Outpatient Clinic in Bydgoszcz. All of the patients were diagnosed with spinal disc herniation. The patients were split into two groups, 15 patients in each one. The first group was treated with traction and the second with massage. Both treatments were carried out daily.

The following ranges of cervical spine motion were tested basing on the methodology of spine mobility tests described by Rosławski and Skolimowski:

- bending forward and straightening backwards,
- bending sideways, left and right,
- head rotation, left and right.

The results were compared to existing norms [9]. Additionally, a test of the subjective feeling of pain based on the VAS pain scale and a test for downward head compression were also carried out. The VAS scale has 10 grades and the patient uses it to grade his/her own pain. Downward head compression checks if the patient does not suffer from narrowed intervertebral foramen or brachialgia and is characterized by negative sensations like pain or paresthesia of the cervical spine [14,15]. Those tests were carried out in both

patient groups. The results are shown on comparative charts.

Traction treatment starts with a traction test which qualifies or disqualifies a patient from the treatment. Next two types of traction are applied to the aching part of the cervical spine. Both types are repeated several times. The traction test is carried out with the patient sitting or lying down.

## RESULTS

### Mobility

In case of bending forward, the motion range is different after massage and after traction. In the case of massage the motion range increased only in 20% of the patients, and traction showed an increase in 78% of the cases. Other results are comparable with an advantage for traction.

Bending backwards shows similar results. Massage provided an increase for 80% of the patients and traction for 87%. In the case of turning the head right the results were: 73% for massage and 87% for traction. Turning right proves to be better in the case of patients treated with massage (93%) than traction (87%). Lateral bend right was better in 100% of patients in both groups. Lateral bend right proved better for 100% of the patients treated with traction and 67% with massage. The results are shown in the charts [fig. 1-7].

As for a statistical analysis of the motions (fig. 9) the following movements show a similar therapeutic value in the case of traction and massage: head and neck bending backwards, turning right and left.

Figure 8, in a comparison of both treatments regarding mobility, shows a clear boundary between the change of mobility for massage and traction during bending forward which is lower in the case of turning right and a lateral bend left.

There is no significant difference between bending backwards and turning left.

### Downward head compression

Present for 27% of patients after massage and 13% of patients after traction. Subsided after therapy.

### VAS pain scale

Lowered for both groups of patients – a 100% lowering for traction and 93% for massage.

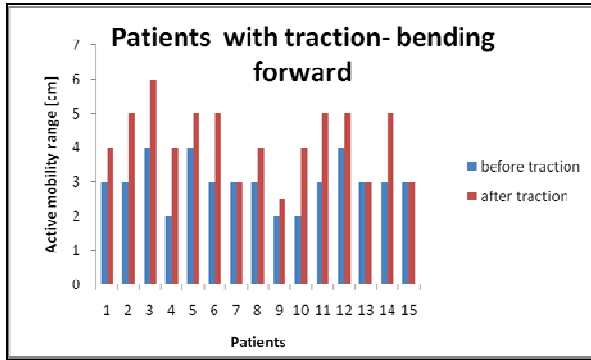


Fig. 1. Active mobility range for patients treated with traction – bending forward [cm]

Ryc. 1. Zakres ruchomości czynnej pacjentów poddanych trakcji podczas skłonu w przód [cm]

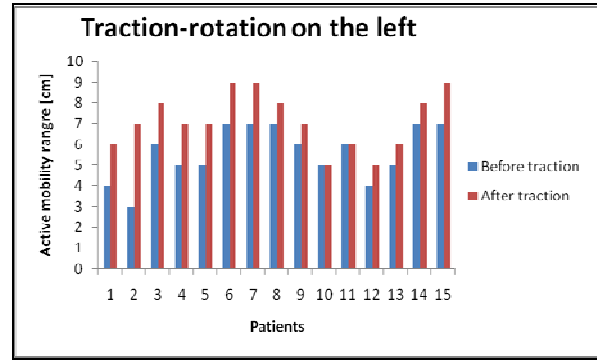


Fig. 4. Active mobility range for head left rotation for patients treated with traction [cm]

Ryc. 4. Zakres ruchomości czynnej trakcji podczas skrętu w lewo [cm]

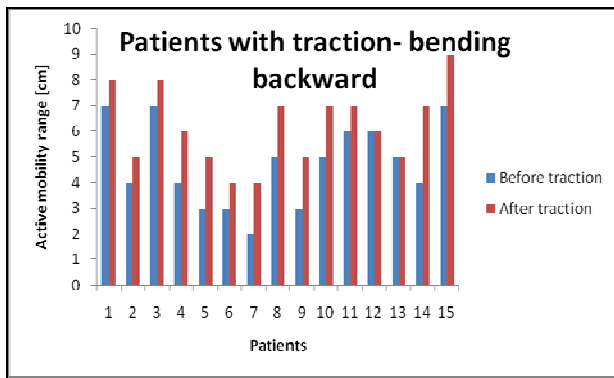


Fig. 2. Active mobility range for patients treated with traction – bending backward [cm]

Ryc. 2. Zakres ruchomości czynnej pacjentów poddanych trakcji podczas skłonu w tył [cm]

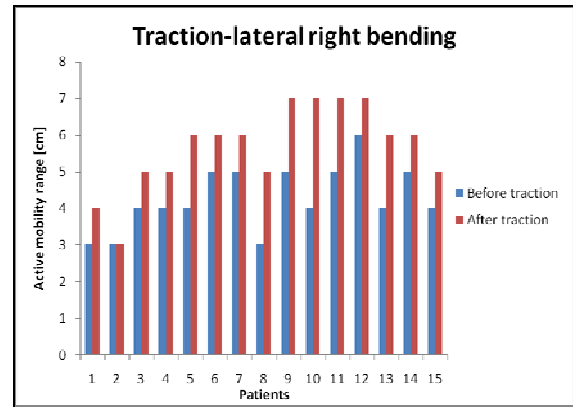


Fig. 5. Active mobility range for lateral right bend for patients treated with traction [cm]

Ryc. 5. Zakres ruchomości czynnej pacjentów poddanych trakcji podczas skłonu bocznego w prawo [cm]

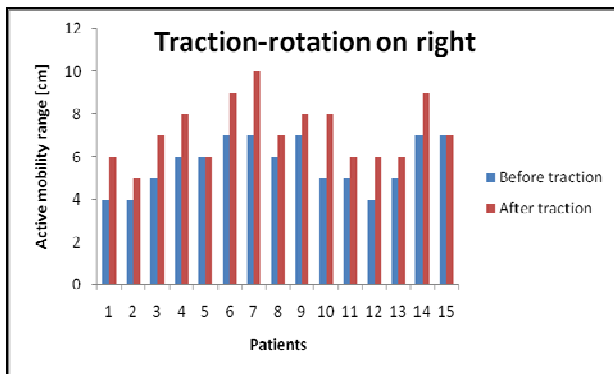


Fig. 3. Active mobility range for head right rotation for patients treated with traction [cm]

Ryc. 3. Zakres ruchomości czynnej pacjentów poddanych trakcji podczas skrętu w prawo [cm]

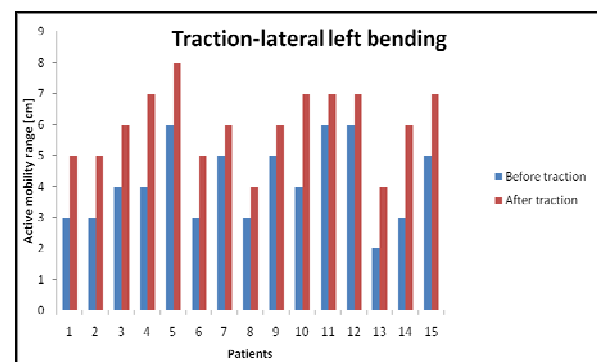


Fig. 6. Active mobility range for lateral left bend for patients treated with traction [cm]

Ryc. 6. Zakres ruchomości czynnej pacjentów poddanych trakcji podczas skłonu bocznego w lewo [cm]

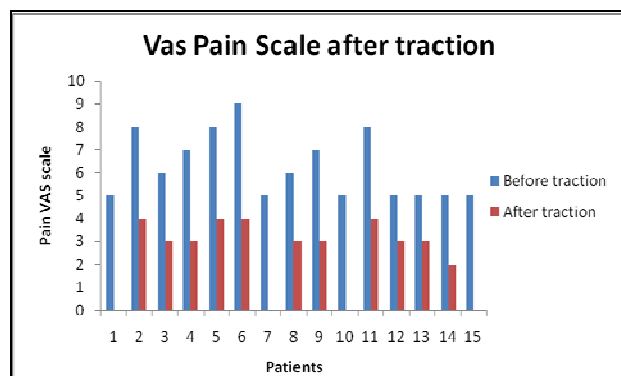


Fig. 7. Patients' pain in VAS scale before and after traction

Ryc. 7. Skala VAS pacjentów przed i po zabiegu trakcji

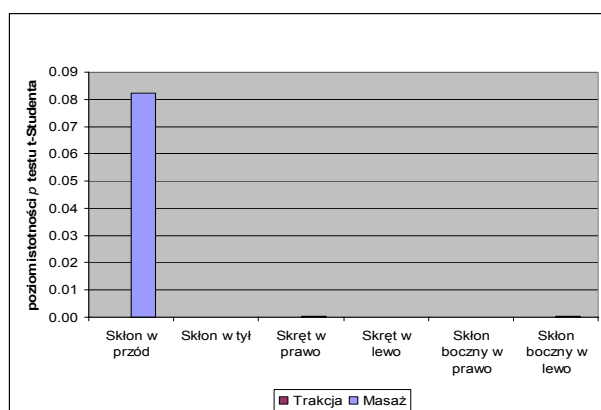


Fig. 8. Statistical analysis showing the influence of massage and traction on mobility change

Ryc. 8. Analiza statystyczna pokazująca wpływ masażu i trakcji na zmianę ruchomości

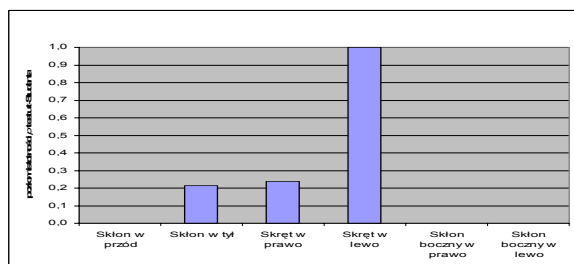


Fig. 9. Statistical analysis of the efficacy of massage and traction

Ryc. 9. Analiza statystyczna skuteczności masażu i trakcji

## DISCUSSION

The presented results show that traction is superior to classical massage when it comes to relieving pain linked with spinal disc herniation. Traction create such an arrangement of the mobile segment that allows the mobilizing force to be aimed at a given joint or muscle, not the whole cervical part of the spine, as it is in the case of classical massage. It is a painless treatment

which relieves ailments by taking the weight off the facet joints, lowering the sensitivity of muscle-joint proprioceptors, lowering muscle tension, improving blood circulation and most importantly, relieving pain [3,4,7].

Study results also show increased cervical mobility on all planes, even the limited ones like head and neck bending and lateral bends. The latter showed 100% improvement. The VAS scale pain was also lowered and downward head compression vanished.

However it must be said that the treatment causes only a temporary unblocking of the facet joints. A permanent unblocking is impossible due to the destroyed facet disc and a permanent coming together of the neighboring joints [5,6,13]. However a significant positive aspect of this treatment is the fact that it brings immediate relief to the patient, even after the first session. Manual traction is better than mechanical traction (Glisson's loop) because it affects the chin and not the occiput, which causes contraction of the scalene and deep muscles of the neck, which in turn shatters the effects of traction. This is why the best choice is manual traction, which allows for a quick change of the pressure exerted on a given part of the spine. The treatments decreased pain and increased psychophysiological efficacy [2,13].

Traction treatment also supports the bolting of the mobile segment of the spine. It must be pointed out that traction is recommended as a treatment for every kind of joint injury as it is a risk-free form of treatment. Patients treated with massage also feel relief but it happens much later, sometimes even after the end of therapy. Mobility of the cervical spine is also improved but the improvement is not as visible as with traction. Massage leads to a loosening of tense ligaments and muscles, especially the trapezius and levator scapulae muscle. Muscle tension is normalized, then an outflow of lymph and the hyperemic effect occur. Both types of treatment have a positive effect on patients but if the same effect can be reached faster, the more effective method should be used.

## CONCLUSIONS

1. Manual tractions are a method which is more effective than massage when it comes to elimination or lessening of pain resulting from cervical disc herniation.
2. Both treatments cause a lessening of excess muscular tension which leads to an increase of the motion



range of the cervical spine.

3. Downward head compression, observed in the case of only several patients, disappeared after traction treatment.
4. The prognosis is better in the cases of patients treated with the use of manual traction.

#### REFERENCES

1. Dziak A.: Bóle i dysfunkcje kręgosłupa. Medicina Sportiva, Kraków 2007.
2. Rakowski A.: Kręgosłup w stresie. Gdańskie Wydawnictwo Psychologiczne, Gdańsk 2002.
3. Frisch H., Roex. J.: Terapia manualna. Poradnik wykonywania ćwiczeń. PZWL, Warszawa 2005.
4. Kaltenborn F.: Kręgosłup, badanie manualne i mobilizacja. Wydawnictwo Rolewski. Kielce 1998.
5. Lewit K.: Leczenie manualne zaburzeń czynności narządu ruchu. PZWL, Warszawa 1983.
6. Frisch H.: Programierte Untersuchung des Bewegungsapparates, Chirodiagnostik. Springer-Verlag, Heidelberg 1983.
7. Neumann H.D.: Manuelle Medizin-ein Einführung in Theorie, Diagnostik-und Therapie, Berlin 1983.
8. Rosłowski A., Skolimowski T.: Badania czynnościowe w kinezyterapii. AWF, Wrocław 1995.
9. Kiwerski J i wsp.: Operacyjne leczenie przepukliny krążka międzykręgowego u chorych w młodym wieku. Nowa Medycyna 8, 13-15, 1997.
10. Kwolek A.: Wybrane zagadnienia z budowy części szyjnej kręgosłupa. Postępy Rehabilitacji 1997, XI, 2.
11. Stodolny J.: Jak chronić swój kręgosłup. ZL NATURA, Kielce 1997.

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**THERAPEUTIC TEAM AND EFFECTIVE MANAGEMENT AS DETERMINANTS  
OF PATIENT CARE IN A HOSPITAL**

**ZESPÓŁ TERAPEUTYCZNY A EFEKTYWNE KIEROWANIE  
JAKO DETERMINANTY OPIEKI NAD CHORYM W SZPITALU**

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**S u m m a r y**

The transformations of the system of health services in Poland create the need for the improvement of the methods of managing staff teams in order to provide optimum results in patient care. Teamwork is an important element of the improvement of the quality of patient care, therefore, studies were undertaken to investigate the functioning of staff teams in Polish hospital wards.

**M e t h o d .** A diagnostic survey was conducted during the period 1999-2001 among 560 physicians and nurses in 29 wards at 4 hospitals with accreditation and 56 wards at 17 health care units without the certificate.

**R e s u l t s .** The study showed that, therapeutic teams which are more effectively managed by the heads/managers

of clinics are present significantly more often in Polish hospitals with accreditation

**C o n c l u s i o n .** The preparation of a hospital for accreditation in order to obtain the quality certificate provides incentives for the staff to work collectively. Managing a therapeutic team in hospitals with the certificate is also more effective, and preconditions a higher level of patient care. Polish physicians and nurses from hospitals with accreditation certificate appreciate the effectiveness of teamwork to a greater extent than the staff of hospitals without the certificate. In hospitals possessing accreditation the effectiveness of managing a team was evaluated in significantly more positive terms.

**S t r e s z c z e n i e**

**T ł o .** Przekształcenia systemu ochrony zdrowia w Polsce powodują potrzebę doskonalenia metod kierowania zespołami pracowniczymi w celu zapewnienia optymalnych wyników opieki nad chorym. W kryteriach akredytacyjnych dla polskich szpitali praca zespołowa jest traktowana jako istotny element poprawy jakości opieki nad chorym. Postanowiono zatem zbadać funkcjonowanie zespołów pracowniczych w polskich oddziałach szpitalnych pod kątem efektywnej współpracy.

**M e t o d y .** Badania przeprowadzono w latach 1999 – 2001 wśród 560 lekarzy i pielęgniarek w 29 oddziałach 4 szpitali z akredytacją (losowo wybranych) i 56 oddziałach w 17 zakładach opieki zdrowotnej bez certyfikatu (losowo wybranych). Metodą badań był sondaż diagnostyczny. Otrzymany materiał poddano analizie statystycznej za pomo-

cą testów:  $\chi^2$  - (chi kwadrat) na jednorodność, testu  $u$  dla różnicy procentów,  $\chi^2$  - (chi kwadrat) na zgodność oraz  $V^2$  - *Cramera*.

**W y n i k i .** Analiza materiału badawczego pozwala stwierdzić, że wśród polskich lekarzy i pielęgniarek w szpitalach z akredytacją istotnie częściej istnieją zespoły terapeutyczne, które są efektywniej kierowane przez ordynatorów/kierowników klinik.

**W n i o s k i .** Przygotowanie szpitala do akredytacji w celu uzyskania certyfikatu jakości wpływa motywująco na pracowników aby pracowali zespołowo. Kierowanie zespołem terapeutycznym w szpitalach z certyfikatem jest również efektywniejsze co warunkuje wyższy poziom opieki nad chorym.

**Key words:** therapeutic team, managing therapeutic team, quality of patient care in a hospital, hospital with quality certificate and without quality certificate

**Słowa kluczowe:** zespół terapeutyczny, kierowanie zespołem terapeutycznym, jakość opieki nad chorym w szpitalu, szpitale z certyfikatem jakości i bez akredytacji

## INTRODUCTION

The improvement of the system of health care in Poland delineates for the management staff the directions for optimising the management of health care units, in order to maintain themselves, develop and provide good quality care. In Poland, despite the 20-year period of health care reform, the pace of development in the direction of providing high quality care is still not satisfactory. In the improvement of management it is important to implement effective methods of work organization, for example, a team form of acting within a hospital.

Many authors define a therapeutic team as a group of people realizing together the process of care. The traits of a therapeutic team are as follows: common goal in acting and responsibility for its achievement, common determination of the scope of tasks, and associated with them individual duties and authorities, an efficient flow of information [1, 2, p.766].

In literature, it is emphasized that the members of a multi-disciplinary team, by joining their knowledge and skills, exchange experiences, thus increasing the potential possibilities of restoring efficiency of a patient. In addition, a therapeutic team, in working out a model of effective co-operation, inspires a patient to participate in the therapeutic process in order to provide the highest level of care [3, 4, 5].

The importance of the improvement of team work in a hospital was emphasized in the Target 29 formulated by the World Health Organization: 'hospitals in all Member States should be providing cost-efficient secondary and tertiary care contribute actively to improving health status and patient satisfaction'. This goal will be achieved if the provision of services is based on cooperation between the professional staff of health services [2, p.45].

In the Polish system of health care, in order to provide better quality care in 1997 under the Act on health units, the Centre for Quality Monitoring in Health Care was created. The main objective was to provide incentives for hospitals to become subjects to voluntary accreditation, and thus guarantee patient care on a level higher than to-date.

In accreditation criteria for Polish hospitals, teamwork is approached as an important element of the improvement of the quality of patient care. The Centre for Quality Monitoring in Health Care emphasizes the importance of teamwork: 'Patient care is a planned process aimed at the provision of an appropriate care adequate to the state of health. Care covers the totality of diagnostic, treatment, nursing and rehabilitation activities. The plan of care covers main actions which a multidisciplinary team intends to undertake for the realization of individual goals of care...' [7, p.53].

The process of effective management and integration of the team depends to a large degree on the leader of the therapeutic team, and his/her role in shaping relations in a desired way. In Polish medical literature, a physician – ward head/manager – is indicated as the leader of the therapeutic team who coordinates the work of a multidisciplinary group. A ward head is endowed, within official duties, with the management of work of the therapeutic team [8]. In Poland, a physician occupies a key position in a therapeutic team; however, all its members actively participate in patient care [9]. In order to attain this goal, team members must co-operate under the management of the ward head. An efficient management of a therapeutic team by a physician becomes especially important in the care of bed-ridden patients, because the multidisciplinary approach decreases the risk of complications and significantly affects the results of treatment. An effective management of a therapeutic team by a ward head is an element of an efficient organization of work in the ward, and conditions the optimisation of the quality of patient care. An effective management of a therapeutic team affects the degree of the usage of knowledge and experience, and exchange of information during the work meetings and consultations, when problems of patient care are brought to light, and a common direction of actions is determined [10, 11].

Considering the fact that accreditation in Poland is a certain *novum*, and only a part of hospitals participate in it, there arise many problems associated with the provision of high quality medical services through the improvement of the organization of work of a therapeutic team. The problems are as follows:

1. Is the functioning of individual employees in the ward perceived as teamwork?

2. How do the employees evaluate the effectiveness of managing a therapeutic team in hospitals with and without the quality certificate?

3. What is the scope of problems discussed during the meetings of a therapeutic team in hospitals with and without accreditation?

In order to provide a reply to the above-mentioned questions, a study was undertaken which covered 560 physicians and nurses at 21 Polish hospitals.

## MATERIAL AND METHODS

The research material was collected during the period from January 1999 to June 2001 at 21 hospitals. These were 4 hospitals with 29 wards possessing accreditation certificate in south-eastern Poland (Group A), and 56 wards at 17 hospitals without accreditation (Group B) in central and south-eastern Poland. The total number of 560 physicians and nurses participated in the study, including 55 ward heads/clinic managers, 72 head nurses, 106 assistant physicians and 327 charge nurses.

The study was conducted by the method of a diagnostic survey, and the technique was a specially designed questionnaire form containing open and closed questions. The following questions were directed to the physicians and nurses in the survey:

- Does the medical staff in the ward create a therapeutic team?
- Who manages the therapeutic team?
- What is the degree of the effectiveness of managing the therapeutic team?
- Are there in the ward common meetings and consultations of the therapeutic team?
- What problems are discussed during the meetings and debates of the therapeutic team?

The material obtained was subject to the statistical analysis by means of the following tests:  $\chi^2$  – chi-square test for uniformity, U-test for the difference in percentage,  $\chi^2$  – chi-square test for conformity, and Cramer's  $V^2$  test.

## RESULTS

Analysis of the collected research material allows the presumption that over 67% of respondents admit that people employed in their ward constitute a therapeutic team. Among the staff, in hospitals with accreditation (Group A) 86.4% of employees reported that

they work as a team, while in Group B – 56.8%. As many as 10.2% of respondents in Group A and almost twice that percentage in Group B – 25.7% had difficulties with providing an answer to this question (Tab. 1).

Tab. 1. *Functioning of a therapeutic team according to respondents' opinions (Groups A and B)*

No.	Presence of therapeutic team	Group A No. = 206		Group B No. = 354		Total No. = 560	
		No.	%	No.	%	No.	%
1.	Yes	178	86.4	201	56.8	379	67.8
2.	No	7	2.4	61	17.2	68	12.1
3.	Difficult to say	21	10.2	91	25.7	112	20.0
4.	Lack of reply	-	-	1	0.3	1	0.1
Total		206	100.0	354	100.0	560	100.0

Yes:  $u = 7.2$   $p < 0.001$   
 No:  $u = -4.8$   $p < 0.001$   
 Difficult to say:  $u = -4.4$   $p < 0.001$

While providing a reply to the question if, according to the respondents, the staff works as a team, it was interesting to discover whether any differences occurred among individual occupational groups - Fig. 1.

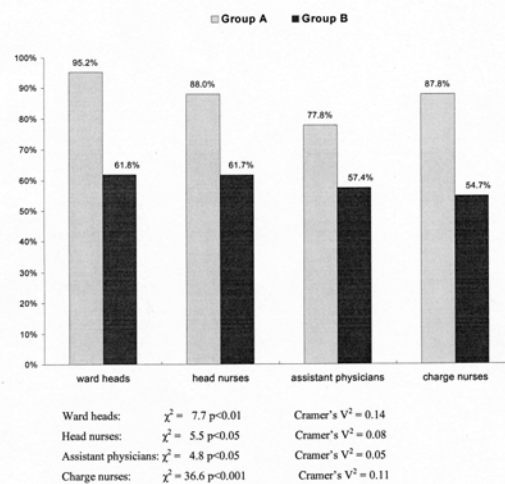


Fig. 1. *Presence of a therapeutic team and type of position held (Groups A and B)*

In Group A, ward head/clinic managers, head nurses, assistant physicians and charge nurses significantly more clearly emphasized the presence of therapeutic teams, compared to Group B (Fig. 1). This especially concerned ward heads/clinic managers from hospitals possessing accreditation (95.2%), compared to those without accreditation (61.8%), and head nurses (Group A – 88.0%; Group B – 61.7%).

Following the information from respondents concerning the presence of the therapeutic team, their opinions pertaining to the effectiveness of managing the therapeutic team was analysed. Ward heads and managers of clinics performed self-evaluation, which consisted in the selection of a specified score value (from 0-6) and marking this value on an axis. The largest number of very good evaluations (44.7%) – 5 scores, good evaluations (30.6%) – 4 scores, and excellent – 13.1% - 6 scores were noted in Group A. In Group B, the evaluation ‘good’ (25.7%) occupied the first position, followed by ‘very good’ (17.8%), and satisfactory (16.1%) – 3 scores (Tab. 2).

Tab. 2. Evaluation of the effectiveness of managing a therapeutic team by ward heads according to respondents' opinions (Groups A and B)

No.	Evaluation	Group A No. = 206		Group B No. = 354		Total No. = 560	
		No.	%	No.	%	No.	%
1.	6 scores	27	13.1	26	7.3	53	9.5
2.	5 scores	92	44.7	63	17.8	155	27.7
3.	4 scores	63	30.6	91	25.7	154	27.5
4.	3 scores	14	6.8	57	16.1	71	12.7
5.	2 scores	4	1.9	43	12.2	47	8.4
6.	1 scores	2	1.0	14	4.0	16	2.8
7.	0 scores	-	-	11	3.1	11	2.0
8.	Lack of evaluation	4	1.9	49	13.8	53	9.4
Total		206	100.0	354	100.0	560	100.0
6, 5, 4 scores: $u = 8.9$ $p < 0.001$							
3, 2, 1, 0 scores: $u = -6.7$ $p < 0.001$							
lack of evaluation: $u = -4.6$ $p < 0.001$							

Statistical analysis shows that physicians and nurses from hospitals with accreditation evaluated the effectiveness of managing therapeutic teams by ward heads in more positive terms; they reported significantly more very good, good and excellent evaluations  $\{U = 8.9$   $p < 0.001\}$ , compared to group B. Low evaluations concerning the managing of a therapeutic team were more often selected by respondents from Group B, which was statistically significant  $\{U = 6.7$   $p < 0.001\}$ . The lack of evaluation occurred significantly more frequently statistically in Group B  $\{U = -4.6$   $p < 0.001\}$ , compared to group A (Tab. 2).

Further analysis of the research material concerning the aspect of the effectiveness of managing a therapeutic team confirmed that ward heads/clinic managers from hospitals with the quality certificate considerably more often initiated meetings in group A (66.5%) than in Group B (36.7%), which was statistically significant on the level  $p < 0.001$ . The staff from hospitals without the certificate more frequently participated in meetings ‘sometimes’, compared to Group A – 35.0% and

17.5%, respectively ( $p < 0.001$ ). It was noted that the lack of meetings of the team dominated among the staff from hospitals without accreditation – 28.3%, compared to Group A – 16.0%. As a justification, some respondents mentioned the lack of time, no such habit in the ward, no initiative on the part of the ward head, poor organization of work in the ward, or they did not know the reason for the lack of common consultations (such a justification was most frequently reported by charge nurses, followed by assistant physicians) – Fig. 2.

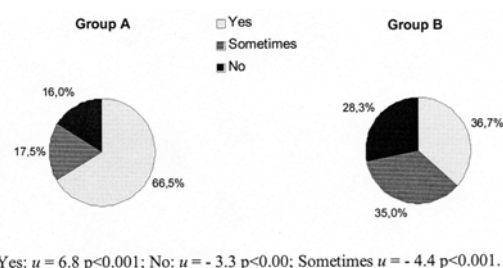


Fig. 2. Meetings of a therapeutic team (Groups A and B)

The flow of information concerning a patient is associated with meetings of a multi-disciplinary team. This flow was also better in hospitals with the certificate, compared to the health units without accreditation (Group A – 81.1%; Group B – 53.4%). The problems of patients were more comprehensively discussed during meetings and consultations in Group A than in Group B.

The results obtained concerning the problems discussed during the meetings of a therapeutic team indicate that most frequently the essence of discussion were diagnostic, therapeutic and nursing problems resulting from patients' complaints (Group A – 40.8%; Group B – 22.6%). A part of the above-mentioned problems were perceived as significantly more serious by Group A, compared to Group B –  $U = 4.6$ ;  $p < 0.001$ . No statistically significant differences between Groups A and B were observed with respect to ‘discussion current problems’ – 12.7% and 8.2% respectively;  $U = -1.6$ ;  $p > 0.05$ . The problems concerning the rehabilitation of patients were more often mentioned by the staff from Group A (7.8%) than Group B (2.3%), and the difference was significant statistically on the level –  $U = 3.1$ ;  $p < 0.01$ . The methods of treatment and prevention of diseases were considerably more often discussed in Group A (5.8%) than in Group B (4.2%); however, the difference was not significant statistically –  $U = 2.8$ ;  $p > 0.05$ . The lack of reply was significantly more frequent in Group B (40.1%), compared to group

A (19.9%) –  $U = -4.9$ ;  $p < 0.001$ . Figure 3 presents the detailed scope of problems discussed during meetings (Fig. 3).

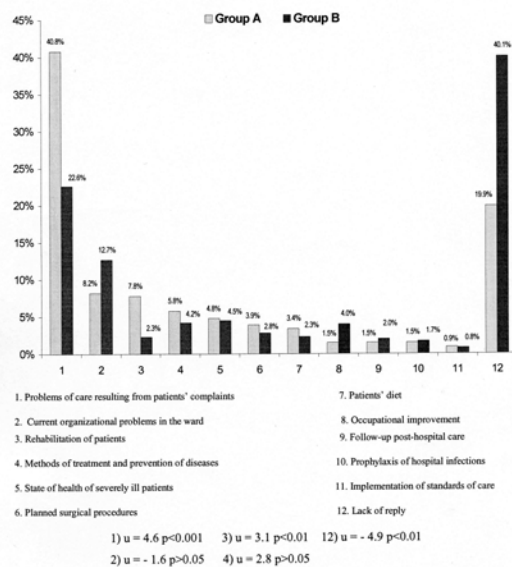


Fig. 3. Problems discussed during meetings of a therapeutic team in respondents' opinions (Groups A and B)

While analysing the respondents' replies concerning the problems discussed during meetings of a therapeutic team, attention was paid to the group which mentioned these problems most comprehensively. The results showed that all ward heads from group A reported the essence of the problems discussed during the meetings of the team (100.0%), followed by charge nurses also from Group A (80.9%). Assistant physicians from hospitals without the quality certificate occupied the third position (73.8%), followed by head nurses (Group A – 72.0%; Group B – 66.0%), and charge nurses from group B (56.1%).

The results of statistical analysis concerning the problems discussed differed according to the position held. The smallest number of problems was reported by charge nurses from Group B (56.1%), compared to Group A – 80.9% -  $\chi^2 = 20.0$ ;  $p < 0.001$ . A statistically significant difference was also observed between ward heads/clinic managers from Group B (79.4%) and group A – 100.0% -  $\chi^2 = 4.9$ ;  $p < 0.05$ . The strongest difference was noted between ward heads/clinic managers from hospitals without the certificate (Cramer's  $V^2 = 0.09$ ), and between charge nurses, also from Group B (Cramer's  $V^2 = 0.06$ ).

## DISCUSSION

The World Health Organization and the Centre for Quality Monitoring in Health Care in Poland perceive teamwork as an important element of the improvement of care of hospitalized patients. Considering the fact that the process of accreditation, the concept of which is to improve the quality of hospital services, has been functioning in Poland for a relatively short time, at attempt was undertaken to investigate whether there occur differences in the quality of functioning of therapeutic teams and the effectiveness of managing these teams between hospital wards possessing the quality certificate and those without this certificate.

Many authors indicate that due to the advantages of teamwork, students of various medical professions should be prepared for teamwork in hospital wards. The process of education in this area should begin as early as in the first year of study. It is recommended that practitioners should be incorporated into this process, which is very difficult [12, 13].

The reports from literature show that the studies conducted on the aspect of teamwork in a hospital confirm that despite the fact that physicians and nurses represent two separate professions and have different tasks to fulfill according the acquired knowledge and skills, their work mutually overlaps and they create therapeutic teams. In turn, skilful communication between the members of a therapeutic team and patients, in the context of patient's co-operation in contemporary complex care, allows patients to solve their health problems [14].

Similar results were obtained in own studies carried out in Polish hospital wards. These results show that 67.8% of physicians and nurses express an opinion that they constitute a therapeutic team. Only 20.0% of respondents reported difficulties with providing an answer to this question, and 12.1% of them consider that they are not a therapeutic team. It is noteworthy that physicians and nurses from hospitals with accreditation (Group A) at all workplaces (ward heads, head nurses, assistant physicians, charge nurses) considerably more clearly underlined that they work as a team (86.4%), compared to the staff from hospitals without the certificate (Group B) – 56.8%. The differences in replies concerning the presence of teamwork between both groups of the staff were statistically significant ( $p < 0.001$ ) in favour of Group A. This indicates that the efforts to integrate staff teams during the preparation of hospitals for accreditation yielded the desired results.

The problem of managing a team is also analysed in the aspect of perception of organizational effectiveness of the manager, on whom depend the results of the whole team. In Polish medical literature, a physician is indicated as the manager of a therapeutic team, who, within the scope of duties, is endowed with the supervision of an adequate flow of information concerning a patient among the members of the team [8, p. 14]. Managing a therapeutic team by a physician becomes especially important in the care of bed-ridden patients, because a multidisciplinary approach decreases the risk of complications, and exerts a considerable effect on the results of care. Own studies show that 96.6% of respondents from Group A indicated a physician as the manager of therapeutic team. In hospitals without accreditation, 81.6% of respondents mentioned that a physician manages a team. The respondents from hospitals with accreditation expressed significantly higher evaluations of the effectiveness of managing a therapeutic team by the ward head, compared to the respondents from hospitals without the certificate ( $p < 0.001$ ). They ascribed the largest number of very good evaluations (44.7%) – 5 scores; good - (30.6%) – 4 scores and excellent - (13.1%) – 6 scores. In Group B, the respondents evaluated managing a therapeutic team in more negative terms; there was a small number of very good evaluations (5 scores) – 17.8%, good (4 scores) – 25.7%, and excellent (6 scores) – only 7.3%.

Gregory and Einstein clearly underline that meetings and consultations, during which team members present their ideas necessary for solving common problems, introduction of changes and establishing the direction of teamwork, are an element of effective work of a team [15, 16]. In addition, the reports from literature also indicate other advantages of the meetings of a team, such as the exchange of information, developing bonds, and delegating authorities and responsibility to other members of the therapeutic team [17]. Own studies confirmed that the meetings of a therapeutic team to a greater extent take place in hospitals with accreditation (66.5%) than in those without the quality certificate (36.7%), the differences between these groups being on the level  $p < 0.001$ . The staff from hospitals without the certificate more frequently reported that they participated in meetings ‘sometimes’, (35.0%) compared to Group A – 17.5%. In respondents’ opinions the lack of meetings in both groups results from the lack of time, fixed habit in the ward, poor organization of work, and lack of initiative on the part of the team leader.

The results of studies conducted by Canales, confirmed by other researchers, indicate that the essence of the consultations of a therapeutic team are the problems of a patient, who shared them with the members of the therapeutic team, as well as the ways of providing a patient the sense of psychological security in a hospital. The participation of the whole therapeutic team in patient care, including a psychologist, exerts a significant effect on the correct course of patient’s emotional processes, may improve the effects of treatment in chronic diseases, and decrease the costs of treatment [18, 19]. Other authors also indicate that the cooperation of a multidisciplinary team, discussing the problems of a patient during the meeting of the team may decrease the cost of medical services and affect the quality of palliative care [20]. Own studies show that the essence of consultations of a therapeutic team in Polish hospital wards are diagnostic, treatment and nursing problems (Group A – 40.8%; Group B – 22.6%). In opinions of physicians and nurses, the essence of the meetings are also current organizational problems in the ward (Group A – 8.2%; Group B – 12.7%), rehabilitation of patients (Group A – 7.8%; Group B – 2.3%), as well as the methods of treatment and prevention of diseases, the state of health of severely ill patients, planned surgical procedures, patients’ diet, vocational improvement, post-hospital follow-up care, prophylaxis of hospital infections and the implementation of the standards of care.

The greatest number of problems discussed in a therapeutic team were mentioned by ward heads (100.0%) and charge nurses (80.0%) from hospitals with accreditation, whereas in the facilities without the certificate – also ward heads (79.4%), followed by assistant physicians (73.8%), head nurses (66.0%), and charge nurses (56.1%). These differences were statistically significant in favour of ward heads ( $p < 0.05$ ) and charge nurses ( $p < 0.001$ ) from hospitals with the certificate, compared to the same group of the staff from hospitals without accreditation.

## CONCLUSIONS

1. Teamwork is more clearly perceived by physicians and nurses in hospitals with accreditation, compared to the hospitals without the certificate.
2. The effectiveness of managing a therapeutic team is evaluated in more positive terms by the respondents from hospitals possessing the accreditation certificate than in the units without accreditation. The re-



spondents indicated that more effective management of the therapeutic team by the ward head in hospitals with the certificate is associated with more frequent meetings and therapeutic consultations, during which various problems concerning patient care are discussed, which results in the improvement of the quality of medical services.

3. It was observed that during meetings of a therapeutic team in hospitals with the quality certificate, diagnostic, treatment and nursing problems resulting from patients' complaints, as well as the problems of rehabilitation of patients, are significantly more often discussed, compared to the teams in hospitals without accreditation. During the consultations of a therapeutic team in hospitals without accreditation, organizational problems in the ward are relatively frequently discussed, which are not directly associated with the provision of a complex care of a patient.
  4. The preparation of a hospital for accreditation, the implementation of accreditation standards in order to obtain the quality certificate provides incentives for the staff to work as a team. The management of a therapeutic team in a hospital with the certificate is also more effective, which conditions a higher level of patient care, and at the same time, indicates the need for the implementation of the process of accreditation in Polish hospitals

## REFERENCES

1. Cofta S.: Interdisciplinary care of patients with pathologies of the respiratory system. *Physician's Guide* 2007; 1: 149 – 152
2. Widomska – Czekajaska T, Górajek – Józwick J. (et al.): *Encyclopaedic guide for nurses*. National Medical Publishers, Warsaw 1996
3. Gedney B J. Partnership and collaboration: what skills are needed? *Online J Issues Nurs* 2005; 10: 1
4. Sierpińska L, Ksykiewicz - Dorota A. Holistic approach to the therapeutic team in patient care. *ANNALES* 2003; 58: 131 – 5
5. Zahradniczek K.: *Introduction to nursing*. National Medical Publishers, Warsaw 1995
6. Health goals in the programme: Health for All. Health Policy in Europe. Medical Centre for Teachers' Training for Secondary Level Medical Education, Warsaw 1993
7. Accreditation programme for hospitals. Centre for Quality Monitoring in Health Care, Cracow 1998
8. Dymalska – Kubasik L, Smyrek T, Olek E.: Ward head – description of the workplace. *Guide of Health Manager* 2000; 4:13 – 6
9. Hebanowski M, Kliszcz J, Trzeciak B.: *Guide for physician-patient communication*. National Medical Publishers, Warsaw 1994
10. Common H.A question of care. *CMAJ*, 1997;146: 541 – 4
11. Klamut M K.: Between bioethical and holistic models of medicine. *Alma Mater*1998; 5: 26 – 9
12. Horsburgh M, Lamdin R, Wiliamson E. Multiprofessional learning: the attitudes of medical, nursing and pharmacy students to shored, learning. *Multiprofessional learning*. *Medical Education* 2001; 35: 876 – 883
13. Mc Nair R P. The case for educating health care students in professionalism as the care of interprofessional education. *Interprofessional learning*. *Medical Education* 2005; 39: 456 – 464
14. Lindeke L L, Siec Kerf A M. Nurse – physician workplace collaboration. *Online J Issues Nurs*. 2005;10: 5
15. Einstein K. Communication. In: Ehrlich A. (et al.): *Management practice*. Polish Economic Publishers, Warsaw 1997, p. 322 – 341
16. Gregory J. Narady.W: Ehrlich A. (et al.): *Management practice*. Polish Economic Publishers, Warsaw 1997, p. 342 – 348
17. Jasiński Z.: *Work management. Organization, planning, motivation, supervision*. 'Placet' Publishing Agency, Warsaw 1999, p. 39 – 41
18. Canales M. Narrative interaction: creating a space for therapeutic communication. *Issues in Mental Health Nursing* 1997; 18: 477 – 494
19. Litaker D, Mion L, Planavsky L, Kippes C, Mehta N, Frolikis J. Physician – nurse practitioner teams in chronic disease management: the impact on costs, clinical effectiveness, and patients' perception care. *J Interprof Care* 2003; 17: 223 – 237
20. Hall P, Weaver L, Gravelle D, Thibault H. Developing collaborative person – centered practice: a pilot project on a palliative care unit. *J Interprof Care* 2007; 21: 69 - 81

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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**INHIBITION OF ACTIVITY OF P-GLYCOPROTEIN BY CYCLOSPORIN  
IN LEUKEMIC CELLS AFTER SHORT-TERM PREDNISOLONE THERAPY *IN VITRO***

**ZMNIEJSZENIE AKTYWNOŚCI GLIKOPROTEINY P PRZEZ CYKLOSPORYNĘ  
W KOMÓRKACH BIAŁACZKOWYCH PODCZAS KRÓTKOTRWAŁEJ TERAPII *IN VITRO*  
Z PREDNIZOLONEM**

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**S u m m a r y**

**Introduction.** Multidrug resistance (MDR) is a major obstacle to chemotherapy success in acute lymphoblastic leukemia (ALL). An increased expression or function of P-glycoprotein (PGP) is regarded as a classical MDR mechanism.

**The objective of the study.** The analysis of *in vitro* function of PGP in children with ALL and lymphoid cell lines with the effect of cyclosporin A (CSA), an inhibitor of PGP activity.

**Patients and methods.** Children with ALL and leukemic/lymphoid cell lines were tested for accumulation and retention of rhodamine 123 (Rh123) without/with the presence of cyclosporine A before and after 3-day incubation with prednisolone. Retention analysis was performed with the use of flow cytometry.

**Results.** Rh123 retention was higher after addition of CSA in all cell lines. Rh123 retention decreased after 3 days

of *ex vivo* prednisolone therapy was lower in the presence of CSA after 72 hours than respective retention before the incubation. PGP inhibition was highest in Raji and Jurkat. The Rh123 retention was lower after 72 hours in 55/60 ( $p < 0,001$ ), and in the presence of CSA in 57/60 ( $p < 0,001$ ) children. An increase of Rh123 retention in the presence of CSA on day "0" correlated with the expression of PGP on day "0" ( $p = 0,030$ ) and with the percentage of PGP-positive cells on day "0" ( $p = 0,017$ ).

**Conclusions.** Rh123 retention is lower in most leukemic samples after short-term incubation with prednisolone. In individual patient samples, the upregulation of PGP function was observed. Rh123 retention was higher in the presence of cyclosporine A, which inhibits PGP function. Lack of this effect in patient samples indicates possibilities of evolving drug resistance during subsequent therapy.

**Streszczenie**

**Wstęp.** Oporność wielolekowa (MDR) jest jedną z głównych przeszkód w terapii ostrej białaczki limfoblastycznej (ALL). Podwyższona ekspresja lub funkcja białka PGP jest uważana za klasyczny mechanizm MDR.

**Cel pracy.** Analiza funkcji PGP u dzieci z ALL i w liniach komórkowych z uwzględnieniem hamującego efektu cyklosporyny A (CSA) po krótkotrwałej terapii *in vitro* z prednizolonem.

**Pacjenci i metodyka.** Określono akumulację i retencję rodaminę (Rh123) bez CSA i w jej obecności u 60 dzieci i w 4 liniach komórkowych. Analiza retencji Rh123 przed i po inkubacji z prednizolonem wykonano metodą cytometrii przepływowej.

**Wyniki.** Retencja Rh123 była wyższa po dodaniu CSA we wszystkich liniach komórkowych i u wszystkich pacjentów. Retencja malała po 3 dniach terapii *ex vivo* z prednizolonem, zarówno bez CSA, jak i w jej obecności, w stosunku do odpowiedniej wartości przez rozpoczęciem inkubacji. Zahamowanie aktywności PGP było największe w liniach Raji i Jurkat. Retencja Rh123 była niższa po 72 godzinach, zarówno bez obecności CSA (u 55/60 pacjentów,  $p < 0,001$ ), jak i w obecności CSA (u 57/60 dzieci,  $p < 0,001$ ). Wzrost retencji Rh123 w obecności CSA w dniu 0 korelował z ekspresją PGP w dniu 0 ( $p = 0,030$ ) i z odsetkiem komórek PGP-pozytywnych w dniu 0 ( $p = 0,017$ ).

Wnioski. Retencja rodamininy jest zmniejszona w większości próbek ALL po krótkotrwałej terapii *in vitro* z prednizolonem. W pojedynczych przypadkach stwierdzono jednak nasilenie aktywności PGP. Retencja rodamininy była

wyższa w obecności cyklosporyny, inhibitora PGP. Brak tego zjawiska w próbkach pacjentów może wskazywać na możliwość rozwoju oporności wielolekowej podczas dalszej terapii.

**Key words:** acute lymphoblastic leukemia, children, PGP function, rhodamine, cyclosporine

**Słowa kluczowe:** ostra białaczka limfoblastyczna, dzieci, funkcja PGP, rodamina, cyklosporyna

## INTRODUCTION

Multidrug resistance (MDR) is still a major obstacle to chemotherapy success in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Recent studies have shown that the expression of certain gene products mediate the development of resistance to chemotherapeutic agents [1, 2]. The best characterized is the multidrug resistance gene MDR-1. With respect to multidrug resistance protein PGP, two forms of presence of this protein might be analyzed: expression and function. The PGP expression is analyzed as the presence of molecule on the cell surface, while function is tested by ability of this protein to efflux xenobiotics out of the cell and ability to stop this process by specific inhibitor of PGP. Classical xenobiotics used in the assays is rhodamine 123, while examples of PGP inhibitors are: verapamil, reserpine (I generation of inhibitors), cyclosporine A (II generation), and zosuquidar (III generation of inhibitors) [3]. Functional activity is a more sensitive predictor of chemoresistance than PGP/170 surface expression [1]. PGP/170 is expressed to a higher degree in leukemic cells and this is greater in relapsed compared to de novo cases and more in AML than ALL blasts [1]. It has been shown in a monocentric study that MDR1 expression in childhood ALL is an independent adverse prognostic factor on outcome, and could be a useful biological marker of response in these patients. Moreover, MDR1 function was also a predictor of response, but only in univariate analysis [2].

This study was aimed to analyze the function of P-glycoprotein/170 (PGP) in children with acute lymphoblastic leukemia and lymphoid cell lines with the effect of Cyclosporin A (CSA) as a modulator of P-glycoprotein functional activity. MDR1 functional activity was performed by a rhodamine (Rho-123) efflux test with or without PGP inhibitor.

## PATIENTS AND METHODS

*Patients.* An *in vitro* analysis was performed on leukemic cells isolated from bone marrow either at the diagnosis or relapse of ALL. A total number of 60

children (30 boys, 30 girls), aged 0.1-17 (median 7.8) years were included into the study. Initial white blood cell count was 2.03-690.0 G/L (median 66.7 G/L). With respect to diagnosis: 8 children were of pre-pre-B-ALL phenotype, 43 with common-ALL, and 9 with T-ALL phenotype; 46 patients had de novo and 14 relapsed ALL.

*Cell lines.* Four cell lines of human acute lymphoid malignancies were used: B-cell lymphoma Raji and Daudi as well as T-cell ALL cell lines CCRF-CEM and Jurkat. The cell lines were obtained from the Institute of Immunology and Experimental Therapy, Wrocław (prof. dr hab. Danuta Duś) and originated from The European Collection of Cell Cultures (ECACC, Salisbury, Wiltshire, UK) [4]. Concentration of cells was  $0.4-0.5 \times 10^6/\text{ml}$ .

*Conception of the study.* Children with ALL and leukemic/lymphoid cell lines were tested for accumulation and retention of rhodamine 123 without/with the presence of cyclosporine A before and after 3-day incubation with prednisolone. Retention analysis was performed with the use of flow cytometry.

*Retention and efflux of rhodamine 123.* Rhodamine (Rh123) is a cationic and lipophilic fluorescent (excitation – 505 nm, emission – 534 nm) [5], accumulating inside mitochondria of living cells. Rh123 is an artificial substrate actively effluxed out of the cells by PGP and is regarded as a measure of its function (but not expression). Rh123 (Sigma, St Louis, USA) solution was used at concentration 200 ng/ml. In parallel, retention of Rh123 in the presence of cyclosporin A (CSA), a known PGP inhibitor was tested. CSA concentration was  $2 \mu\text{M}$ , and time of incubation of cells with  $\text{Rh123} \pm \text{CSA}$  was 30 minutes, and then the samples were assayed by flow cytometry.

*PGP expression.* PGP was tested before and after *in vitro* prednisolone therapy by flow cytometry with the

use of human monoclonal antibodies anti-PGP (Alexis Biochemicals, Lozanna, Szwajcaria, nr cat. 801-004-C250), clone JSB-1, isotype control: mouse immunoglobulins IgG1. Clone JSB-1 reacts with cytoplasmic epitope of surface protein of PGP 170 kDa.

**Statistical analysis.** The differences between Rh123 retention were analyzed by t-Student paired test for cell lines and by Wilcoxon matched-pair test. Correlation was tested by Spearman's rho coefficient.

## RESULTS AND DISCUSSION

**Cell lines.** Rh123 retention was higher after addition of CsA in all cases (Tab. 1). Rh123 retention has decreased after 3 days of *ex vivo* prednisolone therapy, which can be explained either as an increase of PGP activity (even in spite of lack of expression of this protein) or loss of function of mitochondria to efflux the dye. Rh123 retention in the presence of CsA after 72 hours was lower than respective retention on day "0" (Fig. 1A). No correlation was found between Rh123±CsA retention on day „0” and PGP expression, while on day „3”, PGP expression correlated with basal Rh123 retention (Rh123 without CsA, in comparison to day „0”) ( $r=0,446$ ,  $p=0,010$ ) and with decrease of Rh123 retention in the presence of CsA (on day „3”, in comparison to day „0”) ( $r=0,561$ ,  $p=0,001$ ). PGP inhibition, expressed as higher Rh123 retention, after CsA addition, and was the highest in Raji and Jurkat, which is related to expression of PGP in these cell lines. On the other hand, differences between Rh123 retention in CCRF-CEM cell line on day „0” were almost undetectable, which is related to lack of PGP in these cell lines.

Table 1. *Rh123 retention in cell lines*

Tabela 1. *Retencja Rh123 w liniach komórkowych*

		Raji	Daudi	CCRF-CEM	Jurkat
day 0	-CsA	69.9±33.0	21.5±5.4	55.4±13.3	57.4±12.0
	+CsA	294.1±77.1	45.5±12.3	67.4±11.8	109.3±30.3
	p-value	<0.001	<0.01	Ns	<0.01
day 3	-CsA	42.6±14.7	10.9±4.8	9.6±3.4	18.4±4.1
	+CsA	72.1±10.3	23.4±9.5	18.7±5.6	39.5±10.6
	p-value	<0.01	<0.05	<0.02	<0.001

Rh123 retention value is given in MFI, as mean±OS of 3 independent experiments. p - value by t-test

**Patients.** The values of Rh123 retention in patient cells are shown in Tab. 2 and on Fig. 1B. The retention was higher in the presence of CsA in 54/60 children on day „0”, and in 59/60 children on day „3”. In compari-

son to Rh123 retention on day „0”, the retention was lower after 72 hours in 55/60 ALL children ( $p<0.001$ ), and in the presence of CsA was also lower in 57/60 ( $p<0.001$ ) children. In ALL patients, an increase of Rh123 retention in the presence of CsA on day „0” correlated with the expression of PGP on day „0” ( $r=0.281$ ,  $p=0.030$ ) and with the percentage of PGP-positive cells on day „0” ( $r=0.306$ ,  $p=0.017$ ). After 72 hours, Rh123 retention adversely correlated with PGP expression on day „0” ( $r=-0.264$ ,  $p=0.042$ ).

Table 2. *Rh123 retention in patients' cells*

Tabela 2. *Retencja Rh123 w komórkach pacjentów*

		ALL
day 0	-CsA	35.8 (1.4-87)
	+CsA	48.8 (3.2-130)
	p <sub>w</sub> -value	<0.001
day 3	-CsA	5.3 (1.3-18.5)
	+CsA	13.2 (4-889)
	p <sub>w</sub> -value	<0.001

Value of Rh123 retention is provided as MFI (median and range). p<sub>w</sub> - p-value by Wilcoxon matched-pair test

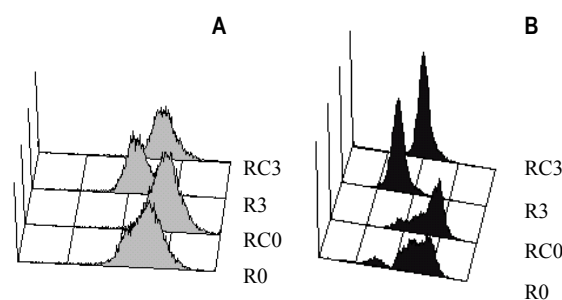


Fig. 1. *Rh123 retention in (A) Jurkat cell line, (B) in patient with ALL. R0 and R3 – Rh123 retention on days „0” and „3”. RC0 i RC3 – Rh123 retention in the presence of CsA on days „0” and „3” is higher than Rh123 retention without CsA, respectively. Logarithmic scale was used*

Ryc. 1. *Histogramy retencji Rh123 w (A) linii Jurkat, (B) u pacjenta z ALL. R0 i R3 – retencja Rh123 odpowiednio w dniu „0” i „3”. RC0 i RC3 – retencja Rh123 w obecności CsA w dniach „0” i „3” jest odpowiednio większa od retencji Rh123 bez obecności CsA. Wykres w skali logarytmicznej*

With respect to leukemic immunophenotype in ALL patient samples, the Rh123 retention in the presence of CsA was higher on day „0” in 8/8 children with pre-pre-B-ALL, 39/44 with c-ALL and 8/9 with T-ALL, and after 72 hours, in 8/8, 41/43 and in 8/9 children, respectively. In comparison to Rh123 retention without CsA on day „0”, after 3 days the retention was lower in 8/8 children with pre-pre-B-ALL ( $p=0,012$ ), 39/43 with common-ALL ( $p<0.001$ ) and in

8/9 with T-ALL ( $p=0.011$ ); and it was lower in the presence of CsA in 8/8 ( $p=0.012$ ), 41/43 ( $p<0.001$ ) and 8/9 ( $p=0.011$ ) children, respectively.

One of the possible causes of treatment failure in acute leukemia is the emergence of multidrug resistance caused by PGP overexpression, although, it is possibly of minor significance in pediatric ALL in comparison to AML in adults [6, 7] We compared a flow cytometric assay using rhodamine 123 to evaluate the PGP function in acute lymphoblastic leukemia. These data suggest that the assessment of the clinical impact of MDR expression in pediatric ALL can be based on methodological approaches capable of providing information extended to the PGP pump function, rather than only on its gene and protein expression.

#### CONCLUSIONS

Rh123 retention is lower in most leukemic samples after short-term incubation with prednisolone. In individual patient samples, the upregulation of PGP function was observed. Rh123 retention was lower in the presence of cyclosporine A, which inhibits PGP function. Lack of this effect in patient samples indicates possibilities of evolving drug resistance during subsequent therapy.

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#### REFERENCES

1. Abd El-Ghaffar H.A., Aladle D.A., Farahat S.E. et al. P-glycoprotein (P-170) expression in acute leukemias. *Hematology* 2006;11:35-41.
2. Casale F., D'Angelo V., Addeo R. et al. P-glycoprotein 170 expression and function as an adverse independent prognostic factor in childhood acute lymphoblastic leukemia. *Oncol Rep* 2004;12:1201-1207.
3. Vasconcelos F.C., Cavalcanti G.B., Jr., Silva K.L. et al. Contrasting features of MDR phenotype in leukemias by using two fluorochromes: implications for clinical practice. *Leuk Res* 2007;31:445-454.
4. <http://www.ecacc.org.uk>. The European Collection of Cell Cultures S, Wiltshire, UK.
5. Petriz J., Garcia-Lopez J. Flow cytometric analysis of P-glycoprotein function using rhodamine 123. *Leukemia* 1997;11:1124-1130.
6. Kamel A.M., El-Sharkawy N., Yassin D. et al. P-gp expression and Rh 123 efflux assay have no impact on survival in Egyptian pediatric acute lymphoblastic leukemia patients. *J Egypt Natl Canc Inst* 2005;17:165-172.
7. Tafuri A., Gregorj C., Petrucci M.T. et al. MDR1 protein expression is an independent predictor of complete remission in newly diagnosed adult acute lymphoblastic leukemia. *Blood* 2002;100:974-981.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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**REDUCTION OF MITOCHONDRIAL TRANSMEMBRANE POTENTIAL IN LEUKEMIC CELLS AFTER SHORT-TERM PREDNISOLONE THERAPY *IN VITRO***

**UTRATA POTENCJAŁU WEWNĄTRZMITOCHONDRIALNEGO W LIMFOBLASTACH BIAŁACZKOWYCH PODCZAS KRÓTKOTRWAŁEJ TERAPII PREDNIZOLONEM *IN VITRO***

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head: prof. dr hab. n. med. Mariusz Wysocki

**S u m m a r y**

**I n t r o d u c t i o n .** During early apoptosis, a reduction in mitochondrial transmembrane potential (MTP) and externalization of phosphatidylserine in cell membrane, followed by cascade of caspases, occurs prior to eventual cell death.

**T h e o b j e c t i v e o f t h e s t u d y .** The analysis of loss of mitochondrial transmembrane function and caspase-3 activity in the model of short-term in vitro glucocorticoid-induced apoptosis in the human lymphoid/leukemic cell lines and in pediatric acute lymphoblastic leukemia samples.

**P a t i e n t s a n d m e t h o d s .** Children with ALL and leukemic/lymphoid cell lines were tested for loss of mitochondrial transmembrane potential (MTP) and caspase-3 expression before and after 3-day incubation with prednisolone. MTP and caspase-3 analysis was performed with the use of flow cytometry.

**R e s u l t s .** Both parameters indicated activation of apoptotic processes in all cases of 72-hour in vitro therapy

with prednisolone. Disturbances in  $\Delta\Psi_m$  and an increase of caspase-3 expression occurred in all patients. In relapsed ALL patients at day „0” median value of loss of MTP was 0,89, while in de novo ALL patients it was 1,17 (ns). The value of FL2 fluorescence (indicating normal MTP) on day „0” in relapsed patients was higher ( $p=0,008$ ). These results show smaller MTP changes in relapsed patients and usually correspond with higher drug resistance observed at leukemia relapse.

**C o n c l u s i o n s .** The detection of loss of mitochondrial transmembrane potential is a marker of activation of intrinsic apoptotic pathway occurring in leukemic cells after short-term in vitro therapy with prednisolone. Caspase-3 expression is increased after induction of extrinsic pathway of apoptosis in leukemic cells caused by prednisolone. Prednisolone induces both intrinsic and extrinsic apoptotic pathway in leukemic and lymphoid cells.

**S t r e s z c z e n i e**

**W s t ę p .** We wczesnym etapie apoptozy dochodzi do indukcji różnych mechanizmów, takich jak: utrata przez błonowego potencjału mitochondrialnego i aktywacja kaskady kaspaz, prowadzące ostatecznie do śmierci komórek.

**C e l p r a c y .** Analiza utraty przez błonowego potencjału mitochondrialnego i ekspresji kaspazy-3 w modelu krótkoterminowej indukcji apoptozy przez glikokortykoidy u dzieci z ostrą białaczką limfoblastyczną (ALL) i w liniach komórkowych

**P a c j e n c i i m e t o d y k a .** Określono utratę przez błonowego potencjału mitochondrialnego i ekspresję kaspazy-3 u 60 dzieci i w 4 liniach komórkowych. Analizę zmian potencjału mitochondrialnego i ekspresji kaspazy-3 przed

i po inkubacji z prednizolonem wykonano metodą cytometrii przepływowej.

**W y n i k i .** Obydwa badane parametry wskazują indukcję procesów apoptozy we wszystkich przypadkach w badanych liniach komórkowych i próbkach pacjentów po krótkotrwałej terapii in vitro z prednizolonem. Zaburzenia potencjału wewnątrzmitochondrialnego i wzrost ekspresji kaspazy-3 wystąpiły u wszystkich pacjentów. We wznowie ALL, mediana zaburzeń potencjału mitochondrialnego wynosiła 0,89, podczas gdy u pacjentów z de novo ALL wynosiła 1,17 (ns). Intensywność fluorescencji FL2 (wskazującej prawidłowy potencjał mitochondrialny) w dniu „0” u pacjentów ze wznową była wyższa ( $p=0,008$ ). Wyniki te pokazują, że mniejsze zaburzenia potencjału wewnątrzmitochondrialnego

występują u pacjentów we wznowie ALL i mogą być związane z większą opornością na cytostatyki w tej fazie choroby.

**Wnioski.** Określenie utraty przez błonowego potencjału mitochondrialnego jest dobrym markerem aktywacji wewnątrzpochodnego szlaku indukcji apoptozy występującej w komórkach ostrej białaczki limfoblastycznej po krótko-

trwałej terapii *in vitro* z prednizolonem. Ekspresja kaspazy-3 jest podwyższona po indukcji szlaku zewnątrzpochodnego apoptozy. Prednizolon indukuje zarówno wewnątrz- jak i zewnątrzpochodny szlak apoptozy w komórkach ostrej białaczki limfoblastycznej.

**Key words:** acute lymphoblastic leukemia, children, mitochondrial transmembrane potential, caspase-3

**Słowa kluczowe:** ostra białaczka limfoblastyczna, dzieci, potencjał wewnątrzmitochondrialny, kaspaza-3

## INTRODUCTION

According to the mitochondrial theory of aging, mitochondrial dysfunction increases intracellular reactive oxidative species production, leading to the oxidation of macromolecules and ultimately to cell death [1]. During early apoptosis, there is a reduction in mitochondrial transmembrane potential (MTP) and externalization of phosphatidylserine (PS) in cell membrane prior to eventual cell death. Flow cytometric detection techniques targeting these changes, reduction of DiOC(6) uptake upon the collapse of MTP and annexin V binding to PS have been successfully used to detect apoptotic cells. These methods gave comparable results when cell lines were used. A comparison of two different techniques, DiOC(6) uptake and Annexin V-propidium iodide co-labeling in the quantification of cytarabine, vincristine and daunorubicin induced apoptosis on three leukemia cell lines (HL-60, CEM, U937), and bone marrow blasts from children with acute myeloid leukemia and acute lymphoblastic leukemia has shown a correlation between the apoptosis rates measured by these two techniques for drug-induced apoptosis in myeloid and lymphoid blasts. This data suggests that reduction of the MTP and PS externalization may be common to many apoptotic pathways and techniques targeting either of these changes may be used in quantification of apoptosis in different clinical samples [2, 3].

We hypothesize that loss of mitochondrial function leads to induction of apoptosis after short-term *in vitro* therapy with prednisolone. We tested this hypothesis using the model of glucocorticoid-induced apoptosis in the human lymphoid/leukemic cell lines and in pediatric acute lymphoblastic leukemia samples.

## PATIENTS AND METHODS

**Patients.** An *in vitro* analysis was performed on leukemic cells isolated from bone marrow either at the diagnosis or relapse of ALL. A total number of 60 children (30 boys, 30 girls), aged 0.1-17 (median 7,8)

years were included into the study. Initial white blood cell count was 2,03-690,0 G/L (median 66,7 G/L). With respect to diagnosis: 8 children were of pre-pre-B-ALL phenotype, 43 with common-ALL, and 9 with T-ALL phenotype; 46 patients had *de novo* and 14 relapsed ALL.

**Cell lines.** Four cell lines of human acute lymphoid malignancies were used: B-cell lymphoma Raji and Daudi as well as T-cell ALL cell lines CCRF-CEM and Jurkat. The cell lines were obtained from the Institute of Immunology and Experimental Therapy, Wrocław (prof. dr hab. Danuta Duś) and originated from The European Collection of Cell Cultures (ECACC, Salisbury, Wiltshire, UK) [4]. Concentration of cells was  $0,4-0,5 \times 10^6/\text{ml}$ .

**Design and conception of the study.** Children with ALL and leukemic/lymphoid cell lines were tested for mitochondrial transmembrane potential (MTP) and caspase-3 expression before and after 3-day incubation with prednisolone. The analysis was performed with the use of flow cytometry.

**Mitochondrial transmembrane potential.** The detection of loss of mitochondrial transmembrane potential (MTP,  $\Delta\Psi\text{m}$ ) was performed with the use of DiSCAM kit (R&D Systems GmbH, Wiesbaden, Germany, nr cat TA700) after 20 minute incubation of tested cells with the dye [5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl carbocyanine iodide] in temperature 37°C and atmosphere 5% CO<sub>2</sub>. This dye has the properties to accumulate in mitochondria and aggregates after polarization of mitochondrial membrane creating orange compound with fluorescent properties. In the case of loss of potential of mitochondrial membrane, this dye does not aggregate, but converts to monomeric form with green fluorescence. The analysis was performed with the use of flow cytometry: normal potential is measured as orange signal by



FL2 fluorescence, and disturbed potential is measured as green signal by FL1. Loss of mitochondrial transmembrane potential is related to release of cytochrom C from damaged mitochondrium [5]. Changes in the ratio of signals FL1/FL2 (green/red) were assumed as parameter of loss of mitochondrial transmembrane potential (MTP,  $\Delta\Psi_m$ ).

**Caspase-3 expression.** Rabbit anti-human monoclonal antibodies anti-caspase-3-PE were used: PE-conjugated monoclonal active caspase-3 antibody apoptosis kit I (PharMingen, Becton Dickinson, nr cat. 550914). This kit includes: monoclonal antibodies, Cytotfix/Cytoperm Solution and Perm/Wash Buffer. Antibodies detect heterodimer 17-12 kDa of active caspase-3, marker of cells undergoing apoptosis. Caspase-3 activity is proportional to fluorescence of expression of this protein [6]. Changes in caspase-3 activity correlate in each time point, thus it is sufficient to perform only one time point measurement [6]. Caspase-3 is a marker of both intrinsic and extrinsic apoptotic pathway i.e. receptor pathway (acc. to Jia et al. [7]).

**Statistic analysis.** The differences in MTP were analyzed by t-Student paired test for cell lines and by Wilcoxon matched-pair test. Correlation was tested by Spearman's rho coefficient.

## RESULTS AND DISCUSSION

**Cell lines.** Both parameters indicated activation of apoptotic processes in all cases of 72-hour in vitro therapy with prednisolone (Tab. 1, Fig. 1). Increase of  $\Delta\Psi_m$  was higher when initial (before incubation) disturbances were small. An increase of caspase-3 expression was also higher when initial (before incubation) expression was low.

Table 1. Loss of mitochondrial transmembrane potential ( $\Delta\Psi_m$ ) and expression of caspase-3 in cell lines

Tabela 1. Zaburzenia przezłonowego potencjału mitochondrialnego ( $\Delta\Psi_m$ ) i ekspresja kaspazy-3 w liniach komórkowych

	Raji	Daudi	CCRF-CEM	Jurkat
$\Delta\Psi_m$	1,20±0,53 2,85±0,81	1,61±0,58 2,47±0,81	0,82±0,58 4,71±1,16	1,38±0,34 3,18±0,93
caspase 3	3,00±0,16 4,35±0,28	6,25±0,65 6,98±0,53	1,75±0,39 5,59±0,51	3,25±0,11 5,53±0,19

The value of  $\Delta\Psi_m$  is expressed as a ratio of signals FL1/FL2. Upper value – expression on day „0”, lower value – expression on day 3, after incubation with prednisolone at concentration of 250 µg/ml. In all cases, MFI value was higher on day „3” (p<0,05, Wilcoxon matched-pair test). Results are given as mean±SD..

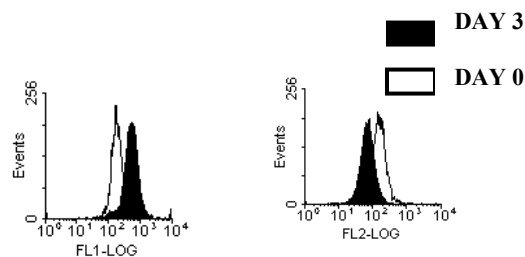


Fig. 1. Loss of transmembrane mitochondrial potential ( $\Delta\Psi_m$ ) after ex vivo therapy with prednisolone, expressed as FL1 increase and FL2 decrease

Ryc. 1. Zaburzenie przezłonowego potencjału mitochondrialnego ( $\Delta\Psi_m$ ) po terapii ex vivo z prednizolone, wyrażające się wzrostem FL1 i zmniejszeniem FL2

**Patients.** Disturbances in  $\Delta\Psi_m$  and an increase of caspase-3 expression occurred in all patients after 72-hour in vitro therapy with prednisolone (Tab. 2). In relapsed ALL patients on day „0” median value of FL1/FL2 ratio was 0,89, while in de novo ALL (n=46) patients it was 1,17 (ns). The value of FL2 fluorescence (indicating normal MTP) on day „0” in relapsed patients was higher (MFI 20,9 vs 13,3, p=0,008). These results show small  $\Delta\Psi_m$  changes in relapsed patients and usually correspond with higher drug resistance observed at leukemia relapse.

Table 2. Loss of mitochondrial transmembrane potential ( $\Delta\Psi_m$ ) and expression of caspase-3 in patient samples

Tabela 2. Funkcja przezłonowego potencjału mitochondrialnego ( $\Delta\Psi_m$ ) i ekspresja kaspazy-3 w komórkach pacjentów

	ALL patients
$\Delta\Psi_m$	1,16 (0,36-4,76) 3,57 (2,00-4,54)
caspase 3	1,05 (0,60-2,13) 2,32 (0,74-8,49)

The value of  $\Delta\Psi_m$  is expressed as a ratio of signals FL1/FL2. Upper value – expression on day „0”, lower value – expression on day 3, after incubation with prednisolone at concentration of 250 µg/ml. In all cases, MFI value was higher on day „3” (p<0,01, Wilcoxon matched-pair test). Results are given as mean±SD..

The loss of mitochondrial transmembrane potential was observed in Jurkat cells after vincristine induced apoptosis. Reactive oxygen species (ROS) can play a regulatory role in the initial phase of a mitochondrial controlled pathway of apoptosis [8]. Possibly, different sensitivities of ALL and AML cells to undergoing spontaneous apoptosis in vitro can be explained also by

ability to lose mitochondrial transmembrane potential. Detection of the early/intermediate, but not the late stage of apoptosis is preferable for correct assignment of spontaneous apoptosis in pediatric acute leukemia [3]. It is speculated that the increase in Coenzyme Q10 concentration during induction treatment may be attributed to the activation of a natural antioxidative defence mechanism, endocrine influence on CoQ10 synthesis from steroid treatment, or a shift in CoQ10 from the damaged cells to the plasma after cell lysis [9].

Our findings indicate that drugs leading to loss of MTP can be promising therapeutic agents for treatment of ALL cases [10]. The examples of compounds with this mechanism of anticancer activity are rotenone and arsenic trioxide [11]. These data suggest that nitric oxide production in the leukemia-derived cells may be a protective response to maintain mitochondrial  $\Delta\Psi_m$  after antioxidant exposure and inhibition of nitric oxide synthase increases the disruption of mitochondrial homeostasis induced by the antioxidants [12].

## CONCLUSIONS

1. The detection of loss of mitochondrial transmembrane potential is a marker of activation of intrinsic apoptotic pathway occurring in leukemic cells after short-term in vitro therapy with prednisolone.
2. Caspase-3 expression is increased after induction of extrinsic pathway of apoptosis in leukemic cells caused by prednisolone.
3. Prednisolone induces both intrinsic and extrinsic apoptotic pathway in leukemic and lymphoid cells.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Cabreiro F., Picot C.R., Perichon M. et al. Overexpression of mitochondrial methionine sulfoxide reductase B2 protects leukemia cells from oxidative stress-induced cell death and protein damage. *J Biol Chem* 2008;283:16673-16681.
2. Ozgen U., Savasan S., Buck S. et al. Comparison of DiOC(6)(3) uptake and annexin V labeling for quantification of apoptosis in leukemia cells and non-malignant T lymphocytes from children. *Cytometry* 2000;42:74-78.
3. Savitskiy V.P., Shman T.V., Potapnev M.P. Comparative measurement of spontaneous apoptosis in pediatric acute

leukemia by different techniques. *Cytometry B Clin Cytom* 2003;56:16-22.

4. <http://www.ecacc.org.uk>. The European Collection of Cell Cultures S, Wiltshire, UK.
5. Moon E.Y., Lerner A. PDE4 inhibitors activate a mitochondrial apoptotic pathway in chronic lymphocytic leukemia cells that is regulated by protein phosphatase 2A. *Blood* 2003;101:4122-4130.
6. Liu T., Raetz E., Moos P.J. et al. Diversity of the apoptotic response to chemotherapy in childhood leukemia. *Leukemia* 2002;16:223-232.
7. Jia W., Yu C., Rahmani M. et al. Synergistic antileukemic interactions between 17-AAG and UCN-01 involve interruption of RAF/MEK- and AKT-related pathways. *Blood* 2003;102:1824-1832.
8. Groninger E., Meeuwse-De Boer G.J., De Graaf S.S. et al. Vincristine induced apoptosis in acute lymphoblastic leukaemia cells: a mitochondrial controlled pathway regulated by reactive oxygen species? *Int J Oncol* 2002;21:1339-1345.
9. Niklowitz P., Wiesel T., Andler W. et al. Coenzyme Q10 concentration in the plasma of children suffering from acute lymphoblastic leukaemia before and during induction treatment. *Biofactors* 2007;29:83-89.
10. Chiarini F., Del Sole M., Mongiorgi S. et al. The novel Akt inhibitor, perifosine, induces caspase-dependent apoptosis and downregulates P-glycoprotein expression in multidrug-resistant human T-acute leukemia cells by a JNK-dependent mechanism. *Leukemia* 2008;22:1106-1116.
11. Styczynski J., Wysocki M. Ex vivo modulation of response to prednisolone in childhood acute lymphoblastic leukaemia. *Br J Haematol* 2006;133:397-399.
12. Kellner C., Zunino S.J. Nitric oxide is synthesized in acute leukemia cells after exposure to phenolic antioxidants and initially protects against mitochondrial membrane depolarization. *Cancer Lett* 2004;215:43-52.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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**MORPHOMETRIC STUDY OF THE AORTIC ISTHMUS DIAMETER  
IN HUMAN FETUSES**

**BADANIA MORFOMETRYCZNE ŚREDNICY CIEŚNI AORTY U PŁODÓW CZŁOWIEKA**

Chair and Department of Anatomy Nicolaus Copernicus University Collegium Medicum in Bydgoszcz

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**S u m m a r y**

**Introduction:** The aortic isthmus, known as the A segment, occurs as a constriction of the aorta, which is situated between the left subclavian artery and the ductus arteriosus. According to the professional literature the aortic isthmus showed more variation than those on the other parts of aorta. The present study was performed on 128 spontaneously aborted human fetuses aged 15-34 weeks to establish normal values for aortic isthmus diameter at varying gestational age. **Material and Methods:** Using anatomical dissection, digital-image analysis (system of Leica Qwin Pro 16) and statistical analysis (ANOVA, regression analysis) the growth of the aortic isthmus diameter was examined during gestation.

**Results:** No significant gender differences were found ( $p > 0.05$ ). Regression plots of the aortic isthmus diameter against gestational age in weeks modeled the linear function  $y = -2.4247 + 0.2333x \pm 0.3983$  ( $r = 0.96$ ,  $P < 0.001$ ). Correlation coefficient between the aortic isthmus diameter and gestational age was statistically significant ( $P < 0.001$ ), and

reached the value  $r = 0.96$ . The aortic isthmus-to-aortic root diameter ratio indicated the intensive increase from  $0.45 \pm 0.10$  to  $0.72 \pm 0.07$  for fetuses aged 15-16 and 21-22 weeks of gestation, respectively. In the remaining fetuses, the ratio of the diameter of the aortic isthmus to that of the aortic root was stable and ranged from  $0.66 \pm 0.10$  to  $0.73 \pm 0.06$  ( $P > 0.05$ ). Similarly, in fetuses aged 15-22 weeks, the aortic isthmus-to-descending aorta diameter ratio increased from  $0.73 \pm 0.10$  for 15-16 week group to  $0.93 \pm 0.11$  for 21-22 week group. In the remainder, the aortic isthmus-to-descending aorta diameter ratio ranged from  $0.88 \pm 0.09$  to  $0.90 \pm 0.07$  ( $P > 0.05$ ).

**Conclusions:**

1. The aortic isthmus measurements should facilitate the prenatal diagnosis of the coarctation of the aorta.
2. Our findings argue against the concept of relatively little fetal blood flow through the aortic isthmus during gestation.

**Streszczenie**

**Cel:** Cieśń aorty znana jako segment A, występuje w postaci zwężenia aorty, które jest usytuowane między tętnicą podobojczykową lewą a przewodem tętniczym. Zgodnie z fachowym piśmiennictwem cieśń aorty wykazuje większą zmienność niż inne części aorty. Badania przeprowadzono na 128 płodach człowieka w wieku 15-34 tyg., pochodzących z samoistnych poronień w celu określenia prawidłowych wartości średnicy cieśni aorty w różnym wieku ciążowym.

**Materiał i metody:** Przy zastosowaniu dysekcji anatomicznej, systemu cyfrowej analizy obrazu (system Leica Q Win Pro 16) i analizy statystycznej (ANOVA, rachunek regresji) zbadano wzrost średnicy cieśni aorty podczas ciąży.

**Wyniki:** Nie stwierdzono różnic płciowych ( $P > 0.05$ ). Naniesione wartości średnicy cieśni aorty względem wieku ciążowego w tygodniach modelowały funkcję liniową  $y = -2.4247 + 0.2333x + 0.3983$  ( $r = 0.96$ ,  $P < 0.001$ ). Współczynnik korelacji między średnicą cieśni aorty a wiekiem ciążowym był istotny statystycznie ( $P < 0.001$ ) i osiągał wartość  $r = 0.96$ . Wskaźnik średnicy cieśni aorty do średnicy początkowej aorty wstępującej wykazywał intensywny wzrost z  $0,45 \pm 0,10$  do  $0,72 \pm 0,07$  dla płodów odpowiednio w wieku 15-16 i 21-22 tyg. ciąży. U pozostałych płodów wskaźnik ten był stabilny i wahał się od  $0,66 \pm 0,10$  do  $0,73 \pm 0,06$  ( $P > 0,05$ ). Podobnie u płodów w wieku 15-22 tyg., wskaźnik cieśni łuku aorty do średnicy początkowej aorty zstępującej wzrastał z  $0,73 \pm 0,10$  w wieku 15-16 tyg. do  $0,93 \pm 0,11$

w wieku 21-22 tyg., u pozostałych płodów wskaźnik ten wahał się od  $0,88 \pm 0,09$  do  $0,90 \pm 0,07$  ( $P > 0,05$ ).

Wnioski:

1. Pomiar cieśni aorty ułatwiają prenatalną diagnostykę koarktacji łuku aorty.

2. Nasze wyniki sprzeciwiają się koncepcji względnie mniejszego przepływu krwi przez cieśń aorty podczas ciąży.

**Key words:** aortic isthmus, external diameter, aortic isthmus-to-aortic root diameter ratio, aortic isthmus-to-descending aorta diameter ratio, human fetuses

**Słowa kluczowe:** cieśń aorty, średnica zewnętrzna, stosunek średnicy cieśni do średnicy aorty wstępującej, stosunek średnicy cieśni do średnicy aorty zstępującej, płody człowieka

## INTRODUCTION

The aortic isthmus, known as the A segment, occurs as a constriction of the aorta, which is situated between the left subclavian artery and the ductus arteriosus. It is the main site for hypoplasia or coarctation of the aorta [1, 2]. Recent high-resolution capabilities of ultrasonographic equipment facilitated quantitative analysis of the development of the fetal aortic isthmus during gestation [3, 4]. According to Makikallio et al. [5], fetuses with retrograde aortic isthmus net blood flow demonstrated a rise in right ventricular afterload with diminished oxygen delivery to cerebral circulation. The aortic isthmus showed more variation than those on the other parts of aorta [6]. Also, the ratio of aortic isthmus to aortic root diameter and to descending aorta diameter increased [7, 8] or decreased [9] with advanced fetal age.

On the basis of available literature data the objectives for the present research were set to examine:

- the normal values for the aortic isthmus diameter at varying gestational ages,
- the developmental growth of the aortic isthmus diameter (growth curve),
- the aortic isthmus-to-aortic root diameter ratio,
- the aortic isthmus-to-descending aorta diameter ratio,
- the influence of sex on the value of the parameters studied.

## MATERIAL AND METHODS

The examinations were carried out on 128 human fetuses of both sexes (63 males, 65 females) from spontaneous abortions or stillbirths. The present study was approved by the University Research Ethics Committee (statement on ethical approval, KB/217/2006). All specimens were relatively unmacerated, and were large enough to evaluate anatomically. Internal and external anatomical malformations were ruled out in all specimens, which were diagnosed

as normal. In no case was the cause of fetal death related to congenital cardiovascular or non-cardiovascular anomalies. The fetal age ranged from 15 to 34 weeks (Table 1). The fetal ages of the specimens were calculated on the basis of the following criteria: 1) known date of the beginning of the last normal menstrual period, 2) gestational age based on measurement of crown-rump length [10], and 3) in some cases corrections regarding fetal age were established by measuring their humeral and femoral bones using USG equipment [11]. Fetuses were grouped into six monthly cohorts, corresponding to the 4<sup>th</sup> - 9<sup>th</sup> month of gestation. The fetal arteries were filled with white latex LBS 3060, without over-distention of the perfused vessels, through a catheter Stericath (diameter of 0.5-1.0 mm), which was introduced by lumbar access into the abdominal aorta. The arterial bed filling was performed under controlled pressure of 50-60 mm Hg, using a syringe infusion pump SEP 11S (Ascor S.A., Medical Equipment, Warsaw 2001). The specimens were immersed in a 10% neutral formalin solution for 4-24 months for preservation, and then dissected, according to standard autopsy techniques, under a stereoscope with Huygens ocular at a magnification of 10. The great vessels of the fetal heart were separated from the lungs, and the cardiovascular blocks removed from the chest cavity. In each fetus, the dissected aortic arch with the millimeter scale was placed perpendicular to the optical lens axis, afterwards recorded using a camera Nikon Coolpix 8400, and digitalized to JPEG images. Next, digital pictures of the aortic arch underwent morphometric analysis using the digital-image analysis system of Leica Qwin Pro 16 (Cambridge), which automatically estimated external diameter of the marked vessels. Because the arteries filled with latex constituted the flexible cylinder, the vessels examined were circular in cross-section.

For each individual, the five following linear measurements and calculations were made:

1. the largest external diameter of the aortic root (mm), at level of the aortic valve annulus,
2. external diameter of the aortic isthmus (mm), immediately distal to the left subclavian origin.
3. external diameter of the descending aorta (mm), immediately below the entry of the ductus arteriosus,
4. the aortic isthmus-to-aortic root diameter ratio,
5. the aortic isthmus-to-descending aorta diameter ratio.

Table 1. Distribution of fetuses studied

Fetal age		Crown-rump length (mm)				Number	Sex	
months	weeks (Hbd-life)	mean	SD	min	max		male	female
4	15	89.4	6.1	85.0	92.0	10	5	5
	16	103.7	6.1	95.0	106.0	7	3	4
5	17	114.9	8.2	111.0	121.0	6	4	2
	18	129.3	6.6	124.0	134.0	8	3	5
	19	142.7	7.7	139.0	148.0	6	3	3
	20	155.3	5.8	153.0	161.0	4	1	3
6	21	167.1	4.7	165.0	173.0	3	2	1
	22	178.1	6.9	176.0	186.0	7	4	3
	23	192.3	6.3	187.0	196.0	9	4	5
	24	202.9	5.7	199.0	207.0	11	6	5
7	25	215.2	4.8	211.0	218.0	7	5	2
	26	224.7	5.2	220.0	227.0	7	4	3
	27	234.1	4.3	231.0	237.0	4	0	4
	28	244.2	5.1	240.0	246.0	5	2	3
8	29	253.8	4.5	249.0	255.0	6	1	5
	30	262.7	3.1	260.0	264.0	6	5	1
	31	270.7	5.2	268.0	275.0	4	1	3
	32	281.4	3.7	279.0	284.0	5	4	1
9	33	290.3	6.1	286.0	293.0	9	4	5
	34	301.4	3.2	296.0	302.0	4	2	2
Total						128	63	65

The external diameters of the aortic isthmus were correlated to fetal age so as to establish their growth. The results obtained were evaluated by one-way ANOVA test for unpaired data and post hoc RIR Tukey test. Regression analysis was used to derive the growth curve of best fit for the plot for the aortic isthmus diameter against gestational age. We correlated data obtained with fetal age instead of the crown-rump length, because in the echocardiographic literature the above-mentioned measurements were related to fetal age, so that we could compare postmortem morphometric data with the echocardiographic data *in utero*. Correlation coefficient (r) between external diameters and fetal age were estimated. A priori level of significance was set at P<0.05.

RESULTS

The native picture of the aortic arch is presented in Figure 1. The statistical analysis of the five parameters examined did not show gender differences (P>0.05). For this reason, the morphometric values obtained, without regard to sex, have been presented in Table 2. During the study period, external diameter of the aortic isthmus ranged from  $0.92 \pm 0.24$  mm for fetuses aged 4 months to  $4.99 \pm 0.58$  mm for fetuses aged 9 months of gestation.

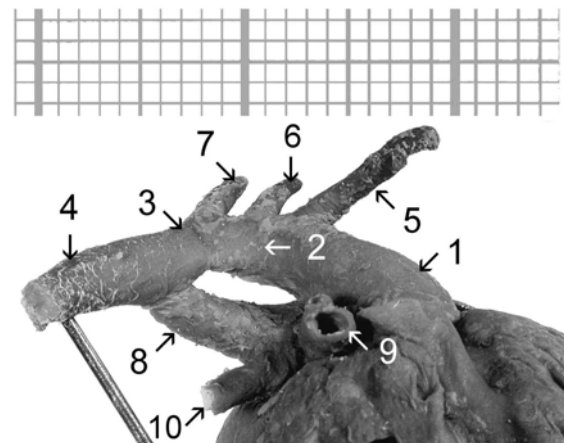


Fig. 1. The great arteries of the fetal heart in male fetus aged 23 weeks (aspectus posterior): 1-ascending aorta, 2-aortic arch, 3-aortic isthmus, 4-descending aorta, 5-brachiocephalic trunk, 6-left common carotid artery, 7-left subclavian artery, 8-ductus arteriosus, 9-right pulmonary artery, 10-left pulmonary artery

Table 2. Block scheme of the statistical analysis of the absolute and relative diameters of the aortic isthmus

Fetal age (months)	n	Aortic root diameter (mm)	Descending aorta diameter (mm)	Aortic isthmus diameter (mm)	Aortic isthmus-to-aortic root diameter ratio	Aortic isthmus-to-descending aorta diameter ratio
4	17	2.02±0.26	1.25±0.28	0.92±0.24	0.45±0.10	0.73±0.10
↓		↓(P<0.001)	↓(P<0.001)	↓(P<0.001)	↓(P<0.001)	↓(P<0.001)
5	24	2.94±0.49	2.07±0.35	1.71±0.31	0.58±0.08	0.82±0.13
↓		↓(P<0.001)	↓(P<0.001)	↓(P<0.001)	↓(P<0.001)	↓(P<0.001)
6	30	3.96±0.57	3.05±0.38	2.84±0.39	0.72±0.07	0.93±0.11
↓		↓(P<0.001)	↓(P<0.001)	↓(P<0.05)	↓(P>0.05)	↓(P>0.05)
7	23	4.91±0.47	3.62±0.55	3.26±0.61	0.66±0.10	0.90±0.07
↓		↓(P<0.001)	↓(P<0.001)	↓(P<0.001)	↓(P>0.05)	↓(P>0.05)
8	21	6.11±0.50	4.84±0.59	4.39±0.58	0.72±0.06	0.90±0.08
↓		↓(P<0.001)	↓(P<0.001)	↓(P<0.05)	↓(P>0.05)	↓(P>0.05)
9	13	6.84±0.63	5.65±0.48	4.99±0.58	0.73±0.06	0.88±0.09

The relation between the aortic isthmus diameter and gestational age is displayed in Figure 2, together with the appropriate correlation coefficient, the curve of the best fit, and the 3<sup>rd</sup> and 97<sup>th</sup> percentile lines. The growth of the aortic isthmus diameter followed the linear function  $y = -2.4247 + 0.2233x \pm 0.3983$ . Correlation coefficient between aortic isthmus diameter and gestational age was statistically significant ( $P < 0.001$ ) and reached the value  $r = 0.96$ . The 97<sup>th</sup> and 3<sup>rd</sup> percentiles for aortic isthmus diameter at varying gestational age were defined by the upper and lower borders, respectively.

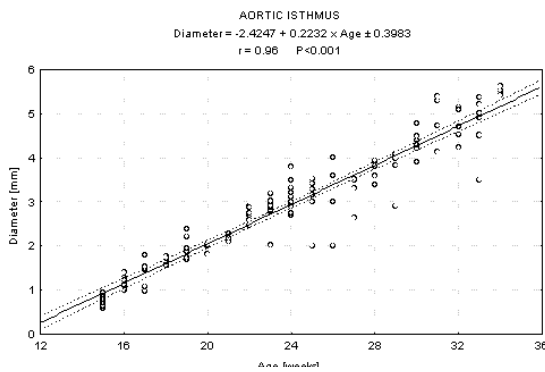


Fig. 2. Regression line for the aortic isthmus diameter (y) versus fetal age (x);  $y = -2.4247 + 0.2232 x \pm 0.3983$

The relative growth of the aortic isthmus diameter is presented in Figures 3 and 4.

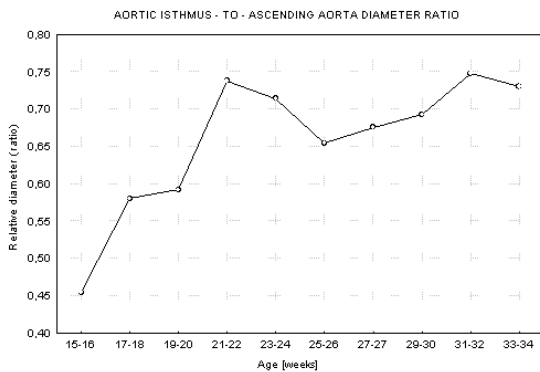


Fig. 3. Developmental growth of the aortic isthmus -to- aortic root diameter ratio in human fetuses

The aortic isthmus-to-aortic root diameter ratio (Fig. 3) indicated the intensive increase from  $0.45 \pm 0.10$  to  $0.72 \pm 0.07$  for fetuses aged 15-16 and 21-22 weeks of gestation, respectively. In the remaining fetuses, the ratio of the diameter of the aortic isthmus to that of the aortic root was stable and ranged from

$0.66 \pm 0.10$  to  $0.73 \pm 0.06$  ( $P > 0.05$ ). Similarly, in fetuses aged 15-22 weeks, the aortic isthmus-to-descending aorta diameter ratio (Fig. 4) increased from  $0.73 \pm 0.10$  for 15-16 week group to  $0.93 \pm 0.11$  for 21-22 week group. In the remainder, the aortic isthmus-to-descending aorta diameter ratio ranged from  $0.88 \pm 0.09$  to  $0.90 \pm 0.07$  ( $P > 0.05$ ).

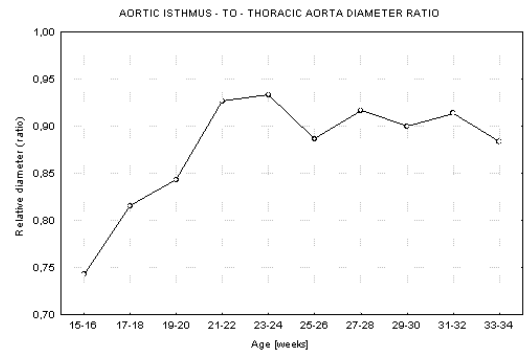


Fig. 4. Developmental growth of the aortic isthmus -to- descending aorta diameter ratio in human fetuses

DISCUSSION

In the present autopsy study the precise digital-image analysis system was used to provide the absolute and relative diameters of the aortic isthmus during gestation. Regression plots of the aortic isthmus diameter against gestational age in weeks modeled the linear function  $y = -2.4247 + 0.2333x \pm 0.3983$  ( $r = 0.96$ ,  $P < 0.001$ ). Similar observations concerning the linear growth of the aortic isthmus diameter were made by Hyett et al. [7] on autopsy study of 61 fetuses at 9-18 weeks of gestation, where this parameter generated the function  $y = 0.123 - 0.644x$  ( $r = 0.871$ ,  $P < 0.001$ ). Van Meurs - van Woezik and Krediet [6] demonstrated that in specimens from 21 weeks of gestation up to 10 years after birth, the increase in body length from 30 to 140 cm was accompanied by a parallel increase of the aortic isthmus diameter, according to the linear function  $y = 0.80 + 0.102x$ . Alvarez et al. [12] confirmed that the internal circumference of the aortic isthmus increased from 0.846 cm to 1.374 cm in fetuses and newborns ranging in body weight from 101 to 4984 g. Regression plots of this parameter against body weight in kg demonstrated the proportional growth  $y = 0.5688 + 0.17x$  ( $r = 0.829$ ,  $P < 0.001$ ). Castillo et al. [13] showed that in fetuses between 13-20 weeks of gestation the aortic isthmus diameter increased proportionally from 1.45 to 3.0 mm. These anatomical findings

are compatible with those of echocardiographic examinations, which have shown linear growth in the diameter of the aortic isthmus [1, 9]. According to Achiron et al. [1] the aortic isthmus diameter grows proportionally, as the function  $y = -1.39 + 0.189x$  ( $r = 0.938$ ,  $P < 0.001$ ). Also, Nomiya et al. [9] found that regression equation for the aortic isthmus diameter against gestational age generated the linear function  $y = 0.132x - 0.059$  ( $r = 0.60$ ,  $P < 0.001$ ).

Our results obtained indicated that in fetuses ranged from 15-16 weeks to 21-22 weeks of gestation, the relative diameter of the aortic isthmus increased with advanced fetal age. The ratio of the aortic isthmus diameter to that of the aortic root or the descending aorta increased from  $0.45 \pm 0.10$  to  $0.72 \pm 0.07$ , and from  $0.73 \pm 0.10$  to  $0.93 \pm 0.11$ , respectively. In the remaining fetuses both the ratios were stable and ranged from  $0.66 \pm 0.10$  to  $0.73 \pm 0.06$ , and from  $0.88 \pm 0.09$  to  $0.90 \pm 0.07$ , respectively. This fact was confirmed by Hyett et al. [7], because in their study the ratio of the diameter of the aortic isthmus to that of the aortic root increased with gestation from about 0.6 at 9 weeks to about 0.8 at 18 weeks, according to the regression line  $y = 0.444 + 0.018x$  ( $r = 0.346$ ,  $P < 0.001$ ). Also, Ursell et al. [8] observed that in fetuses aged 10-26 weeks, the ratio of aortic isthmus to descending aorta diameter and to aortic root diameter increased from 0.71 to 0.78, and from 0.55 to 0.58, respectively. According to Castillo et al. [13] in fetuses between 13-20 weeks of gestation, the ratios obtained between the aortic isthmus diameter and the ascending and descending aortas ranged between 0.66- 0.93. Nomiya et al. [9] revealed that the ratio of the aortic isthmus to the descending aorta was found to decrease in a linear fashion throughout late gestation, according to the function  $y = -0.007x + 1.002$  ( $r = 0.26$ ,  $P < 0.01$ ). Narrowing of the aortic isthmus during gestation attributed this finding to large cranial blood flow through the aortic arch, resulting in less flow through the isthmus into descending aorta [14]. In this aspect, my findings closely correlate with results obtained by Hyett et al. [7] and Ursell et al. [8], as well, and disagree with observations of Nomiya et al. [9]. Our observations argue against the concept of relatively little fetal blood flow through the aortic isthmus. The differences between these data resulted probably from the fact, that the aortic isthmus diameter showed more variable than those on the other parts of the aorta. Therefore, van Meurs-van Woezik and Krediet et al. [15] noted significant isthmus narrowing in 10 of 21 perinatal cases, with a ratio of aortic isthmus to descending

aorta diameter that varied from 0.24 to 0.56, whereas in the remaining 10 cases, the same ratio exceeded 0.60. Angelini et al. [16] also demonstrated a significant difference in aortic isthmus diameter to other aortic arch segments from postmortem measurements, with the isthmus being 25% smaller than the descending aorta, which was in turn 20% smaller than the ascending aorta. Machii and Becker [17] reported that in neonates and infants, the aortic isthmus-to-descending aorta diameter ratio increased rapidly. A significant increase ( $P = 0.0107$ ) was observed between newborns ( $0.78 \pm 0.15$ ) and babies from 1 month to 1 year ( $0.96 \pm 1.13$ ). Morrow et al. [18] demonstrated that diameters of the aortic isthmus and transverse arch (between the left carotid and left subclavian arteries) were significantly smaller ( $P < 0.001$ ) in coarctation patients than in control subjects. In coarctation the ratio of the aortic isthmus diameter to that of the aortic root diameter and the descending aorta reached the values  $0.48 \pm 0.08$  and  $0.55 \pm 0.13$ , respectively. In control neonates these ratios were greater as follows:  $0.61 \pm 0.10$  and  $0.77 \pm 0.17$ , respectively.

## CONCLUSIONS

1. The aortic isthmus measurements should facilitate the prenatal diagnosis of coarctation of the aorta.
2. Our findings argue against the concept of relatively little fetal blood through the aortic isthmus during gestation.

## REFERENCES

1. Achiron R., Zimand S., Hegesh J., Lipitz S., Zalel Y., Rotstein Z.: Fetal aortic arch measurements between 14 and 38 weeks' gestation: in utero ultrasonographic study. *Ultrasound Obstet. Gynecol.* 2000; 15: 226-230.
2. Gersony W.M. Coarctation of the aorta. In: Adams F.H., Emmanouilides G.C., Riemenschneider T.A.: *Moss' Heart Disease in Infants, Children, and Adolescents*. Baltimore: Williams & Wilkins, 1989; pp. 243-255.
3. Gembruch U., Shi C., Smrcek J.M.: Biometry of the fetal heart between 10 and 17 weeks of gestation. *Fetal Diagn. Ther.* 2000; 15: 20-31.
4. Hornberger L.K., Weintraub R.G., Pesonen E., Murilo-Olivas A., Simpson I.A., Sahn C., Hagen-Ansert S., Sahn D.J.: Echocardiographic study of the morphology and growth of the aortic arch in the human fetus. Observations related to the prenatal diagnosis of coarctation. *Circulation*, 1992; 86: 741-747.
5. Makikallio K., Jouppila P., Rasanen J.: Retrograde aortic isthmus net blood flow and human fetal cardiac function

- in placental insufficiency. *Ultrasound Obstet. Gynecol.* 2003; 22: 351-357.
6. van Meurs-van Woezik H., Krediet P.: Measurements of the descending aorta in infants and children: comparison with other aortic dimensions. *J. Anat.* 1982; 135: 273-279.
  7. Hyett J., Moscoso G., Nicolaidis K.: Morphometric analysis of the great vessels in early fetal life. *Hum. Reprod.* 1995; 10: 3045-3048.
  8. Ursell P.C., Byrne J.M., Fears T.R., Strobino B.A., Gersony W.M.: Growth of the great vessels in the normal human fetus and in the fetus with cardiac defects. *Circulation* 1991; 84: 2028-2033.
  9. Nomiya M., Ueda Y., Toyota Y., Kawano H.: Fetal aortic isthmus growth and morphology in late gestation. *Ultrasound Obstet. Gynecol.* 2002; 19: 153-157.
  10. Iffy L., Jakobovits A., Westlake W., Wingate M.B., Caterini H., Kanofsky P., Menduke H.: Early intrauterine development: I. The rate of growth Caucasian embryos and fetuses between the 6<sup>th</sup> and 20<sup>th</sup> weeks of gestation. *Pediatrics* 1975; 56: 173-186.
  11. Szpinda M., Szwesta A., Szpinda E.: Morphometric study of the ductus arteriosus during human development. *Ann. Anat.* 2007; 189, 47-52.
  12. Alvarez L., Aranega A., Saucedo R., Contreras J.A., Lopez F., Aranega A.: Morphometric data concerning the great arterial trunks and their branches. *Int. J. Cardiol.* 1990; 29: 127-139.
  13. Castillo E.H., Arteaga-Martinez M., Garcia-Pelaez I., Villasis-Keever M.A., Aguirre O.M., Moran V., Vizcaino Alarcon A.: Morphometric study of the human fetal heart. I. Arterial segment. *Clin. Anat.* 2005; 18: 260-268.
  14. Rudolph A.M., Heymann M.A., Spitznas U.: Hemodynamic considerations in the development of narrowing of the aorta. *Am. J. Cardiol.* 1972; 30: 514-525.
  15. van Meurs-van Woezik H., Debets T., Klein W.: Internal diameter of the ventriculo-arterial junction and great arteries of normal infants and children: a data base for evaluation of congenital cardiac malformations. *Int. J. Cardiol.* 1989; 23: 303-308.
  16. Angelini A., Allan L.D., Anderson R.H., Crawford D.C., Chita S.K., Ho S.Y.: Measurements of the dimensions of the aortic and pulmonary pathways in the human fetus: a correlative echocardiographic and morphometric study. *Br. Heart J.* 1988; 60: 221-226.
  17. Machii M., Becker A.E.: Morphologic features of the normal aortic arch in neonates, infants, and children pertinent to growth. *Ann. Thorac. Surg.* 1997; 64: 511-515.
  18. Morrow W.R., Huhta L.C., Murphy D.J., McNamara D.G.: Quantitative morphology of the aortic arch in neonatal coarctation. *J. Am. Coll. Cardiol.* 1986; 8: 616-620.

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**QUANTITATIVE ANALYSIS OF THE AORTIC ARCH IN HUMAN FETUSES**

**ANATOMIA ILOŚCIOWA ŁUKU AORTY U PŁODÓW CZŁOWIEKA**

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**S u m m a r y**

**Introduction:** The present study was performed on 128 spontaneously aborted human fetuses aged 15-34 weeks to compile normative data for aortic arch dimensions at varying gestational age.

**Material and Methods:** Using anatomical dissection, digital-image analysis (system of Leica QWin Pro 16) and statistical analysis (ANOVA, regression analysis) the growth of the length, external diameter, and volume of the aortic arch was examined.

**Results:** No significant gender differences were found ( $P > 0.05$ ). During the study period, both the length and external diameter of the aortic arch showed a linear model with advanced gestational age. The values of the aortic arch length ranged from  $3.93 \pm 0.57$  mm for the 4<sup>th</sup> month to  $15.25 \pm 1.98$  mm for the 9<sup>th</sup> month of gestation. With regard to fetal age, the aortic arch length increased according to the linear function  $y = -6.079 + 0.6370x \pm 1.1133$ , with a correlation coefficient  $r = 0.96$ . The results obtained were statistically significant ( $P < 0.001$ ) for each age group. The aortic arch diameter ranged from  $1.61 \pm 0.24$  to  $6.13 \pm 0.49$  mm for

groups of 4 and 9 months of gestation, respectively. The external diameter of the aortic arch showed a proportional increase with advanced fetal age, according to the linear function  $y = -2.413 + 0.2532x \pm 0.3532$ . Correlation coefficient between aortic arch diameter and gestational age was statistically significant ( $P < 0.001$ ) and attained the value  $r = 0.97$ . The values of the aortic arch volume ranged from  $8.84 \pm 2.90$  mm<sup>3</sup> to  $453.51 \pm 125.54$  mm<sup>3</sup> during the study period. The volumetric growth of the aortic arch was dependent on fetal age in weeks, according to the quadratic function  $y = 513.4 - 58.464x + 1.704x^2 \pm 49.254$ . The correlation of determination between the aortic arch volume and fetal age attained the value  $R^2 = 0.90$ . The normal values for aortic arch growth provided in this autopsy study are new and these data provide the background for future autopsy and Doppler studies of the aortic arch abnormalities.

**Conclusions:** The developmental dynamic of both the length and external diameter of the aortic arch follows according to the linear function, whereas the volumetric dynamic - according to the quadratic model.

**S t r e s z c z e n i e**

**Cel:** Badania przeprowadzono u 128 płodów człowieka w wieku 15-34 tygodni, pochodzących z poronień samoistnych w celu określenia normatywnych danych rozmiarów łuku aorty dla różnych wieków ciąży.

**Materiał i metody:** Przy zastosowaniu dysekcji anatomicznej, cyfrowej analizy obrazu (system Leica Q Win Pro 16) i analizy statystycznej (ANOVA, analiza regresji) zbadano wzrost długości, średnicy zewnętrznej i pojemności łuku aorty.

**Wyniki:** Nie stwierdzono różnic płciowych ( $P > 0.05$ ). W badanym przedziale wieku zarówno długość, jak i średnica zewnętrzna łuku aorty wykazywały liniowy model wzrostu. Wartość długości łuku aorty wzrastała z  $3,93 \pm 0,57$  mm w 4 mies. do  $15,25 \pm 1,98$  mm w 9 mies. ciąży. W stosunku

do wieku płodu długość łuku aorty wzrastała zgodnie z funkcją liniową  $y = -6,079 + 0,6370x + 1,1133$  ze współczynnikiem korelacji  $r = 0,96$ . Uzyskane wyniki były istotnie statystyczne ( $P < 0,001$ ) dla każdej grupy wieku. Średnica łuku aorty wzrastała z  $1,61 \pm 0,24$  do  $6,13 \pm 0,49$  mm odpowiednio dla grupy wieku 4 i 9 m. ż. pł. Średnica zewnętrzna łuku aorty wykazywała proporcjonalny wzrost z wiekiem płodu, zgodnie z funkcją liniową  $y = -2,413 + 0,2532x \pm 0,3532$ . Współczynnik korelacji między średnicą łuku aorty a wiekiem płodu był istotny statystycznie ( $P < 0,001$ ) i osiągał wartości  $r = 0,97$ . W badanym przedziale wieku wartości pojemności łuku aorty wzrastały z  $8,84 \pm 2,90$  mm<sup>3</sup> do  $453,51 \pm 125,54$  mm<sup>3</sup>. Wzrost pojemności łuku aorty zależał od wieku płodowego w tygodniach, zgodnie z funkcją kwa-

dratową  $y = 513,4 - 58,5 x + 1,7 x^2 \pm 49,3$ . Współczynnik determinacji między pojemnością łuku aorty a wiekiem płodu osiągał wartość  $R^2 = 0,90$ . Wartości normatywne wzrostu łuku aorty uzyskane w tej pracy są nowe i stanowią tło dla przyszłych badań autopsyjnych i dopplerowskich dotyczących nieprawidłowości łuku aorty.

**Key words:** aortic arch measurements; length; external diameter; volume

**Słowa kluczowe:** pomiary łuk aorty; długość; średnica zewnętrzna; pojemność

## INTRODUCTION

The aortic arch lies wholly in the superior mediastinum. As a continuation of the ascending aorta it begins at the level of the upper border of the 2<sup>nd</sup> right sternocostal joint. It first runs upward, diagonally back and to the left over the anterior surface of the trachea, then back across its left side, and finally passes downward left of the 4<sup>th</sup> thoracic vertebral body, continuing at its lower border as the descending aorta. The fetal aortic lumen narrows between the origin of the left subclavian artery and the attachment of the ductus arteriosus forming an aortic isthmus [1]. Recent high-resolution capabilities of ultrasonographic equipment facilitated detailed quantitative analysis of the development of the fetal aortic arch during gestation [2, 3]. Thus, it is important to define the normal growth patterns for all cardiovascular structures to recognize abnormal development as early, as possible. Morphometric studies on the aortic arch have been conducted previously in relation to its diameter only, using echocardiographic [3-5] and autopsy [1, 5-9] methods. In the opinion of these authors, the aortic arch diameter presented linear growth during gestation. Moreover, the limited anatomical data suggested that length and diameter of the aortic arch increased linearly, whereas the volumetric growth was expressed by a square root function [10].

Our objectives were to investigate:

- the normal values for the length, external diameter, and volume of the aortic arch at varying gestational ages,
- the influence of sex on the value of the parameters examined,
- the developmental growth of the morphometric features (growth curves).

## MATERIAL AND METHODS

The material examined consisted of 128 human fetuses of both sexes (63 males, 65 females) from spontaneous abortions or stillbirths.

Wniosek: Dynamika rozwojowa długości i średnicy zewnętrznej łuku aorty przebiega zgodnie z funkcją liniową, podczas gdy dynamika rozwojowa pojemności łuku aorty – zgodnie z modelem kwadratowym.

The gestational age ranged from 15 to 34 weeks (Table 1). The study protocol was approved by the University Research Ethics Committee (KB/217/2006). In no case was the cause of fetal death related to congenital cardiovascular anomalies. The fetal ages of the specimens were calculated on the basis of the following criteria: 1) known date of the beginning of the last normal menstrual period, and 2) gestational age based on measurement of crown-rump length [11]. Fetuses were grouped into six monthly cohorts, corresponding to the 4<sup>th</sup> - 9<sup>th</sup> month of gestation. The fetal arteries were filled with white latex LBS 3060, at an amount of approximately 15-30 ml (without over-distention of the perfused vessels), through a catheter Stericath (diameter of 0.5-1.0 mm), which was introduced by lumbar access into the abdominal aorta. According to our experience, the arterial bed filling was performed under controlled pressure of 50-60 mm Hg, using a syringe infusion pump SEP 11S (Ascor S.A., Medical Equipment, Warsaw 2001). Specimens were immersed in a 10% neutral formalin solution for 4-24 months for preservation, and then dissected, according to standard autopsy techniques, under a stereoscope at a magnification of 10-25. The great arteries of the fetal heart were separated from the lungs and the cardiovascular blocks removed from the chest cavity. In each fetus, the dissected aortic arch with the millimeter scale was placed perpendicular to the optical lens axis, afterwards recorded using a camera Nikon Coolpix 8400, and digitalized to JPEG images (Fig. 1). Next, digital pictures of the aortic arch underwent morphometric analysis using digital image analysis system of Leica QWin Pro 16 (Cambridge), which automatically estimated length, external diameter and volume of the marked vessel. Automatic measurements of the parameters examined were derived by assuming that the filled arteries constituted the flexible cylinder, could be divided into small irregular cylinders of varying diameter and height. The sum of volumes of such cylinders

approximating the vessel was given in  $\text{mm}^3$  as the aortic arch volume.

Table 1. Age, number and sex of fetuses studied

Fetal age		Crown-rump length (mm)				Number	Sex	
Months	Weeks (Hbd-life)	Mean	SD	Min	Max		male	female
4	15	89.4	6.1	85.0	92.0	10	5	5
	16	103.7	6.1	95.0	106.0	7	3	4
5	17	114.9	8.2	111.0	121.0	6	4	2
	18	129.3	6.6	124.0	134.0	8	3	5
	19	142.7	7.7	139.0	148.0	6	3	3
	20	155.3	5.8	153.0	161.0	4	1	3
6	21	167.1	4.7	165.0	173.0	3	2	1
	22	178.1	6.9	176.0	186.0	7	4	3
	23	192.3	6.3	187.0	196.0	9	4	5
	24	202.9	5.7	199.0	207.0	11	6	5
7	25	215.2	4.8	211.0	218.0	7	5	2
	26	224.7	5.2	220.0	227.0	7	4	3
	27	234.1	4.3	231.0	237.0	4	0	4
	28	244.2	5.1	240.0	246.0	5	2	3
8	29	253.8	4.5	249.0	255.0	6	1	5
	30	262.7	3.1	260.0	264.0	6	5	1
	31	270.7	5.2	268.0	275.0	4	1	3
	32	281.4	3.7	279.0	284.0	5	4	1
9	33	290.3	6.1	286.0	293.0	9	4	5
	34	301.4	3.2	296.0	302.0	4	2	2
Total						128	63	65

For each fetus the eight following measurements and calculations were made:

1. the aortic root diameter (mm), at the level of the aortic valve annulus,
2. length of the aortic arch (mm), from its origin (just proximal to the brachiocephalic trunk origin) to its ending (just proximal to the entry of the ductus arteriosus),
3. external diameter of the aortic arch (mm), distal to the left common carotid origin,
4. volume of the aortic arch ( $\text{mm}^3$ ),
5. the external descending aorta diameter (mm), immediately below the entry of the ductus arteriosus,

The length, external diameter and volume of the aortic arch, were correlated to fetal age so as to establish their growth. The results obtained were evaluated by one-way ANOVA test for unpaired data and then post-hoc intergroup comparisons were performed using RIR Tukey test. Regression analysis was used to derive the line of best fit for the plot for each parameter examined against gestational age. Correlation coefficients ( $r$ ) between length or external diameters and fetal age, and coefficient of determination ( $R^2$ ) between volume and fetal age were estimated. A priori level of significance was set at  $P < 0.05$ .

## RESULTS

The native picture of the aortic arch is presented in Figure 1. The statistical analysis of the morphometric

features of the aortic arch did not show gender differences ( $P > 0.05$ ). Hence the morphometric values obtained, without regard to sex, are presented in Table 2. The aortic arch parameters increased significantly with gestational age. The relations between the aortic arch parameters and gestational age are displayed in Figures 2-4 together with the appropriate correlation coefficients for length and external diameters, or the coefficient of determination for volume, the curves of the best fit, and the 3<sup>rd</sup> and 97<sup>th</sup> percentile lines. The 97<sup>th</sup> and 3<sup>rd</sup> percentiles for each morphometric feature at varying gestational age were defined by the upper and lower borders respectively, of the 95% confidence limits around each regression analysis.

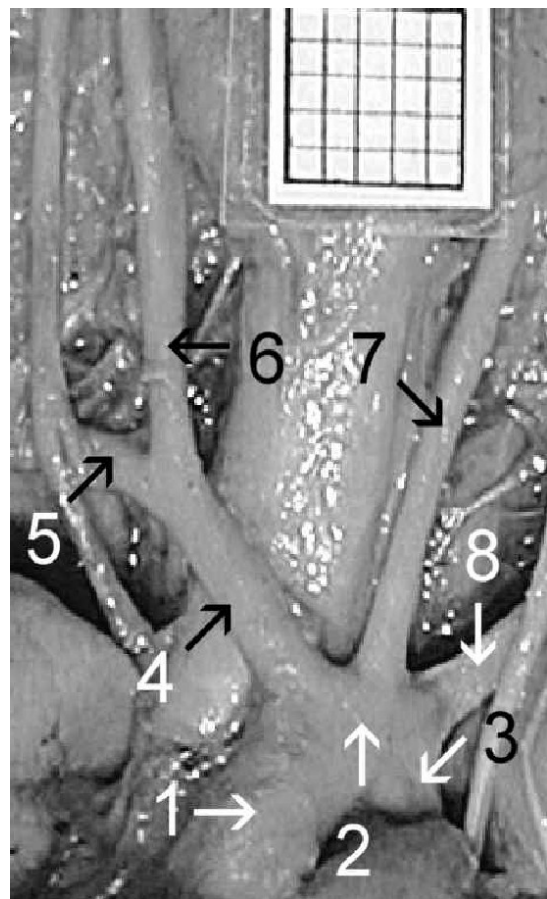


Fig. 1. The great chest arteries (in situ) in male fetus aged 26 weeks (aspectus anterior): A-heart, B-right lung, C-left lung, D-trachea, 1-ascending aorta, 2-aortic arch, 3-aortic isthmus, 4-brachiocephalic trunk, 5-left common carotid artery, 6-left subclavian artery, 7-right subclavian artery, 8-right common carotid artery, 9- pulmonary trunk, 10-ductus arteriosus, 11-right vagus nerve, 12-left vagus nerve, 13-right recurrent laryngeal nerve, 14- left phrenic nerve

Table 2. Block scheme of the statistical analysis of the morphometric parameters (length, external diameter, volume) of the aortic arch

Fetal age [month]	n	Length [mm] (mean ± SD)	External diameter [mm] (mean ± SD)	Volume [mm <sup>3</sup> ] (mean ± SD)
4	17	3.93 ± 0.57 ↓ (P<0.01)	1.61 ± 0.24 ↓ (P<0.001)	8.84 ± 2.90 ↓ (P>0.05)
5	24	5.56 ± 0.89 ↓ (P<0.001)	2.32 ± 0.45 ↓ (P<0.001)	25.69 ± 13.67 ↓ (P<0.05)
6	30	8.55 ± 1.08 ↓ (P<0.001)	3.23 ± 0.45 ↓ (P<0.001)	74.60 ± 27.21 ↓ (P<0.01)
7	23	10.22 ± 1.30 ↓ (P<0.001)	4.10 ± 0.42 ↓ (P<0.001)	140.04 ± 42.75 ↓ (P<0.001)
8	21	13.56 ± 1.55 ↓ (P<0.01)	5.41 ± 0.47 ↓ (P<0.001)	322.67 ± 84.66 ↓ (P<0.001)
9	13	15.25 ± 1.98	6.13 ± 0.49	453.51 ± 125.54

During the study period, both the length and external diameter of the aortic arch showed a linear fashion with advanced gestational age. The values of the aortic arch length ranged from  $3.93 \pm 0.57$  mm for the 4<sup>th</sup> month to  $15.25 \pm 1.98$  mm for the 9<sup>th</sup> month of gestation. With regard to fetal age, the aortic arch length increased according to the linear function  $y = -6.079 + 0.6370 x \pm 1.1133$ , with a correlation coefficient  $r = 0.96$  (Fig. 2). The results obtained were statistically significant ( $P < 0.001$ ) for each age group. The aortic arch diameter ranged from  $1.61 \pm 0.24$  to  $6.13 \pm 0.49$  mm for groups of 4 and 9 months of gestation, respectively.

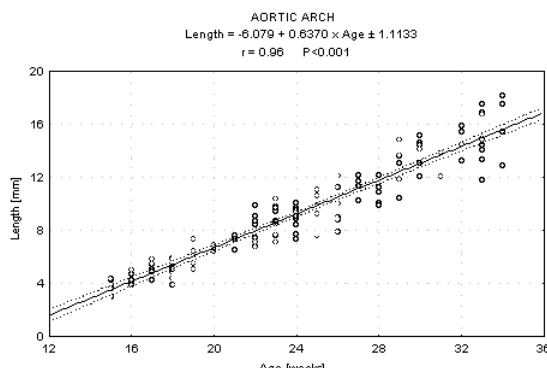


Fig. 2. Regression line for the length (y) of the aortic arch versus fetal age (x);  $y = -6.079 + 0.6370 x \pm 1.1133$  ( $r = 0.96, P < 0.001$ )

The external diameter of the aortic arch showed a proportional increase with advanced fetal age, according to the linear function  $y = -2.413 + 0.2532 x \pm 0.3532$  (Fig. 3). Correlation coefficient between aortic arch diameter and gestational age was statistically significant ( $P < 0.001$ ) and attained the value  $r = 0.97$ .

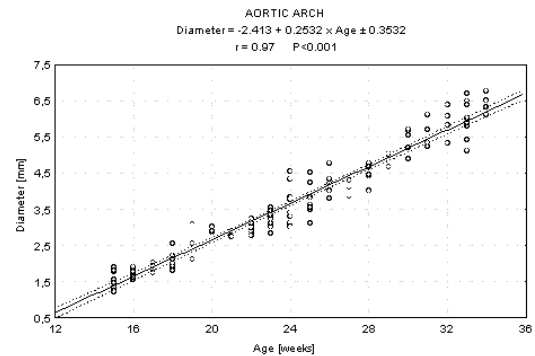


Fig. 3. Regression line for the external diameter (y) of the aortic arch versus fetal age (x);  $y = -2.413 + 0.2532 x \pm 0.3532$  ( $r = 0.97, P < 0.001$ )

The values of the aortic arch volume ranged from  $8.84 \pm 2.90$  mm<sup>3</sup> to  $453.51 \pm 125.54$  mm<sup>3</sup> during the study period. The volumetric growth of the aortic arch was dependent on fetal age in weeks, according to the quadratic function  $y = 513.4 - 58.464 x + 1.704 x^2 \pm 49.254$  (Fig. 4). The correlation of determination between the aortic arch volume and fetal age attained the value  $R^2 = 0.90$ .

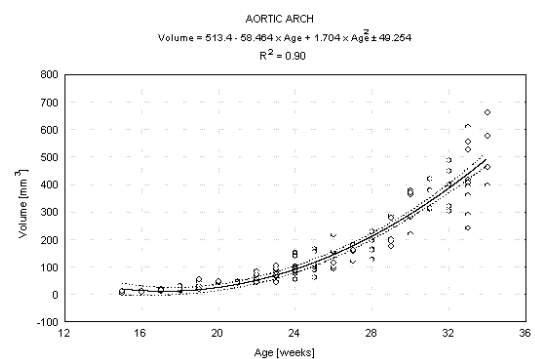


Fig. 4. Regression line for the volume (y) of the aortic arch versus fetal age (x);  $y = 513.4 - 58.464 x + 1.704 x^2 \pm 49.254$  ( $R^2 = 0.90$ )

## DISCUSSION

The delineation of fetal cardiovascular anatomy has been substantially assisted by the advent of high-resolution echocardiography [2, 3]. References data for

dimensions of the aortic arch, as determined using both echocardiography and anatomical dissection are scarce in human fetuses. In the present autopsy study, the precise digital image analysis was used to provide normal morphometric parameters of the aortic arch during gestation. The present data indicated that growth of the length and diameters of the aortic arch and aortic isthmus appears to be linearly related to gestational age. The values of the aortic arch length generated the linear function  $y = -6.079 + 0.6370x \pm 1.1133$ . Also, external diameters of the aortic arch increased proportionally with advanced age, as follows  $y = -2.413 + 0.2532x \pm 0.3532$ . Linear growth of the length and diameter of the aortic arch was suggested by Gielecki et al. [10], although in their material the correlation coefficients between length or diameter and gestational age were much lower ( $r = 0.72$  for length,  $r = 0.79$  for diameter) in comparison to our results ( $r = 0.96$  for length,  $r = 0.97$  for diameter).

In this study, measurements of the aortic arch volume clearly show its strong dependence on fetal age, in accordance with the quadratic function  $y = 513.4 - 58.464x + 1.704x^2 \pm 49.254$ . In the fetuses examined the aortic arch volume increased 51-fold, from  $8.84 \pm 2.90$  to  $453.51 \pm 125.54 \text{ mm}^3$ . This result was obtained from the product of the length and the squared diameter, that both increased approximately 3.9-fold. The quadratic function, expressed as parabola was the best model ( $R^2 = 0.90$ ,  $P < 0.001$ ) for the volumetric growth of the aortic arch. Therefore, we disagree with opinion of Gielecki et al. [10], who suggested that the volumetric growth of the aortic arch followed a square root function. The square root function is related to the quadratic function, as its inverse.

The lack of statistically significant gender differences concerning the aortic arch parameters was observed, as well. In this aspect, our results are in accordance with the findings of some authors [12-14]. According to Poutanen et al. [13], although in young adults the absolute aortic diameters were greater in males than in females, nevertheless these diameters were similar in both genders when indexed to BSA.

In the present study we characterized the growth pattern of the aortic arch in fetuses from 15 to 34 weeks of gestation. In conclusion, we hope that normative data of the aortic arch obtained in this study provide the background for future autopsy and Doppler studies of the aortic arch abnormalities.

## CONCLUSION

The developmental dynamic of both the length and external diameter of the aortic arch follows according to the linear function, whereas the volumetric dynamic - according to the quadratic model.

## REFERENCES

1. Ursell P.C., Byrne J.M., Fears T.R., Strobino B.A., Gersony W.M.: Growth of the great vessels in the normal human fetus and in the fetus with cardiac defects. *Circulation* 1991; 84: 2028-2033.
2. Gembruch U., Shi C., Smrcek J.M.: Biometry of the fetal heart between 10 and 17 weeks of gestation. *Fetal Diagn. Ther.* 2000; 15: 20-31.
3. Hornberger L.K., Weintraub R.G., Pesonen E., Murilo-Olivas A., Simpson I.A., Sahn C., Hagen-Ansert S., Sahn D.J.: Echocardiographic study of the morphology and growth of the aortic arch in the human fetus. Observations related to the prenatal diagnosis of coarctation. *Circulation* 1992; 86: 741-747.
4. Achiron R., Zimand S., Hegesh J., Lipitz S., Zalel Y., Rotstein Z.: Fetal aortic arch measurements between 14 and 38 weeks' gestation: in utero ultrasonographic study. *Ultrasound Obstet. Gynecol.* 2000; 15: 226-230.
5. Angelini A., Allan L.D., Anderson R.H., Crawford D.C., Chita S.K., Ho S.Y.: Measurements of the dimensions of the aortic and pulmonary pathways in the human fetus: a correlative echocardiographic and morphometric study. *Br. Heart J.* 1988; 60: 221-226.
6. Alvarez L., Aranega A., Saucedo R., Contreras J.A., Lopez F., Aranega A.: Morphometric data concerning the great arterial trunks and their branches. *Int. J. Cardiol.* 1990; 29: 127-139.
7. Castillo E.H., Arteaga-Martinez M., Garcia-Pelaez I., Villasis-Keever M.A., Aguirre O.M., Moran V., Vizcaino Alarcon A.: Morphometric study of the human fetal heart. I. Arterial segment. *Clin. Anat.* 2005; 18: 260-268.
8. Hyett J., Moscoso G., Nicolaidis K.: Morphometric analysis of the great vessels in early fetal life. *Hum. Reprod.* 1995; 10: 3045-3048.
9. Szpinda M., Brazis P., Elminowska-Wenda G., Wiśniewski M.: Morphometric study of the aortic and great pulmonary arterial pathways in human fetuses. *Ann. Anat.* 2006; 188: 25-31.
10. Gielecki J.S., Syc B., Wilk R., Musiał-Kopiejka M., Piwowarczyk-Nowak A.: Quantitative evaluation of aortic arch development using digital-image analysis. *Ann. Anat.* 2006; 188: 19-23.
11. Iffy L., Jakobovits A., Westlake W., Wingate M.B., Caterini H., Kanofsky P., Menduke H.: Early intrauterine development: I. The rate of growth Caucasian embryos and fetuses between the 6<sup>th</sup> and 20<sup>th</sup> weeks of gestation. *Pediatrics* 1975; 56: 173-186.
12. Nidorf S.M., Picard M.H., Triulzi M.O., Thomas J.D., Newell J., King M.E., Weyman A.E. New perspectives in

- the assessment of cardiac chamber dimensions during development and adulthood. *J. Am. Col. Cardiol.* 1992; 19: 983-988.
13. Poutanen T., Tikanoja T., Sairanen H., Jokinen E.: Normal aortic dimensions and flow in 168 children and young adults. *Clin. Physiol. Funct. Imaging* 2003; 23: 224-229.
14. Roman M.J., Devereux R.B., Kramer-Fox R., O'Loughlin J.: Two-dimensional echocardiographic aortic root dimensions in normal children and adults. *Am. J. Cardiol.* 1989; 64: 507-512.

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