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REVIEW / PRACA POGLĄDOWA

Wojciech J. Baranowski

THE ACTIVITY OF THE HUMAN DIGESTIVE TRACT – A NEW INTERPRETATION OF OLD FACTS

CZYNNOŚCI PRZEWODU POKARMOWEGO CZŁOWIEKA – STARE FAKTY W NOWEJ INTERPRETACJI

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S u m m a r y

Secretion and absorption processes in the human digestive tract are described as membrane processes. The mucus-covered wall of the bowel is a asymmetric (composite) membrane. The force driving the permeation is the gradient of the electrochemical potential. This gradient depends mostly on the gastrointestinal motility and velocity of blood transport through the capillary network of submucosa.

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

Procesy wydzielania i wchłaniania w ludzkim przewodzie pokarmowym przedstawiono jako procesy membranowe. Pokryta śluzem ściana jelita jest membraną asymetryczną (złożoną). Siłą napędową permeacji jest gradient potencjału elektrochemicznego. Gradient ten zależy przede wszystkim od aktywności motorycznej przewodu pokarmowego i szybkości przepływu krwi przez sieć naczyń włosowatych błony podśluzowej.

Key words: secretion, absorption, mucus, gut, membrane processes
Słowa kluczowe: wydzielanie, wchłanianie, śluz, przewód pokarmowy, procesy membranowe

Nutrition has the purpose of delivering to the organism the nutrients which are the source of energy and body mass. For that reason the secretion of digestive juices and absorption of nutrients must be advantageous for the organism in terms of energy. In this sense the ideal would be an absorption of nutrients independent of energy usage. Is it possible that the human organism attempts to achieve this ideal? The purpose of this paper is a discussion of membrane systems as well as their use by the organism with regard to the digestive tract.

Membrane processes have long been known and are widely applied for practical uses such as in hemodialysers. The principle of their action are processes occurring in semi-permeable membranes or simply membranes. The membrane separates two streams of different solutions/suspensions (or two phases), between which mass transfer (permeation) occurs, as shown in Figure 1.

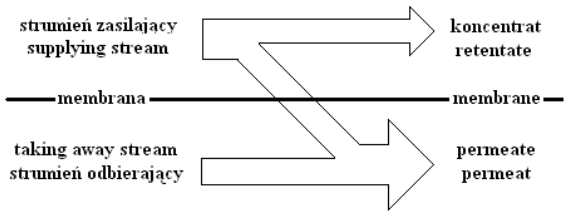

Fig. 1. *Permeation through membrane*
Ryc. 1. *Proces permeacji przez membranę*

The mass (called permeant or penetrant) is made up of the entities (solutes or suspended solids) from a phase in contact with one of the membrane surfaces that passes through the membrane with a certain amount of solvent. In this system the membrane is

a dynamic filter. In this way the supplying stream becomes the retentate, since its volume is reduced in favour of the taking away stream that becomes permeate. Membranes are either convective that is porous or solution-diffusion that is dense (non-porous). There is also the distinction between symmetric and asymmetric membranes. Symmetric membranes (homogeneous membranes) have a uniform structure along the whole transverse cross-section. In contrast asymmetric membranes (composite membranes) are built of at least two layers: the so-called matrix (or carrying layer), and the skin (or active) layer. The matrix is usually a porous membrane, and the skin layer is a dense membrane, which determining the permeability of the asymmetric membrane. In terms of their state of matter, membranes can be either solid or fluid.

The force driving the permeation is the gradient of their electrochemical potential, by which is understood the differences in concentration, pressures or electric charge on both sides of the membrane. Of course at a given time various driving forces can act, and therefore under the right conditions permeation can occur in both directions. In porous membranes the driving force of permeation is most often the pressure differential, though in dense membranes it can be any gradient of electrochemical potential. Membrane processes are characterized by flux and selectivity. Flux means the amount of mass passing through a unit of membrane surface in a unit of time, while selectivity means that only specific permeant can passes through the membrane. It should be noted that selectivity depends on structure and especially on the chemical composition of the membrane, but not on its thickness. The membrane thickness determines only the flux: the thinner the membrane, the faster the process and the greater its flux. From membrane technology we know that dense asymmetric membranes have even 100 times greater flux than symmetric membranes of this type. In technical practice it has been shown that membrane processes are separation methods which depend exclusively on low-energy physical processes, thanks to which the permeate is not subjected to chemical changes. From a technological point of view the weakness of membranes is their limited chemical, mechanical durability and thermal stability. Another important disadvantage is the deposition on the membrane surface of particles which can change the membrane's technical parameters or even damage it. These phenomena are known as fouling, scaling and concentration polarization.

Fouling is the process resulting in loss of performance of a membrane due to the deposition of suspended substances at its pore openings. Scaling is similar to fouling, but it is due to the crystallisation of dissolved substances within pores of membrane. Concentration polarization causes a reverse diffusion and reduction in the force of the permeation process due to concentration profile that has a higher level of solute nearest to the supplying stream membrane surface compared with the well-mixed bulk fluid far from the membrane surface.

The membrane processes as well as structure and systematic of membranes are broadly discussed in chemical engineering, physical chemistry, physics and biophysics textbooks and especially in relevant monographs [1].

The digestive tract is a membrane system because in its anatomic structure can be identified the membrane and the two phases divided by it. The membrane is part of the digestive tract wall which divides blood from the intestinal lumen through which flows the chyme. Figure 2 is a schematic representation of the intestinal wall structure.

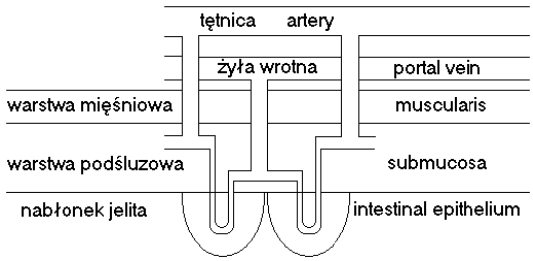

Fig. 2. *Schema of the structure of the digestive tract wall*
Ryc. 2. *Schemat budowy ściany przewodu pokarmowego*

The schema shows that the epithelium covered with mucus is "washed" by the chyme from the lumen side of the digestive tract, and from the other side by the blood in the network of capillaries of the submucosa.

The epithelium forms the matrix, and the mucus forms skin layer of the asymmetric membrane. Such an assumption has its justification in the construction of the epithelium, which is a porous membrane, as in the properties of mucus, which forms a dense membrane.

It should be stressed that the cell membrane of enterocyte fulfills the criteria for it to be qualified a dense symmetric membrane. From membrane technology it is known that flux through dense symmetric membranes is up to 100 times slower than flux through asymmetric membranes. For that reason permeation through the epithelium covered with mucus is one-hundred times

faster than permeation through membranes of cell membranes of specific enterocytes.

Currently it is assumed that in the digestive tract occur four types of processes of the transport of mass, and these are:

1. membrane passive transport (simple diffusion) - a passive process of equalizing the substance concentration on both sides of a membrane;
2. membrane carrier-mediated transport (facilitated diffusion), which equalizes the concentrations on both membrane sides thanks to the activity of non-energy-using carriers;
3. membrane active transport, which carries the substance through the membrane against the concentration gradient with the help of energy-using carriers;
4. endocytosis, which is performed through the consumption of certain substances by cells.

The natural consequence of such an interpretation of the absorption process is the presumption that in the human organism the transport of mass occurs exclusively by the transcellular path, which is false. It is not difficult to notice that that processes described above can involve only cells. It should be stressed that the metabolism of a multicellular organism is not the simple sum of the metabolisms of its individual cells. Processes of transport of mass described on a micro-scale are different from processes occurring on a macro-scale. Cell membranes are not the only type of membrane occurring in multicellular organisms, including the human. Also, biological membranes are built from cells by tissue specialists. Such membranes necessarily have to be counted as porous membranes. Exactly such a membrane occurs in the digestive tract in the form of mucous membrane.

Physico-chemical knowledge clearly shows that transport between cells (intercellular, paracellular) is privileged in the digestive tract, and the idea of transport through cells contradicts many facts - the most important of which is that transcellular transport would disturb the basic activity of enterocytes, which is the production of mucus (ectoenzymes). Beyond that, this transport requires penetration two times through the cell membrane, which happens by the action of so-called pumps and other carrying systems, which require energy supply and have a particular efficiency. A decisive argument against the theory of transcellular transport are the mathematical calculations showing that the efficiency of these pumps is less than the actual efficiency of transporting a mass through the intestinal membrane [2]. In addition, transcellular transport is contradicted by the fact of the peeling off of enterocytes into the intestinal lumen [3], which from the point of view of the organism is a waste of energy.

The driving force of permeation in the membrane system of the digestive tract is the gradient of the electrochemical potential. This gradient depends above all on the motor activity of the digestive tract, that is, the change in pressure in the intestine in relation to the blood pressure in the vessels of the submucosa of the intestine. Other forces driving permeation are related to blood circulation, the pH difference between chyme and blood, and the difference of concentrations exchanging substances between the chyme and blood.

Motor activity of the digestive tract consists of regular and synchronous contractions of its muscularis. Until now it has been thought that the purpose of these movements was only the maceration and mixing of food with digestive juices, facilitating the contact of the chyme with the mucous membrane [4,5], as well as the timely secretion of digestive juices and nutrient absorption. The visible differentiation of the architecture of the mucous membrane in the various sections of the digestive tract, which until now has been associated only with the extent of its absorptive surface, permits an active alteration in the membrane structure synchronized with the intestinal movements. At this point it is worth recalling two interesting facts: in the stomach there occurs almost only secretion, and in the last portions of the large intestine, almost only absorption. In both cases the mucous membrane has a similar form, but there is a marked difference in the motor activity of these two intestinal tract sectors.

After the introduction of food into the stomach, its mucous membrane undergoes increased blood flow, but its muscles do not exhibit any motor activity except for pressing the walls on the food, thanks to which this gathers on the gastric fundus and adheres tightly to the mucous membrane. The first peristaltic stomach movements appear only after about 30 minutes, during which time secretion of stomach juices (stomach acid) begins – a consequence of blood flow in the stomach's mucous membrane, and the lack of motor activity. The stomach motor activity which does appear at this time has a low frequency – a contraction wave occurs about every 20 seconds. The chyme created by food and stomach juices, moving into the intestine, irritates it, evoking increased blood flow through the network of capillary vessels of the submucosa [6], as well as inducing intestinal movement [4]. These movements are: segmental, pendular and peristaltic. Segmental (sepa-

rating) movements appear as the consequence of round-muscle spasms in sections of a few centemeters' length, with simultaneous relaxation of the long muscles. The circular contraction lasts a few seconds, after which the circular muscles which were contracting relax, and those which were relaxed, contract. Pendular movements have a similar effect, while peristaltic (propulsive) movements are the effect of a 'wandering' contraction of the circular muscles, which pushes the chyme in the direction of the large intestine. In the large intestine the motor activity diminishes through the disappearance of the pendular movements [4]. The contraction frequency also slows. In the last sections of the large intestine appear mass movements. These movements cause a significant speeding up of blood circulation in the net of capillary blood vessels of the submucosa, and these are responsible for absorbing water, i.e. desiccating the stool mass.

It has been long known that water and aqueous solutions pass freely through the membrane in accordance with the hydrostatic pressure gradient [7]. Beyond that it has been established experimentally that changes of intestinal blood-vessel pressure lead to a change in the direction of transport of the mass [8, 9]. Intestinal motor activity is not taken into account as a force driving processes of absorption and secretion in the digestive tract because traditionally those processes have not been associated with membrane processes. It has been recognized in the meantime that the intestine's movements assure the active adjustment of its capacity to the amount of content filling it, and also cause temporary changes in lumen pressure. An awareness of this fact and association of it with the anatomical structure allows demonstration of the process responsible for absorption and secretion in the intestinal tract, a process that can be called the "intestinal pump".

Segmental movement, and in the case of the stomach circular contractions, evoke pressure changes not only in the intestinal lumen, but also in the blood-vessel network located between the muscularis and intestinal epithelium. Under the influence of those movements the speed of blood transport through the intestinal vessels, and especially through the intestinal villi, changes. In accordance with Bernoulli's Law, a faster flow of blood is accompanied by the absorption process, and a slower – secretion. Those processes occur simultaneously in one intestinal segment and at the same values of pressure gradients driving them, which vary in direction. In that respect the amount of intestinal juice secreted into the intestinal lumen is equal to the amount of solution of nutrients which are absorbed from the intestinal lumen. It has been already mentioned that in the stomach mainly secretion occurs, and in the large bowel, absorption. This phenomenon depends above all on the motor activity of those intestine sectors, which influences the speed of blood transport through their blood vessels.

The model system for the phenomena described in this article is the suckling's alimentary tract which consumes only liquid foods. Ingested milk is gathered in the stomach, where it is mixed with stomach juices. The resulting mixture, chyme, is injected portion-wise into the duodenum, becoming the source of the supplying stream. With the intestinal motor activity the liquid chyme undergoes the gradual passage of its aqueous part, with its dissolved or suspended nutrient elements, to the blood, which is here the taking away stream. Simultaneously a second membrane process occurs in which the blood is the supplying stream, and the chyme is the taking away stream. This happens thanks to the segmental movements, by which the same amount of water which passed out together with nutrients returns to the intestine in the form of blood permeate. In this way the continuation and great efficiency of absorption is assured: in each subsequent segment the chyme is increasingly poor in nutrients, because they have passed through the intestinal membrane of previous segments. At the same time this segmentation prevents the problems of fouling, scaling and concentration polarization mentioned earlier. The propulsive movements prevent the retention of chyme in any of the segments by pushing it in the direction of the large intestine. In the large intestine the now nutrient-poor chyme is thickened by the rapid passage of blood through the dense net of capillary blood vessels of the submucosa. The effect of this process is that the thickened chyme forms a concentrate of indigestible food components which are retained by the membrane of the intestinal lumen. In this case blood is again the taking away stream. The indigestible food components remaining in the intestine form a concentrate of chyme, which is then excreted in the form of stool.

This process of secretion and absorption is characterized by its low energy requirement, and depends on vegetative activity of the organism. In light of the above, the decades-old view of the complexity of the intestinal surface structure on the lumen side as related only to the necessity of increasing the surface area for absorption of nutrients, should be modified. The ali-

mentary tract is a membrane system driven by the mechanism of the “intestinal pump” which depends on the motor activity of the intestinal tract.

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REVIEW / PRACA POGLĄDOWA

Dorota Gregorowicz-Warpas

THE BENEFITS RESULTING FROM INTRODUCTION OF MICROBIOLOGICAL SCREENING STANDARD ON THE DAY OF ADMITTING A PATIENT TO THE SPECIALIZED HOSPITAL IN KOŚCIERZYNA

KORZYŚCI WYNIKAJĄCE Z WPROWADZENIA STANDARDU MIKROBIOLOGICZNYCH BADAŃ PRZESIEWOWYCH W DNIU PRZYJĘCIA PACJENTA DO SZPITALA SPECJALISTYCZNEGO W KOŚCIERZYNIE

Specialized Hospital in Kościerzyna
Director DDS Andrzej Steczyński

S u m m a r y

The aim of introducing an examination standard to perform on the day of admitting a patient to the hospital is (early) detection of alarming microorganisms (germs) as well as recognition of the (possible) epidemiological situation. The range of different examinations made in our hospital is the result of characteristic features of germs, their virulence, resistance to antibiotics, epidemiological and endemic character of the microorganisms, as well as recommendation (and advice) of different specialists.

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

Wprowadzenie standardu badań mikrobiologicznych w dniu przyjęcia pacjenta do szpitala ma na celu wczesne wykrycie drobnoustrojów alarmowych oraz rozpoznanie sytuacji epidemiologicznej. Zakres wykonywanych badań wynika z cech charakterystycznych (zjadliwość, charakter endemiczny i epidemiczny, lekooporność) drobnoustrojów oraz zaleceń i rekomendacji środowisk szpitalnych różnych specjalności.

Key words: Methicillin Resistant *Staphylococcus aureus, HBV, Treponema pallidum, Streptococcus agalactiae, Streptococcus pyogenes*

Słowa kluczowe: Methicillin Resistant *Staphylococcus aureus, HBV, Treponema pallidum, Streptococcus agalactiae, Streptococcus pyogenes*

INTRODUCTION

A microbiological screening applied on the day of admitting a patient to the hospital gives measurable profits resulting from identification of a carrier of infection caused by alert pathogens.

Such a diagnosis of epidemiological situation enables application of suitable action plan by implementation of patient's isolation depending on infection transmission and possible application of evidence-based antibiotic therapy.

An examination performed during the first day of patient's presence in a hospital facilitates correct qualification of infection and consequently makes it easier to avoid patient's claims in a court.

The effectiveness of implemented examination standard and more precisely the range of examinations included in the standard is temporarily verified and evaluated by the Committee of Hospital Infection Control.

THE RANGE OF MICROBIOLOGICAL SCREENING UNDERGONE BY PATIENTS DURING THE DAY OF ADMISSION TO THE SPECIALIZED HOSPITAL IN KOŚCIERZYNA

1. Testing for Methicillin resistant *Staphylococcus aureus* (MRSA) is performed on the basis of smear tests taken from nasal atrium, the throat, groin and skin changes in patients:
   - admitted from other hospitals and social welfare centers and (or) treated in other hospital or exposed to invasive diagnostic treatment during the period of 3 months before admitting to the Specialized Hospital in Kościerzyna,
   - treated in the Specialized Hospital in Kościerzyna during the period of last 3 months – the decision of performing the examination is made by attending physician taking into consideration the risk of infection and results of examination made so far,
   - In case of patients admitted from different hospitals and social welfare centers according to plan, there is a need to present current examination results concerning the possibility of carrying MRSA. That examination should be performed 3 days before the planned admission.
2. Testing for Hepatitis B Virus applies to patients from the following risk groups:
   - patients admitted from other hospitals and several times hospitalized in different centers of Health Service in a period of last 6 months,
   - patients exposed do dialysis treatment,
   - patients who have hemophilia, treated with blood derivatives in the wards,
   - blood donors and donors of different cells, tissues and organs,
   - recipients of transfusion (especially when repeated),
   - patients prepared for operation (does not apply to patients of pediatric wards, the decision is made by an attending physician after taking into consideration the medical documentation describing prophylactic vaccination),
   - patients working as medical and support personnel in hospitals, in outpatient clinics and other healthcare institutions,
   - the drug-addicted patients,
   - homosexuals,
   - patients with reduced immunology.
3. Testing for syphilis is performed:
   - in each newly-admitted patient from psychiatric department,
   - in patients from other departments the examination is performed when ordered by an attending physician.
4. Compulsory smear tests (samples collected from the throat, vagina and rectum) in order to affirm *Streptococcus agalactiae* and *Streptococcus pyogenes* presence are performed in female patients from gynecological and obstetric ward before each operation.

TESTING FOR MRSA

A depiction of testing for MRSA among patients admitted to the Specialized Hospital in Kościerzyna mainly aims at:
- rapid and correct identification of microorganism species and resistance mechanisms in microbiological laboratory and in effect rapid identification of patients colonized by MRSA strains,
- prevention from the proliferation of MRSA in a unit,
- reduction of costs connected with treatment of patients,
- reduction of costs connected with testing of personnel for MRSA.

In a period of 3 months before admission to the Specialized Hospital in Kościerzyna (agreed by Committee of hospital's Infection Control), the patients of other healthcare institutions must undergo examination concerned with MRSA.

Staphylococcus aureus also known as golden staph is one of the most common hospital infection etiologies, causing infections of operated place and postrespiratory pneumonia [1]. Golden staph is responsilbe for hospital infections among the sick treated mainly in surgical wards. Epidemical character of those microorganisms and the ability to survive outside the living organism during a long period of time (7 days to 7 months) on a surface of equipment and medical apparatus often causes hispital epidemy encompassing several departments [2, 3, 4].

The most common phenomenon is golden staph carried in front nares and it occurs constantly in about 20-35% of healthy people but in 3-70% of population there occurs a transitory carrying [1].

The Recommended material for examinations of golden staph carrying are two smear tests from nasal atrium (left and right). It is worth mentioning that

colonization may occur also in the throat, armpit, groins and anus [2].

In a hospital, Methicillin-resistant *Staphylococcus aureus* is of particular importance. According to data obtained from healthcare policy program of the Ministry of Health, realized by the Center of Microbiology and Infection Diseases of the National Institute of Public Health, the percentage of MRSA in Polish hospitals ammounts to 10-13% of all *Staphylococcus aureus* strains [1].

The most common source of MRSA infection in hospital conditions is an infected patient and medical personnel (especially when inflammation process is going on, for instance pus formation on skin) and the reservoir is created on surfaces, apparatus, furniture or bedding. The main problem connected with transmission of MRSA strains comes from hands of medical personnel. The percentage of MRSA strains carriers amounts to 1-9% of population [6].

As a part of infection prophylaxis it is essential to perform routine examination towards MRSA strain carriers among admitted patients and periodical examination of medical personnel [7, 8, 9, 10].

In order to prevent cruciform transmission of MRSA strains, up to the moment of obtaining the result of microbiological examination, it is recommended to aply contact isolation of a patient. If a presence of golden staph strain (resistant to methiciline) is detected in the material coming from the patients, there is an obligation for a hospital to introduce very restrictive rules in order to localize the infection and prevent proliferation of dangerous strains [11].

TESTING FOR HEPATITIS B

Hepatitis B is an infection disease caused by *hepatitis B virus* (HBV) coming from a *Hepadnaviridae* family [12]. In majority of cases there are no symptoms but in case of 5-10% of sick persons there is no HBV elimination and the disease transforms into chronic state which in turn may lead to cirrhosis and primary liver cancer.

In Poland spreading of HBV infecion is caused mainly by medical operations followed by infridgement of tissue continuity. About 60% of HBV infections take place in Health Service institutions. The main cause of infection is lack of habits to obey the rules of workplace safety by medical personnel, lack of habits to wash hands, incorrect dealing with medical equipment, ineffective process of sterilization and inadequate hospital hygiene [12].

"Each year there is a growing number of compensation cases against healthcare institutions coming into Polish courts. The majority of them (above 70%) concern claims for hospital infection caused by Hepatitis B and C virus" – Prof. M. Nestorowicz.

The statute of 6th September 2001 (Dz.U. [Journal of Laws] No. 126, Item 1384) defines the meaning of hospital's infection as "an infection which was acquired during patient's presence in healthcare institution (..) and the one which was not in a state of incubation in time of admitting a patient to that institution"

Infection is a typical reason for compensation claims in civil process. Current court practice in cases concerned with infection was created on the basis of alleged fault of healthcare institution as for infection initiation (provisions of Art. 231 of Code of Civil Procedure. It means that the burden of proving the detriment is transmitted from a patient (who normally had to prove it) to a defendant (hospital or a doctor). The last ones have to prove with a large probability level that the infection came into being in other place and for different reason than their action or lack of it [13].

The HBV tests performed while admitting a patient to a hospital may serve as a basis to refute allegation of real fault of which the hospital is responsible for in case of infection arising [13].

TESTING FOR TREPONEMA PALLIDUM

Syphilis is a widespread sexually transmitted disease caused by the *Treponema pallidum* spirochete. In some countries of Western Europe, a number of infections has a growing tendency. In Poland in the years between 1969 and 1999 an incidence rate for primary siphilis decreased from 251,8 to 1,3 per 100 thousand thanks to introduction of preventive program [14]. However, there is a serious threat due to very high incidence rate in the neighbouring countries from the east, irregularities in healthcare functioning after the reform and lack of resources for prevention and health education. The highest incidence rate has proven to be in people aged between 19 and 25 [14].

In 2003 there was observed a series of negative phenomena; drastically low – in comparison with the nineties – number of serological examinations towards syphilis; very low index of immediate treatment so-

called contacts in case of syphilis and gonorrhea; relative increase of women number, where syphilis during pregnancy or childbirth was recognized; childbirths with innate syphilis [15]. Inadequate recognition of latent syphilis is a result of restrictions in performing screening towards syphilis in pregnant women and blood donors. An obligation of women to be examined twice during pregnancy is not fully realized. At present syphilis is affirmed in the same number of pregnant women as when the number of childbirths was 4 times higher. It is worth mentioning that the decrease of registered (but not actual) incidences of sexually transmitted diseases takes place due to an oversight of doctors of different specialties although reporting incidences is a statutory duty [15].

The testing for syphilis is recommended for pregnant women. In the field of pre-delivery care for normal pregnancy, Polish Gynecological Society Management recommended in 2005 examination of VDRL (flocculation reagin with cardiolipin antigen) as as a mandatory. It is recommended to perform VBRL examination during the first visit between 7th and 8th week of pregnancy up to 10th week of pregnancy. In a group of women with increased population or individual risk of infection another examinations should be performed between 33rd and 37th week of pregnancy. The course of innate syphilis may vary depending on its escalation. Innate syphilis may cause fetal atrophy or premature birth of ill and unable to live child, or an infant is seemingly healthy and only positive serological reaction confirms that infection took place in mother's womb. In that case the changes may occur after many years or not occur at all [14].

A program of hospital accreditation worked out by the Health Protection Quality Monitoring Center assumes fulfillment of determined standards influencing the quality of healthcare given to patients. In order to complete the standards of hospital infection control for psychiatric healthcare there is a need to work out effective mechanisms allowing early detection of spreading sexually transmitted diseases.

The second standard concerns realization of a program which promotes body and mouth cavity hygiene [16].

Center of Diagnosis and Treatment of Sexually Transmitted Diseases in Warsaw in a letter to the hospital recommends to perform screening tests for syphilis especially in patients hospitalized in obstetrical, gynecological, psychiatric and neurological wards.

TESTING FOR STREPTOCOCCUS AGALACTIAE I STREPTOCOCCUS PYOGENES

At the beginning of the seventies of the 20th century invasive infections caused by *Streptococcus agalactiae* turned out to be a leading factor causing mortality of neonates and infants in the USA. That alarming information in the eighties led to a series of clinic examinations utilizing chemoprophylaxis in order to diminish or eliminate incidence rate. The examinations proved that intradelivery chemoprophylaxis application in pregnant carriers of *Streptococcus agalactiae* essencially protected newborn infants against incidence [17, 18].

In 1996 the Center for Disease Control and Prevention in cooperation with the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics worked out a prophylactic recommendation for women during pregnancy, serving to prevent infections from *Streptococcus agalactiae* in neonates and infants [17, 18,19].

The pattern recommends to applicate one of the two prevention methods: the first – applying antibiotic therapy based on the risk evaluation (risk-based strategy) and the second – utilizing microbiological screening (screening strategy). The doctors using the first method qualify a woman to intradelivery chemoprofylaxis when one of the following risk factors is affirmed: childbirth before 37th week of pregnancy, body temperature during delivery $\geq$38°C or when time which elapsed from fetal membrane fracture exceeded 18 hours. In case of the second method it is recommended to perform microbiological examinations: inoculation from vagina and smear test from anus in all pregnant women between 35th and 37th week of pregnancy. Positive infection test result determines serving antibiotics during delivery [17, 18, 19].

The conditions in urinary and sexual tracts appearing during pregnancy, the vicinity of anus, chronic inflammation processes, the vicinity of delivery channel are the factors which predestine to infections coming from vagina microflore. A serious problem are infections of neonates, which are closely connected with the bacteria colonizing mother's delivery channel . Bacteremia usually appears during the first week of life but meningitis in the course of 2-3 weeks. Inflammation caused by microflore may be a result of the fetal bladder's injury and also may

appear during the passage of an infant through delivery channel [20].

The factors causing infection in mother's womb are overruning deliveries, infection of amniotic fluid and premature fracture of fetal bladder. Delivery channel may be a starting point of lethal sepsis for an infant [20].

Streptococcus agalactiae is a basic etiologic factor of infections in neonatal period and its carrying in vagina of healthy women amounts to 50-75% [22]. The fact of colonization in pregnant woman is a key risk factor of infant infection [22]. It is believed that among the etiologic factors of bacterimia and neonate sepsis, the second place after coagulase-negative staphylococci is assigned to streptococci. It was proved that infections spreading out in a hospital were in those departments, where there was a high level of carrying among women giving birth [22].

Infections from *Streptococcus agalactiae* in neonates and infants were characterized as 2 distinct syndromes. Early syndromes appeared in neonates up to 7 days of life and its symptoms are sepsis, pneumonia, seldom meningitis.

Mortality in such cases is very high and can amount to 50 % [17,18, 22]. Subsequent syndrome occur in neonates and infants between 7 days and 3 months of life. It is manifested first of all by meningitis. In order to prevent infant infection essential meaning is ascribed to perinatal chemoprophylaxis in female carriers [22].

In pregnant women or after delivery *Streptococcus agalactiae* is responsible for infection of urinal tracts, fetal membrane infection, uterus infection, septic infection, seldom meningitis [17].

In Poland there are still no epidemiological data, which would allow to evaluate the problem's scale and to make coordinated efforts in aid of introducing the action procedures in case of perinatal infection caused by *Streptococcus agalactiae* [17].

Streptococcus pyogenes is a cause of hospital infections mainly in obstetric and infant wards. The source of those microorganisms may be respiratory paths, alimentary tract or vagina in women (carrying). The carrying factor may amount to 15-20% [22].

Streptococcus pyogenes as a cause of septic infection in parturients (so called postnatal fever) was known as early as in the 20th century. It may also cause an infection in neonates.

Those bacteria predominantly infect a stump of umbilical cord and may be carried by the hands of nursing personnel [22, 21].

The decision of the hospital, which concerns performing tests for *Streptococcus pyogenes* in the patients of obstetrical ward was caused among other things by the level of carrying and mainly by virulence of those microorganisms, especially in the context of known septic infection incidents of such etiology followed by lethal effect.

CONCLUSIONS

A meticulous realization of all the activity encompassed by the standard of screening leads to:

1. Identification of the infection source and introduction of antibiotic therapy, thanks to rapid microbiological diagnosis .
2. Introduction of active isolation of the patient.
3. Cost reduction connected with antibiotic therapy.
4. Cost reduction of treatment and consequently shortening of patient's stay in hospital
5. Improvement of the sanitary state of a hospital by reducing the number of hospital infections.
6. Control of application of selected antibiotics, i.e. vancomycin, teicoplanin, imipenem
7. Reduction of a hospital's costs due to possible compensations resulting from infections and consequently an increase of insurance rates.

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REVIEW / PRACA POGLĄDOWA

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THE HISTORY AND THE PRESENT OF HERNIOLOGY

HISTORIA I DZIEŃ DZISIEJSZY HERNIOLOGII

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S u m m a r y

The paper focuses on the history and the present day of herniology. The milestones in the development of this area of surgery are discussed, as well as the role of major herniology specialists throughout history. Hernias have accompanied humanity since its origins, and their exact etiology remains to be discovered. Medical scripts of early civilizations have been found to contain descriptions of the condition and methods of treatment, which until the works of Bassini were based more on intuition and experiment than solid anatomical and physiological research. Bassini's operation was the first breakthrough in hernia surgery, the second one being the introduction of synthetic materials. Currently, intensive research into the etiopathogenesis of all types of hernias continues, largely influencing the choice of appropriate treatment.

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

Praca przedstawia historię oraz dzień dzisiejszy herniologii. Omówiono najważniejsze dla rozwoju tej dziedziny chirurgii wydarzenia i rolę najwybitniejszych lekarzy herniologów na przestrzeni dziejów. Przepukliny, których etiologia nie została do dziś ostatecznie poznana, znane są ludzkości od zarania dziejów. Pisma medyczne wczesnych cywilizacji zawierają opisy zarówno samej choroby, jak i jej leczenia, które do czasów Bassiniego oparte było bardziej na działaniu intuicyjno-doświadczalnym niż na rzetelnych podstawach anatomicznych i fizjologicznych. Operacja Bassiniego była pierwszym przełomem w herniologii, zaś wprowadzenie materiałów syntetycznych drugim. Współcześnie trwają intensywne badania naukowe mające na celu ustalenie etiopatogenezy wszystkich rodzajów przepuklin, co w znacznym stopniu implikuje stosowanie odpowiednich metod leczniczych.

Key words: ventral hernias, history, methods of treatment
Słowa kluczowe: przepukliny brzuszne, historia, metody operacji

HISTORY

Hernias have been one of the most frequent ailments, known for millennia. The name is derived from the ancient Latin *hira* or the Indo-European *ghere*, meaning "intestine". Aulus Cornelius Celsus used the word in his writings, stating that it is a part of common vernacular vocabulary, *coele* being the preferred term for *hernia* in the medical language of his age. In later texts (including modern-age ones) the term *ruptura* (fracture, rupture) appears, in line with Galen's theory of hernia resulting from a rupture of the peritoneum. The Greek word *hernia* meant „bud" or „budding" [1].

In ancient medical texts, descriptions of the condition and proposed treatment thereof constitute a large part. In Egypt, in the age of the pharaohs, a few of whom suffered from hernias, bandaging was the preferred treatment. The Ebers papyrus (approx. 1552 B.C.) contains a description of the principles of physical examination of an inguinal hernia. Hippocrates was able to differentiate an inguinal hernia from a hydrocoele by transillumination and reduce incarcerated

hernias. Hernia belts were in use in Rome; in case of incarceration the spermatic cord and testis were removed via an incision in the scrotum and the wound left to heal by granulation. Incarceration was not the only indication for surgery in ancient times - herniotomy was also performed for persistent pain. Paul of Aegina operated scrotal hernias by ligating both the hernial sac and the spermatic cord – sacrificing the testis. Celsus attempted to spare the testis while operating [2].

During the Middle Ages there has been little advance in hernia surgery, even though some of the most renowned physicians of that era took an interest in that area. William of Saliceto followed the path Celsus had taken thirteen centuries before him, striving to spare the testis while performing surgery for inguinal hernia. Guy de Chauliac was able to discern between femoral and inguinal hernias and used the Trendelenburg position during hernia reduction.

The wonderful advancement of science during the Renaissance era concerned medicine as well. Antonio Benivieni (1440-1502), one of the founding fathers of pathology, wrote extensively about various hernias in his "De abditis morborum causis („On the hidden causes of diseases"). The greatest Renaissance surgeon, Ambrose Pare, gave a detailed description of hernia repair techniques, including drawings. In his practice he used golden wire as a suturing material. His technique included ligation of the hernial sac, its reduction into the peritoneal cavity and closure of the parietal peritoneum in certain cases. Pare warned against traveling herniotomists and barbers, who almost universally castrated their patients during hernioplasty. This practice was far from marginal, as shown by the example of Jacques Beaulieu, a XVIIth century traveling lithotomist, who performed over 2000 herniotomies and approximately 4500 cystolithotomies [2, 3]. In 1556 Pierre Franco, a Swiss surgeon, introduced a dissector of his own invention to expand the inguinal ring in incarcerated hernia. He recommended a reduction of the sac contents and closure with linen sutures [2].

Autopsies, performed since the Renaissance, have led to a vast improvement in the knowledge of human anatomy. In 1559 Kaspar Stromayr first distinguished between direct and indirect hernia. Advances in other areas of science have led to an accumulation of knowledge on human anatomy, physiology and pathology. During the following decades, both theoretical research and attempts at new operative techniques continued. In 1721 Chesleden successfully operated an incarcerated scrotal hernia, while Percival Pott published a report on the pathogenesis of incarceration in 1757 [2,4].

The XVIIIth century was a period of intense investigations of inguinal anatomy. Many names of the researchers of that era, such as Cooper, Skarpa, Gimbernat have entered the language of anatomy forever. Gimbernat advised dissection of the inguinal ring laterally rather than cephalad in cases of strangulated hernia, which led to life-threatening hemorrhages and damage to the inguinal ligament. Despite the significant advances in theoretical knowledge, the outcomes of surgical treatment did not improve markedly, partially due to the lack of the rules of aseptic and antiseptic surgery. The introduction of the latter coincided with the advent of a new era of herniology heralded by Bassini. Earlier, in 1871, Marcy, who was a student of Lister, performed the first antiseptic hernioplasty. In 1874 Steele reported a „radical hernia operation" which consisted of hernia reduction and closure of the superficial inguinal ring. Lucas-Championniere was the first to open the inguinal canal in 1881 (through an incision in the external oblique aponeurosis) and excise the hernial sac to the level of the deep inguinal ring. Five years later, Mac Ewen folded the peritoneum of the sac and placed it as a „plug" inside the deep inguinal ring, which was additionally reinforced by sutures [2, 5, 6].

Despite the use of general anesthesia, aseptic and high sac ligation, the outcomes of inguinal hernia surgery in the latter half of the XIXth surgery were unfavorable both in Europe and the USA. Mortality rates due to sepsis, hemorrhage and other causes reached 2-7% of the cases and the recurrence rate was practically 100% after 4 years. As Billroth stated in 1890, most surgeons at that time left the wound to heal by secondary intention after sac ligation, believing that the resulting scar would reinforce the abdominal wall, preventing recurrence. By the end of the XIXth century routine resection and primary anastomosis were introduced in cases of gut necrosis due to strangulation [7].

BREAKTHROUGH

Prior to his famous operation, Eduardo Bassini used numerous techniques to treat inguinal hernias. Through analyzing his failures, he came to understand the principle of correct inguinal hernia repair: instead of closing the deep inguinal ring, one should strive to recreate physiological anatomical relationships be-

tween the elements of the inguinal canal and to reinforce its posterior wall. His original technique (which has, over time, spawned numerous modifications) was, similarly to the later introduced Shouldice repair, based on a longitudinal incision of the transverse fascia ranging from the pubic tubercle to approximately 2.5 cm above the deep ring. Thus, he gained wide access to the preperitoneal space, which allowed for high ligation of the hernial sac. The medial non-absorbable silk sutures ran through the rectus sheath. Bassini was the first to closely follow up his patients. In 1887, three years after his initial operation, he presented the outcomes of his treatment at the congress of Italian surgeons in Genoa. A beautifully illustrated monograph, published in 1889 and translated into German in 1890, spawned a tremendous interest in the new method. Soon, Bassini's position as the founding father of modern herniology was unchallengeable [8].

At roughly the same time, William Halsted presented his method of inguinal hernia repair. The main difference from Bassini's technique was the placement of the spermatic cord (often with the cremaster muscle and pampiniform plexus resected) above the closed external oblique anastomosis. Both these great surgeons have set the fourth principle of successful inguinal hernia repair. They have added reinforcement of the posterior wall of the inguinal canal to the three principles already known: aseptic/antiseptic surgery, high sac ligation and reduced diameter of the deep inguinal ring. they have also stressed the importance of the transverse fascia [8, 9].

The basic drawback to Bassini's repair was the tension arising along the suture line, causing pain and recurrence. To reduce the tension, in 1892 Wolfer performed an incision of the anterior layer of the rectus sheath. Berger made a similar incision, but he fastened the lateral flap of the incised rectus sheath to Poupart's ligament. The idea was approved by Halsted, who discarded his previous principle of spermatic cord thinning, developing a new type of hernia repair (the Halsted II technique). This type of inguinal hernia repair was further studied and developed by McVay and Anson, who have confirmed its usefulness on a large group of patients [10].

The use of foreign materials was the next logical stage in inguinal hernia repair. This solution was pioneered by Marcy, who implanted kangaroo tendons to cover a tissue defect as early as 1887. He also experimented with fasciae of other animals. In 1901 McArthur initiated the era of fascial repair, using a vascularized flap of the external oblique aponeurosis. This concept was revisited 80 years later in India by Mohan Desarda [11, 12, 13]. The external oblique aponeurosis was soon considered insufficient, which led to the use of the fascia lata as a free or pedicle flap. This method was popularized in England by Geoffrey Keynes, who used it in femoral hernias as well (suturing the flap to Cooper's ligament). In later years, reports on various biological materials had been published up to 1975, when Sames proposed the use of the vas deferens as suturing material [2, 3].

The use of human skin for inguinal hernia repair forms a separate chapter. This material, being autogenous, has been considered infection-resistant. Loewe was one of the pioneers of its use, implanting human skin in seven patients, including one with a postoperative hernia, in 1913 [14]. The procedure was popularized by Rehn, who prepared the skin by scraping off the epidermis to prevent fistula and cyst formation. In Poland human skin was introduced to herniology by Jankowski [15]. One of the ways to prepare the skin flap was exposure to high temperature and epidermis removal, described by Hoffman in 1970 [16]. This method was in use in our Clinic, but long-term outcomes have proven far from perfect. The introduction of synthetic materials has practically eliminated human skin as prosthetic material [17].

The ancient concept of metal as an implant was also revisited. The materials used included silver - „silver mesh filigree"(Witzel and Goepel), tantalum (Burke) , steel (Babcock) and gold. The initial enthusiasm waned when complications in the form of cysts, tissue damage and high recurrence rates became apparent. These materials remained in use until the early 1960's [2].

By the end of the XIXth century, the luminaries in the field of surgery gained certainty that the road to successful hernia repair led through the use of synthetic materials. In a 1878 letter Billroth wrote to Czerny: „If we learn to manufacture artificial tissues with the properties of fasciae and tendons, we will solve the problem of radical hernia repair" [4].

In 1935 nylon was synthesized. Its biocompatibility was soon appreciated and it was introduced to surgery, including herniology. Melick developed the „nylon darn" technique, which remains in use today.

Based on the considerations on nylon, the problem of the ideal hernia prosthesis arose. The desired material should meet the criteria set by Schumpelick [18]:

– properties must not be altered by exposure to bodily

fluids and tissues.
- must be chemically inactive, must not induce foreign body response or ‚inflammatory reaction.
- must not be carcinogenic and hypoallergenic.
- must show mechanical strength, be sterilizable and infection resistant.

A material meeting all of the above criteria has not yet been synthesized, however many materials approach this ideal (polyester, polypropylene, Teflon-PTFE).

In 1944, French surgeons Aquaviva and Bounet reported their experiences with nylon meshes shaped similarly to present-day implants. Over time, nylon has proved to be less than perfect: it loses its properties due to hydrolysis, denaturations and frequent infections [19].

Teflon, or polytetrafluoroethylene (PTFE) was accidentally synthesized in 1938 at the Du Pont chemical plant. It has been perfected in 1963 in Japan, and made ready to use in medicine. Its mechanical properties and ideal smoothness have made it indispensable in many fields of surgery.

Usher is universally considered the pioneer of synthetic mesh use. In 1955 he took an interest in a new product – polypropylene, trade-named „Marlex". It displayed many of the characteristics of an ideal prosthesis. After a series of experiments, in 1958 he used Marlex for hernia repair. He published over 20 reports, presenting many technical innovations. Above all, he desisted from covering the defect with the implant, using the mesh as a „bridge" over the defect with an appropriate margin around it [20].

Around that time, Dacron (ethylene polytereftalane) was introduced to hernia surgery. However, its properties were inferior to those of Marlex and Goretex (PTFE).

PRESENT DAY

Many of the methods described above remain in use today. Even though synthetic materials were in use earlier, the greatest role in the popularization of „tension-free" techniques was played by Irving Lichtenstein. In 1989 he reported a series of 1000 patients operated by his technique under local anesthesia in a „day-case" setting. Although the procedure was technically simple, Lichtenstein did not recommend that hernioplasty should be performed by any surgeon in any center. Parviz Amid, a student and follower of Lichtenstein, recollects two distinct periods in the evolution of his technique: the years 1984-1988 and the period after 1988, when the shape and size of the implant were changed. The size was increased to help prevent recurrence due to mesh shrinkage. Lichtenstein's repair became the „gold standard" against which all new hernioplasty techniques are compared [21].

Modern „open" surgery of inguinal hernia gravitates toward outpatient procedures. Local anesthesia is considered safe and sufficient for the majority of hernias. The concept of local anesthesia dates back to late 1800's, hen it was introduced by Cushing and popularized by Halsted. According to Amid, anesthesia is one of the key elements of successful hernioplasty.

Other concepts of hernia repair developed simultaneously. The "plugging" concept is quite old. In the 1830's Pierze Nicholas Gerdy used an inverted flap of scrotal skin to fill the inguinal canal, suturing it closed and inducing inflammation. Wutzer proposed placing foreign bodies, such as wooden plugs, in the inguinal canal to close it through induced inflammation. [2, 3]. In modern times, Irving Lichtenstein pioneered the plug technique, introducing a cigarette-shaped Marlex implant in 1968 to treat femoral and recurrent hernias with favorable results. In 1987 Bendavid used an "umbrella-shaped" mesh; a shape which was also used by Gilbert who abandoned his experiments with a cigar-shaped implant after unfavorable results [2, 3, 4].

Many of the implants manufactured presently come in ready-to-use sets, provided in several sizes. One of them is the „Perfix" system, formed according to Rutkow's recommendations, consisting of an „umbrella" and a patch similar to Lichtenstein's. The „PHS/UHS" (Prolene Hernia System) – two flat mesh implants connected by a plug (in a fashion similar to a cufflink) has also been gaining popularity due to favorable outcomes.

Synthetic materials have been criticized for the increased likelihood of infection, mesh migration and even carcinogenesis. This last allegation is probably false, and implant migrations are rare [22]. Infections can be prevented by maintaining a proper aseptic regime and using antibiotic prophylaxis. "Light meshes" have been in use for a few years, consisting of an absorbable (Vicryl) and non-absorbable part (polypropylene). After healing and remodeling of the inguinal region, the absorbable part undergoes biodegradation while the polypropylene provides a sufficient scaffolding to reinforce tissues. The patient does not feel a foreign body, which is especially important in large

postoperative hernia repair. [23]

The problem of recurrent and bilateral hernia repair has remained unsolved for many years. Repeated operations using the same approach did not work under altered anatomical conditions among weakened tissues.

Lichtenstein's and Rutkow's repairs are two representatives of „tension-free" inguinal hernia repair (flat and 3D implant, respectively). A third tension-free method has been described by Rene Stoppa, a French surgeon. In 1975 he reported a series of cases of recurrent hernias repaired with the use of Dacron mesh. The important difference lay in a completely different choice of approach to the hernial defect. The preperitoneal space was accessed via an inferior midline incision, The contents of the hernial sac were reduced into the abdominal cavity, giving excellent view of the defect, which was then covered by a relatively large implant, covering both musculopectineal orifices. The chevron-shaped mesh secured the area of the surgical incision as well. It was fixed in place in 2-3 places only to prevent folding, the main force fixating the mesh in place being the abdominal pump, acting according to Pascal's law. The preperitoneal space proved to be the ideal location for the implant. Mesh placement in a space unaccessed during previous repair attempts was an excellent solution especially for patients with numerous recurrencies [24].

Even though preperitoneal repairs evoke the name of Stoppa, the history of this approach is longer. It was probably first used by Thomas Annandale of Edinburgh in 1876. Two reports exist of operations utilizing this approach as early as 1743 [2]. The idea was revisited in early 1900's in the USA. Cheatle performed operations using the midline incision in the Trendelenburg position in 1920. After separating the rectus muscles, he dissected the peritoneum off the anterior pelvic wall and bladder, reduced the contents of the hernia to the abdominal cavity, leaving a part of the transected sac in the inguinal canal, and partially closed the deep ring. He used this approach on inguinal and femoral hernias. Cheatle also utilized the Pfannenstiel incision. His ideas were popularized by A. Henry. The preperitoneal approach gained popularity in the 1950's in the USA. Mc Evedy utilized an oblique incision within the rectus sheath, gaining excellent view of the preperitoneal space. Read and McVay also operated in this fashion, but it was Nyhus who performed thorough anatomical and clinical research on the subject. In 1959 he was the first to use a synthetic implant via a preperitoneal approach. His idea was further developed by Rignault and Stoppa [25].

In spite of the successful introduction of synthetic materials into hernia surgery in the latter half of the XXth century, „pure tissue repair" techniques were not abandoned. The most well-known of these, besides Bassini's repair which remained to be used and modified, was Shouldice's method, known as "Canadian repair". Developed in the early 1950's, this technique was perfected in Shouldice's clinic, where the recurrent rates did not exceed 2%. In other centers the results were less spectacular, and the technique is considered difficult to perform. In many other centers – including Polish ones – tension methods such as Bassini's, Halsted's and others are still used [26].

A remarkable method has been proposed by the above-mentioned M. Desarda. He used a deep external oblique aponeurosis as a natural "mesh". The results given by the author and confirmed by our Department seem favorable [13].

The wonderful development of videosurgery did not omit hernia surgery. The concept of intraperitoneal approach to inguinal hernia has been conceived in the late 1970's at the Albert Einstein College of Medicine in the USA. The idea was based on the reduction of hernial defect size by clips in order to prevent the migration of the viscera into the inguinal canal. Initial results, performed during laparotomy for unrelated diseases, appeared inviting. At that time, the laparoscopic techniques were insufficiently developed to allow hernia repair. A series of procedures performed in 1982 was less successful. Bogojavlenski initiated laparoscopic hernia treatment in 1989, using a synthetic mesh. The development of this technique was again halted by the lack of proper equipment. In 1990, Schultz introduced a technique of plug insertion into the deep ring after a peritoneal incision. The implant was not fixed in place and the recurrence rates after 2 years reached 25%. Therefore, the technique evolved toward larger implants fixated by clips. Early experiences with the onlay technique were equally poor (intestinal adhesions and recurrences) and it is infrequently used today. Contemporarily, two techniques of laparoscopic herniotomy are used: TAPP (transabdominal preperitoneal) in which the mesh is placed under the peritoneum and TEPA (total extraperitoneal approach) – in which the preperitoneal space is dissected by a special balloon device and a mesh placed there. The laparoscopic approach, particularly the TEPA technique, is a development of the classical Stoppa technique [2].

Those in favor of laparoscopy stress swift rehabilitation and diminished pain after these procedures (important e.g. in sportsmen). However, the procedure carries a risk of typical laparoscopy complications (including fatal ones) and requires general anesthesia. The problem is subject to discussion [27].

Laparoscopic techniques are also used in postoperative and parastomal hernia repair. The large size of implants required (the defect is covered with a 5cm margin) render these procedures rather costly. Previous laparotomies are other factors limiting the application of laparoscopic techniques.

BASIC RESEARCH

Basic research has played a crucial role in the development of herniology. Its origin lies in the uncovering of human anatomy. Galen connected hernia with peritoneal rupture. Until Renaissance, the awareness of human anatomy in general and the relationships within the inguinal canal was low. The greatest advances in understanding the anatomical foundations of hernia formation took place in the XVIIIth and XIXth centuries. Even though the anatomical and physiological relations within the inguinal region were fully understood, the reason for hernia formation remained unclear. In the early 1900's, Harrison focused on the connective tissue of the fascia and its abnormalities as a possible causative factor. He observed the incidence of hernia to grow with age. It was only in the latter half of the XXth century that Harrison's suspicions were confirmed. Immunohistochemical, histological and genetic research has shown significant differences in the ultrastructure of the fascia forming hernial defects in comparison to healthy subjects. Moreover, these alterations were soon found to encompass even tissues lying beyond the actual hernia and be gene-related. The alterations include disrupted synthesis and maturation of collagen and elastic fibers, as well as increased expression of enzymes degrading these structures [29, 30, 31].

It has to be stressed, however, that not every aspect of the pathogenesis of hernia has been fully explained, and the research continues. According to contemporary theories, hernias have a complex etiology, definitely including congenital factors, concerning connective tissue structure and metabolism.

THE FUTURE

The future of herniology is to be sought in improved synthetic materials (composite, partially absorbable), as well as perfected surgical techniques. Even today it is no longer the recurrence rate, but other complications, such as chronic pain, hematomas, seromas or postoperative testicular edema that are the measure of correct treatment. Recurrence rates have been reduced to approximately 1-1,5% and, after elimination of surgical errors, are attributed to connective tissue abnormalities. A return to certain types of all-tissue repair or the continued use of the techniques presently utilized cannot be excluded. There are still many surgeons who distrust synthetic materials, with the economic aspect being significant in certain regions.

An important problem appears to be the possibility to assess the quality of the connective tissue prior to surgery. An outcome of such a test would influence the choice of the repair technique. If connective tissue abnormalities would be found, a synthetic material would be used, and if the tissue would be assessed as healthy, all-tissue repair would be justified.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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CATHETER – RELATED INFECTIONS AMONG THE PATIENTS OF THE DEPARTMENT OF PEDIATRICS, HEMATOLOGY AND ONCOLOGY OF THE DR A. JURASZ UNIVERSITY HOSPITAL IN BYDGOSZCZ, POLAND – AN ANALYSIS OF BLOOD CULTURES OBTAINED FROM THE BROVIAC CATHETER AND PERIPHERAL VEIN

ZAKAŻENIA ODCEWNIKOWE U DZIECI Z KLINIKI PEDIATRII, HEMATOLOGII I ONKOLOGII SZPITALA UNIWERSYTECKIEGO IM. DR. A. JURASZA W BYDGOSZCZY NA PODSTAWIE ANALIZY POSIEWÓW KRWI POBRANEJ Z ŻYŁY I BROVIACA

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Summary

Background. The aim of the study was to assess the incidence of Broviac catheter-related infections among the patients of the Department of Pediatrics, Hematology and Oncology of the dr A. Jurasz University Hospital in Bydgoszcz, Poland.

Materials and methods. 2941 blood samples obtained from peripheral veins and Broviac catheters from 519 patients were included in the study. Microbial species identification was performed with the use of a culture media set and by biochemical feature analysis. The antibiotic resistance was determined by Kirby-Bauer disk diffusion, according to the recommendations of the CLSI and the National Reference Center for Antimicrobial Susceptibility.

Results. Isolation of the same microorganism from blood cultures obtained simultaneously from the catheter and peripheral vein in a patient with no other apparent infection source was considered to be evident of a catheter-related bloodstream infection. Catheter-related bacteriemia was found in 21.9% of the patients. 87 strains were isolated (57.5% thereof were Gram – negative and 42.5% Gram-positive bacteria). *Klebsiella oxytoca* was the most frequently isolated microorganism. The most common Gram-positive bacteria were staphylococci. None of the Gram-negative rod strains produced extended-spectrum β-lactamases (ESβL's). Two imipenem-resistant strains of non-fermenting rods were isolated. In almost 90% of the coagulase-negative stapylococcal strains a resistance to β-lactams (methicillin resistance) was detected.

Conclusions. Despite the study shows low percentage (4,6%) of catheter-related infection, Broviac catheters may be related to a serious risk of bacteriemia among the children with neoplastic diseases. Correct microbiological evaluation of catheter-related infections should be based on the analysis of blood samples harvested simultaneously from the catheter and a peripheral vein.

Streszczenie

Wstęp. Celem pracy była ocena częstości zakażeń związanych ze stosowaniem cewników typu Broviac u pacjentów Kliniki Pediatrii, Hematologii i Onkologii Szpitala Uniwersyteckiego im. dr. A. Jurasza w Bydgoszczy.

Materiał i metody. Badaniem objęto 2941 prób krwi pobranej z żyły i Broviaca od 519 pacjentów. Przynależność gatunkową drobnoustrojów określano przy użyciu zestawu pożywek i na podstawie cech biochemicznych. Antybiotykooporność oznaczano metodą krążkowo-dyfuzyjną według Kirby-Bauera zgodnie z rekomendacjami podanymi przez CLSI i Krajowy Ośrodek Referencyjny ds. Lekowrażliwości Drobnoustrojów.

Wyniki. Za zakażenie odcewnikowe uznawano wyhodowanie tego samego drobnoustroju z prób krwi pobranych jednocześnie z Broviaca i żyły od pacjenta, u którego nie stwierdzono innego źródła zakażenia. Odcewnikową bakteriemię stwierdzono u 21,9% pacjentów. Izolowano 87 szczepów (57,5% stanowiły bakterie Gram-ujemne, 42,5% -

Gram-dodatnie). Najczęściej izolowanym drobnoustrojem była *Klebsiella oxytoca*. Wśród bakterii Gram-dodatnich dominowały gronkowce. Żaden ze szczepów Gram-ujemnych pałeczek nie wytwarzał beta-laktamaz o poszerzonym spektrum substratowym (ESβLs). Wśród pałeczek niefermentujących stwierdzono 2 szczepy oporne na imipenem. U prawie 90% szczepów gronkowców koagulazoujemnych wykryto oporność na beta-laktamazy (meticylinooporność).

W n i o s k i . Pomimo że w badaniach wykazano niewielki odsetek (4,6%) zakażeń odcewnikowych, cewniki typu Broviac mogą stanowić poważne ryzyko bakteriemii wśród dzieci z chorobami nowotworowymi. Prawidłowa diagnostyka mikrobiologiczna zakażeń odcewnikowych powinna opierać się na analizie krwi pobranej równocześnie z żyły i Broviaca.

Key words: bacteriemia, catheter-related infection, Broviac
Słowa kluczowe: bakteriemia, zakażenia odcewnikowe, Broviac

INTRODUCTION

Patients with neoplastic diseases frequently require placement of a permanent external venous catheter or implantation of a subcutaneous venous port (totally implanted device, TID) [1]. The introduction of treatment methods based on central venous catheters has many advantages, one of them being the diminished risk of chemotherapy - related skin inflammation and reduced need for venipuncture [2]. On the other hand, permanent central catheters have many disadvantages related to technical complications during placement (catheter rupture; malfunction due to thrombosis at the tip or within the lumen; catheter migration), skin infection at placement site, deep venous thrombosis or bloodstream infections (catheter-related bloodstream infections, CRBSI) [3, 4, 5, 6]. The development of catheter-related infection in pediatric patients with neoplastic diseases carries a high risk, primarily due to impaired immune response [2].

The aim of this study has been to attempt to assess the incidence of catheter-related infections among the children treated at the Department of Pediatrics, Hematology and Oncology of the dr A. Jurasz University Hospital in Bydgoszcz, Poland as well as to perform an analysis of the microorganisms isolated from blood cultures harvested simultaneously from the Broviac catheter and a peripheral vein.

MATERIAL AND METHODS

The study included 2941 blood samples obtained from peripheral veins and Broviac catheters from 519 patients of the Department of Pediatrics, Hematology and Oncology of the dr A. Jurasz University Hospital in Bydgoszcz, in the period of one and a half years. All of the samples were harvested according to recommended protocols and referred to the Dept. of Microbiology.

The blood cultures were analyzed with the use of the BacT/Alert (bioMerieux) and Bactec (Becton Dickinson) automated systems. The samples indicated as positive by the system were cultured on a set of media: Columbia Agar Base with 5% sheep blood, chocolate agar, Pyocyanosel agar, Sabouraud Agar (bioMerieux), MacConkey agar (Becton Dickinson). The Petri dishes were incubated at 37^{o}C for 24-48 hours in an oxygen atmosphere and in an atmosphere of 5% CO_2 (chocolate agar cultures). Colony morphology and the production of bound and free coagulase were taken into consideration in identification of staphylococci. Species determination was based on biochemical features, with the use of the following assays: API 20 STREP, ID 32 STAPH, ID 32 E, ID 32 GN, API CORYNE (bioMerieux) which were read by using an ATB Expression instrument and ATB Expression software (version 2.8.8, bioMerieux).

Antibiotic susceptibility was determined by the Kirby-Bauer disk diffusion method, according to the recommendations of the CLSI (Clinical and Laboratory Standards Institute) [7] and the National Reference Center for Antimicrobial Susceptibility [8]. A McFarland 0.5 inoculum strength was used on Mueller-Hinton II Agar (Becton Dickinson). The antibiogram dishes were incubated for 18-20 hours at 35^{o}C. Methicillin resistance of staphylococci was assessed with the use of 1μg/ml oxacillin disks (bioMerieux). In order to detect extended-spectrum β-lactamases, the two disk test was performed according to CLSI [7] and the National Reference Center for Antimicrobial Susceptibility [8] standards. The results were read after 18-20 hours of incubation at 35^{o}C. The growth inhibition zones around the disks were interpreted according to the current CLSI tables [7].

RESULTS

Of the total number of 2941 blood samples obtained from 519 patients of the Department of Pediatrics, Hematology and Oncology, 2008 were harvested from a peripheral vein and 993 from a Broviac catheter. In 105 patients (20.2%) proper CRBSI diagnostics has been performed. 663 blood samples have been simultaneously harvested from each source.

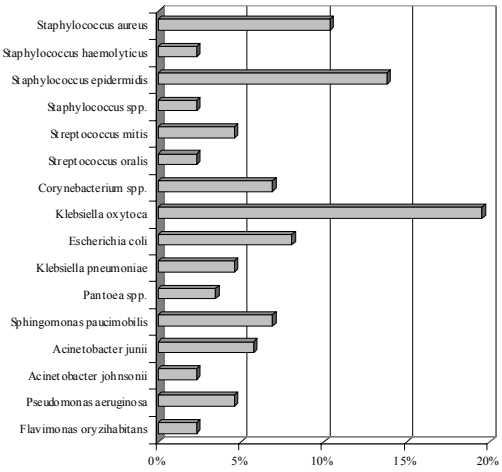
The percentage of positive cultures of blood harvested from the Broviac catheter and peripheral vein was 4.6%. A correlation between the infection and the presence of a Broviac catheter was found in 23 (21.9%) of 105 patients. The analysis of clinical diagnoses of the patients in the study group revealed no other apparent source of inflammation that could cause bloodstream infection. The predominant diagnosis was acute lymphoid leukemia (26.1%). Patients with fever of unknown origin constituted an equally large portion of the study group. A detailed analysis of clinical diagnoses is presented in Table 1.

Table I. *Clinical diagnosis in patients of the Dept. of Pediatrics, Hematology and Oncology*

Tabela I. *Rozpoznanie kliniczne u pacjentów Kliniki Pediatrii, Hematologii i Onkologii*

Jednostka chorobowa/ Diagnosis	Liczba chorych/ Nº of patients	Odsetek [%]/ Percentage [%]
Gorączka o nieznanej etiologii/ Fever of unknown origin	6	26.1
Ostra białaczka limfatyczna/ Acute lymphoid leukemia	6	26.1
Mięsak prążkowanokomórkowy/ Rhabdomyosarcoma	2	8.7
Mięsak prążkowanokomórkowy zarodkowy/ Rhabdomyoblastoma	1	4.3
Guz Ewinga/ Ewing's sarcoma	1	4.3
Guz terminalny jądra prawego/ Terminal tumor of the right testicle	1	4.3
Szyszyniak/ Pinealoma	1	4.3
Neuroblastoma/ Neuroblastoma	1	4.3
Guz jamy brzusznej/ Abdominal tumor	1	4.3
Guz mózgu/ Cerebral tumor	1	4.3
Nefroblastoma/ Nephroblastoma	1	4.3
Hemofilia/ Hemophilia	1	4.3

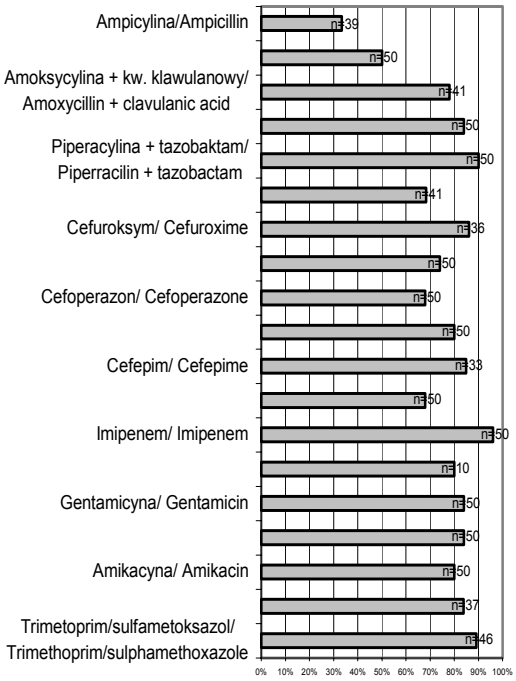
Fifty strains of Gram-negative rods and 37 Gram-positive strains have been isolated. Among the Gram-negative microorganisms, 57.5% were rods of the *Enterobacteriaceae* family, predominantly of the species *Klebsiella oxytoca* (34.0%). Among the Gram-positive microorganisms (42.5%) staphylococci were the prevalent group (67.6%), 64% of which were coagulase-negative (*Staphylococcus epidermidis* – 14 strains, *S.haemolyticus* – 2 strains). The data on the distribution of microorganisms in the analyzed material is depicted in Fig. 1.



Ryc. 1. *Skład gatunkowy bakterii (n=87) wyosobnionych z krwi żylnej i krwi z Broviaca dzieci Kliniki Pediatrii, Hematologii i Onkologii*

Pic. 1. *Strain distribution (n=87) of bacteria isolated from venous and Broviac blood samples from the patients of the Department of Pediatrics, Hematology and Oncology*

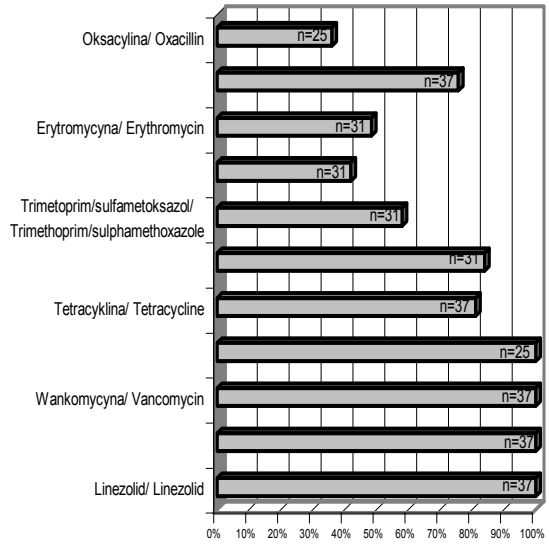
Among the Gram-negative rods, 96% of the strains were susceptible to imipenem, 90% to piperacillin + tazobactam and 89.1% to trimethoprim/ sulphamethoxazole. The susceptibility to other antibiotics ranged from 33.4% for ampicillin to 86.1% for cefuroxime (Fig. 2). No ESβL-producing strains were found.



Ryc. 2. *Lekowrażliwość bakterii Gram-ujemnych wyosobnionych z krwi żylnej i krwi z Broviaca dzieci Kliniki Pediatrii, Hematologii i Onkologii*

Pic. 2. *Antimicrobial susceptibility of Gram-negative bacteria isolated from venous and Broviac blood samples from the patients of the Department of Pediatrics, Hematology and Oncology*

In the coagulase-negative staphylococci group, 87.5% of the strains showed methicillin resistance. No MRSA (methicillin-resistant *Staphylococcus aureus*) strains were isolated. All strains of Gram-positive bacteria were susceptible to glycopeptides and linezolid (Fig. 3).



Ryc. 3. *Lekowrażliwość bakterii Gram-dodatnich wyosobnionych z krwi żylnej i krwi z Broviaca dzieci Kliniki Pediatrii, Hematologii i Onkologii*

Pic. 3. *Antimicrobial susceptibility of Gram-positive bacteria isolated from venous and Broviac blood samples from the patients of the Department of Pediatrics, Hematology and Oncology*

DISCUSSION

It is estimated that 14-51% of central venous lines placed in patients of oncology wards lead to bacteremia and 35% result in implantation-site infection [3, 4, 5, 6]. Stamou *et al.* [9] have observed 27 (9.7%) cases of bacteremia in children with neoplastic diseases and Hickman-Broviac type catheters. Fratino *et al.* [10] have identified venous line as the cause of bloodstream infection in 5.8% of the patients. In our material, positive cultures of blood samples harvested from the Broviac catheter and peripheral vein were found in 21.9% of the patients. The low percentage (4,6%) of probable CRBSI may result from differences in primary conditions of the analyzed patients, the advancement thereof and the antimicrobial therapy used.

The most frequent source of external colonization of a Broviac-type catheter is the physiological flora of the skin of the patient or of the staff member's hands. Thus, the primary cause of CRBSI are coagulase-negative staphylococci. The fibrin layer developing on the surface of the deeper portion of the catheter may be a location for the development of microorganisms present in the deep layers of the skin. Less frequently, the lumen of the catheter may be colonized by microorganisms derived from infusion fluids [2, 11]. In our material, *Staphylococcus epidermidis* was the dominant species among the Gram-positive bacteria and constituted 48% of all isolated staphylococci. Flynn *et al.* reported CRBSI due to *S. epidermidis* in 60% of all pathogens [12].

In our study, Gram-negative rods were the microorganisms most frequently isolated from blood cultures (57%). Superiority in numbers of Gram-negative microorganisms has been reported by Flynn *et al.* in patients of pediatric oncology wards [12]. The probable cause of this fact is the impairment of mucosal membrane function in children receiving long-term parenteral alimentation, which facilitates the migration of microorganisms from the gastrointestinal tract [13]. A similar percentage of Gram-negative bacteria in catheter-related infections in children treated in oncology and hematology wards found Viscoli *et al.* [14]. The incidence of these infections was 20% in neutropenic patients and 55% in others.

Most of the Gram-negative rods responsible for CRBSI are acquired from the hospital environment, e.g. *Pseudomonas aeruginosa, Acinetobacter* spp. [2]. In the presented studies the percentage of these microorganisms was high and constituted 22% of all Gram-negative bacteria.

Gram-positive rods of the genus *Corynebacterium* can also participate in catheter-related infections [2], which has been confirmed by our findings. Although no fungi were isolated from our material, the species colonizing the hands of the staff, such as *Candida albicans* and *Candida parapsilosis,* are an important etiological factor of CRBSI's [2]. According to Prager *et al.* [15], the genus *Candida* was the most common microorganism colonizing central venous lines, leading to fungemia.

The available literature yields no information on the drug resistance of microorganisms isolated from catheter-related infections. No ESβL-producing bacteria were isolated from our material. Only single *P.aeruginosa* strains resistant to carbapenems have been isolated from the analyzed samples, despite the reported increase of this resistance [16]. The high percentage (87.5%) of methicillin-resistant coagulase-negative staphylococci might be alarming. This fact

eliminates the use of β-lactams in as many as 90% of catheter-related infections of this etiology.

CONCLUSIONS

Despite the study shows low percentage of catheter-related infection, Broviac catheters may be related to a serious risk of bacteriemia among the children with neoplastic diseases. An analysis of the strains isolated from blood samples collected simultaneously from the catheter and peripheral vein has shown a domination of microorganisms of the genus *Staphylococcus* and *Klebsiella.* In therapy of catheter-related infections, antibiotics active against methicillin-resistant coagulase-negative staphylococci must be included, as these microorganisms were frequently isolated. Multiplication of the number of samples collected simultaneously from the vein and Broviac catheter enables proper interpretation of microbiology test results.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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CHEMICAL ELEMENTS IN THE BLOOD OF WHITE STORK *CICONIA CICONIA* CHICKS IN DIFFERENTIAL POLAND REGIONS

PIERWIASTKI CHEMICZNE WE KRWI PISKLĄT BOCIANA BIAŁEGO *CICONIA CICONIA* W ZRÓŻNICOWANYCH ŚRODOWISKACH POLSKI

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S u m m a r y

The aim of study was to compare the ecophysiological basis for developing White Stork *Ciconia ciconia* chicks in differential Poland environments. We examined the level of Ca, Mg, Fe, Zn, Cu, Mn, Co, Cd and Pb in the blood of growing chicks, which were grows and feeds in the variety of environmental pollution. These regions were also represents the variety of biogeochemical backgrounds for soil and foraging properties. The investigations were carried out during stork breeding season 2006. Blood samples were collected from young storks developing in relatively pure environment and treated as a control (Kłopot; SW Poland (52°07'56,3" N, 14°42'10,4" E). It was compared with Czarnowo (52°02'03,7" N, 14°57'24,7" E), located 20 km away from Zielona Góra (SW Poland), and treated as suburbs, and near Głogów (51°39'32,6" N, 16°04'49,9" E; SW Poland), where a copper smelter is situated. We have also conduct our research in Cecenowo, a small Pomeranian village near Słupsk (N Poland; 54°38'34,5" N, 17°32'31" S).

In total of 182 of White Stork chicks from 33 jacks have been surveyed. The age of birds changed from 19 up to 56 days. Samples of investigated wing venous blood were taken for analyses of chemical element concentration. The content of elements were then determined using AAS method.

We have stated differences in the concentration of all investigated elements, besides calcium, in studied regions. We also found a high level of cadmium, both in Pomeranian region, and polluted area, however lead concentration was high only in Głogów area.

Simultaneously we observed a high level of Ca, Mg and Fe both in Pomeranian and polluted areas. Na, K, and Ca were in most concentration in suburbs and polluted regions, while Zn and Co - in suburbs and polluted, and Cu, and Mn – in polluted and Pomeranian regions. Thus we can observe the high intensity of the degree of environmental pollution in the Pomeranian region.

It is evidence for importance of anthropogenic activity in the environment in the past, which influenced the course of biogeochemical processes and caused bioaccumulation of toxic heavy metals locally. This case took place in Pomeranian village Cecenowo, which we investigated. We can concluded that use of blood research with accompanied chemical element level studies is helpful to assess the condition of birds, and given the positive association with miscellaneous environmental loads. We can also suggested that anthropogenic processes and activities may plays an important role in bioaccumulation and economy of free radicals in various types of environment, but it has not any connection with the type of this region.

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

Zamierzeniem pracy było określenie bazy ekofizjologicznej dla rozwoju piskląt bociana białego *Ciconia ciconia*, w zróżnicowanych środowiskach Polski, w sezonie lęgowym 2006. Oznaczono koncentracje Ca, Mg, Fe, Zn, Cu, Mn, Co, Cd i Pb we krwi piskląt rozwijających się w regionach o różnych podstawach biogeochemicznych. Krew pobierano z żyły skrzydłowej od piskląt ze środowisk czystych (Kłopot; 52°07'56,3" N, 14°42'10,4" E; kontrola), terenów podmiejskich Zielonej Góry (52°02'03,7" N, 14°57'24,7" E), na terenie huty miedzi i ołowiu k/Głogowa (51°39'32,6" N, 16°04'49,9" E) i na Pomorzu k/Słupska (54°38'34,5" N, 17°32'31" S). Przebadano 182 pisklęta, w wieku 19-56 dni, pochodzące z 33 gniazd. Poziom pierwiastków we krwi oznaczano metodą AAS.

Stwierdzono różnice koncentracji wszystkich analizowanych pierwiastków, z wyjątkiem wapnia, w badanych środowiskach. Stwierdzono wysoki poziom kadmu w regionie pomorskim i na terenach skażonych k/Głogowa, chociaż stężenie ołowiu było wysokie tylko w tym regionie skażonym. Zanotowano również wysoki poziom Ca, Mg i Fe na Pomorzu i w okolicach Głogowa. Koncentracja pozostałych badanych pierwiastków była wysoka przeważnie w regionie skażonym i na terenach podmiejskich, chociaż poziom Cu i Mn był też wysoki w regionie pomorskim. Może to świadczyć o wysokim zanieczyszczeniu badanego regionu na Pomorzu. Można więc wnioskować o istnieniu procesów antropopresji na tych terenach w niedalekiej przeszłości, która zapewne spowodowała tam bioakumulację metali toksycznych. Ma to istotne znaczenie lokalne, co pozostaje w związku z procesami ekofizjologicznymi stwierdzonymi przez nas wcześniej u piskląt bociana z tych terenów. Możemy wnioskować o ważności badań enzymatycznych krwi piskląt, które mogą dać odpowiedź nt. kondycji piskląt bociana rozwijających się w tych środowiskach pomorskich.

Key words: chemical elements, heavy metals, blood, chicks, White Stork, *Ciconia ciconia*

Słowa kluczowe: pierwiastki chemiczne, metale ciężkie, krew, pisklęta, bocian biały, *Ciconia ciconia*

INTRODUCTION

According to the last investigations it can be stated significant changes in the number and dynamics of White Stork population in Poland and West Europe particularly, and increases in the mortality rate amongst chicks, which have been linked to the pollution of their environment by heavy metals [1, 2, 3]. Toxic heavy metals have their unfavourable impact upon the course of lipoperoxidation processes in living bird. Research by these authors has linked concentrations of toxic metals in the organs of birds with higher mortality amongst chicks, and with a fall in fecundity. This indicates a necessity to determine the stages and mechanisms by which pollutants enter birds during the time of their development in the nest. Metals act to increase the mortality rate of birds, reducing productivity of their populations in types of regions. In addition, they may give rise to many pathological abnormalities, and to improper functioning of immunological system [2, 4, 5, 6, 7]. Unfortunately, there is the lack of these studies in field conditions. E.g. [8] investigated the reduction of erythrocyte catalase and superoxide dismutase activities in male inhabitants of cadmium-polluted areas in Jinzu river (Japan). We can also find several research of more widespread, generally, i.e. biogeochemical and element-enzyme interactions. Thus some papers have analyzed biogeochemical interactions affecting hepatic trace elements in aquatic birds [9].

Among others, [10] studied metal-metal interactions in rat liver and kidney and their relations with thioneins activity. The remaining papers assume effects of laboratory and field investigations concerning toxic metals intoxication during particular physiological periods in birds and mammals. E.g. [1] studied ecological determinations of trace elements in blood collected from birds feeding in the area affected by toxic spill.

The aim of this study was to compare the ecophysiological basis for developing stork chicks in various Poland environments. Thus we examined the level of physiological elements Ca, Mg, Fe, microelements Zn, Cu, Mn, and Co, and toxic heavy metals Cd and Pb in the blood of growing chicks, which all were grows and feeds in the variety of environmental pollution. These regions were also represents the variety of biogeochemical backgrounds for soil and foraging properties.

STUDY AREA

The investigations were carried out in stork breeding season of 2006. Blood samples were collected from young storks developing in relatively pure environment and treated as a control (Kłopot village with absolutely lack of any manufactures in the radius of 150 km around [11]; SW Poland (52°07'56,3" N, 14°42'10,4" E). It was compared with Czarnowo (52°02'03,7" N, 14°57'24,7" E), a village located 20 km away from

Zielona Góra (51°56'26,1" N, 15°30'38,9" E; SW Poland), and treated as suburbs, and near Głogów (51°39'32,6" N, 16°04'49,9" E; SW Poland), where a copper smelter is situated, with copper manufacture. It produced copper and lead from lead fields. Głogów plant copper leads an active proecological activity. Green fields consist about 50% of protective areas of this manufacture complex. The forests present 32% of this area. Acid soils are subjected by calcification. One of numerous proecological ventures of the manufacture was desulphuring installation and modernize of sulphur acid manufacture. These innovations have contributed towards rapid decrease of sulphur dioxide. Now the process of modernization of lead department is continued. We have also conduct our research in Cecenowo, a small and relatively pure Pomeranian village near Słupsk (N Poland; 54°38'34,5" N, 17°32'31" S).

MATERIAL AND METHODS

In total of 91 of White Stork chicks from 33 jacks have been surveyed in 2006. The age of birds changed from 19 up to 54 days from an output from an egg. For elimination of diurnal rhythm changes all examinations were started at 10 and ended at 12 am. Samples of investigated wing venous blood were taken for analyses of chemical element concentration. The content of elements were then determined with use of Perkin-Elmer atomic absorption spectrophotometer [12]. Standard curves were prepared using standardized Merck samples. Concentration of elements were given in terms of $\mu g*g^{-1}$d.w. (ppm). We collected blood samples via veni-puncture of the brachial vein of stork chicks. They were retrieved from the nest and placed into individual ventilated cotton sacks. Blood (5 ml) was collected using 5 ml syringe washed up with EDTA. Samples were kept in a chilled cooler before transporting to the laboratory. After centrifugation, plasma samples were frozen at -20^{o}C and stored until analysis. Our behavioral observations as well as physical examinations of the birds suggested that all of them were physically healthy.

Statistical Analysis. The results are expressed as mean ± S.D. Significant differences among the means were measured using a multiple range test at min. $P<0.05$. Data not having a normal distribution were log transformed. Student t-tests with 95% confidence intervals ($\alpha = 0.05$) were applied to determine the significance of differences between element concentrations in types of regions. Significance of differences in element level and enzyme activity in regions studied was examined using ANOVA for correlation test. Differences between element concentration in the blood of White Stork chicks from differentiated environments were determined by RIR Tukey test for non equal numbers. Arithmetic mean concentrations of elements and enzymes activity in blood were estimated by using two-way ANOVA (Scheffe multiple range test).

RESULTS

We have stated statistically significant differences in the concentration of all investigated elements, besides calcium, in the blood of young storks nesting in all regions which have been studied (Tab.. I).

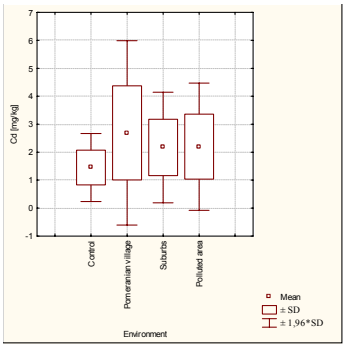
Tabłe I. *Differences between environments in respect of elements concentration in blood of White Stork Ciconia ciconia chicks in Poland (p<0.05 - *; p<0.01 - **; p<0.01 - ***).*

Tabela I. *Różnice pomiędzy badanymi regionami Polski pod względem koncentracji pierwiastków we krwi piskląt bociana białego Ciconia ciconia (p<0.05 - *; p<0.01 - **; p<0.01 - ***).*

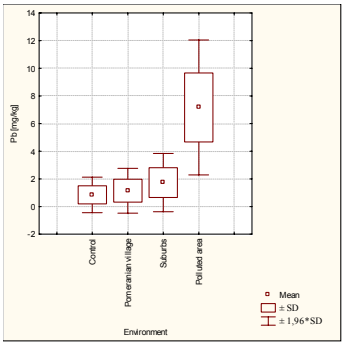
Element	Differences between environments	p
Na	Control: Suburbs	**
	Control: Polluted area	**
	Pomeranian village : Suburbs	***
	Pomeranian village: Polluted area	***
K	Control: Suburbs	***
	Pomeranian village : Suburbs	***
	Pomeranian village: Polluted area	***
Mg	Control: Suburbs	***
	Control: Polluted area	***
	Control: Pomeranian village	***
	Suburbs: Polluted area	***
	Suburbs: Pomeranian village	***
	Polluted area: Pomeranian village	***
Fe	Control: Polluted area	***
	Control: Pomeranian village	*
	Suburbs: Control	***
	Polluted area: Pomeranian village	**
Zn	Control: Suburbs	***
	Control: Polluted area	**
	Control: Pomeranian village	**
Cu	Control: Polluted area	**
	Control: Pomeranian village	**
	Suburbs: Polluted area	***
	Suburbs: Pomeranian village	***
	Polluted area: Pomeranian village	**
	Suburbs: Control	
Mn	Control: Polluted area	**
	Suburbs: Polluted area	***
Co	Control: Polluted area	***
	Suburbs: Polluted area	***
	Polluted area: Pomeranian village	***
Cd	Control: Pomeranian village	**
Pb	Polluted area: Control	***
	Polluted area: Suburbs	***
	Polluted area: Pomeranian village	***

We also found a high level of cadmium in the blood of chicks, either from Pomeranian region, or from Głogów polluted area (Fig. 1), however lead concentration was high in chicks from Głogów area (Fig. 2). Simultaneously we observed a relatively high level of macroelements Ca, Mg and Fe in White Stork chicks

both in Pomeranian (Cecenowo) and polluted (Głogów) areas (Figs. 3, 4, Tab. II). The remaining macroelements Na, K, and Ca were in most concentration in chicks from suburbs and polluted regions, while the level of microelements Zn, and Co - in suburbs and polluted, and Cu, and Mn – in chicks from polluted and Pomeranian regions (Tab. II). Thus we can observe the high intensity of degree of environmental pollution in the Pomeranian region, which was studied in this paper.



1



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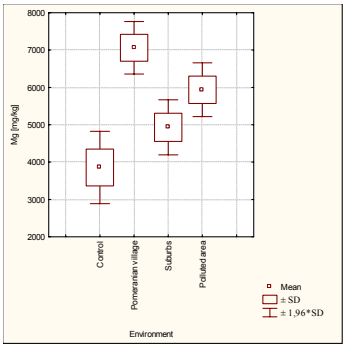

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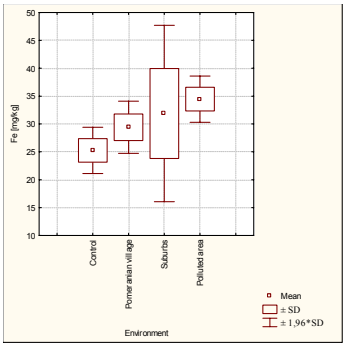

4

Control – teren kontrolny
Pomeranian village – wieś pomorska
Suburbs – tereny podmiejskie
Polluted area – teren zanieczyszczony
Environment – środowisko
Mean – średnia
SD – odchylenie standardowe

Figs. 1-4. *Mean and SD concentration of Cd, Pb, Mg, and Fe in blood of White Stork Ciconia ciconia chicks in differentiated Poland regions.*

Ryc. 1-4. *Średnie arytmetyczne i odchylenia standardowe koncentracji kadmu, ołowiu, magnezu i żelaza we krwi piskląt bociana białego Ciconia ciconia w zróżnicowanych regionach Polski*

Table II. *Mean and SD concentration of elements in blood of White Stork Ciconia ciconia chicks in different Poland regions*

Tabela II. *Średnie arytmetyczne i odchylenia standardowe koncentracji pierwiastków we krwi piskląt bociana białego Ciconia ciconia w zróżnicowanych regionach Polski*

	Control		Pomeranian village		Suburbs		Polluted area	
Na [mg/kg]	147.810	4.4567	150.833	3.1669	143.318	2.2759	143.222	5.0707
K [mg/kg]	3.612	0.6754	3.304	0.7087	4.649	0.7798	3.824	0.5958
Ca [mg/kg]	112.496	14.2104	122.193	29.5120	126.805	28.2778	115.893	18.2428
Mg [mg/kg]	3856.556	493.7636	7060.556	359.0185	4933.048	376.8301	5938.278	367.2127
Fe [mg/kg]	25.263	2.1198	29.409	2.3916	31.884	8.0717	34.463	2.1202
Zn [mg/kg]	6.872	4.8774	9.626	0.3084	10.151	0.2958	9.664	0.2650
Cu [mg/kg]	7.609	3.4453	11.044	1.4891	4.037	1.1779	10.879	4.6693
Mn [mg/kg]	38.189	9.1337	42.187	6.6311	37.782	7.6669	47.611	4.8838
Co [mg/kg]	2.413	1.6977	1.752	0.8950	2.713	1.1492	5.584	1.8477
Cd [mg/kg]	1.452	0.6195	2.691	1.6818	2.168	1.0076	2.197	1.1607
Pb [mg/kg]	0.844	0.6546	1.147	0.8274	1.731	1.0750	7.167	2.4899

Significantly higher Fe level in the blood of young storks from polluted areas as compared with those from controls was likely to be related to lower concentration of toxic heavy metals in chicks from the control. It was also higher in chicks from unpolluted areas, which may be indicative of their better development.

The results of present studies show that concentration of hardly toxic heavy metals gradually increased over nestling development, and in polluted areas were about twice as high as in a control. This was probably due to a higher contamination of soils in polluted regions.

It is evidence for importance of anthropogenic activity in the environment in the past, which influenced the course of biogeochemical processes and caused bioaccumulation of toxic heavy metals locally. This case took place in Pomeranian village Cecenowo, which we investigated. We can concluded that use of hematological researches assess the health and condition of birds is questionable, and given the positive association with miscellaneous environmental loads.

DISCUSSION

Changes of chemical elements concentration in nestlings depends not only on their concentration in the environment and development strategy, but also on mutual interactions between elements. Moreover, soft tissues, bones and feathers are the organs which are helpful for permanent regulation of chemical elements homeostasis in chicks starting from hatching.

The results of studies presented in this paper provide evidence that White Stork from control areas has better conditions for growth and development than in polluted ones or even suburbs.. They also show that it is necessary to know the stages of growth to understand bioaccumulation processes of elements in chicks. But it is evidence for importance of anthropogenic activity in the environment in the past, which influenced the course of biogeochemical processes and caused bioaccumulation of toxic heavy metals locally. This case took place in storks developing in Pomeranian region, which we investigated, where cadmium level was relatively high (Fig. 1, Tab. II).

The parameters which most affect trace elements accumulation in precocial and semiprecocial birds are species (i.e. ecological form of bird) and its trophic situation, sex, time of exposure and biomass [1]. These authors stated e.g., that in the unpolluted areas Zn and Cu occurred at higher levels in blood than in polluted ones. Concentrations of Pb and Cd are higher in polluted areas. They simultaneously emphasized, that metals level in blood of chicks may be influenced by physiological response of species to distinct metals, and by the greater or lesser bioavailability of these metals. The reference values should also be interpreted with care, since they do not refer to the same types of species in particular environments [1]. In our studies on White Stork chicks we also stated these relations, particularly between element level in blood and environmental stress [13].

The results of our studies show that concentration of cadmium is high in blood of White Stork chicks both in the Pomeranian and polluted environments (Tab. II, Fig. 1). This was probably due to a higher contamination of soils with cadmium in these regions. Cd is accumulated in the blood of chicks during growth, mostly in bones and feathers, which was found by [14], and its toxic effects on an organism are intensified with age. Cd and other hardly toxic metals primarily disturb growth, reduce the hemoglobin content, displace biologically necessary elements, and have antagonistic effect on metabolism of these elements [15, 16, 17].

Among various mechanisms of chemical elements economy in birds there is an important role of blood calcium levels and correlated mechanisms of pesticide-induced eggshell thinning [18]. These authors concluded about physiological mechanisms determining the impact of pesticides and organochlorines in the environment on the blood calcium levels in both altricial and precocial birds. These findings are consistent with pesticide conversion ways actions involving inhibition of shell gland function, but not with those involve decreased calcium supply to the gland [19]. Furthermore, these relationships caused widely bird responses to environmental stress. E.g. gross pathology of skeletal forms is supported by histopathology, which showed that bone remodeling activity is grater in the deformed storks. It also has more irregular subperiosteal bone, and tend to have higher residual islets of cartilage in their metaphyses, which, in turn is related to metal contaminant residues. Both Ca and P in bones are independently affected by metals. Deformed birds have lower serum bone alkaline phosphatase. Bone malformations, measured by leg asymmetry, are only partially explained by bone metals, indicating that a combination of factors is involved with abnormal development in young storks [19]. However, the respectively connections with heavy metals and hemoglobin content and blood indices development was stated by [1] and [20].

Metals exert toxic effects if they enter into biochemical reactions in which they are not normally involved. The threshold concentration at which such deleterious effects occur is usually higher for essential elements than for non-essential although the "window for essentiality" for some ones is quite narrow. Unlike many elements can not be broken down into less toxic components. When released into environment, they have long residence times in soils and may continue to exert harmful effects on the environment long after the source of pollution has ceased to operate [21, 22, 3]. This phenomenon is actual in the Pomeranian village, studied in this paper, which is traditionally stork burden in northern Poland, where the level of cadmium is relatively high (this paper).

However, the interaction between chemical elements and antioxidant enzymatic activity plays an important role in physiological response of chicks in their environment.

Various heavy metals and disturb of macroelements transfer in the environment have their differential ecophysiological impact upon the course of the level of pro- and antioxidant activity of enzymes and on the development of lipoperoxidation processes. We can find some attempts for explanation of these interactions, but they are concerned laboratory conditions and raising animals.

The widely dependences of metal content in blood of birds and their condition, which expressed by their

hemoglobin and hematocrit quality, was stated by various authors [23, 24, 25, 26, 27]. They emphasized the effects of dietary heavy metal concentrations upon the plasma lipid content both in altricials and precocials. Their health and immunocompetence also depends on calcium and toxic heavy metals interactions. Pb and Cd exposure are significantly suppress secondary humoral immune response towards sheep red blood cells, but only additional Ca source is not available. This effect is found rather only in females, suggesting sexual differences in susceptibility of humoral immunity to lead treatment [27]. It was also stated besides that plasma cholesterol concentration is significantly affected by interaction between dietary Cu and Cr. In addition, plasma triglyceride level is affected by Cu-Cr and Cu-Zn interaction effects [26]. Our investigations on White Stork chicks indicates on the role of element-element interactions impact upon the definite image of hemoglobin content and the values of red blood picture [28]. However, hemoglobin and hematocrit are depended not only upon element content in the environment, but also on the time of day, ambient temperature, food resources, level of blood infection with parasites [29, 23, 24, 25].

Our investigations on White Stork chicks indicate the role of element-element interactions impact upon the condition of bird. So we can thus conclude that use of blood enzymatic researches can be helpful for assess the health and condition of birds, and given the positive association with miscellaneous environmental loads. We can also suggested that anthropogenic processes and activities may plays an important role in bioaccumulation and transfer of chemical elements in various types of environment, but it has not any connection with the type of this region. They also show the necessity to know the stages of growth to understand bioaccumulation processes of elements in chicks. Any changes of chemical elements metabolism in metabolic pathways of homoiotherms reflecting by environmental stress, cause significant ecophysiological and population responses of their organisms. This phenomenon is especially concerns for altricial and semiprecocial birds, which nestlings are directly depends upon immediately environmental impact. Our results exhibit that the level of microelements in blood of White Stork chicks shows such large differences between areas (Tab. I). Differences in the level of these elements in blood between polluted and control areas could be due to a physiological role of these metals in the protein and carbohydrate metabolism, which is depend on environmental stress. The results of studies presented provide evidence that White Stork from control areas has better conditions for growth and development than in polluted ones. They also show the necessity to know the stages of growth to understand bioaccumulation processes of elements in chicks. Elements concentration in blood of chicks may be influenced by physiological response of species to distinct metals, and by the greater or lesser bioavailability of these metals. In our studies we stated the correlation of elements concentration in blood and the type of environment, particularly between element level and environmental stress.

CONCLUSIONS

1. It is evidence for importance of anthropogenic activity in the environment in the past, which influenced the course of biogeochemical processes and caused bioaccumulation of toxic heavy metals locally. It can cause non successful development of White Stork in Pomeranian region.
2. The use of blood research with accompanied chemical element level is helpful to assess the condition of birds, and gives the positive association with miscellaneous environmental loads.
3. Elements concentration in blood of chicks may be influenced by physiological response of species to distinct metals, and by the greater or lesser bioavailability of these metals. In our studies we stated the correlation of elements concentration in blood and the type of environment, particularly between element level and environmental stress.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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IMPACT OF MANDATORY VACCINATION PROGRAM AGAINST HBV ON EPIDEMIOLOGY OF HBV AND HCV INFECTIONS IN CHILDREN WITH MALIGNANCIES

ZNACZENIE SZCZEPIENIA PRZECIWKO HBV W EPIDEMIOLOGII ZAKAŻEŃ HBV I HCV U DZIECI Z CHOROBAMI NOWOTWOROWYMI

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Summary

Introduction. Children with malignancy are at high risk of hepatitis B and C infections, often with unfavorable course of the disease. Before a mandatory vaccination program against HBV was introduced in Poland, HBV and HCV infections were found, respectively, in 62,2% and 54,3% of children during anti-cancer therapy. Currently, ocurrence of hepatitis in Poland is estimated to be 1-1,5% for HBV and 1,5% for HCV.

Aims. The purpose of this study was to analyze epidemiology of HBV and HCV infections among children with malignancy, with respect to mandatory vaccination program against HBV of neonates and infant, which was introduced in 1995.

Patients and methods. The study included 305 children with malignant diseases, hospitalized between 2004-2008 in the department of pediatric hematology and oncology. 146 patients out of 305 (48%) were born prior to 1995. All patients were screened for serological markers of HBV and HCV infections during hospitalizations. An infection with HBV was diagnosed when a presence of HBsAg or anti-HBc-IgM antibodies was detected. HCV infection was diagnosed when the anti-HCV tests were positive.

Results. Among 305 patients, 3 were found to be HBV positive (0,98%). All these infections were observed already at the time of first admission to the department. These patients were born prior to 1995. Among children undergoing mandatory vaccination program, presence of anti-HBs antibodies was detected in 150/159 (94%) cases. In 72 cases a protective level of the antibodies was observed. HCV infections occurred in 4 cases (1.3%). Out of these, 3 patients were infected during anticancer treatment. Three out of 4 anti-HCV positive patients were born prior to 1995.

Conclusions. (1) Introduction of routine vaccination against HBV helped to control HBV infections among children with malignancy. (2) Coinciding reduction of HCV infections shows the importance of non-specific prophylaxis. (3) Currently, the risk of HBV and HCV infections during anticancer treatment does not exceed general population risk.

Streszczenie

Wstęp. Dzieci z chorobami nowotworowymi należą do grupy wysokiego ryzyka zakażenia wirusami zapalenia wątroby, a zakażenia HBV i HCV często mają u nich niekorzystny przebieg. Przed wprowadzeniem szczepień ochronnych przeciwko zakażeniom HBV, odsetek zakażeń HBV i HCV wśród dzieci z chorobami nowotworowymi sięgał odpowiednio 62,2% i 54,3%. Obecnie odsetek zakażeń w populacji ogólnej w Polsce wynosi odpowiednio 1-1,5% i 1,5%.

Cel pracy. Analiza epidemiologiczna zakażeń HBV i HCV u dzieci z chorobami nowotworowymi, z uwzględnieniem wprowadzenia obowiązkowego szczepienia przeciwko HBV w 1995 roku.

P a c j e n c i i m e t o d y k a . Badaną grupę stanowiło 305 dzieci z chorobami nowotworowymi, leczonymi w latach 2004-2008 w Klinice Pediatrii, Hematologii i Onkologii. 146/305 pacjentów było urodzonych przed 1995 rokiem. Przeanalizowano obecność w surowicy krwi markerów zakażenia wirusami HBV i HCV oznaczonych w trakcie hospitalizacji. Zakażenie HBV rozpoznawano na podstawie obecności antygenu HBsAg lub przeciwciał anty-HBc-IgM, natomiast zakażenie HCV na podstawie obecności przeciwciał anty-HCV.

W y n i k i . W badanej grupie 305 dzieci u 3 (0,98%) stwierdzono zakażenie wirusem HBV, wszyscy należeli do grupy urodzonych przed 1995 rokiem. We wszystkich przypadkach zakażenia wykryto przed przyjęciem do Kliniki, przy czym 2/3 zakażonych pacjentów pochodziło z innych ośrodków. Ponadto u 150/159 (94,3%) dzieci objętych programem szczepień stwierdzono obecność przeciwciał anty-HBs; w 72 przypadkach stężenie ochronne. U 4 (1,3%) pacjentów stwierdzono zakażenie wirusem HCV: u 3 urodzonych przed 1995 rokiem i u 1 urodzonego później. U 3/4 dzieci zakażenie nastąpiło w trakcie terapii.

W n i o s k i . (1) Wprowadzenie szczepień ochronnych przeciwko zakażeniom HBV przyczyniło się do opanowania endemii zakażeń HBV wśród dzieci z chorobami nowotworowymi. (2) Równocześnie odsetek zakażeń HCV w tej grupie pacjentów uległ obniżeniu, co przemawia za dużym znaczeniem również niespecyficznych metod profilaktycznych. (3) Aktualne ryzyko zakażenia HBV i HCV w trakcie terapii przeciwnowotworowej jest porównywalne z ryzykiem w populacji ogólnej.

Key words: HBV, HCV, vaccination, cancer, children, epidemiology
Słowa kluczowe: HBV, HCV, szczepienia, nowotwór, dzieci, epidemiologia

INTRODUCTION

Children with malignancy carry a high risk of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Factors that promote the infections include: frequent contact with a highly endemic environment due to many hospitalizations, all necessary diagnostic and therapeutic manipulations that injure tissues [1,2]. Immunosuppression and treatment harmful to liver which patients has to undergo, might cause an unfavorable progression of hepatitis. Although hepatitis is usually asymptomatic during anticancer treatment, it can rapidly progress to fulminant type after cessation of chemotherapy and cause liver necrosis with hepatic coma [2]. The high rate of the infection might progress to the chronic phase, which is further associated with the development of liver cirrhosis and hepatocellular carcinoma [3]. Thus, HBV and HCV infections can play an adverse prognostic role in criteria of disease-free survival [4]. It can essentially abolish the effects of treatment of the basic disease or become the final cause of death [3].

Prior to 1995, viral hepatitis infections among children with cancer were a serious clinical problem in Poland. At that time, HBV infections were found in 62,2% of oncological patients in our Department, while 54,3% were infected with HCV [1]. This situation forced introduction of many prophylaxis methods, which included the use of new-generation disposal equipment, strict hygienic rules in patients nursing, proper blood donors screening, and specific passive and active immunization [2].

Apart from that, an obligatory vaccination of all neonates in Poland was introduced. It started in 1994 and covered the whole country in 1996. It was introduced in Bydgoszcz area in the beginning of 1995 [3].

The aim of this study was to analyze present epidemiology of HBV and HCV infections among children with malignancy, with respect to mandatory vaccination program against HBV of neonates and infants introduced in 1995.

MATERIALS AND PATIENTS

A retrospective study was carried out on 305 children with malignant diseases that were hospitalized between August 2004 and January 2008 in the Department of Pediatric Hematology and Oncology in Bydgoszcz, Poland. 146 patients out of 305 (48%) were born prior to 1995. The remaining 159 (52%) patients were born in 1995 or later and had therefore been immunized according to mandatory vaccination schedule (3 doses of vaccine administrated at the first days of life, and then at 2^{nd} and 6^{th} months after birth). The patients diagnosis were as follows: leukemia 122 (40%), lymphoma 57 (19%), brain tumors 31 (10%), nephroblastoma 18 (6%), and other solid tumors (25%).

All patients were screened for hepatitis B and C viruses by testing for presence of HBsAg, anti-HBs, anti-HBe, HBeAg, anti-HBc-IgM, anti-HBc-IgG and anti-HCV in serum. The tests were performed at the beginning of first admission to the hospital and were then repeated during further therapy. A patient was consid-

ered infected with HBV if presence of HBsAg antigen or anti-HBc-IgM antibodies were observed in two consecutive tests. HCV infection was diagnosed on a basis of two consecutive positive results for anti-HCV antibodies.

RESULTS

Among 305 patients, 3 were found to be HBV positive, which was at the level of 0,98% of the total number of patients. All of these patients were already infected at the time of first admission to the hospital. No HBV infection occurred during anticancer treatment in our department. Two out of the 3 patients had been transferred from another hospital. All HBV infected patients were born before 1995, thus no HBV infections were observed among patients included in the mandatory vaccination program against HBV of neonates and infants.

HCV infections occurred in 4 cases (1,3%). Out of these, 3 patients were infected during anticancer treatment. Three out of 4 HCV positive patients belonged the group of children born prior to 1995 (Fig. 1). Simultaneous HBV and HCV infections were not observed.

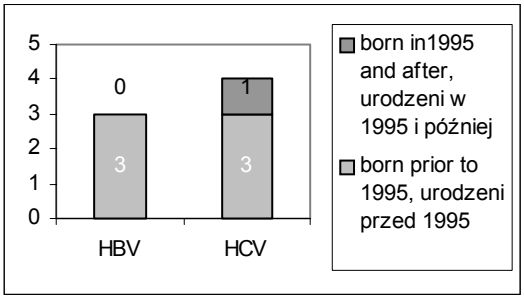

Fig. 1. *The infected patients among the group of patients born prior to 1995 and among the group of born afterwards*

Ryc. 1. *Zakażeni pacjenci z grupy urodzonych przed rokiem 1995 i z grupy urodzonych później*

In order to evaluate the significance of vaccination prior to neoplastic disease, titers of anti-HBs antibodies were assessed. Out of 159 patients, which underwent routine vaccination against HBV as neonates and infants, 150 (94%) children were anti-HBs positive. The protective level of anti-HBs, as considered by the titer >100 IU/L, was observed in 72 patients, i.e. in 48% of anti-HBs positive patients. Only 9 out of 159 vaccinated patients did not respond to vaccination having anti-HBs titer <10 IU/L.

DISCUSSION

The study shows that occurrence of HBV and HCV infections in pediatric oncology ward has been under control since the vaccination program of neonates and infants was introduced. The number of HBV infections has diminished from 62,6% to 0,98%, while occurrence of HCV infections was reduced from 54,3% to 1,3% (Fig. 2).

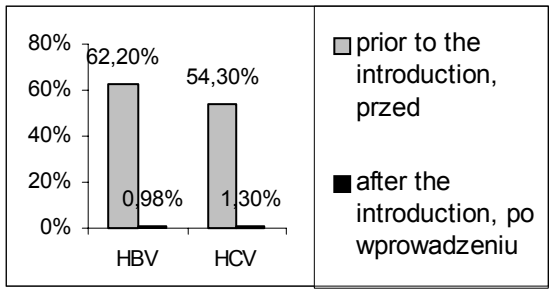

Fig. 2. *The reduction of HBV and HCV infections after the introduction of the mandatory vaccination program of neonates and infants*

Ryc. 2. *Obniżenie odsetka zakażeń HBV i HCV po wprowadzeniu programu obowiązkowych szczepień noworodków i niemowląt*

Comparing these data with the frequency of hepatitis in Poland, which is estimated to be 1-1.5% for hepatitis B and 1.5-5% for hepatitis C, reveals that anticancer therapy no longer increases the risk of infection for HBV or HCV above the risk in the Polish population [4].

The problem to be raised, is whether these good results were achieved due to mandatory vaccination program against HBV or not. The group of children born prior to 1995 and thereby not subjected to the vaccination as neonates, came within other prophylaxis methods: vaccinations after the diagnosis of neoplastic disease or passive immunoprophylaxis with hepatitis B immunoglobulin, while non-specific prophylaxis methods were the same for both groups. In the group of patients who were vaccinated as neonates, no HBV infection was detected, compared to 4/146 (2,7%) HBV infection rate in the other group. The positive influence of vaccination prior to neoplastic disease seems to be confirmed by presence of anti-HBs antibodies in most of vaccinated patients (94%), despite a high probability of immune dysfunction at the moment of testing. It has recently been confirmed that in comparison to vaccination after cancer diagnosis, a higher

response of immunization rate was achieved when vaccination took place before cancer diagnosis [5].

Another important issue is the decreased rate of HCV-infected patients. The use of serological methods for the diagnosis of HCV infection is not optimal, because of a risk of delayed seroconversion due to immunosuppression. However, the same method was used in both analyzed groups. Despite the lack of specific HCV prophylaxis, it was significantly reduced, comparably to the rate of HBV infections. This observation highlights the importance of non-specific methods in preventing HCV transmission. The non-specific prophylaxis can play an important role in reducing frequency of HBV infections, in addition to vaccinations.

In previous decade, HBV and HCV infections among children with malignancy in Poland were considered to occur mainly during or following the treatment of neoplastic disease [6]. Contrary to this observation, all HBV infections in the last analyzed period were present at the beginning of treatment among the examined patients. No new HBV infections were noted during therapy over last 4 years.

World Health Organization (WHO) recommends routine vaccination of all infants against HBV infections as an integral part of national immunization schedules worldwide [7]. The main purpose of this strategy is to protect the population from chronic HBV infections, which might be followed by liver cirrhosis and hepatocellular cancer. At the same time the mandatory vaccinations help to prevent hepatitis B in high risk group, such as children with malignancy.

CONCLUSIONS

1. Introduction of routine vaccination against HBV helped to control HBV infections among children with malignancy.
2. Coinciding reduction of HCV infections shows the importance of non-specific prophylaxis.
3. Currently, the risk of HBV and HCV infections during anticancer treatment in children does not exceed general population risk.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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BONE TURNOVER DURING PREGNANCY

WSKAŹNIKI PRZEBUDOWY KOŚCI PODCZAS CIĄŻY

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Summary

The mechanisms involved in bone turnover during pregnancy remain partly unknown. Increase of osteoprotegerin with elevated bone turnover is supposed to be a homeostatic mechanism limiting bone loss. The aim of the study was to assess if OPG may be involved in the regulation of bone turnover during pregnancy. Osteocalcin (OC), beta-crosslaps (CTx), osteoprotegerin (OPG), vitamin 25 OH D_3 , parathormone (PTH), and calcium (Ca) were determined in 30 healthy women at 1st and at 3rd trimester of pregnancy and 27 healthy age-matched non pregnant women.

During pregnancy a significant increase in serum OPG and CTx concentrations with concomitant decrease of calcium level was observed. OPG correlated positively with OC and Ca only at 1st trimester. Serum OPG and to a lesser extent CTx already during 1st trimester seemed to indicate elevated bone turnover whereas osteocalcin level remained within the reference range during pregnancy.

In pregnancy bone turnover increases mainly due to enhanced bone resorption. The determination of osteocalcin at the beginning of pregnancy seems to be of limited clinical use. Instead, measuring CTx and/or osteoprotegerin may have a good predictive value for later pregnancy-associated bone loss.

Streszczenie

Mechanizmy zaangażowane w proces przebudowy kości u kobiet w ciąży nie są dokładnie poznane. Wzrost stężenia osteoprotegeryny towarzyszący nasileniu przebudowy tkanki kostnej jest prawdopodobnie elementem mechanizmu homeostazy ograniczającym utratę masy kostnej. Celem pracy była ocena związku między osteoprotegeryną a procesem przebudowy tkanki kostnej w przebiegu prawidłowej ciąży. Oznaczono stężenie osteokalcyny (OC), beta-crosslaps (CTx), osteoprotegeryny (OPG), witaminy 25 OH D_3 , parathormonu (PTH) i wapnia (Ca) u 30 zdrowych kobiet w pierwszym i trzecim trymestrze ciąży oraz u 27 zdrowych kobiet niebędących aktualnie w ciąży, dobranych pod względem wieku.

W przebiegu ciąży zaobserwowano istotny wzrost stężenia w surowicy OPG i CTx oraz obniżenie stężenia wapnia. OPG korelowała dodatnio z OC i Ca tylko w pierwszym trymestrze ciąży. Stężenie OPG i w mniejszym stopniu CTx już w trakcie pierwszego trymestru mogło wskazywać na nasilenie przebudowy kości, podczas gdy stężenie osteokalcyny pozostawało w zakresie wartości referencyjnych przez cały czas trwania ciąży.

W ciąży obserwuje się nasilenie procesu przebudowy kości, przede wszystkim jednak zwiększenie resorpcji. Wydaje się, że oznaczanie na początku ciąży stężenia osteokalcyny-wskaźnika tworzenia kości ma ograniczone zastosowanie, natomiast wczesne oznaczanie CTx i/lub osteoprotegeryny, odzwierciedlających resorpcję, może mieć znaczną wartość predykcyjną dla oceny utraty masy kostnej w późniejszym okresie ciąży.

Key words: osteoprotegerin, bone turnover, pregnancy

Słowa kluczowe: osteoprotegeryna, wskaźniki przebudowy kości, ciąża

INTRODUCTION

The attainment of peak bone mass in women typically takes place in the early 30s but pregnancy and lactation occur mostly during or before this period of life. It is considered that pregnancy could affect peak bone mass and increase the risk of developing osteoporosis later in life [1]. Bone loss during pregnancy may result in pregnancy-associated osteoporosis and vertebral fractures [2, 3].

During pregnancy, about 30 g of calcium is transferred to a full term neonate [4]. Approximately 80% of calcium accumulates during the third trimester, when the fetal skeleton is rapidly mineralizing. Although maternal adaptations designed to meet the calcium needs of the fetus might begin early in pregnancy, they are most needed in the third trimester [5-6]. Calcium homeostasis appears to be attained by increased dietary intake with or without increased efficiency of absorption, decreased urinary excretion as a result of increased tubular calcium resorption and by elevated bone turnover with bone loss [7].

Several studies showed a decrease in BMD during pregnancy even up to 5%. Thus, there seems to be a good evidence that during pregnancy calcium is mobilized from the maternal skeleton to that of developing fetus. Development of biochemical markers enabled to asses bone turnover during normal pregnancy, when radiography or densitometry cannot be used [8-10].

The mechanisms regulating bone turnover during pregnancy are not well known [11]. RANK - a cellular receptor activator of NF-kappaB, RANK-ligand and osteoprotegerin (OPG) constitute a novel cytokine system that regulate activity of bone cells. Osteoprotegerin, is a soluble decoy receptor that inhibits bone resorption by binding to receptor activator of nuclear factor NF-kappaB ligand (RANKL) and in consequence inhibits osteoclast's maturation and activation [12]. RANKL produced by osteoblastic lineage cells and activated T lymphocytes is the essential factor for osteoclastogenesis, fusion, activation and survival of osteoclasts, thus effecting on bone resorption and bone loss. RANKL activates its specific receptor-RANK, located on osteoclasts and its signalling cascade involves stimulation of osteoclasts action. The effects of RANKL are counteracted by OPG which acts as a soluble neutralizing receptor.

RANKL and OPG are regulated by various hormones (glucocorticoids, vitamin D, estrogens), cytokines (tumour necrosis factor alpha, interleukins 1,4,6,11 and 17) and various mesenchymal transcription factors. RANKL and OPG are also important regulators of vascular biology and calcification and of the development of a lactating mammary gland during pregnancy. OPG was also found in placenta [13]. All this indicates a crucial role for this system in extraskeletal calcium handling [14]. The discovery and characterization of RANKL, RANK, OPG and subsequent studies have changed the concept of bone and calcium metabolism.

The objective of the study was to assess bone turnover in pregnancy by measuring biochemical bone markers in the serum in relation to osteoprotegerin level.

PARTICIPANTS AND SAMPLE COLLECTION

Thirty healthy, pregnant women during their first visit for prenatal care participated in our study. Exclusion criteria included assisted conception or any diseases or use of medication known to affect bone metabolism. All pregnant women were primiparas of mean age 24.5±3.8 yrs (20-36 yrs) and body mass index (BMI) before pregnancy 20.3±2.8 kg/m2 (16.7-30.9). Most of women fulfilled 50-75% of recommended daily calcium requirement.

27 healthy, non pregnant women, before first pregnancy, (mean age, 25±3.4 yrs; range 21-33 yrs, mean BMI 20.9±2.9 ; range 17.6-29.8) served as controls. The average calcium intake in most of non-pregnant women was on the level of 50-75% of daily requirement.

The study protocol was approved by the local Bioethical Committee of Collegium Medicum, N.C. University in Bydgoszcz. All participants gave their informed written consent.

MATERIALS AND METHODS

Fasting blood samples from pregnant women were collected, between 8-9 am, at 1st trimester (6-14 wks) and at 3rd trimester (31-37 wks) of pregnancy. In control group fasting blood samples were taken once in autumn/winter season. Serum was immediately sepa-

rated after blood clotting and kept deep frozen until assayed. Osteoprotegerin and vitamin 25 OH D_3 were assayed by ELISA (Biomedica, Austria). Reference value for OPG at age 20-36 yrs was 44.5 ± 21.2 pg/ml, reference range vitamin 25 OH D_3 in winter and summer were 14-42 ng/ml and 15-80 ng/ml, respectively. N-mid osteocalcin (OC), a bone formation marker and beta-Crosslaps (βCTx), a bone resorption marker were determined by ECLIA (Roche Diagnostics). Reference values for OC in premenopausal women were 4-35 ng/ml and for βCTx 0.299 ± 0.137 ng/ml. Intact PTH was assayed by ECLIA (Roche Diagnostics), expected values were 15-65 pg/ml. Serum calcium was measured by colorimetric method (Roche Diagnostics) and accepted reference values were 2.15-2.55 mmol/L.

STATISTICAL ANALYSIS

Data were expressed as means (SD). Pearson correlation tests were performed. The data collected at 1st trimester and during 3rd trimester were compared by Wilcoxon test. P values equal to or less than 0.05 were considered statistically significant.

RESULTS

Mean concentrations of CTx, OPG and calcium were elevated in pregnant women comparing to expected reference values (Table I). At 3rd trimester serum CTx and calcium levels were significantly higher than in age-adjusted non pregnant women (p<0,004; p<0,001 respectively).

Table I. *Mean (± SD) of biochemical parameters measured in pregnant and non-pregnant women*

	OC (ng/ml)	CTx (ng/ml)	OPG (pg/ml)	PTH (pg/ml)	Vit 25OH D_3 (ng/ml)	Ca (mmol/L)
1st trimester (6-14 wks) n= 30	20.6± 9.1	0.391 ± 0.185	86 ± 50	15.7± 8	71.5 ± 28	2.52± 0.22
3rd trimester (31-37 wks) n= 30	23.2±9.6	0.543 * ±0.216	113 *** ± 58	18.5±8.8	87.5 ± 38	2.46± 0.15
Non-pregnant women n= 27	17.9±6.5	0.348 **±0.144	69 **** ±19	24.4 ± 11.6	59.4 ± 46	2.24***** ± 0.25

*1st vs 3rd trimester p<0.002; ** 3rd vs non-pregnant p<0,004; *** 1st vs 3rd p<0,004; **** 3rd vs non-pregnant p<0,03; *****1st, 3rd vs non-pregnant p<0,001

The average OC concentrations were only slightly increased during pregnancy and comparable with these in non-pregnant women. Serum vitamin D3 in pregnant women was found to be in the upper reference range whereas PTH was in the lower. A strong relationship between both markers of bone turnover OC and CTx (r=0,76; p<0,00001) and positive but weak correlations between OPG and OC (r=0,54; p<0,04), OPG and Ca (r=0,55; p<0,03) were found at 1st trimester.

Serum CTx and OPG significantly increased during pregnancy (p<0,002; p<0,004) whereas calcium slightly decreased. At 3rd trimester no correlation between OPG and OC or Ca was found, but there was still a strong positive relationship between OC and CTx (r=0,69; p<0,00002).

Serum OPG and to a lesser extent CTx already during 1st trimester seemed to indicate elevated bone turnover whereas osteocalcin level remained within the reference range during pregnancy.

DISCUSSION

During pregnancy dynamic changes occur in maternal bone and calcium metabolism, but the effect of pregnancy upon the bone mass is not fully understood [15]. Two mechanisms: intestinal calcium absorption and urinary calcium excretion help to satisfy the increased demand for calcium during pregnancy. But they are not sufficient enough, because there is evidence that pregnancy affects also bone mass. Many authors infer that pregnancy is followed by loss in bone mass up to 5% [8, 9, 11, 16]. Some pregnant women become prone to excessive bone loss and even fractures [17].

It is not known whether osteoprotegerin is involved in the regulation of bone turnover during pregnancy. In earlier study Uemura et al have found that circulating OPG levels increased with gestational age and especially before the delivery, after 36weeks [18]. The tissue source of OPG in pregnancy is unknown, but the placental source was suggested [19]. The breast is also a potential source of maternal serum OPG and the RANK-RANKL signalling pathway appears to be involved in the development of lactating mammary tissue [20,21]. The presence of OPG in human breast milk was previously described [22]. However, the rapid postpartum decline in maternal OPG toward preconception values in both breast- feeding and non-breast-feeding women suggests that the breast is not the primary contributor to maternal serum OPG during pregnancy [23].

In our study serum OPG concentration in non pregnant women and those at 1st trimester of pregnancy was

similar, what suggests that OPG levels gradually increased as gestational age progressed [18,23]. This may be related with the increasing level of estradiol found during pregnancy. In postmenopausal women a significant, positive relationship between OPG and estradiol was found [24,25], but such a correlation was not confirmed in pregnant women [19]. Contrary to the others [18,19] we found a weak positive correlation between OPG and OC but only at the 1st trimester.

We noticed a significant increase in OPG during pregnancy. It is consistent with previous observations in women [18,19]. Similarly to earlier findings [18], we observed much higher rise in OPG at the end of 3rd trimester with concomitant decline of serum calcium.

Data on bone turnover markers during pregnancy are inconsistent. Among bone formation markers bone alkaline phosphatase was shown to rise with gestational age [10,18,19] whereas osteocalcin did not change or similarly to N-terminal propeptide of collagen type I showed a biphasic pattern with decrease from 1st to second trimester, followed by increase in the 3rd [10,16,18]. We have measured biochemical markers in fasting morning samples only twice, at 1st and 3rd trimester and observed the elevation in OC during pregnancy, especially at 36-37 wks.

Bone resorption, reflected by serum CTx, increased significantly during pregnancy with peak levels at the end of 3rd trimester that confirms data by other authors [5, 10, 11, 19]. This was accompanied by a decrease in serum calcium, especially before the delivery (36-37wks).

Serum CTx and OPG seemed to be the only parameters to indicate elevated bone turnover. The nomogram proposed for the Polish premenopausal women indicated that serum CTx value over 0,490 ng/ml and OC > 34 ng/ml (>95th percentile) reflect the elevated bone turnover [26]. From our data it may be concluded that, at least, increrased CTx during 1st trimester may be a good predictor for faster bone loss during pregnancy.

Our results confirm that serum OPG and bone turnover markers levels increase during pregnancy and clearly show that bone resorption precedes bone formation. In pregnancy many factors known to influence on the bone mass undergo changes: increased calcium demand, change in nutritional habits, changes in body weight and fat content, changed levels of physical activity and hormones with potential to affect bone metabolism [27]. This may be the main reason for difficulties in finding the exact role of OPG in relation to bone turnover during pregnancy. While the determination of osteocalcin at the beginning of pregnancy, seems to be of limited clinical use, measuring OPG as a factor related to bone turnover or a bone resorption marker, such as CTx, may have a good predictive value for later pregnancy-associated bone loss or osteoporosis.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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QUANTITATIVE ANALYSIS OF CHANGES IN EXPRESSION OF LEUKEMIC MARKERS DURING SHORT-TERM PREDNISOLONE THERAPY *IN VITRO*

ILOŚCIOWA ANALIZA ZMIAN EKSPRESJI ANTYGENÓW BIAŁACZKOWYCH PODCZAS KRÓTKOTRWAŁEJ TERAPII PREDNIZOLONEM *IN VITRO*

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Summary

Introduction. Response to treatment in childhood acute lymphoblastic leukemia (ALL) depends on numerous variables, including the clinicobiologic features of the disease, chemotherapy regimens and interactions, and the ability of individual patients to metabolize antileukemic drugs.

The objective of the study. The analysis of *in vitro* changes in expression of leukemic markers in leukemic/lymphoid cell lines and in children with ALL after shot-term incubation with prednisolone.

Patients and methods. Sixty children with ALL and four leukemic/lymphoid cell lines were tested for immunophenotype changes occurring after three-day incubation with prednisolone. Immunophenotype analysis was performed with the use of flow cytometry.

Results. After 72 hours of incubation with prednisolone, the expression of CD19 decreased in Raji and Daudi cell lines, while CD10 expression increased in both cell lines. The expression of dominating immunophenotype in T-lineage CCRF-CEM and Jurkat cell lines decreased during the culture. In patient samples, the expression of specific leukemic markers decreased after 3-day prednisolone therapy in B-lineage samples. In common-ALL, CD10 and CD19 expression decreased 2-fold ($p<0,001$) and 1,7-fold ($p<0,001$), respectively. In T-ALL, expression of CD2, CD4 and CD8 decreased 1,5-fold, 1,8-fold and 1,6-fold, respectively (ns). In individual cases, upregulation of leukemic antigens was observed.

Conclusions. Short-term *in vitro* therapy of leukemic cells with prednisolone is sufficient to induce downregulation of leukemic immunophenotype in patient samples. This strategy was not effective in leukemic and lymphoid cell lines. Flow cytometry might be an important method in analysis of minimal residual disease in pediatric acute lymphoblastic leukemia.

Streszczenie

Wstęp. Odpowiedź na terapię u dzieci z ostrą białaczką limfoblastyczną (ALL) zależy od wielu czynników, takich jak cechy kliniczne i biologiczne choroby, rodzaje chemioterapii oraz indywidualna zdolność pacjentów do metabolizowania leków przeciwbiałaczkowych.

Cel pracy. Analiza zmian immunofenotypu białaczkowego w liniach komórkowych i u dzieci z ALL po krótkotrwałej terapii *in vitro* z prednizolonem.

Pacjenci i metodyka. Określono zmiany ekspresji antygenów białaczkowych u 60 dzieci i w 4 liniach komórkowych. Analiza immunofenotypu przed i po inkubacji z prednizolonem została wykonana metodą cytometrii przepływowej.

Wyniki. Po 72 godzinach inkubacji z prednizolonem, ekspresja CD19 obniżyła się w liniach Raji i Daudi, podczas gdy ekspresja CD10 uległa podwyższeniu w obydwu liniach. Ekspresja dominującego immunofenotypu w liniach T-komórkowych CCRF-CEM i Jurkat obniżyła się w czasie inkubacji. W próbkach pacjentów z B-liniowym fenotypem, ekspresja markerów białaczkowych obniżyła się podczas 3-dniowej terapii. W common-ALL, ekspresja CD10 i CD19 obniżyła się odpowiednio 2-krotnie ($p<0,001$) i 1,7-krotnie

(p<0,001). W T-ALL, ekspresja CD2, CD4 i CD8 obniżyła się odpowiednio 1,5, 1,8 i 1,6-krotnie (ns). W pojedynczych przypadkach obserwowano zwiększenie ekspresji antygenów białaczkowych.

W n i o s k i. Krótkotrwała terapia komórek białaczkowych *in vitro* z prednizolonem jest wystarczająca do wyindukowania zmian immunofenotypu białaczkowego u pacjentów. Ta strategia nie była jednak skuteczna w liniach komórkowych. Cytometria przepływowa może być ważną metodą w analizie minimalnej choroby resztkowej u dzieci z ostrą białaczką limfoblastyczną.

Key words: acute lymphoblastic leukemia, children, immunophenotype, minimal residual disease

Słowa kluczowe: ostra białaczka limfoblastyczna, dzieci, immunofenotyp, minimalna choroba resztkowa

INTRODUCTION

Response to treatment in childhood acute lymphoblastic leukemia (ALL) depends on numerous variables, including the clinicobiologic features of the disease, chemotherapy dosages and interactions, and the ability of individual patients to metabolize antileukemic drugs [1, 2]. Among consistently useful prognostic indicators that reflect the collective impact of such variables is leukemia cytoreduction, defined as the rate of clearance of leukemic cells during remission induction chemotherapy, leading to disappearance of cells with typical leukemic markers [3]. Persistence of circulating lymphoblasts after 1 week of chemotherapy, or the detection of blast cells in the bone marrow by morphologic criteria after completion of remission induction therapy, are associated with an increased risk of relapse [4, 5].

Bone marrow normal lymphoid progenitors (CD19+, CD10+, and/or CD34+) are exquisitely sensitive to corticosteroids and other antileukemic drugs. Since most leukemic children with ALL are of precursor-B-lineage phenotype, we hypothesized that the rate of leukemic clearance is high and it might occur within 3 days. The objective of the study is the analysis of *in vitro* changes in expression of leukemic markers in leukemic/lymphoid cell lines and in children with ALL after shot-term incubation with prednisolone.

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). According 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) [6]. Concentration of cells was $0,4\text{-}0,5\times10^6$/ml.

Isolation of mononuclear cells. Bone marrow was collected with the use of heparin (15-20 U/ml bone marrow) from patients at the day of diagnosis or at relapse. Lymphoblasts were isolated on Ficoll gradient (Gradisol L, Aqua Medica, Łódź), washed twice in RPMI-1640 (Sigma, St Louis, USA). Erythrocytes were lysed by ammonium chloride. Isolated lymphoblasts were resuspended in culture medium at the concentration $\geq2,0\times10^6$ cells/ml. Cell viability was assessed by 0,4% trypan blue assay (Sigma); all samples had viability ≥95%. The morphology of lymphoblasts was assessed on cytospins performed on centrifuge (type 3-15, Sigma) and stained by May-Grunwald-Giemsa method in light microscopy (Nikon Eclipse E600).

Media. RPMI-1640 was the medium used in all experiments. For each bottle of 500 ml of medium, a 10 ml of solution of penicillin, streptomycin and amphotericin (PSF), at final concentrations: 100 U/ml, 100 μg/ml and 0,125 μg/ml, respectively. Fetal calf serum (FCS, Fetal Calf Serum, Gibco BRL Life Technologies, UK) was added at concentration 1% to obtained washing medium. Culture medium contained RPMI-1640 with PSF, 20% inactivated FCS, 200 μg/ml gentamicin, 2 mM glutamine (Sigma), insulin 5 μg/ml, transferine 5 μg/ml and sodium selenite 5 ng/ml (ITS, Insulin-Transferin-Selenite, Sigma). Inactivation of FCS was done in order to inactivate complement by incubation in temperature 56°C for 30 minutes.

Conditions of cell line culture and incubation of leukemic cells. Cells were cultivated in culture medium in incubator Forma Scientific Inc. model 3110 (Marietta, Ohio, USA), in atmosphere of 5% CO_2 and temperature 37°C and humidity 90%. Cell culture was performed in culture flaks (Corning Incorporated, Corning, NY, USA, nr cat. 430639) with area 25 cm^2. All experiments were prepared in laminar chamber (AURA 2000 M.A.C., Bioair Instruments, Opera, Italy).

Flow cytometry. Analyses were done with flow cytometry EPICS XL (Coulter, Miami, FL, USA). This device is equipped with argon laser of power of 15 mW, which causes fluorochrome excitation with wavelength of 488 nm. These fluorochromes are detected on detectors at following wavelengths: 515-535 nm (fluorescence 1, FL1), 565-585 nm (FL2) and 610-630 nm (FL3). Cells were gated based on their FSC/SSC (forward scatter, side scatter) signal. For each sample, all assays were performed twice: before and after 72 hours of incubation with prednisolone.

Immunophenotype analysis. Immunophenotyping was performed according to guidelines of the National Institute of Health, USA (Proteins Review On Web. Bethesda USA, http://www.ncbi.nlm.nih.gov/prow/guide/guide/45277084.htm), with the use of mouse monoclonal antibodies. Immunophenotyping was done on isolated cell suspension. 25 µl of cell suspension was used, and 5 µl of monoclonal antibodies stained with fluorochrome was added. Cells were incubated in the dark for 20 minutes, washed in PBS without calcium and magnesium, and then analyzed by flow cytometry. For each type of assay isotype control was done. The value of mean fluorescence intensity (MFI) was calculated as the difference between MFI of tested sample and MFI of isotype control. All patient samples were tested for the expression of following cell surface antigens: CD2, CD3, CD4, CD8, CD10, CD19 (Table I).

Table I. *Reagents, monoclonal antibodies and isotype controls*

Tabela I. *Zastosowane odczynniki, przeciwciała i kontrole izotypowe*

Reagent Odczynnik	Producer Producent	Code Kod	Isotype control Kontrola izotypowa	Antibody clone Klon przeciwciał
CD2-FITC/CD19-RPE	DakoCytomation	FR894	IgG1, κ	MT910+HD37
CD10-FITC/CD19-RPE	DakoCytomation	FR883	IgG1, κ	SS2/36+HD37
CD3-FITC/CD4-RPE/CD8-Cy5	DakoCytomation	TC641	IgG1, κ	DK25+MT310+UCHT1
CD5-FITC/CD20-RPE	DakoCytomation	FR729	IgG1, κ	DK23+B-Ly1

Quantitative analysis of CD10 antigen (Qifikit). Quantitative analysis of expression of CD10 antigen was performed with the use of Qifikit (nr cat. K0078, DakoCytomation, Glostrup, Dania), based on non-stained mouse monoclonal antibodies Anti-Human-CD10-CALLA (DakoCytomation, nr M0727), goat secondary stained antibodies Goat-anti-Mouse Ig-FITC (DakoCytomation, nr F0479) and isotype control Mouse IgG1 (DakoCytomation, nr X0931) according to producer's protocol (Figure 1).

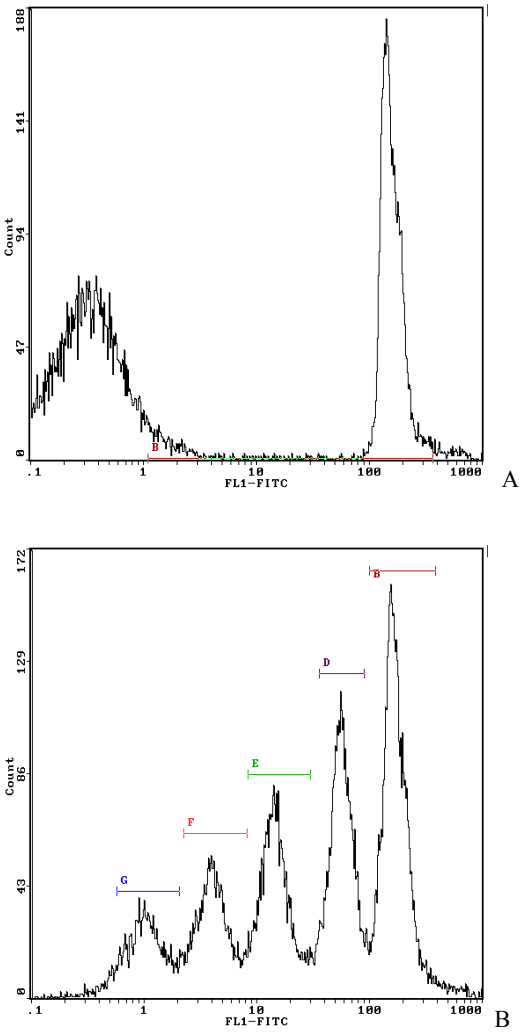

Fig. 1. *Control histograms for quantitative analysis by Qifikit. (A) Negative and positive control. (B) Fluorescence of standard beads enabling calibration line formation*

Ryc. 1. *Histogramy kontrolne zestawu Qifikit. (A) Kontrola ujemna + kontrola dodatnia, (B) Fluorescencja wzorcowych kulek służących do określenia linii kalibracyjnej*

RESULTS AND DISCUSSION

Cell lines. After 72 hours of incubation with prednisolone in B-lineage cell lines, the expression of CD19 decreased, in Raji cell line from MFI=12,99 to 10,89 ie. by 16%, and in Daudi cell line from MFI=7,82 to 5,88 ie. by 25%; while expression of CD10 increased in the same period, in Raji from 1,80 to 2,47, and in Daudi from 2,66 to 3,86. Since the number of cells with CD10 expression has decreased (by 5% and 3%, respectively), the upregulation of the expression of CD10 antigen was dependent on the increase of mean number of CD10 molecules on cell surface (from 6151 to 8441 in Raji, and from 9090 to 13191 in Daudi, respectively). On the other hand, expression of dominating immunophenotype in T-lineage CCRF-CEM and Jurkat cell lines decreased during *in vitro* therapy with prednisolone (Fig. 2).

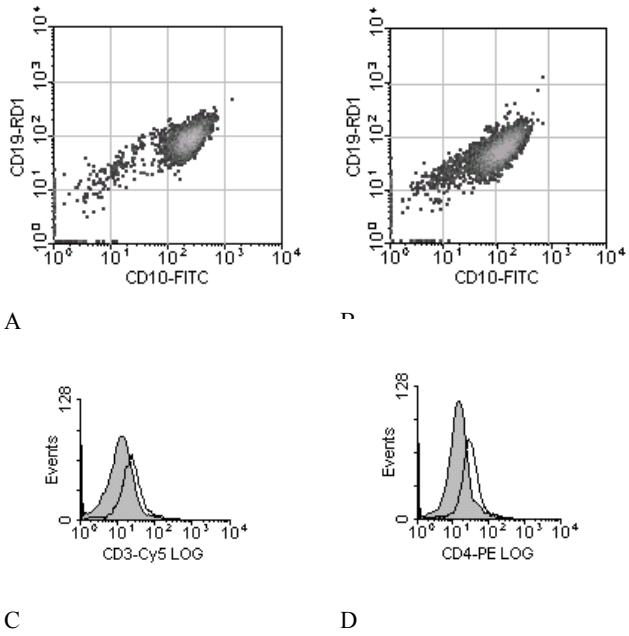

Fig. 2. *Immunophenotype changes in cell lines after 72 hours of incubation with prednisolone (final concentration: 250 μg/ml). Expression of CD10 and CD19 in Raji before (A) and after (B) prednisolone therapy. (C) CD3 downregulation in Jurkat cell line. (D) CD4 downregulation in CCRF-CEM cell line. Contour area – expression on day „0", grey area – expression on day „3"*

Ryc. 2. *Zmiany w immunofenotypie w liniach komórkowych po 72 godzinach inkubacji z prednizolonem w stężeniu 250 μg/ml. Ekspresja CD10 i CD19 w linii Raji (A) przed i (B) po terapii z prednizolonem. (C) Spadek ekspresji CD3 w linii Jurkat. (D) Spadek ekspresji CD4 w linii CCRF-CEM. Obszar zakonturowany odpowiada ekspresji w dniu „0", a obszar zacieniowany – ekspresji w dniu „3"*

Changes in immunophenotype in patient samples. After 3-day prednisolone therapy, expression of specific leukemic markers was decreased. The only exception was an increase of CD3 expression in T-ALL, however a large variability of expression of this antigen was observed among patients with leukemia. CD10 expression measured by MFI in common-ALL was decreased 2-fold ($p<0,001$), and CD19 expression in pre-pre-B- and common-ALL decreased 1,7-fold ($p<0,001$) after 72 hours (Fig. 3A,B). In T-ALL, expression of CD2, CD4 and CD8 decreased 1,5-fold, 1,8-fold and 1,6-fold, respectively (ns) (Fig. 3. C,D). In selected cases, an increase of expression of leukemic antigens was observed: in 1/8 patients with pre-pre-B-ALL upregulation of CD19; in 4/43 common-ALL an increase of CD10, and in T-ALL an increase of CD2 and CD8 in 3/9 and 4/9 patients, respectively.

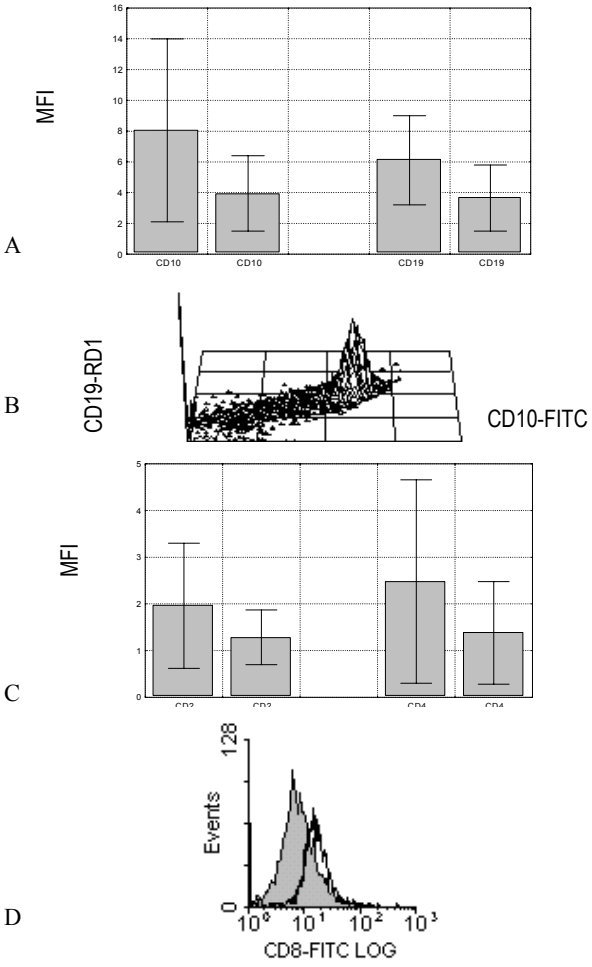

Fig. 3. *Downregulation of lineage-specific CD antigens in ALL after ex vivo prednisolone therapy. (A) CD10 and CD19 in common-ALL, (B) 3-dimensional chart of CD10 and CD19 expression in common-ALL. (C) CD2 and CD4 and (D) CD8 in T-ALL*

Ryc. 3. *Zmniejszenie ekspresji antygenów CD liniowo-specyficznych w ALL po terapii ex vivo z prednizolonem. (A) CD10 i CD19 w common-ALL, (B) wykres przestrzenny ekspresji CD10 i CD19 w common-ALL. (C) CD2 i CD4 oraz (D) CD8 w T-ALL*

The initial response of leukemia cells to treatment has consistently been shown to be a reliable prognostic indicator, and its evaluation has been significantly enhanced by methods that allow detection of submicroscopic levels of leukemia, defined as minimal residual disease (MRD) [7-9]. Universal application of MRD assays would be highly desirable, but so far progress towards it has been slow because of the complexity and high costs of MRD evaluation [10]. So far, it has been shown that the morphologic detection of leukemic cells in day-15 or day-33 in bone marrow is prognostically important [3]. The rationale for the use of this strategy based on flow cytometry with normal immature CD19+ cells (ie, those expressing CD10 and/or CD34) is the interesting option for bone marrow samples collected from children with ALL after beginning of remission induction chemotherapy, because of their exquisite sensitivity to glucocorticoids and other antileukemic drugs. We therefore tested the possibility of disappearance of leukemic markers within 3 days of prednisolone *in vitro* therapy. We have found that in most cases, this short-term prednisolone therapy significantly reduces the leukemic load both in precursor-B-lineage and T-lineage pediatric ALL. This was also observed in tested leukemic lymphoid cell lines. This approach might be useful to identify patients who are highly sensitive to remission induction therapy [2] and also those who are susceptible to the presence of MRD. We suggest that the simplified flow cytometric assay described here would provide the valuable information.

CONCLUSIONS

1. Short-term *in vitro* therapy of leukemic cells with prednisolone is sufficient to induce downregulation of leukemic immunophenotype in patient samples. This strategy was not effective in leukemic and lymphoid cell lines.
2. Flow cytometry might be an important method in analysis of minimal residual disease in pediatric acute lymphoblastic leukemia.

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ANALYSIS OF IMMUNOPHENOTYPE AT SECOND RELAPSE OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

ANALIZA IMMUNOFENOTYPU W DRUGIEJ WZNOWIE OSTREJ BIAŁACZKI LIMFOBLASTYCZNEJ U DZIECI

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Summary

Introduction. Childhood acute lymphoblastic leukemia (ALL) is the single most common childhood malignancy. Despite substantial improvements in therapy, cases in which relapse occurs are still more common than newly diagnosed cases of many other childhood cancers.

The objective of the study. Analysis of immunophenotype of leukemic blasts at second relapse, in comparison to previous stages of acute lymphoblastic leukemia.

Patients and methods. Six children with ALL, who suffered for second bone marrow relapse of the disease, were analyzed for specific changes in lymphoblast immunophenotype. Diagnosis of leukemia was made by morphological, cytochemical and immunological methods. Immunophenotype analysis was performed with the use of flow cytometry.

Results. The basic immunophenotype was similar at second relapse in comparison to first diagnosis or first relapse. No immunological shift between T-lineage and B-lineage or between lymphoid and myeloid lineage was observed. However, changes in the expression of CD38 antigen was observed. Its expression was relatively high at second relapse in 5/6 patients. Expression of CD10 antigen dissapper in one patient, while it appeared in another one at second relapse.

Conclusions. Second relapse of acute lymphoblastic leukemia reveals tendency to occurrence of more immature phenotype of blasts. Phenotypic changes in ALL from diagnosis to relapse and subsequent second relapse, might indicate a certain limitation for the immunological detection of minimal residual disease.

Streszczenie

Wstęp. Ostra białaczka limfoblastyczna (ALL) jest najczęstszym nowotworem wieku dziecięcego. Pomimo znacznych postępów w terapii, ciągle wznowy tej choroby są częstszym zjawiskiem niż nowe rozpoznania wielu innych nowotworów u dzieci.

Cel pracy. Analiza immunofenotypu blastów białaczkowych podczas drugiej wznowy oraz porównanie z rozpoznaniem i pierwszą wznową ostrej białaczki limfoblastycznej.

Pacjenci i metodyka. U sześciorga dzieci z ALL, u których doszło do drugiej wznowy szpikowej choroby, przeprowadzono analizę immunofenotypu blastów w trzech fazach choroby. Rozpoznanie ostrej białaczki limfoblastycznej oparto na metodach badania morfologicznego, cytochemicznego i immunologicznego. Immunofenotyp oznaczano metodą cytometrii przepływowej.

Wyniki. Podstawowy immunofenotyp blastów podczas drugiej wznowy był porównywalny z immunofenotypem przy pierwszym rozpoznaniu i podczas pierwszej wznowy. Nie obserwowano zmian fenotypu pomiędzy liniami B- i T-komórkowymi, ani pomiędzy liną limfoidalną i mieloidalną. Stwierdzono zmiany ekspresji CD38, wyrażające się wzrostem ekspresji tego antygenu u 5/6 pacjentów. Ekspresja CD10 zanikła u jednego pacjenta, a pojawiła się też u jednego.

W n i o s k i . Druga wznowa szpikowa u dzieci z ostrą białaczką limfoblastyczną wykazuje tendencję do bardziej niedojrzałego immunofenotypu blastów. Zmiany immunofenotypu w ALL pomiędzy rozpoznaniem, pierwszą i drugą wznową wskazują, że u niektórych pacjentów metoda wykrywania minimalnej choroby resztkowej w tej chorobie ma pewne ograniczenia przy wykorzystaniu metody cytometrii przepływowej.

Key words: acute lymphoblastic leukemia, children, immunophenotype, relapse
Słowa kluczowe: ostra białaczka limfoblastyczna, dzieci, immunofenotyp, wznowa

INTRODUCTION

Childhood acute lymphoblastic leukemia (ALL) is the single most common childhood malignancy. Despite substantial improvements in therapy, cases in which relapse occurs are still more common than newly diagnosed cases of many other childhood cancers. The survival of patients who relapse despite improved therapy is still unsatisfactory. Relapsed ALL is a disease as frequent as neuroblastoma and more frequent than Wilms tumor, Hodgkin lymphoma or non-Hodgkin lymphoma [1]. Survival after relapse is dependent to the period of relapse (very early, early or late), immunophenotype (T-ALL vs non-T-ALL), localization of relapse (bone marrow, extramedullary) and varies from 5% up to 57% (reviewed by Gaynon [2]). For most relapsed patients allogeneic hematopoietic stem cell transplantation is the only curative option. Among those who achieve second remission, some will eventually experience second relapse before transplantation is available. According to protocol ALL-REZ BFM 2002, relapsed patients are stratified to groups S1-S4 (Table I), with therapeutic implications, indicating necessity of prompt hematopoietic stem cell transplantation (HSCT) in S3-S4 groups, chemotherapy or HSCT in group S2 and no HSCT in S1 group.

Table I. *Definition of therapeutic groups S1 to S4*
Tabela I. *Określenie grup leczniczych S1 do S4*

	Immunophenotype non-T			Immunophenotype pre-T/T		
Localisation (Lokalizacja) Time (Czas)	Extramedullar Pozaszpikowa Isolated Izolowana	Bone marrow Szpikowa Combined Kombinowana	Bone marrow Szpikowa Isolated Izolowana	Extramedullar Pozaszpikowa Isolated Izolowana	Bone marrow Szpikowa Combined Kombinowana	Bone marrow Szpikowa Isolated Izolowana
Very early Bardzo wczesna	S2	S4	S4	S2	S4	S4
Early Wczesna	S2	S2	S3	S2	S4	S4
Late Późna	S1	S2	S2	S1	S4	S4

Several authors indicated that lymphoblast immunophenotype at first relapse tends to show more immature blast biology, expressing as disappearance of more mature markers [3-6]. Thus, biology of lymphoblasts at second relapse continues to be of great interest.

The objective of the study was analysis of immunophenotype of leukemic blasts at second relapse, in comparison to previous stages of acute lymphoblastic leukemia.

PATIENTS AND METHODS

Out of total number of 151 children aged 0.09-19.1 years (median 5.5 years), newly-diagnosed for acute lymphoblastic leukemia (ALL) and treated in our Departament over a period of 10 years, 24 had bone marrow relapse. Six of 24 relapsed patient suffered from the second marrow relapse. Children with first relapse of ALL were treated according to ALL-BFM-REZ-96 protocol up to 31.12.2003 and then with ALL-BFM-REZ-02 protocol [7]. Detailed analysis of immunophenotype changes at first relapse was presented in our previous report [8].

Acute lymphoblastic leukemia was diagnosed when bone marrow contained at least 25% of lymphoblasts. Except bone marrow, usually peripheral blood and cerebro-spinal fluid was tested. The analysis was performed within 3 hours after collection from patient. Diagnostic profile of ALL was performed for each patient (blast morphology, cytochemistry, immunophenotype, DNA ploidy and cytogenetic and molecular studies). Immunophenotype analysis was performed by flow cytometry in each case of new diagnosis and bone marrow relapse.

Flow cytometry studies were performed with EPICS XL (Coulter, Miami FL, USA) system. This cytometer is equipped with argon laser of power 15 mW, which excitates fluorochromes with wavelength of 488 nm. Triggered fluorescence (FL) was detected by sensors for following wavelengths: 515-535 nm (FL1), 565-585 nm (FL2) and 610-630 nm (FL3). Cells were gated based on FSC/SSC (forward scatter/side scatter) signal. From the year 2005, the analysis was performed with Cytomics FC 500 (Beckman Coulter, Miami FL, USA) flow cytometer.

Immunophenotyping was performed using cell suspension 0.5-2.5 × 10^6/ml. Cells were incubated with 1-3 monoclonal antibodies bound up with fluorochromes for 15 minutes in the darkness. Afterwards, erythrocytes were lysed with UtiLyse (DakoCytomation, Glostrup, Dannemark) or FACS Lysing Solution (Becton Dickinson, Heidelberg, Germany), washed in PBS, and analyzed by flow cytometry.

Following monoclonal antibodies and fluorochromes were used: CD2-FITC/CD19-RPE, CD10-FITC/CD19-RPE, CD3-FITC/CD4-RPE/CD8-Cy5, CD5-FITC/CD20-RPE, TdT-FITC, CD7-FITC/CD13-RPE/CD33-RPE, CD34-PerCP-Cy5, CD38-FITC, HLA-DR (DakoCytomation or Becton Dickinson). Respective isotype controls required for the analysis were used.

In each case 5-10 thousands of cells were analyzed with System II (Coulter) or CXP (Beckman Coulter) software. Subtypes of ALL were classifies as: pre-pre-B-ALL, common-pre-B-ALL, B-ALL and T-ALL.

RESULTS

Bone marrow relapse has occurred in 24/151 patients, including 13 girls (54.1%) and 11 boys (45.9%) with median age 10.5 years (range: 0.4-19.2 years). These patients relapsed after 0.3-5.5 years (median 1.7 years) from the initial diagnosis. Very early relapse ocurred in 11 patients, early relapse in 5 patients and late relapse in 8 patients. With respect to immunophenotype, 20 patients had precursor-B-lineage ALL, 3 had T-ALL and 1 was undifferentiated, both on first diagnosis and at relapse.

In 6/24 patients (5 girls, 1 boy) subsequent, second bone marrow relapse of ALL has occurred. Analysis of immunophenotype of lymphoblasts on first diagnosis, first and second relapse is presented in Table II.

The basic immunophenotype was similar at second relapse in comparison to first diagnosis or first relapse. No immunological shift between T-lineage and B-lineage or between lymphoid and myeloid lineage was observed. However, changes in the expression of CD38 antigen was observed. Although the analysis for presence of CD38 was not done in all cases, its expression was relatively high at second relapse in 5/6 patients, and this antigen was usually present on almost all leukemic cells, while it was not often observed on earlier phases of disease. Expression of CD10 antigen dissapper in one patient (UPN 140), while it appeared in another one (UPN 156) at second relapse. Expression of CD34 dissappeared in 2 patients.

Table 2. *Immunophenotype of lymphoblasts. Expression of each antigen is given as a percentage of cells expressing this marker*

Tabela 2. *Immunofenotyp blastów. Ekspresja każdego antygenu jest podana jako odsetek komórek wykazujących ekspresję danego antygenu*

UPN UPN	Immunophenotyp Immunofenotyp	Diagnosis Rozpoznanie	First relapse Pierwsza wznowa	Second relapse Druga wznowa
52	Common-ALL	CD19 82.6 CD10 83.0 CD34 86.0 CD38 ND	CD19 92.4 CD10 91.8 CD34 91.9 CD38 ND	CD19 71.2 CD10 71.2 CD34 71.6 CD38 ND
101	Common-ALL	CD19 98.2 CD10 99.5 CD34 99.4 CD38 ND	CD19 96.9 CD10 73.1 CD34 10.8 CD38 97.0	CD19 95.0 CD10 83.8 CD34 4.33 CD38 93.8
115	Pro-B-ALL with CD2 coexpression	CD19 98.8 CD10 4.28 CD34 75.5 CD38 ND CD2 35.5	CD19 82.7 CD10 0.4 CD34 16.9 CD38 ND CD2 66.1	CD19 94.0 CD10 9.6 CD34 0.0 CD38 87.0 CD2 79.4
140	Common-ALL	CD19 85.8 CD10 82.1 CD34 4.98 CD38 ND	CD19 90.7 CD10 85.6 CD34 0.08 CD38 6.60	CD19 80.4 CD10 3.1 CD34 1.79 CD38 51.5
156	Pro-B-ALL	CD19 92.2 CD10 0.3 CD34 0.1 CD38 ND	CD19 72.0 CD10 0.4 CD34 0.03 CD38 81.0	CD19 94.7 CD10 94.0 CD34 1.11 CD38 92.8
163	Pro-B-ALL	CD19 61.9 CD10 0.05 CD34 0.12 CD38 58.3	CD19 81.4 CD10 0.10 CD34 2.26 CD38 87.0	CD19 69.9 CD10 0.30 CD34 ND CD38 86.8

UPN – unique patent number (specyficzny numer pacjenta)
ND – not done (nie oznaczono)

DISCUSSION

In 24/151 (15.9%) of patients, bone marrow relapse has occurred. In six of them subsequent relapse took place. In immunophenotype analysis, a tendency to appearance of more immature antigens was observed. Increase of CD38 antigen and decrease of CD10 was observed in most of patients. Various changes in immunophenotype at relapse of ALL are relatively frequent phenomenon. Relapse of acute lymphoblastic leukemia frequently reveals more immature phenotype of blasts and occurrence of unfavorable and complex cytogenetic abnormalities [3, 9, 10]. According to the overall phenotype of the blast cells, phenotypic changes usually reflect a trend towards more immature antigenic profile in most cases [3, 6, 11, 12]. Both in our previous report [8] and current analysis, the changes from diagnosis to relapse and then to the second relapse, affected mostly only one antigen. The variations were not associated with any clear maturation pattern. Thus, marker-shifts were found in most of

analyzed patients. We did not observe an intra-lineage shift, however, myeloid markers were usually not tested. Our results show that the lineage of leukemia usually remained unchanged while other individual phenotypic features frequently changed. Phenotypic changes in ALL from diagnosis to relapse and subsequent second relapse, seemed to present a certain limitation for the immunological detection of minimal residual disease. Introduction of new generation, 6-8-color flow cytometers will probably overcome this problem.

There are three general mechanisms for immunophenotype changes in leukemia. First, chemotherapy appears to eradicate the dominant clone present at diagnosis, permitting expansion of a secondary clone with a different phenotype. The second mechanism is related to drug-induced changes in the original clone, which may either amplify or suppress differentiation programs so that phenotypic shift is possible [13]. The third mechanism is based the clonal evolution of subclones within a single cell population [6, 11, 12].

Our analysis shows that relapsed leukemia seems tends to present blasts with immunophenotype being close to the leukemia initiating cell in ALL. Leukemic stem cells (LCSs) present immunophenotype based on CD34+/CD38- expression, both in AML [12] and ALL [14]. The next stage of immunophenotype is CD34+/CD38+, followed by subsequent differentiation.

Although myeloid leukemias have been the proving ground for most of the theories concerning LSCs, recent studies suggest that lymphoid leukemias may also arise from a subset of cells with stem/progenitor cell characteristics. ALL, like AML, is a heterogeneous disease with close to 80% of ALL involving B lineage cells. Reports studying the involvement of the HSC compartment in these B-ALLs have failed to provide an unequivocal demonstration that LSCs are derived from normal hematopoietic stem cells, though studies in different types of ALL suggest that ALL originates from a primitive cell rather than from committed progenitors [15].

CONCLUSIONS

1. Second relapse of acute lymphoblastic leukemia reveals tendency to occurrence of more immature phenotype of blasts.
2. Phenotypic changes in ALL from diagnosis to relapse and subsequent second relapse, might indicate a certain limitation for the immunological detection of minimal residual disease.

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MEASURES OF DIAGNOSTIC ACCURACY: BASIC DEFINITIONS

MIARY PRECYZJI DIAGNOSTYCZNEJ: PODSTAWOWE DEFINICJE

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S u m m a r y

Diagnostic accuracy relates to the ability of a test to discriminate between the target condition and health. This discriminative potential can be quantified by the measures of diagnostic accuracy such as sensitivity and specificity, predictive values, likelihood ratios, the area under the ROC curve, Youden's index and diagnostic odds ratio. Different measures of diagnostic accuracy relate to different aspects of diagnostic procedure: while some measures are used to assess the discriminative property of the test, others are used to assess its predictive ability. Measures of diagnostic accuracy are not fixed indicators of a test performance, some are very sensitive to the disease prevalence, while others to the spectrum and definition of the disease. Furthermore, measures of diagnostic accuracy are extremely sensitive to the design of the study. Studies not meeting strict methodological standards usually over- or under-estimate the indicators of test performance as well as they limit the applicability of the results of the study. STARD initiative was a very important step toward the improvement of the quality of reporting of studies of diagnostic accuracy. STARD statement should be included into the Instructions to authors by scientific journals and authors should be encouraged to use the checklist whenever reporting their studies on diagnostic accuracy. Such efforts could make a substantial difference in the quality of reporting of studies of diagnostic accuracy and serve to provide the best possible evidence to the best for the patient care. This brief review outlines some basic definitions and characteristics of the measures of diagnostic accuracy.

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

Precyzja diagnostyczna dotyczy zdolności testu do różnicowania pomiędzy chorobą a zdrowiem. Te możliwości różnicowania można określić ilościowo za pomocą miar dokładności diagnostycznej, takich jak: czułość i specyficzność, wartości predykcyjne, wskaźniki wiarygodności, wartość pola pod krzywą ROC, wskaźnik Youdena i diagnostyczny iloraz szans. Różne miary dokładności diagnostycznej odnoszą się do różnych aspektów postępowania diagnostycznego: podczas gdy niektóre miary wykorzystywane są do oceny właściwości różnicującej testu, innych używa się do oceny zdolności predykcyjnej danego testu. Miary dokładności diagnostycznej nie są stałymi wskaźnikami wydajności testu, niektóre zależą od częstości występowania choroby, a inne od definicji choroby. Ponadto, miary dokładności diagnostycznej zależą od sposobu zaprojektowania badań. Badania niespełniające ściśle określonych standardów metodologicznych zazwyczaj przeceniają lub niedoceniają wskaźników wydajności testu, jak również ograniczają możliwości zastosowania wyników badań. Inicjatywa STARD była bardzo ważnym krokiem w kierunku polepszenia jakości raportów na temat badania dokładności diagnostycznej. W instrukcjach dla autorów, zawartych w czasopismach naukowych, powinno być umieszczone oświadczenie STARD oraz powinno się zachęcać do korzystania z listy kontrolnej podczas pisania raportów ze swoich badań nad dokładnością diagnostyczną. Starania te mogłyby wprowadzić znaczną zmianę w jakości raportowania badań nad dokładnością diagnostyczną i posłużyłyby do zapewnienia możliwie najlepszej dokumentacji z korzyścią dla pacjenta. Niniejsza praca przedstawia w zarysie podstawowe definicje oraz charakterystykę miar dokładności diagnostycznej.

***Key words**:* diagnostic accuracy, sensitivity, specificity, likelihood ratio, DOR, AUC, predictive values, PPV, NPV
Słowa kluczowe: precyzja diagnostyczna, czułość, specyficzność, wskaźnik wiarygodności, DOR, AUC, wartości predykcyjne, PPV-wartość predykcyjna wyniku dodatniego, NPV – wartość predykcyjna wyniku ujemnego

INTRODUCTION

Diagnostic accuracy of any diagnostic procedure or a test gives us an answer to the following question: "How well this test discriminates between certain two conditions of interest (health and disease; two stages of a disease etc.)?". This discriminative ability can be quantified by the measures of diagnostic accuracy:

- sensitivity and specificity,
- positive and negative predictive values (PPV, NPV),
- likelihood ratio,
- the area under the ROC curve (AUC),
- Youden's index,
- diagnostic odds ratio (DOR).

Different measures of diagnostic accuracy relate to the different aspects of diagnostic procedure. Some measures are used to assess the discriminative property of the test, others are used to assess its predictive ability [1]. While discriminative measures are mostly used by health policy decisions, predictive measures are most useful in predicting the probability of a disease in an individual [2]. Furthermore, it should be noted that measures of a test performance are not fixed indicators of a test quality and performance. Measures of diagnostic accuracy are very sensitive to the characteristics of the population in which the test accuracy is evaluated. Some measures largely depend on the disease prevalence, while others are highly sensitive to the spectrum of the disease in the study population. It is therefore of utmost importance to know how to interpret them as well as when and under what conditions to use them.

SENSITIVITY AND SPECIFICITY

A perfect diagnostic procedure has the potential to completely discriminate between subjects with and without disease. Values of a perfect test which are above the cut-off always indicate the disease, while the values below the cut-off always exclude the disease. Unfortunately, such a perfect test does not exist in real life and therefore diagnostic procedures can make only partial distinction between subjects with and without disease. Values above the cut-off are not always indicative of a disease since subjects without disease can also sometimes have elevated values. Such elevated values of a certain parameter of interest are called false positive values (FP). On the other hand, values below the cut-off are mainly found in subjects without disease.

However, some subjects with the disease can have them too. Those values are false negative values (FN). Therefore, the cut-off divides the population of examined subjects with and without disease into four subgroups considering values of a parameter of interest:

- true positive (TP) –subjects with the disease with the value of a parameter of interest above the cut-off,
- false positive (FP) –subjects without the disease with the value of a parameter of interest above the cut-off,
- true negative (TN) –subjects without the disease with the value of a parameter of interest below the cut-off,
- false negative (FN) –subjects with the disease with the value of a parameter of interest below the cut-off.

The first step in the calculation of sensitivity and specificity is to make a 2x2 table with groups of subjects divided according to a gold standard or (reference method) in columns, and categories according to test in rows (Table I).

Table I. *2x2 table*

	Subjects with the disease	Subjects without the disease
positive	TP	FP
negative	FN	TN

Sensitivity is expressed in percentage and defines the proportion of true positive subjects with the disease in a total group of subjects with the disease (TP/TP+FN). Actually, sensitivity is defined as the probability of getting a positive test result in subjects with the disease (T+|B+). Hence, it relates to the potential of a test to recognise subjects with the disease.

Specificity is a measure of a diagnostic test accuracy, complementary to sensitivity. It is defined as a proportion of subjects without the disease with negative test result in total of subjects without disease (TN/TN+FP). In other words, specificity represents the probability of a negative test result in a subject without the disease (T-|B-). Therefore, we can postulate that specificity relates to the aspect of diagnostic accuracy that describes the test's ability to recognise subjects without the disease, i.e. to exclude the condition of interest.

Neither sensitivity nor specificity are influenced by the disease prevalence, meaning that results from one study could easily be transferred to some other setting

with a different prevalence of the disease in the population. Nonetheless, sensitivity and specificity can vary greatly depending on the spectrum of the disease in the study group.

PREDICTIVE VALUES

Positive predictive value (PPV) defines the probability of having the state/disease of interest in a subject with positive result (B+|T+). Therefore PPV represents a proportion of patients with positive test result in total of subjects with positive result (TP/TP+FP).

Negative predictive value (NPV) describes the probability of not having a disease in a subject with a negative test result (B-|T-). NPV is defined as a proportion of subjects without the disease with a negative test result in total of subjects with negative test results (TN/TN+FN).

Unlike sensitivity and specificity, predictive values are largely dependent on disease prevalence in examined population. Therefore, predictive values from one study should not be transferred to some other setting with a different prevalence of the disease in the population. Prevalence affects PPV and NPV differently. PPV increases, while NPV decreases with the increase of the prevalence of the disease in a population. Whereas the change in PPV is rather substantial, NPV is somewhat less influenced by the disease prevalence.

LIKELIHOOD RATIO (LR)

Likelihood ratio is a very useful measure of diagnostic accuracy. It is defined as the ratio of expected test result in subjects with a certain state/disease to the subjects without the disease. As such, LR directly links the pre-test and post-test probability of a disease in a specific patient [3]. Simplified, LR tells us how many times more likely particular test result is in subjects with the disease than in those without disease. When both probabilities are equal, such test is of no value and its LR = 1.

Likelihood ratio for positive test results (LR+) tells us how much more likely the positive test result is to occur in subjects with the disease compared to those without the disease (LR+=(T+ | B+)/(T+ | B-)). LR+ is usually higher than 1 because it is more likely that the positive test result will occur in subjects with the disease than in subject without the disease. LR+ can be simply calculated according to the following formula: LR+ = sensitivity / (1-specificity).

LR+ is the best indicator for ruling-in a diagnosis. The higher the LR+ the more indicative of a disease the test is. Good diagnostic tests have LR+ > 10 and their positive result has a significant contribution to the diagnosis.

Likelihood ratio for negative test result (LR-) represents the ratio of the probability that a negative result will occur in subjects with the disease to the probability that the same result will occur in subjects without the disease. Therefore, LR- tells us how much less likely the negative test result is to occur in a patient than in a subject without disease. (LR-=(T- | B+)/(T- | B-)). LR- is usually less than 1 because it is less likely that negative test result occurs in subjects with than in subjects without disease. LR- is calculated according to the following formula: LR- = (1-sensitivity) / specificity.

LR- is a good indicator for ruling-out a diagnosis. Good diagnostic tests have LR- < 0,1. The lower the LR- the more significant contribution of the test is in ruling-out, i.e. in lowering the posterior probability of the subject having the disease.

Since both specificity and sensitivity are used to calculate the likelihood ratio, it is clear that neither LR+ nor LR- depend on the disease prevalence in examined groups. Consequently, the likelihood ratios from one study are applicable to some other clinical setting, as long as the definition of the disease is not changed. If the way of defining the disease varies, none of the calculated measures will apply in some other clinical context.

ROC CURVE

There is a pair of diagnostic sensitivity and specificity values for every individual cut-off. To construct a ROC graph, we plot these pairs of values on the graph with the 1-specificity on the x-axis and sensitivity on the y-axis (Figure 1).

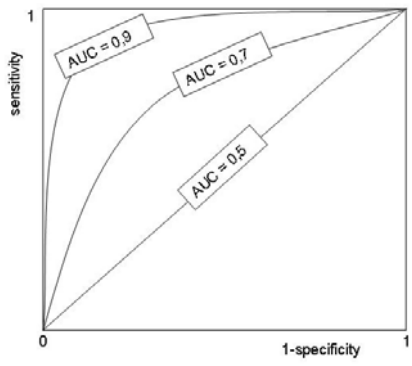

Fig. 1. *ROC curve*

The shape of a ROC curve and the area under the curve (AUC) helps us estimate how high the discriminative power of a test is. The closer the curve is located to upper-left hand corner and the larger the area under the curve, the better the test is at discriminating between diseased and non-diseased. The area under the curve can have any value between 0 and 1 and it is a good indicator of the reliability of the test. A perfect diagnostic test has the AUC 1.0. whereas a nondiscriminating test has an area 0.5. Generally we can say that the relation between AUC and diagnostic accuracy applies as described in Table II.

Table II. *Relationship between the area under the ROC curve and diagnostic accuracy*

area	**diagnostic accuracy**
0.9 – 1.0	excellent
0.8 - 0.9	very good
0.7 - 0.8	good
0.6 - 0.7	sufficient
0.5 - 0.6	bad
< 0.5	test not useful

AUC is a global measure of diagnostic accuracy. It tells us nothing about individual parameters, such as sensitivity and specificity. Out of two tests with identical or similar AUC, one can have significantly higher sensitivity, whereas the other significantly higher specificity. Furthermore, data on AUC state nothing about predicative values and about the contribution of the test to ruling-in and ruling-out a diagnosis. Global measures are there for general assessment and for comparison of two or more diagnostic tests. By the comparison of areas under the two ROC curves we can estimate which one of two tests is more suitable for distinguishing health from disease or any other two conditions of interest. It should be pointed that this comparison should not be based on visual nor intuitive evaluation [4]. For this purpose we use statistic tests which evaluate the statistical significance of estimated difference between two AUC, with previously defined level of statistical significance (P).

DIAGNOSTIC ODDS RATIO (DOR)

Diagnostic odds ratio is also one global measure for diagnostic accuracy, used for general estimation of discriminative power of diagnostic procedures and also for the comparison of diagnostic accuracies between two or more diagnostic tests. DOR of a test is the ratio of the odds of positivity in subjects with disease relative to the odds in subjects without disease [4]. It is calculated according to the formula: DOR = (TP/FN)/(FP/TN).

DOR depends significantly on the sensitivity and specificity of a test. A test with high specificity and sensitivity with low rate of false positives and false negatives has high DOR. With the same sensitivity of the test, DOR increases with the increase of the test specificity. For example, a test with sensitivity > 90% and specificity of 99% has a DOR greater than 500.

DOR does not depend on disease prevalence; however like sensitivity and specificity it depends on criteria used to define disease and its spectrum of pathological conditions of the examined group (disease severity, phase, stage, comorbidity etc.).

DIAGNOSTIC EFFECTIVENESS (ACCURACY)

Another global measure of diagnostic accuracy is so called diagnostic accuracy (effectiveness), expressed as a proportion of correctly classified subjects (TP+TN) among all subjects (TP+TN+FP+FN). Diagnostic accuracy is affected by the disease prevalence. With the same sensitivity and specificity, diagnostic accuracy of a particular test increases as the disease prevalence decreases. This data, however, should be handled with care. In fact, this does not mean that the test is better if we apply it in a population with low disease prevalence. It only means that in absolute number the test gives more correctly classified subjects. This percentage of correctly classified subjects should always be weighed considering other measures of diagnostic accuracy, especially predictive values. Only then a complete assessment of the test contribution and validity could be made.

YOUDEN'S INDEX

Youden's index is one of the oldest measures for diagnostic accuracy [6]. It is also a global measure of a test performance, used for the evaluation of overall discriminative power of a diagnostic procedure and for comparison of this test with other tests. Youden's index is calculated by deducting 1 from the sum of test's sensitivity and specificity expressed not as percentage but as a part of a whole number: (sensitivity + specificity) – 1.

For a test with poor diagnostic accuracy, Youden's index equals 0, and in a perfect test Youden's index equals 1. Youden's index is not sensitive for differences in the sensitivity and specificity of the test,

which is its main disadvantage. Namely, a test with sensitivity 0,9 and specificity 0,4 has the same Youden's index (0,3) as a test with sensitivity 0,6 and specificity 0,7. It is absolutely clear that those tests are not of comparable diagnostic accuracy. If one is to assess the discriminative power of a test solely based on Youden's index it could be mistakenly concluded that these two tests are equally effective.

Youden's index is not affected by the disease prevalence, but it is affected by the spectrum of the disease, as are also sensitivity specificity, likelihood ratios and DOR.

DESIGN OF DIAGNOSTIC ACCURACY STUDIES

Measures of diagnostic accuracy are extremely sensitive to the design of the study. Studies suffering from some major methodological shortcomings can severely over- or under-estimate the indicators of test performance as well as they can severely limit the possible applicability of the results of the study. The effect of the design of the study on the bias and variation in the estimates of diagnostic accuracy can be quantified [7]. STARD initiative published in 2003 was a very important step toward the improvement of the quality of reporting of studies of diagnostic accuracy [8, 9]. According to some authors, the quality of reporting of diagnostic accuracy studies did not significantly improve after the publication of the STARD statement [10, 11], whereas some others hold that the overall quality of reporting has at least slightly improved [12], but there is still some room for potential improvement [13, 14].

Editors of scientific journals are encouraged to include the STARD statement into the Journal Instructions to authors and to oblige their authors to use the checklist when reporting their studies on diagnostic accuracy. This way the quality of reporting could be significantly improved, providing the best possible evidence for health care providers, clinicians and laboratory professionals; to the best for the patient care.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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QUANTITATIVE ANATOMY OF THE AORTIC ARCH BRANCHES IN HUMAN FETUSES: AN ANATOMICAL, DIGITAL AND STATISTICAL STUDY

ANATOMIA ILOŚCIOWA GAŁĘZI ŁUKU AORTY: ANALIZA ANATOMICZNA, CYFROWA I STATYSTYCZNA

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Summary

Introduction: The present study was performed to characterize the growth patterns for the absolute and relative diameters of the aortic arch branches during gestation.

Material and Methods: Using anatomical dissection, digital-image analysis and statistical methods a range of diameters for the aortic arch branches in 128 spontaneously aborted human fetuses aged 15-34 weeks was examined.

Results: No significant gender differences were found. The brachiocephalic trunk diameter ranged from 1.15 ± 0.14 to 4.69 ± 0.58 mm, according to the function y = -1.9835 + 0.1948x ± 0.3728. The values of the left common carotid artery diameter increased from 0.72 ± 0.18 to 3.28 ± 0.40 mm and generated the linear model y = -1.5228 + 0.1428x ± 0.2749. The absolute diameter of the left subclavian artery showed an increase in diameter from 0.68 ± 0.16 to 2.89 ± 0.29 mm, according to the linear pattern y = - 1.2169 + 0.1233x ± 0.2389. The values of the brachiobicarotid trunk diameter revealed a proportional increase in values from 1.49 ± 0.17 to 6.27 ± 0.72 mm, in accordance with the linear function y = - 3.034 + 0.2845x ± 0.4253. The relative diameters increased with advanced fetal age: from 0.569 ± 0.091 to 0.686 ± 0.097 for the brachiocephalic trunk, from 0.356 ± 0.062 to 0.480 ± 0.101 for the left common carotid artery, from 0.337 ± 0.064 to 0.423 ± 0.103 for the left subclavian artery, and from 0.738 ± 0.089 to 0.916 ± 0.088 for the brachiobicarotid trunk.

Conclusions: 1. The developmental dynamic of the absolute diameters of the aortic arch branches follows according to the linear model. 2. The relative diameters of the aortic arch branches increase gradually during gestation. 3. The normal growth curves for the diameters of the developing aortic arch branches should facilitate the prenatal diagnosis of the aortic arch abnormalities, particularly aortic coarctation.

Streszczenie

Cel: Badania wykonano w celu charakterystyki wzrostu średnic bezwzględnych i względnych gałęzi łuku aorty podczas ciąży.

Materiał i metody: Przy zastosowaniu dysekcji anatomicznej, cyfrowej analizy obrazy i analizy statystycznej zbadano zakres średnic gałęzi łuku aorty u 128 płodów człowieka w wieku od 15 do 34 tyg. ciąży.

Wyniki: Nie zaobserwowano różnic płciowych. Średnica pnia ramienno-głowowego wynosiła od 1,15 ± 0,14 do 4,69 ± 0,58 mm, zgodnie z funkcją y = -1,9835 + 0,1948x ± 0,3728. Wartości średnicy tętnicy szyjnej wspólnej lewej wzrastały od 0,72 ± 0,18 do 3,28 ± 0,40 mm i generowały model liniowy y = -1,5228 + 0,1428x ± 0,2749. Średnica bezwzględna tętnicy podobojczykowej lewej wykazywała wzrost średnicy od 0,68 ± 0,16 do 2,89 ± 0,29 mm, zgodnie z wzorcem liniowym y = - 1,2169 + 0,1233x ± 0,.2389. Wartości średnicy pnia ramienno-dwuszyjnego wykazywały proporcjonalny wzrost wartości od 1,49 ± 0,17 do 6,27 ± 0,72 mm, zgodnie z funkcją liniową y = -3,034 + 0,2845x ± 0,4253. Średnice względne wzrastały wraz z wiekiem płodu: od 0,569 ± 0,091 do 0,686 ± 0,097 dla pnia ramienno-głowowego, od 0,356 ± 0,062 do 0,480 ± 0,101 dla tętnicy szyjnej wspólnej lewej, od 0,337 ± 0,064 do 0,423 ± 0,103 dla tętnicy podobojczykowej lewej i od 0,738 ± 0,089 do 0,916 ± 0,088 dla pnia ramienno-dwuszyjnego.

Wnioski: 1. Dynamika rozwojowa średnic bezwzględnych gałęzi łuku aorty następuje zgodnie z funkcją liniową. 2. Średnice względne łuku aorty wzrastają stopnio-

wo podczas ciąży. 3. Krzywe prawidłowego wzrostu średnic rozwijających się gałęzi łuku aorty pozwalają na diagnostykę prenatalną nieprawidłowości łuku aorty, zwłaszcza koarktacji aorty.

***Key words**:* original external diameter, brachiocephalic trunk, left common carotid artery, left subclavian artery, brachiobicarotid trunk, digital image analysis

Słowa kluczowa: początkowa średnica zewnętrzna, pień ramienno-głowowy, tętnica szyjna wspólna lewa, tętnica podobojczykowa lewa, pień ramienno-dwuszyjny, cyfrowa analiza obrazu

INTRODUCTION

Knowledge of normal dimensions of the aortic arch branches and their relationship to one another is important in the management of children with congenital heart disease. During fetal development the morphometric data of the aorta presented the proportional increase in the diameter, closely correlated to the linear function [1-5]. However, reference data for dimensions of the aortic arch branches are scarce in fetuses and children. Generally, the growth of arteries is proportional to the amount of blood carried by them [6].

This study was undertaken to clarify the relationship between both the absolute or relative diameters of the aortic arch branches and gestational age. The objectives for the present study were set to examine, as follows:

- the normal values for the original external diameters of the aortic arch branches at varying gestational age,
- the influence of sex on the value of the diameters examined,
- the growth curves for the absolute diameters of the aortic arch branches during gestation,
- the developmental trend of the relative diameters of the aortic arch branches (aortic arch branch-to-aortic root diameter ratio).

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 Ludwik Rydygier University Research Ethic Committee (KB/217/2006). 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 I). The fetal ages of the specimens were calculated on the basis of the following criteria: 1) gestational age based on measurement of crown-rump length [7], 2) known date of the beginning of the last normal menstrual period, and 3) in some cases corrections regarding fetal age were established by measuring of their humeral and femoral bones using USG equipment [8]. Fetuses were grouped into six monthly cohorts, corresponding to the 4-9th month of gestation.

Table I. *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 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 under a stereoscope with Huygens ocular at a magnification of 10.

In each fetus, the dissected aortic arch branches with the millimeter scale were placed perpendicular to the optical lens axis, afterwards recorded using a camera Nikon Coolpix 8400, and digitalized to JPEG images. Next, digital picture of the aortic arch branches underwent morphometric analysis using digital image analysis system of Leica QWin Pro 16 (Cambridge), which automatically estimated external diameters of the marked vessel. Automatic diameter measurement was derived by assuming that the vessels filled with latex were circular in cross-section.

For each individual, the four following measurements were made: 1. aortic root diameter (mm) measured at the level of the aortic valve annulus, 2-4. the original external diameters of the brachiocephalic trunk, the left common carotid artery, and the left subclavian artery. In 27 individuals (14 males, 13 females) the original external diameter of the brachiobicarotid trunk was measured.

The original external diameters of the aortic arch branches 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 curves of best fit for the plot for each aortic arch branch diameter against fetal age. To compensate for age, the diameter of each branch of the aortic arch was divided by that of the aortic root and expressed as a ratio (relative diameter).

RESULTS

The native pictures of the aortic arch branches are displayed in Figures 1-3. The statistical analysis of the absolute and relative diameters of the aortic arch branches did not show gender differences ($P \geq 0.05$). Hence the values obtained, without regard to sex, have been presented in Table II.

The absolute diameter of each branch of the aortic arch increased significantly ($P < 0.05$) with advanced gestational age in weeks, according to the linear fashion (Fig. 4). The brachiocephalic trunk diameter ranged from 1.15 ± 0.14 mm for 4 month group to 4.69 ± 0.58 mm for 9 month group, according to the function $y = -1.9835 + 0.1948x \pm 0.3728$ ($r = 0.95$; $P < 0.001$). The values of the left common carotid artery diameter increased from 0.72 ± 0.18 to 3.28 ± 0.40 mm and generated the linear model $y = -1.5228 + 0.1428x \pm 0.2749$ ($r = 0.95$; $P < 0.001$). The absolute diameter of the left subclavian artery showed an increase in diameter from 0.68 ± 0.16 to 2.89 ± 0.29 mm for fetuses aged 4 and 9 months, respectively. Plots showing the left subclavian artery diameter were modelled the linear fashion $y = -1.2169 + 0.1233x \pm 0.2389$ ($r = 0.95$; $P < 0.001$). The values of the brachiobicarotid trunk diameter revealed a proportional increase in values from 1.49 ± 0.17 to 6.27 ± 0.72 mm, in accordance with linear function $y = -3.034 + 0.2845x \pm 0.4253$ ($r = 0.97$; $P < 0.001$).

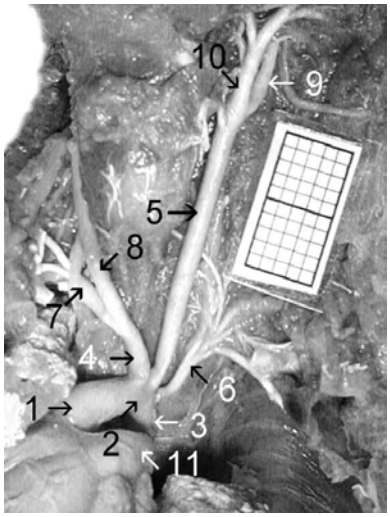

Fig. 1. *The usual branching of the aortic arch (type I of Anson) in a male fetus aged 20 weeks: 1-ascending aorta, 2-aortic arch, 3-aortic isthmus, 4-brachiocephalic trunk, 5-left common carotid artery, 6-left subclavian artery, 7-right subclavian artery, 8-right common carotid artery, 9-left internal carotid artery, 10-left external carotid artery, 11-pulmonary trunk*

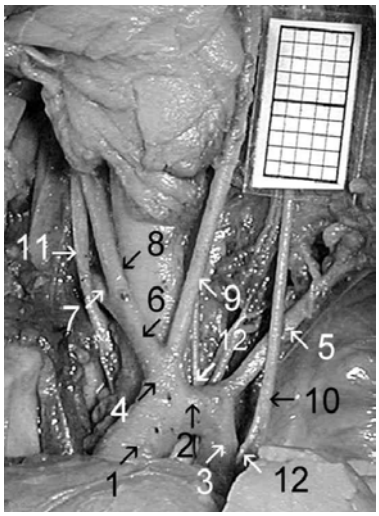

Fig. 2. *Reduction to two derived branches of the aortic arch (type II of Anson) in a female fetus aged 25 weeks: 1-ascending aorta, 2-aortic arch, 3-aortic isthmus, 4-brachiobicarotid trunk, 5-left subclavian artery, 6-brachiocephalic trunk, 7-right subclavian artery, 8-right common carotid artery, 9-left common carotid artery, 10-left vagus nerve, 11-right vagus nerve, 12-left recurrent laryngeal nerve*

Table II. *Block scheme of the statistical analysis of the absolute diameters of the aortic arch branches (measured at their origins*)

Fetal age (month)	Aortic root diameter (mm) (mean ± SD)	Brachiocephalic trunk (mm) (mean ± SD)	Left common carotid artery (mm) (mean ± SD)	Left subclavian artery (mm) (mean ± SD)	Brachiobicarotid trunk (mm) (mean ± SD)
4	2.02 ± 0.26 ↓ (P<0.001)	1.15 ± 0.14 ↓ (P<0.01)	0.72 ± 0.18 ↓ (P<0.01)	0.68 ± 0.16 ↓ (P<0.01)	1.49 ± 0.17 ↓ (P<0.01)
5	2.94 ± 0.49 ↓ (P<0.001)	1.68 ± 0.34 ↓ (P<0.001)	1.13 ± 0.19 ↓ (P<0.001)	1.07 ± 0.18 ↓ (P<0.001)	2.01 ± 0.16 ↓ (P<0.01)
6	3.96 ± 0.57 ↓ (P<0.001)	2.29 ± 0.55 ↓ (P<0.001)	1.75 ± 0.36 ↓ (P<0.001)	1.60 ± 0.30 ↓ (P<0.001)	3.43 ± 0.59 ↓ (P<0.01)
7	4.91 ± 0.47 ↓ (P<0.001)	3.12 ± 0.41 ↓ (P<0.001)	2.23 ± 0.37 ↓ (P<0.001)	2.01 ± 0.31 ↓ (P<0.001)	4.39 ± 0.87 ↓ (P<0.01)
8	6.11 ± 0.50 ↓ (P<0.01)	3.91 ± 0.41 ↓ (P<0.001)	2.85 ± 0.40 ↓ (P<0.05)	2.52 ± 0.40 ↓ (P<0.05)	5.62 ± 0.46 ↓ (P<0.05)
9	6.84 ± 0.63	4.69 ± 0.58	3.28 ± 0.40	2.89 ± 0.29	6.27 ± 0.72

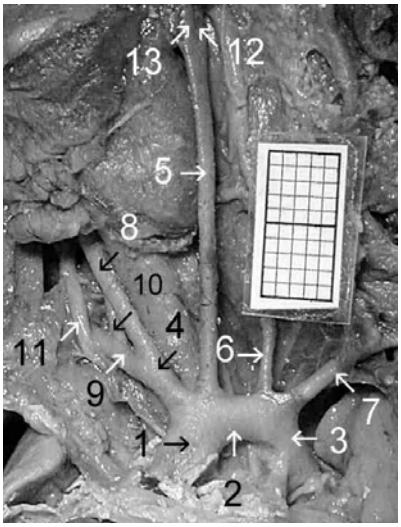

Fig. 3. *Derivation of the four branches from aortic arch (type III of Anson) in a male fetus aged 19 weeks: 1-ascending aorta, 2-aortic arch, 3-aortic isthmus, 4-brachiocephalic trunk, 5-left common carotid artery, 6-left vertebral artery, 7-left subclavian artery, 8-right common carotid artery, 9-right subclavian artery, 10-right vertebral artery, 11-right* vagus *nerve, 12-left internal carotid artery, 13- left external carotid artery*

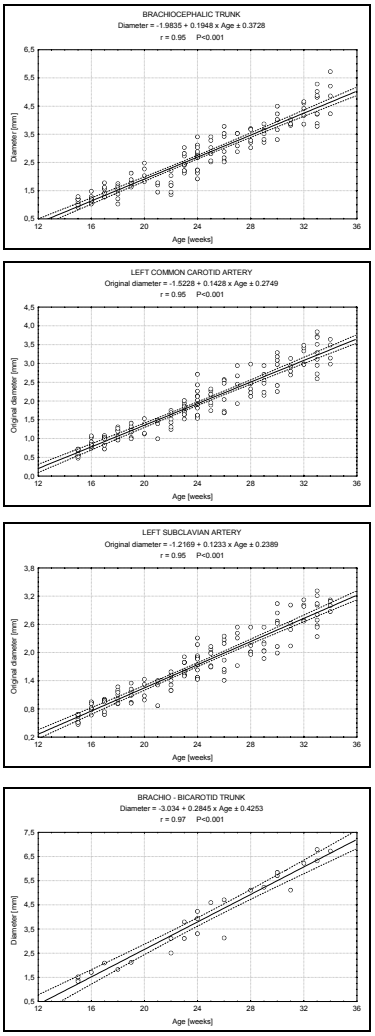

Fig. 4. *Regression lines for the original external diameters (y) of the aortic arch branches vs. gestational age (x)*

Parallel to the absolute increase in the values of the aortic arch branches, their relative diameters increased with advanced fetal age (Table III). In the examined age range the brachiocephalic trunk-to-aortic root diameter ratio increased from 0.569 ± 0.091 to 0.686 ± 0.097. The relative diameter of the brachiocephalic trunk increased significantly (P<0.05) between 6 and 7, and between 8 and 9 months of gestation, as well. The left common carotid artery-to-aortic root diameter ratio ranged from 0.356 ± 0.062 for 4 month group to 0.480 ± 0.101 for 9 month group. The left subclavian artery-to-aortic root diameter ratio showed an increase in values from 0.337 ± 0.064 to 0.423 ± 0.103, between 4 and 9 month groups, respectively. The brachiobicarotid trunk-to-aortic root diameter ratio increased from 0.738 ± 0.089 to 0.916 ± 0.088. It should be noted, that the significant increase in the relative diameters of the left common carotid artery, the left subclavian artery and the brachiobicarotid trunk was found between 5 and 6 month groups, only (P<0.05). The ratios for all aortic arch branches in the remaining age ranges increased gradually with age, although they did not reach levels of statistical significance (P>0.05).

Table III. *Block scheme of the statistical analysis of the relative diameters of the aortic arch branches (measured at their origins)*

Fetal age (month)	Brachiocephalic trunk (mean ± SD)	Left common carotid artery (mean ± SD)	Left subclavian artery (mean ± SD)	Brachiobicarotid trunk (mean ± SD)
4	0.569 ± 0.091 ↓(P>0.05)	0.356 ± 0.062 ↓(P>0.05)	0.337 ± 0.064 ↓(P>0.05)	0.738 ± 0.089 ↓(P>0.05)
5	0.571 ± 0.079 ↓(P>0.05)	0.384 ± 0.121 ↓(P<0.05)	0.364 ± 0.075 ↓ (P<0.05)	0.684 ± 0.102 ↓ (P<0.05)
6	0.578 ± 0.098 ↓(P<0.05)	0.442 ± 0.092 ↓(P>0.05)	0.404 ± 0.087 ↓(P>0.05)	0.866 ± 0.090 ↓(P>0.05)
7	0.635 ± 0.109 ↓ (P>0.05)	0.454 ± 0.111 ↓(P>0.05)	0.409 ± 0.095 ↓(P>0.05)	0.894 ± 0.097 ↓(P>0.05)
8	0.640 ± 0.102 ↓(P<0.05)	0.466 ± 0.098 ↓(P>0.05)	0.412 ± 0.098 ↓ (P>0.05)	0.920 ± 0.071 ↓(P>0.05)
9	0.686 ± 0.097	0.480 ± 0.101	0.423 ± 0.103	0.916 ± 0.088

DISCUSSION

Digital-image analysis is a well-established method for evaluating angiometric parameters providing quantitative data in assessment of the growth of cardiovascular structures. It is obviously accepted that growth of the arterial diameter relates to blood flow.

The normative data we have produced for the absolute diameters of the aortic arch branches indicate the proportional increase with advanced fetal age, according to the linear fashion. A particular strength of this study is the large number of normal specimens used to

generate the growth curves. In material under examination, the original external diameters of the aortic arch branches increased as follows: y = -1.9835 + 0.1948x ± 0.3728 for the brachiocephalic trunk, y = -1.5228 + 0.1428x ± 0.2749 for the left common carotid artery, y = -1.2169 + 0.1233x ± 0.2389 for the left subclavian artery, and y = -3.034 + 0.2845x ± 0.4253 for the brachiobicarotid trunk. The above-mentioned linear functions were the best model for the growth of the diameters examined, because correlation coefficients between the diameters examined and fetal age were very high (r = 0.95; 0.97) and statistically significant (P<0.001). The earlier study performed by Szpinda et al. [9] showed developmental growth in the diameter of the brachiocephalic trunk, correlated with a linear function y = -1.056 + 0.554 x (r = 0.83; P<0.01). Flisiński et al. [10] have provided measurements of the dimensions of the common carotid arteries in fetuses aged 4-9 months, but without growth curves for the normal growth of the parameters studied. It should be noted, that the growth curves for the normal development of the left common carotid artery, the left subclavian artery and the brachiobicarotid trunk have not been previously reported in the professional literature. Flisiński et al. [10] reported that the values of the original external diameter of the left common carotid artery ranged from 0.84 ± 0.14 to 3.13 ± 0.40 mm. However, in their material the correlation coefficient between original external diameter and gestational age was much lower (r = 0.84) in comparison to our results (r = 0.95). Hornberger et al. [11] demonstrated that the internal diameter of the left common carotid artery was found to increase proportionally throughout the second and third trimesters. Machii and Becker [12] emphasized the significant increase of the diameters of the aortic arch branches in specimens aged 0-4 years. The average absolute diameter of each branch increased significantly with age as follows: from 4.6 ± 0.6 to 7.8 ± 0.8 mm for the brachiocephalic trunk, from 3.4 ± 0.7 to 5.9 ± 0.5 mm for the left common carotid artery, and from 3.4 ± 0.3 to 6.2 ± 0.6 mm for the left subclavian artery. Morphometric features of the brachiobicarotid trunk have not been previously reported, neither in prenatal nor postnatal life.

In the present study was found, that the relative diameters of the aortic arch branches reveal their distinct remodelling throughout gestation. In our opinion, an increase of the relative diameters of the aortic arch branches resulted from the increased proportion of blood received by human brain and upper limbs with advanced fetal age. The relative diameters of the aortic arch branches were increased from 0.569 ± 0.091 to 0.686 ± 0.097 for the brachiocephalic trunk, from 0.356 ± 0.062 to 0.480 ± 0.101 for the left common carotid artery, from 0.337 ± 0.064 to 0.423 ± 0.103 for the left subclavian artery, and from 0.738 ± 0.089 to 0.916 ± 0.088 for the brachiobicarotid trunk. Similar observations concerning the relative growth of the three typical aortic arch branches in children aged 0-4 years, were made by Machii and Becker [12]. According to these authors, absolute diameters of the aortic arch branches were expressed as the ratios (absolute diameter divided by the proximal diameter of the descending aorta). In the study period, the ratios were increased from 0.68 ± 0.08 to 0.81 ± 0.10 for the brachiocephalic trunk, from 0.50 ± 0.09 to 0.61 ± 0.07 for the left common carotid artery, and from 0.52 ± 0.06 to 0.64 ± 0.03 for the left subclavian artery. Morrow et al. [13] demonstrated that in coarctation neonates the original diameters of the aortic arch branches were greater than in control subjects, as follows: 5.0 ± 0.8 *vs.* 4.3 ± 0.6 mm for the brachiocephalic trunk (P<0.02), 3.4 ± 0.6 *vs.*2.6 ± 0.4 for the left common carotid artery (P<0.001), and 3.0 ± 0.4 *vs.* 2.8 ± 0.5 for the left subclavian artery (P<0.26). Moreover, the diameters of the left common carotid artery, the left subclavian artery and the aortic arch were essentially equal in coarctation patients. In contrast, in the normal neonate the diameters of the left carotid and left subclavian arteries were approximately 50% of the diameter of the aortic arch.

The lack of statistically significant gender differences (P>0.05) concerning the original external diameters of the aortic arch branches was observed in material under examination. Also, in the opinion of Macchi et al. [14] in adults, the average diameters of these arteries in the women examined were lower than that in men. However, the statistical difference in the arterial diameters of the two sexes was not significant, with the exception of the left subclavian artery, that was larger in men.

CONCLUSIONS

1. The developmental dynamic of the absolute diameters of the aortic arch branches follows according to the linear model.
2. The relative diameters of the aortic arch branches increase gradually during gestation.

3. The normal growth curves for the diameters of the developing aortic arch branches should facilitate the prenatal diagnosis of the aortic arch abnormalities, particularly aortic coarctation.

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VOLUMETRIC GROWTH OF VARIOUS AORTIC SEGMENTS IN HUMAN FETUSES

WZROST POJEMNOŚCI RÓŻNYCH SEGMENTÓW AORTY U PŁODÓW CZŁOWIEKA

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S u m m a r y

Introduction: This study delineates normal volumetric growth for various aortic segments (ascending aorta, aortic arch, thoracic aorta) in human fetuses, taking into consideration fetal sex and age.

Material and Methods: Using anatomical dissection, digital-image analysis (system of Leica Q Win Pro 16) and statistical analysis (ANOVA, regression analysis) a range of volume measurements for the ascending aorta, aortic arch and thoracic aorta in 128 spontaneously aborted human fetuses aged 15-34 weeks was examined.

Results: No significant gender differences were found ($P>0.05$). The volume of the ascending aorta ranged from 7.56 ± 2.65 to 370.99 ± 105.42 mm^3, according to the quadratic function $y = 373.1 - 43.38x + 1.30x^2 \pm 24.51$. The aortic arch volume increased from 8.84 ± 2.90 to 453.51 ± 125.54 mm^3, in accordance with the quadratic model $y = 513.4 - 58.464x \pm 1.704x^2 \pm 49.254$. The volume of the thoracic aorta ranged from 15.75 ± 8.06 to 1158.01 ± 301.85 mm^3, according to the quadratic pattern $y = 1376.2 - 154.42x + 4.419x^2 \pm 125.6$. The sum of the volumes of these aortic segments generated the quadratic function $y = 2262 - 256x + 7.423x^2 \pm 212$. The relationships between the volumes of the various aortic segments revealed the linear regressions: $y = 3.9647 + 0.8098x \pm 12.372$ (for the ascending aorta *vs.* the aortic arch), $y = 4.4740 + 0.3973x \pm 20.002$ (for the aortic arch *vs.* the thoracic aorta), and $y = 7.3242 + 0.3225x \pm 18.523$ (for the ascending aorta *vs.* the thoracic aorta). The volumes of ascending aorta, aortic arch and thoracic aorta also increased proportionally (5:6:15) during gestation.

Conclusions: 1. The volumetric growth of the various aortic segments proceeds according to the quadratic regression. 2. The various aortic segments indicate proportional growth in volume during gestation.

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

Cel: Praca ta określa prawidłowy wzrost pojemności różnych segmentów aorty (aorta wstępująca, łuk aorty, aorta piersiowa) u płodów człowieka, z uwzględnieniem płci i wieku płodu.

Materiał i metody: Przy zastosowaniu dysekcji anatomicznej, cyfrowej analizy obrazu (system Leica Q Win Pro 16) i analizy statystycznej (ANOVA, rachunek regresji) zbadano zakres wartości pojemności aorty wstępującej, łuku aorty i aorty piersiowej u 128 płodów człowieka w wieku od 15 do 34 tyg. pochodzących z samoistnych poronień.

Wyniki: Nie stwierdzono różnic płciowych ($P\geq0,05$). Pojemność aorty wstępującej wynosiła od $7,56 \pm 2,65$ do $370,99 \pm 105,42$ mm^3, zgodnie z funkcją kwadratową $y = 373,1 - 43,38x + 1,30x^2 \pm 24,51$. Pojemność łuku aorty wzrastała od $8,84 \pm 2,90$ do $453,51 \pm 125,54$ mm^3, zgodnie z modelem kwadratowym $y = 513,4 - 58,464x \pm 1,704x^2 \pm 49,254$. Pojemność aorty piersiowej wynosiła od $15,75 \pm 8,06$ do $1158,01 \pm 301,85$ mm^3, zgodnie z funkcją kwadratową $y = 1376,2 - 154,42 x + 4,419 x^2 \pm 125,6$. Suma pojemności tych segmentów aorty generowała funkcję kwadratową $y = 2262 - 256x + 7,423x^2 \pm 212$. Relacje między pojemnościami różnych segmentów aorty wykazywały regresje liniowe: $y = 3,9647 + 0,8098x \pm 12,372$ (aorta wstępująca *vs.* łuk aorty), $y = 4,4740 + 0,3973x \pm 20,002$ (łuk aorty *vs*. aorta piersiowa) i $y = 7,3242 + 0,3225x \pm 18,523$ (aorta wstępująca *vs.* aorta piersiowa). Podczas ciąży pojemności aorty wstępującej, łuku aorty i aorty piersiowej wzrastały proporcjonalnie (5:6:15).

Wnioski: 1. Wzrost pojemności różnych segmentów aorty następuje zgodnie z regresją kwadratową. 2. Podczas ciąży różne segmenty aorty wykazują proporcjonalny wzrost pojemności.

***Key words**:* ascending aorta, aortic arch, thoracic aorta, volume
Słowa kluczowa: aorta wstępująca, łuk aorty, aorta piersiowa, pojemność

INTRODUCTION

Quantitative studies on the fetal aorta have been focused previously on its diameter only, using echocardiographic [1-3] and autopsy [2, 4-7] techniques.

Until now, there has been no information concerning both the volume of the different aortic segments in human fetuses and their proportions. Gielecki et al. [8] suggested that volume of the aortic arch increased according to the square root function as fetal age advanced.

The present study was undertaken to clarify the increase in volume of the various aortic segments in human fetuses. Our objectives were to investigate:

- the reference ranges of the volume for various aortic segments at varying gestational ages,
- the growth curves for the volume of various aortic segments *vs.* fetal age,
- the relationships between the volumes of the ascending aorta, aortic arch and thoracic aorta,
- the influence of sex on the value of the volumes studied.

MATERIAL AND METHODS

The examinations were carried out on 128 spontaneously aborted human fetuses of both genders (63 males, 65 females) whose age varied from 15 to 34 weeks (Table I).

Table I. *Age and number of fetuses studied*

Fetal age		Crown-rump length (CRL) (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 crown-rump length (CRL) measurements were taken as the basis for determining gestational age, according to Iffy et al. [9]. The study was approved by the research ethics committee of the Nicolaus Copernicus University (KB/217/2006). Specimens that had detectable visible malformations were excluded from the study. Fetuses were divided into 6 monthly cohorts, from 4^{th} to 9^{th} month of gestation.

The fetal arteries were injected with white latex LBS 3060 through the abdominal aorta, under controlled pressure of 50-60 mm Hg, using a syringe infusion pump SEP 11S (Ascor S.A. Medical Equipment, Warsaw 2001). The fetuses were immersed in a 10% neutral formalin solution for 4-24 months, and then dissected under a stereoscope with Huygens ocular at a magnification of 10. In each specimen, the dissected ascending aorta, aortic arch and thoracic aorta with the millimeter scale were placed perpendicular to the optical lens axis, recorded and digitalized to JPEG images. Next, digital pictures of the fetal aorta were analyzed by the digital-image analysis system of Leica Q Win Pro 16 (Cambridge). Automatic volume measurements of the various aortic segments were derived by assuming that the filled aorta can be divided into small irregular cylinders of varying diameter and varying height. The sum of volume of such cylinders approximating the vessel was given in mm^3 as the volume of the different parts of the aorta was.

For each fetus, the three following volume measurements were performed:

1. the volume of the ascending aorta - from its origin (at the level of the aortic valve annulus) to its ending (just proximal to the brachiocephalic trunk origin),
2. the volume of the aortic arch - from its origin (just proximal to the brachiocephalic trunk origin) to its ending (just proximal to the entry of the ductus arteriosus),
3. the volume of the thoracic aorta - from its origin (just proximal to the entry of the ductus arteriosus) to its ending (at the level of the diaphragm).

The results obtained were analyzed 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 volume examined against gestational age,
- to calculate the relationships between volumes of the various aortic segments.

Correlation coefficients (r) or coefficients of determination (R^2) between parameters examined were estimated. A priori level of significance was set at $P<0.05$.

RESULTS

The native pictures of the various aortic segments are presented in Figures 1 and 2. The volume values of various aortic segments were similar in both genders ($P>0.05$). Hence the morphometric values obtained, without regard to sex, have been presented in Table II.

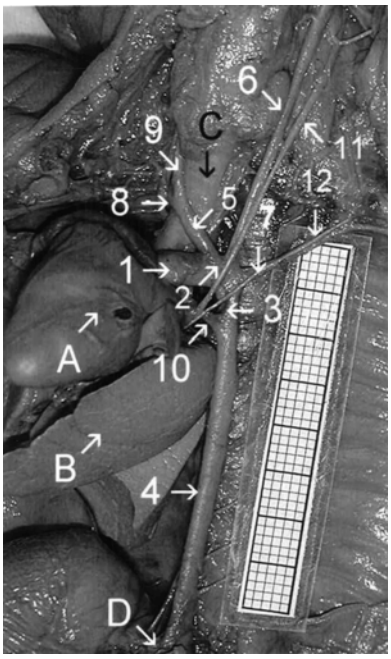

Fig. 1. *The great chest arteries (in situ) in a male fetus aged 28 weeks (aspectus lateralis): A-heart, B-right lung, C-trachea, D-abdominal diaphragm, 1-ascending aorta, 2-aortic arch, 3-aortic isthmus, 4-thoracic aorta, 5-brachiocephalic trunk, 6-left common carotid artery, 7-left subclavian artery, 8-right subclavian artery, 9-right common carotid artery, 10-ductus arteriosus, 11-left vagus nerve, 12-left phrenic nerve*

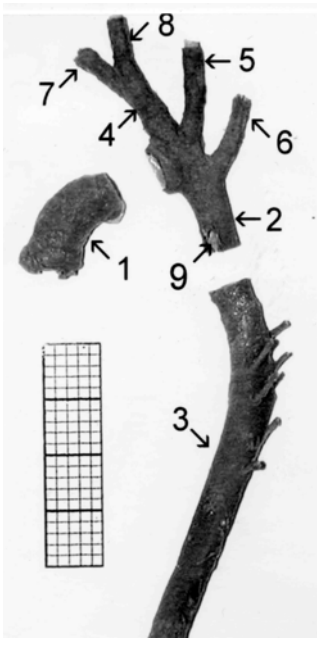

Fig. 2. *Proportions of the various aortic segments in a female fetus aged 28 weeks: 1-ascending aorta, 2-aortic arch, 3-thoracic aorta, 4-brachiocephalic trunk, 5-left common carotid artery, 6-left subclavian artery, 7-right subclavian artery, 8-right common carotid artery, 9-entry of the ductus arteriosus*

Table II. *Block scheme of the statistical analysis of the volume of the various aortic segments*

Fetal age (months)	Volume (mm^3)		
	ascending aorta (mean ± SD)	aortic arch (mean ± SD)	thoracic aorta (mean ± SD)
4	*7.56 ± 2.65 ↓(P>0.01)*	*8.84 ± 2.90 ↓(P>0.05)*	15.75 ± 8.06 ↓(P>0.05)
5	*23.13 ± 11.41 ↓(P<0.05)*	*25.69 ± 13.67 ↓(P<0.05)*	60.28 ± 29.77 ↓ (P<0.05)
6	*65.80 ± 24.51 ↓(P<0.001)*	*74.60 ± 27.21 ↓(P<0.01)*	190.23 ± 60.92 ↓(P<0.05)
7	*122.80 ± 38.07 ↓ (P<0.001)*	*140.04 ± 42.75 ↓(P<0.001)*	331.16 ± 120.56 ↓(P<0.001)
8	*262.19 ± 70.23 ↓(P<0.001)*	*322.67 ± 84.66 ↓(P<0.001)*	763.35 ± 229.41 ↓ (P<0.001)
9	*370.99 ±105.42*	*453.51 ± 125.54*	1158.01 ± 301.85

Several transformations concerning the volume against fetal age were generated, but it was proved a nonlinear correlation being best described as a parabola (Fig. 3).

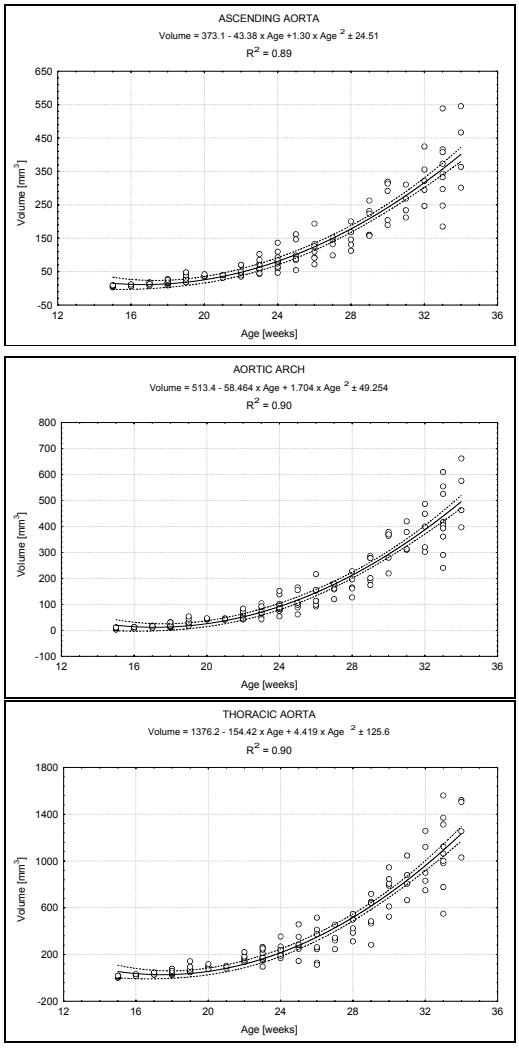

Fig. 3. *The volumes of the various aortic segments vs. fetal age*

During the study period, volumes of the aortic segments presented a quadratic pattern with advanced fetal age. The values of the ascending aorta volume ranged from 7.56 ± 2.65 to 370.99 ± 105.42 mm^3 for groups of 4 and 9 months of gestation, respectively. The results showed that the ascending aorta volume as a function of fetal age was expressed by the quadratic regression $y = 373.1 – 43.38x + 1.30x^2 ± 24.51$ ($R^2 = 0.89$; $P<0.001$). The increase in the volume of the aortic arch ranged from 8.84 ± 2.90 to 453.51 ± 125.54 mm^3, according to the quadratic function $y = 513.4 – 58.464x + 1.704x^2 ± 49.254$ ($R^2 = 0.90$; $P<0.001$). The thoracic aorta volume increased from 15.75 ± 8.06 to 1158.01 ± 301.85 mm^3, and modelled the quadratic regression $y = 1376.2 + 154.42x + 4.419x^2 ± 125.5$ ($R^2 = 0.90$; $P<0.001$). It was found that, the sum of the volumes of these aortic segments generated the quadratic function $y = 2262 – 256x + 7.423x^2 ± 212$ ($R^2 = 0.90$; $P<0.001$) (Fig. 4).

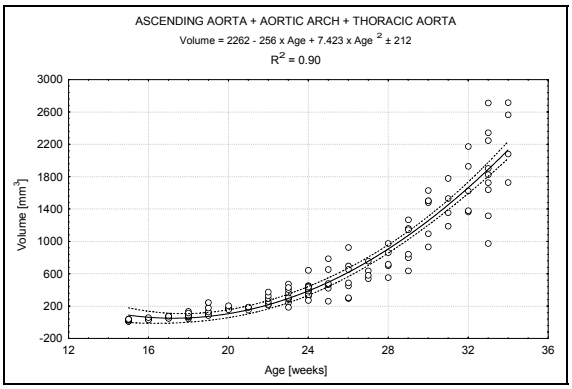

Fig. 4. *Sum of the volumes of the various aortic segments vs. fetal age*

The relationships between volumes of the various aortic segments indicated the following linear regressions (Fig. 5): $y = 3.9647 + 0.8098x ± 12.372$ (for the ascending aorta *vs.* the aortic arch), $y = 4.4740 + 0.3973x ± 20.002$ (for the aortic arch *vs.* the thoracic aorta), and $y = 7.3242 + 0.3225x ± 18.523$ (for the ascending aorta vs. the thoracic aorta). Analysis of the variance revealed that these linear models were highly statistically significant (for each model $P<0.001$). The value of $r ≈ 1.0000$ confirmed a strong positive correlation between volumes of the different aortic segments. Therefore, the regression analysis proved that the volume of the ascending aorta, aortic arch, and thoracic aorta as well, increased proportionally in the ratio 5:6:15 respectively.

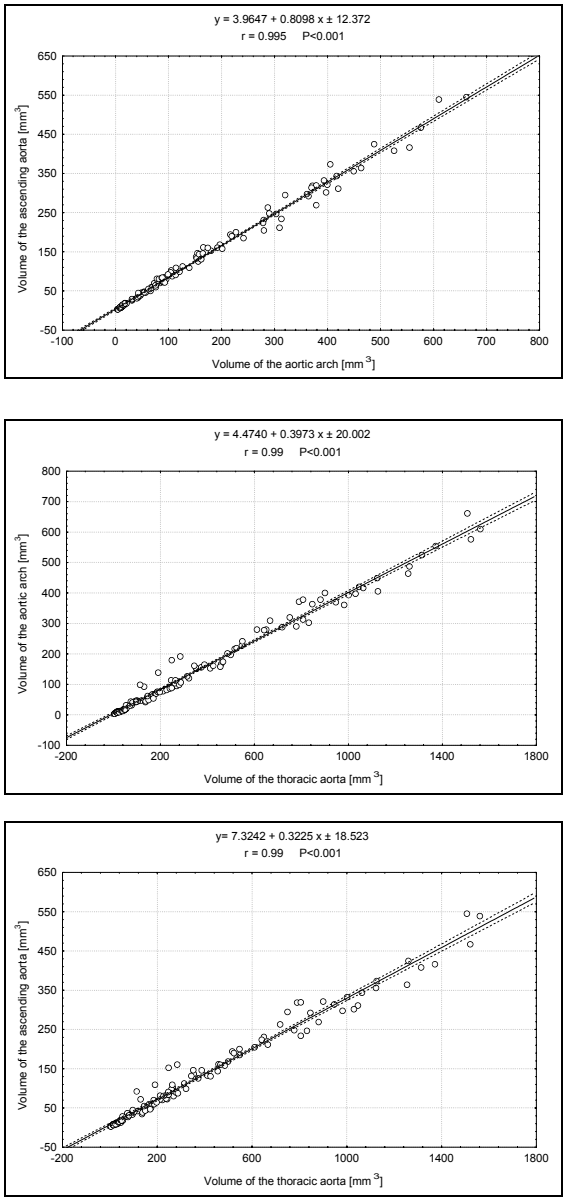

Fig. 5. *Relative proportions of the volumes of the various aortic segments*

DISCUSSION

Reference data for the volumes of various aortic segments in human fetuses and children are still scarce. This study presents novel data regarding the normal volumes of the fetal aorta in its various parts and their relationships to one another. Normal ranges for the volumes of the ascending aorta, aortic arch and thoracic aorta in fetuses were determined as follows: from 7.56 ± 2.65 to 370.99 ± 105.42 mm^3, from 8.84 ± 2.90 to 453.51 ± 125.54 mm^3, and from 15.75 ± 8.06 to 1158.01 ± 301.85 mm^3, respectively. There were no significant differences between genders in volume of the different aortic segments ($P>0.05$). Generally, as

reported by some authors dimensions of the aorta have been found to be independent of gender [10, 11].

The present data show that the volumetric growth of all portions of the aorta related to fetal age increased according to the quadratic function. The volume of the various aortic segments increased as fetal age advanced, according to the following functions: $y = 373.1 - 43.38x + 1.30x^2 \pm 24.51$ for the ascending aorta, $y = 513.4 - 58.464x + 1.704x^2 \pm 49.254$ for the aortic arch, and $y = 1376.2 - 154.42x + 4.419x^2 \pm 125.6$ for the thoracic aorta. It should be noted, that the aortic volume summarized increased in accordance with the model $y = 2262 - 256x + 7.423x^2 \pm 212$. These quadratic functions were the best model for the volumetric growth of the different aortic segments, because the coefficients of determination (R^2) between volume and fetal age reached the values from 0.89 to 0.96 ($P<0.001$). However, Gielecki et al. [8] suggested that the volumetric growth followed the square root function.

This study showed prenatal volumetric growth of the aorta to be uniformly distributed over the different segments, because their relationships to one another were constant. The volumes of the ascending aorta, aortic arch and thoracic aorta were in a ratio 5:6:15, and did not change throughout gestation. This fact seems to disagree with the common opinion that the aortic arch grows with age more than other segments [1, 3, 12]. Hirata [12] performed an anatomical study on the fetal aorta in 20 specimens aged 6-8 months, and claimed that growth rates of the aortic subdivisions were different from one another. Proportions of the aortic arch to the whole aorta increased in fetuses aged 6-7 months. On the other hand, the proportion of the thoracic aorta decreased during this period. However, during the 7-8 month period the proportions of each subdivision were unvaried. Therefore, the present results are in accordance with the findings of Hirata's study only in fetuses aged 7-8 months.

In the professional literature there has been a paucity of quantitative anatomic data concerning the volume of the different aortic segments addressed by this study. The volumes presented of the various aortic segments in human fetuses may serve as reference data for further studies on this subject.

CONCLUSIONS

1. The volumetric growth of the various aortic segments proceeds according to the quadratic regression.
2. The various aortic segments indicate the proportional growth in volume during gestation.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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EFFECT OF LOW LEVEL LASER THERAPY AND HIGH INTENSITY LASER THERAPY ON ENDOTHELIAL CELL PROLIFERATION *IN VITRO* - PRELIMINARY COMMUNICATION

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Summary

Background. A lot of investigators tried to assume an influence of Low Level Laser Therapy (LLLT) on endothelial cells proliferation in vitro. Endothelial cell (EC) plays a key role in the process of tissue repair. The main purpose of this work was to evaluate the influence of LLLT 660 nm, 670 nm, 820 nm and HILT 808 nm on the proliferation of EC.

Material and methods. The tests were done on human umbilical vein endothelial cells (HUVEC). Cultures were exposed to laser irradiation 660 nm and 670 nm at different dosages, power output was 10 – 40 mW, 820 nm with power 100 mW, 808 nm with power 1500 nm. Energy density was from 0.28 to 11.43 J/cm^2. Cells proliferation of control and tested culture was evaluated with colorimetric device to detect alive cells. The tests were repeated 8 times.

Results. We observed good effects of LLLT on alive isolated EC and no effects in experiments on previous deep-frozen cultures. Also HILT stimulated the proliferation of HUVEC.

Conclusion. Endothelial cells play a key role in vascular homeostasis in human. We observed the stimulatory effect of LLLT and HILT on proliferation of HUVEC.

A lot of factors influence the proliferation of EC so is it necessary to continue the experiment with different doses, intensity, cell concentration.

***Key words**:* low level laser therapy (LLLT), high intensity laser therapy (HILT), vascular endothelial cells (EC), HUVEC

INTRODUCTION

Laser is a device enabled to generate electromagnetic radiation characterized by extraordinary properties. The attempts of explanation of it's mechanisms were taken within 40 years of it's application in medicine. It is said, the basics are formed at the cellular and molecular level [1.2.3]. Endothelium plays the significant role in a vascular homeostasis maintenance within the human organism. Growth factors produced by the endothelial cells are responsible for tissues and healing processes as well as neoangiogenesis [4-7]. Favourbale conditions within the blood vessels lead to the rheological blood properties, increase blood flow velocity and decrease the risk of thrombotic embolism.

The absorption and diffusion processes that take place between blood vessels and interstitial tissue are improved at the same time, what reduces tissue ischaemia, stimulates metabolism and thus activates regeneration processes [8-11].

MATERIAL AND METHOD

The experiments were carried out basing on the vascular endothelial cells of HUVEC line. The endothelial cells were placed on 96-dot plater characterized by the hole surface of 0.35 cm^2. To evaluate the influence of the various factors which determine proliferation in the experiments, the number of cell within the culture was being altered, the parameters of radiation

were being changed, living as well as frozen cells were used, single, double and triple exposure of radiation were applied.

First five experiments (1-5) revealed 15 thousand cells within the single hole, 10 thousand in 6th experiment, 5 thousand in 7th and 8th experiment. There were freshly isolated cells used in experiments, except of the 6th sample in which the cells were previously frozen. The cells of 1st-4th and 6th experiment were subjected to single irradiation. In 7th experiment the cells were irradiated three times, two times in case of 5th and 6th experiment. The interval between exposure to radiation amounted 24 hours. The laser irradiation parameters applied in the experiments are introduced in table I. Using the colorimetric detection set of living cells, the proliferation of investigated group (subjected to irradiation) as well as the control group was determined. The cells proliferation was denoted indirectly by means of evaluation of absorption value measured by ELISA factor.

Achieved absorption values are directly proportional to number of living cells. The final relative percentage values were received after correlating the particular results with mean value achieved within the control group.

Table 1. *Laser irradiation parameters applied in the experiments*

Number	Wavelenght	Power	Eds – Power Density
Experiment1	660 nm	40 mW	1 - 5.5 J/cm^2
Experiment 2	670 nm	20 mW	0.28 – 5.71 J/cm^2
Experiment 3	670 nm	20 mW	2.86 - 11.43 J/cm^2
Experiment 4	670 nm	10mW i 20 mW	2.86 - 11.43 J/cm^2
Experiment 5	670 nm	20 mW	2 - 8 J/cm^2
Experiment 6	670 nm	10mW i 20 mW	2 - 8 J/cm^2
Experiment 7	820 nm	100 mW	2 - 8 J/cm^2
Experiment 8	808 nm	1500 mW	2 - 8 J/cm^2

RESULTS

The examinations were to be carried out in a form of eight examinations. There were three trials of irradiation of energy dose of 1-5.5 J/cm$^{2.}$ power of 40 mW and wavelength of 660 nm introduced in the first experiment. The results are shown in table II. It was affirmed, each energy dose by the constant power of 40mW, stimulates the cells proliferation, in comparison with the control group which was not subjected to irradiation. The most significant increase of proliferation (196.6%) was observed by the energy dose of 4.5 J/cm$^{2.}$ the slightest proliferation (104%) was achieved by the energy dose of 5.5 J/cm^2 in case of the control group.

Table II. *Results of experiment 1*
Tabela II. *Wyniki eksperymentu 1*

Parameters	40 mW									
Eds [J/cm^2]	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5
t [s]	10	15	20	25	30	35	40	45	50	55
Test 1	0.84	0.739	0.939	0.891	1.01	0.978	1.021	0.892	1.301	0.853
Test 2	1.663	1.655	1.039	1.789	1.091	1.776	1.784	1.911	1.144	0.314
Test 3	0.977	1.781	1.279	1.723	1.572	1.412	1.423	1.99	1.611	1.365
Medium	1.16	1.392	1.086	1.468	1.224	1.389	1.409	1.598	1.352	0.844
Control	1.265	0.857	0.767	0.751	0.748	0.796	0.871	0.736	0.681	0.654
%	142.8	171.3	133.6	181	151	171	173.4	196.6	166.4	103.9

There were five trials of irradiation of energy doses within the range of 0.28 – 5.71 J/cm^2 and the power decreased by a half (20 mW), wavelength of 670 nm introduced in the second experiment. The results are shown in table III.

Table III. *Results of experiment 2*
Tabela III. *Wyniki eksperymentu 2*

Parametry	20 mW			
Eds [J/cm^2]	0.28	1.43	5.71	Control
t [s]	5s	25s	100s	
Test 1	1.82	1.95	2.021	1.876
Test 2	1.915	1.943	2.101	2.06
Test 3	1.835	2.043	2.025	1.801
Test 4	1.664	1.922	1.948	1.942
Test 5	1.389	1.75	1.944	1.784
Medium	1.72	1.92	2.01	1.89
Control %	91.25	101.67	106.23	
p	NS	NS	NS	

p – statistical importance
NS – non important

In case of energy dose of 0.28 J/cm$^{2.}$ the proliferation was not as significant as it was noticed in a control group, which was not subjected to irradiation. Energy doses of 1.43 J/cm^2 and 5.71 J/cm^2 produced not large increase of proliferation of cells, which was more significant by the energy dose of 5.71 J/cm^2. After decreasing power of laser irradiation, the proliferation of cells decreased significantly.

Basing on the results of the previous examinations, the energy intensity increased from 2.86 to 11.43 J/cm^2 in a third one, by the same power as well as the wave-

length. Four trials of irradiations were carried out. The results are shown in table IV. The application of energy dose of 2.86 J/cm^2 caused the decrease of proliferation with reference to the control group, which was not subjected to irradiation. Energy doses of 5.71 J/cm^2 and 11.43 J/cm^2 caused not large increase of the cells proliferation. Achieved results were similar and amounted in turn about 104% and 107%. So, the increase of energy intensity by the constant power did not influent the increase of proliferation – the results came out to be worse than it was notices in the first experiment. The change of wavelength from 660 nm to 670 nm may also modify the stimulation of epithelium cells.

Table IV. *Results of experiment 3*
Tabela IV. *Wyniki eksperymentu 3*

Parameters	20 mW			
Eds [J/cm^2]	2.86	5.71	11.43	Control
t [s]	50s	100s	200s	
Test 1	1.083	1.566	1.661	1.311
Test 2	1.257	1.573	1.509	1.624
Test 3	1.314	1.596	1.781	1.773
Test 4	1.339	1.642	1.589	1.407
Medium	1.248	1.594	1.635	1.529
Control%	81.64	104.27	106.93	
p	0.0119	NS	NS	

There were five trials of irradiation of energy doses within the range of 2.86-11.43 J/cm$^{2.}$ power of 10 mW and 20 mW and the wavelength characterized by 670 nm introduced in the fourth experiment. The results are shown in table V.

Table V. *Results of experiment 4*
Tabela V. *Wyniki eksperymentu 4*

Parameters	10 mW			Control	20mW			Control
Eds [J/cm^2]	2.86	5.71	11.43		2.86	5.71	11.43	
t [s]	100s	200s	400s		50s	100s	200s	
Test 1	1.056	1.247	1.331	1.36	0.763	1.453	1.588	0.848
Test 2	1.355	1.792	1.711	1.293	1.373	1.86	1.846	1.297
Test 3	1.122	1.78	1.763	1.072	1.412	1.84	1.99	1.267
Test 4	0.935	1.926	1.86	1.017	1.451	1.827	1.53	1.096
Test 5	-	1.668	1.814	-	1.367	1.673	1.671	0.939
Medium	1.117	1.6826	1.6958	1.1855	1.2732	1.7306	1.725	1.0894
Control %	94.22	141.93	143.05		116.87	158.86	158.34	
p	NS	0.037	0.0039		NS	0.005	0.004	

The application of energy dose of 2.86 J/cm^2 and a power characterized by 10 mW caused the decrease of proliferation with reference to the control group. However the same energy dose but the power of 20 mW caused the increase of cells proliferation – 117% in comparison with the control. The results were not as significant as it was noticed by power of 40 mW. Energy doses of 5.71 J/cm^2 and 11.43 J/cm^2 caused the increase of the cells proliferation by power of 10 mW as well as 20 mW. However, best result was achieved in case of 20 mW – 160% of control group. So, the number of cells within the culture exposured to radiation increased along with the increase of the power of laser irradiation.

Another examination (5) was anticipated to be carried out in a form of double irradiation of the cell culture, using the energy doses within the range of 2 - 8 J/cm^2 with a one-day interval. The results are shown in table VI.

Table VI. *Results of experiment 5*
Tabela VI. *Wyniki eskperymentu 5*

Parameters	20 mW			
Eds [J/cm^2]	2	4	8	Control
t [s]	35s	70s	140s	
Test 1	1.657	1.531	1.912	1.275
Test 2	1.801	1.792	1.926	1.528
Test 3	1.952	1.574	1.771	1.612
Test 4	1.553	1.755	1.671	1.571
Test 5	1.517	1.382	1.547	1.763
Test 6	1.475	1.219	1.097	1.11
Medium	1.659	1.542	1.654	1.477
Control %	112.37	104.45	112.02	
p	NS	NS	NS	

The results were similar to these from examination no.3. i.e. by comparable intensity doses of energy but singe exposure to irradiation. So, the influence of double irradiation on the increase of the cells proliferation of HUVEC line was not revealed. The most significant increase of proliferation (112%) was observed by the energy dose of 2 J/cm^2 and 8 J/cm^2. The application of energy dose of 4 J/cm^2 caused the increase of cell proliferation, which amounted 104% in case of the control group.

There were six trials of irradiation of energy doses within the range of 2-8 J/cm$^{2.}$ by the power of 10 mW and 20 mW performed in the sixth experiment, however the researches were carried out using frozen cells. The results are shown in table VII. The significant difference within the living cell culture after the termination of test was observed – number of cells de-

creased. Decrease of the cells number used in the experiments up to 10 000 – greater distances between cells and smaller possibility of interaction – could influence the results. It was the only test on cells which was previously frozen. Another experiments were carried out basing on freshly separated cells.

Table VII. *Results of experiment 6*
Tabela VII. *Wyniki eksperymentu 6*

Parameters	10 mW			Control	20mW			Control
Eds [J/cm^2]	2	4	8		2	4	8	
t [s]	70s	140s	280s		35s	70s	140s	
Test 1	0.967	0.985	0.947	0.992	1.081	1.076	0.887	1.111
Test 2	0.943	0.976	0.939	0.990	1.014	0.995	0.950	1.083
Test 3	0.993	0.937	0.953	1.004	1.042	0.982	1.114	1.147
Test 4	0.845	1.095	0.938	0.907	1.028	1.156	1.043	1.011
Test 5	0.892	0.835	0.823	0.802	0.997	0.933	1.000	0.925
Test 6	0.619	0.75	0.725	0.776	0.887	0.863	0.852	0.879
Medium	0.877	0.930	0.888	0.912	1.008	1.001	0.974	1.026
Control %	96.13	101.96	97.33		98.26	97.55	94.96	
p	NS	NS	NS		NS	NS	NS	

In next, the seventh experiment, the wavelength within the range of infrared and deeper penetration – 820 nm was applied. Six trials were carried out with power of 100 mW and energy doses within the range of 2-8 J/cm^2. Triple exposure to radiation was performed with one-day intervals. The results are shown in table VIII. The increase of proliferation (128% and 117%) was noticed, as a result of the energy doses of 4 J/cm^2 and 8 J/cm^2 application. However such increase, as it was revealed in the first experiment was not achieved. In this trial the number of cells was decreased up to 5 000 per hole.

There were six trials of irradiation characterized by the wavelength of 808 nm and application of high-intensity laser of a power of 1500 mW as well as energy doses within the range of 2-8 J/cm^2 introduced in another experiment. Double exposure of radiation was performed with a one-day interval. The results are shown in table IX. The high-intensity laser application characterized by the power of 1500 mW caused the increase of endothelial cell proliferation which amounted in turn about 108% (for energy dose of 2 J/cm^2) and 117% (for energy dose of 4 J/cm^2). The increase of proliferation was not noticed by the energy dose of 8 J/cm^2. The dose might have been to great, what inhibited multiplication of vascular endothelial cells.

Table VIII. *Results of experiment 7*
Tabela VIII. *Wyniki eksperymentu 7*

Parametry	100 mW			
Eds [J/cm^2]	2	4	8	Control
t [s]	7s	14s	28s	
Test 1	1.32	2.092	1.739	2.035
Test 2	1.837	1.774	1.751	1.788
Test 3	1.565	1.866	1.639	1.204
Test 4	1.175	2.042	1.705	1.298
Test 5	0.574	1.386	1.643	0.948
Test 6	0.422	0.638	0.472	0.391
Medium	1.149	1.633	1.492	1.277
Control %	89.94	127.84	116.77	
p	NS	NS	NS	

Table IX. *Results of experiment 8*
Tabela IX. *Wyniki eksperymentu 8*

Parameters	1500 mW			
Eds [J/cm^2]	2	4	8	Control
t [s]	2.3s	4.6s	9.3s	
Test 1	2.027	2.031	1.537	1.938
Test 2	2.126	2.12	2.003	1.869
Test 3	2.071	2.189	1.81	1.877
Test 4	2.01	2.279	1.793	1.848
Test 5	1.78	2.039	1.769	1.795
Test 6	1.697	2.015	1.466	1.527
Medium	1.952	2.112	1.730	1.809
Control %	107.90	116.76	95.61	
p	NS	0.0047	NS	

DISCUSSION

The photobioactivation phenomenon, i.e. energy absorption by photoreceptors needs to occur to enable the laser irradiation to modulate the cellular processes. It is possible to observe the laser irradiation absorption (in microscale) by the components of respiratory chain (inter alia: cytochrome oxidase, NAD), what causes numerous of photochemical and photobiological changes. The change of oxidation-reduction state within mitochondria as well as cytoplasm follows. The irradiation within the visible range (red) and near infrared which influences these structures contributes to the synthesis modulation of DNA and RNA, what further causes the cells proliferation [3]. Many researchers made an efforts to evaluate the influence of LLLT on *in vitro* cell cultures. The research works concern usually the fibroblast [12-18] and osteoblasts cultures [19-21]. Not many works are devoted to the effect of laser

irradiation on the vascular endothelial cells [22.23]. Schindl'a et al. [22] evaluated the effect of endothelial cells HUVEC irradiation by laser beam characterized by the wavelength of 670 nm. It was affirmed, the energy doses within the range of 2-8 J/cm$^{2.}$ by the constant power of 20mW/cm^2 stimulate the cells proliferation with comparison to the control group, which was not subjected to irradiation. The increase of proliferation was achieved, significantly stressed by the power intensities of 20 and 65mW/cm$^{2.}$ constant energy dose of 8J/cm^2 and variable power within the range of 10-65mW/cm^2. The research works carried out by Kipshidze et al. [12] evaluated the influence of LLLT characterized by the wavelength of 632.8nm (helium neon laser) on the growth of smooth muscles cells, fibrolasts as well as cardiomyocytes *in vitro* and also on the secretion of VEGF. The cells were subjected to irradiation using the energy doses of 01-6.3J/cm^2. The increase of VEGF secretion was observed; the most significant for smooth muscle cells by a dose of 0.5J/cm$^{2.}$ for fibroblasts by 2.1J/cm^2 as well as for cardiomyocytes by a dose of 1.05J/cm^2. The maximal increase of endothelial cells growth was also observed by irradiation characterized by a dose of 1.05J/cm^2 for smooth muscle cells as well as the growth of fibroblasts after a dose of 2.1J/cm^2 application. Khadra et al. [13] examining the influence of lasertherapy on the human fibroblasts proliferation and searching for the optimal irradiation parameters, underlines the effect of recurrent stimulations, in order to achieve the profitable effects of proliferation. Single exposure to irradiation did not meet the expectations.
Power as well as a dose affect the proliferation of irradiated fibroblasts. The Authors underline, higher doses lead to inhibition of cellular processes. Kreisler M et al. [18] examined the influence of low-power laser irradiation on fibroblasts proliferation using laser diode characterized by the wavelength of 808 nm, power of 10 mW and the energy dose of 1.96 J/cm^2-7.84 J/cm^2. The number of irradiations oscillated from 1 to 3. The proliferation was denoted after 24. 48 and 72 hours after the exposure to irradiation. The statistically significant increase of proliferation was denoted after 72 hours from irradiation.

CONCLUSIONS

Nowadays neoangiogenesis presents widely analyzed marker of healing of tissues, thus the examinations concerning methods of tissues proliferation increase are needed. It will help to develop the blood vessels network in case of treatment of hard-to-heal wounds.

As it follows from above mentioned results of examinations, the low-intensity laser irradiation stimulates the cells proliferation *in vitro* as well as causes the increase of secretion of angiogenous factors. Our examinations revealed, that the best results were achieved in case of lasers emitting radiation of 660 nm, the power characterized by 40 mW and the energy dose from 1 to 5J/cm^2. Increase of dose above the value of 5J/cm^2 inhibited proliferation. Number of cells, radiation power as well as fresh cell cultures influent the results of experiment. The process of cells freezing decreased the effects of proliferation in comparison with the same parameters of fresh cells irradiation. There was high-intensity laser used in at the final experiment that was carried out by our team. The examination revealed increase of cell proliferation, however the results were not such profitable as it was given in the first test. Another experiments need to be done to confirm our theses.

The researchers are unanimous, the value of proliferation is dependant on applied radiation parameters. To recapitulate, it is need to be affirmed, the low-intensity laser radiation causes the increase of vascular epithelium cells proliferation derived from fresh cultures. Increase of proliferation of vascular epithelium cells was also observed after the high-intensity laser application. Increase of proliferation was not revealed in a course of the sixth experiment, in which the exposure to irradiation concerned decreased number of cells – 10 thousand in a hole – it was the only one batch of cells derived from previously frozen cells. The continuation of tests is needed to evaluate the optimal conditions of the experiment as well as parameters of laser irradiation which stimulate the growth of vascular endothelial cells *in vitro*, what will contribute to the find out the basics of wounds healing process more accurately.

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CASE REPORT / PRACA KAZUISTYCZNA

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BODY WEIGHT SUPPORT DURING TREADMILL THERAPY IN PATIENTS AFTER SCI – CASE STUDY

SYSTEM DYNAMICZNEGO ODCIĄŻENIA W TERAPII CHODU NA BIEŻNI U PACJENTA PO URAZIE RDZENIA KRĘGOWEGO – PREZENTACJA PRZYPADKU

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Summary

Introduction. Body weight support therapy is a concept of rehabilitation that uses an external device to support a percentage of the patient's body weight allowing them to perform a variety of therapeutic activities in an upright and safe environment. Typically used with Neurological Pathologies, the patient's body weight is supported between 20-40% to assist with developing proper gait patterns and improvements in cardiovascular and muscular endurance with less physical demand. The ability to initiate exercise early in the rehabilitation process can benefit the patient by allowing development of neural pathways through muscular patterning. The purpose of our study was to present a case study of a young patient – male after spinal cord injury at the level T12 who had gait therapy on a treadmill in dynamic unweighting. He was admitted to The Rehabilitation Department 5 months after injury and he had one month training.

Material and methods We supported his weight on a level of 20%, time of therapy was dependent of patients capacity, we started from 5 minutes and stopped at 30 minutes. We used special scales for evaluation of patients mobility and disability: ASIA scale, WISCI II, TWT, spirometry, HR and others.

Conclusions. The main benefits of this kind of therapy are: the increase of muscle strength of hip adductors and quadriceps bilaterally, the increase of time without fatigue, we didn't observed any complains from cardiac system.

Streszczenie

Wstęp. Dynamiczne odciążenie, czyli system odciążenia pacjenta podczas reedukacji chodu na bieżni lub na otwartej przestrzeni (korytarz) jest systemem rehabilitacji, w którym wykorzystuje się urządzenia do podtrzymania masy ciała pacjenta, aby umożliwić pacjentowi wykonywanie ćwiczeń w pozycji wyprostowanej, z dużym poczuciem bezpieczeństwa. Ten system terapii zwiększa możliwości funkcjonalne pacjenta z niekompletnym urazem rdzenia kręgowego, niektórymi chorobami neurologicznymi, po urazie czaszkowo-mózgowym. W schorzeniach neurologicznych stosuje się odciążenie w zakresie 20-40% masy ciała, aby umożliwić wykonanie prawidłowego wzorca chodu, poprawić wytrzymałość mięśniową oraz zmniejszyć obciążenia krążeniowo-oddechowe. Wczesne rozpoczęcie reedukacji chodu u pacjentów ze schorzeniami neurologicznymi może przynieść korzyści w postaci stymulacji szlaków nerwowych i rozwoju prawidłowych wzorców ruchowych.

Celem pracy była wstępna ocena miesięcznej terapii chodu na bieżni w systemie dynamicznego odciążenia u pacjenta po urazie rdzenia kręgowego.

Materiał i metoda. Przedstawiono przypadek pacjenta lat 32 po urazowym uszkodzeniu rdzenia kręgowego na poziomie Th12. Terapię rozpoczęto 5 miesięcy po urazie. Stosowano odciążenie 20% i czas terapii uzależniony od możliwości pacjenta, początkowo pacjent tolerował sesje po 5 minut, ostatecznie po 4 tygodniach terapii, pacjent chodził na bieżni 30 minut dziennie. Ocena pacjenta obejmowała badanie wg skali ASIA, WISCI, ocenę parametrów chodu, spirometrię, badanie ciśnienia tętniczego krwi, AS, ankietę.

Wyniki. pacjent wydłużył dystans chodu, wzrosła siła mięśni przywodzicieli uda z 1 na 2 w skali Lovetta, oraz mięśni czworogłowych uda z 1-2 do 3 w skali Lovetta.

***Key words**:* SCI, BIODEX, body weight support, treadmill, gait
Słowa kluczowe: uraz rdzenia kręgowego, BIODEX, system dynamicznego odciążenia, trening chodu na bieżni, chód

INTRODUCTION

The annual value of spinal cord injuries oscillates within the range from 7000 to 10000 and concerns usually the age group of 16 to 30 year old. The amount of accidents increases along with the development of means of transport as well as technology. Most patients after spinal cord injury (SCI) still want to walk, hence questions directed to physicians, physiotherapists, nurses – what is my chance to walk?

Body weight support (BWS) treadmill training presents one of the method for gait therapy. The method is based on neurobiological principle that part of recovery process depends on neuroplasticity as well as specific and unspecific activity of uninjured nervous system. The training, electrostimulation and pharmacology contribute to improvement of treatment results in patients suffering from acute spine cord injury.

Body weight support therapy applied in patients with adynamia (muscular weakness) improves the gait motor activity within free environment. The examinations concerning therapy will allow to disseminate the method as well as evolve the standards of therapeutic management.

Most patients suffering from SCI aim to accomplish vertical position and a gate, thus new methods of therapy are searched to achieve the intended aim – walking. So far, there is no effective method of treatment that would result in spinal cord regeneration. However, number of the functional therapy methods allow to take efforts to create the same methods of compensation and influence the plasticity of central nervous system. The early tilting the patient to erect position and walk therapy influence the activation of spinal cord generator, prevent from muscular athropy and circulatory as well as vascular complications and also increase the general fitness.

Many researches concerning the body weight support therapy were conducted within last view years in patients suffering from spinal cord and central nervous system injuries, Parkinson's disease and after strokes to evaluate it's superiority in various models application. The questions concern therapy and individual session duration, moment of it's beginning, the velocity of a gait as well as extent of body weight support. The functional tests, neurophysiological examinations including EMG, PW as well as physical efficiency test predominate in methodology. They show the effectiveness of the method and encourage to the further examinations.

Dynamic body weight support during reeducation of walk on treadmill or open environment (corridor) presents a concept of rehabilitation that uses an external device to support patient's body weight that allow to perform a variety of therapeutic activities in an upright and safe environment. The body weight support increases the functional abilities of patients suffering from incomplete spinal cord injury. Typically used with neurological pathologies, the patient's body weight is supported to extent of 20-40% to assist developing of proper gait patterns and improving cardiovascular and muscular endurance with less physical demand at the same time.

The physiological benefits are following:

- Symmetric body weight support of both limbs enabling the equal length of step as well as duration of the limbs support phase what influences the proper biomechanics of gait and regeneration of the proper gait patterns.
- Decrease of parasympathetic activity – reduction of muscular tone (decrease of spastic reflexes) as well as increase of range of movement within joints.
- Decreased load of both circulatory and respiratory system, what is of great importance in case of patients with decreased efficiency, after long-lasting lying and injuries. 40% body weight support lessen the oxygen absorption what makes the long-lasting exercises possible.
- The body weight support enables the therapy at a very early stage after serious injuries, surgical procedures within joints, spinal fracture. It allows to adjust the body weight supporting to medical recommendation.
- Axial traction enables management of a patient suffering from spinal ailments.
- Medical cover in a system of body weight supporting enables rehabilitation of patients suffering from dysequlibrium and dyssynergia decreasing the possibility of fall. Somatosensory stimulation secures the proprioceptive feedback in body location over the base of gate, which also release the correct gait patterns.
- Give the sense of security.

The aim of research project was evaluation of benefits resulting from gait regular rehabilitation supported by treadmill ambulation training in BIODEX system of

dynamic unweighting for patients suffering from spinal cord injury located at the level of Th12.

MATERIAL AND METHOD

The case of 32 year old patient after incomplete spinal cord injury located at the level of Tf12 was introduced. The therapy procedures were applied within 5 months after injury.

Criteria of application: spinal cord injury, sitting without support, lack of contraindications for tilting the patient to erect position, cooperation, motivation, lack of joint contracture and periarticular ossifications. MEP as well as ENG examination were carried out. In MEP examination: lack of tibial muscles response – bilateral, lack of quadriceps muscle of thigh response at right side, trace respond from quadriceps muscle of thigh at the left side. NCS motor: correct amplitudes of motor potential as well as adequate velocity of conductivity within tibial nerves – bilateral, minimal amplitude of motor potential from peroneal nerve at the right side, lack at the left side.

The patient was subjected to rehabilitation supported by GAIT TRAINER 2 treadmill ambulation within 5 days a week, during 4 weeks, starting from 5 minutes (for the sake of intolerance symptom) up to 30 minutes per day (without intolerance symptoms). The intensity of BIODEX body weight support was adjusted optimally to affect the patient in a small extent (20% of the body mass) and to enable the economical gait at the same time

The evaluation included examinations in accordance with ASIA and WISCI scale, encompassed estimation of the gait parameters, spirometry, examination of arterial blood pressure, AS, questionnaire of therapy evaluation.

RESULTS

The initial time of therapy amounted 5 minutes. After this, patient felt tired and the training was interrupted. At first, the active commitment of two therapists was needed. The therapists were moving lower extremities of the patient according to the rhythm of a treadmill. The patient was equipped with orthopedic orthoses preventing from foot drop (photo 1). After few sessions, the patient wearing ortheses was able to move his limbs at a rate of it's possibilities. After 30 minutes, there were no symptoms of physical fatigue (photo 2).

Photo 1. *Beginning of therapy assisted by 2 therapists*

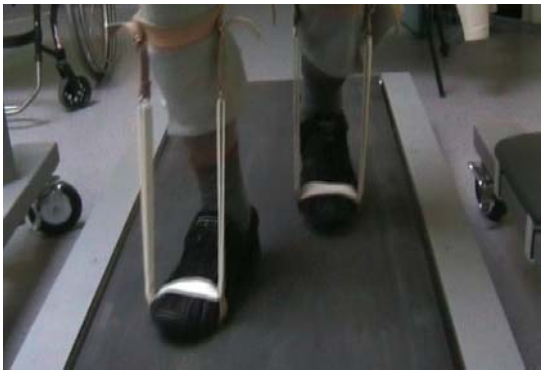

Photo. 2. *At the end of therapy – independent gait with orthopedic ortheses*

The evaluation of patient, according to ASIA scale, before and after therapy is shown in table Ia and Ib.

Table Ia. *Evaluation according to ASIA scale, before therapy*
Tabela Ia.

Level	Motion R	Motion L	Tactile sensation R/L	Algaesthesia R/L
C3				
C4				
C5	5	5	2/2	2/2
C6	5	5	2/2	2/2
C7	5	5	2/2	2/2
C8	5	5	2/2	2/2
Th1	5	5	2/2	2/2
Th2			2/2	2/2
Th3			2/2	2/2
Th4			2/2	2/2
Th5			2/2	2/2
Th6			2/2	2/2
Th7			2/2	2/2
Th8			2/2	2/2
Th9			2/2	2/2
Th10			2/2	2/2
Th11			2/2	2/2
Th12			2/2	2/2
L1			2/2	1/1
L2	4	4	2/2	1/1
L3	1	1	1/1	1/1
L4	0	0	1/1	0/0
L5	0	0	1/1	0/0
S1	0	0	1/1	0/0
S2			1/1	0/0
S3			0/0	0/0
S4-5			0/0	0/0

Table Ib. *Evaluation according to ASIA scale, after therapy*

Level	Motion R	Motion L	Tactile sensation R/L	Algaesthesia R/L
C3				
C4				
C5	5	5	2/2	2/2
C6	5	5	2/2	2/2
C7	5	5	2/2	2/2
C8	5	5	2/2	2/2
Th1	5	5	2/2	2/2
Th2			2/2	2/2
Th3			2/2	2/2
Th4			2/2	2/2
Th5			2/2	2/2
Th6			2/2	2/2
Th7			2/2	2/2
Th8			2/2	2/2
Th9			2/2	2/2
Th10			2/2	2/2
Th11			2/2	2/2
Th12			2/2	2/2
L1			2/2	1/1
L2	4	4	2/2	1/1
L3	3	2	1/1	1/1
L4	0	0	1/1	1/1
L5	0	0	1/1	0/0
S1	0	0	1/1	0/0
S2			1/1	0/0
S3			0/0	0/0
S4-5			0/0	0/0

In accordance with Lovett scale (before therapy): adductor muscles of hip joint: 1/5, quadriceps muscle of thigh R and L: 1/5. The strength of adductor muscles of hip joint increased: 2/5 as well as the strength of quadriceps muscle of thigh (2/5) at the left side and (3/5) at the right side. The patient reported burning sensation at the external side of left calf. Diseased had no sensations around this area before.

In accordance with WISCI scale, before therapy, nine points were admitted (>10 metres, walker, ortheses, without assistant), 12 after therapy (>10 metres, 2 crutches, ortheses, without assistant). The gait time has decreased at the examined distance.

In accordance with gait parameters evaluation: acceptable time period: 5 minutes, average gait velocity: 0,46 m/s, average step length: R 1.01 m, L 0.93 m, support time: R-60%, L-40%.. After therapy acceptable time: 30 minutes and more, average gate velocity: 0.55 m/s, average step length: R 0.66m, L 0.8 m, support time: R-60%, L-40%.

The evaluation of circulatory and respiratory efficiency is shown in table IIa and IIb.

Table IIa. *AS, arterial blood pressure and number of breaths before therapy*

Parameter	beginning	end
Heart rhythm	66	78
Artierial blood pressure (mmHg)	130/90	160/100
Number of breaths	12	24

Table IIb. *AS, arterial blood pressure and number of breaths after therapy*

Parameter	beginning	end
Heart rhythm	60	66
Artierial blood pressure (mmHg)	130/90	130/90
Number of breaths	12	20

The inspiration volume of patient increased; flow-volume parameters did not reveal the significant changes; number of breaths decreased after each session (table 2a).

The patient assessed the sense of security as very good so as the results of therapy. The patient suggested to perform therapy twice a day and revealed a will to cooperation.

DISCUSSION

Shepherd & Carr (1999) show three advantages of the method (20):

1. It enables rehabilitation of the whole gate cycle.
2. It improves the pace as well as the step length.
3. It presents the optimal form of aerobic exercises for patients with spinal cord injury.

Nymark et al. (1998) confirmed that patient suffering from incomplete spinal cord injury reveal effective results of this form of therapy. Gardner et al. (1998), Wernig (1999) proved the improvement of independent gait of a patient even in a few year after injury. Behrman & Harkema (2000) described the range of sensory signals needed for correct gait reciprocal patterns of patients after SCI during rehabilitation supported by treadmill ambulation training:

- The correct response of walking induced by a speed of treadmill.
- The maximal body weight support applied during the standing phase.
- Full extension of trunk and a head.
- Nearly standard kinematics of gate cycle for hip, knee and tarsal joint.
- Time synchronization of extension and load of extremity which adopts the body weight with simultaneous body weight support of the other one.
- Motion of upper extremities during the gate (thanks to the body weight support).

The examinations concern BWS system as a profitable method of therapy in patients after spinal cord injury [3,7,8] as well as after cerebral stroke [4,5]. The best results are achieved within the first 12 months after the injury, when neuroplasticity is the greatest.

Wirz, Zemmon, Rupp et al. described the pulse as well as arterial blood pressure during the gate. The decrease of arterial blood pressure as well as increase of pulse occurred [19]. The improvement of gate parameters and general fitness were noticed [10,14,15]. The authors underline the importance of therapists in a process of correct positioning of the lower extremities as well as walking (one therapist at each side of the body and third one to stabilize the pelvis, if necessary). The motion evoked by therapists is not symmetric, hence new robots for gate automation are being constructed [1,4,5]. It limits the application of Biodex, however if advanced devices are not available and therapists assist carefully, it could be used as a sufficient method of a gait treatment in patients suffering from neurological dysfunctions.

Rehabilitation of gait supported by treadmill ambulation training contributes to improvement of general fitness, gate, increase of muscle force and enables the independent shifting of lower extremities supported by orthoses preventing from foot drop.

CONCLUSIONS

BWS therapy contributed to extension of gate distance as well as enabled crutched supported gate (WISCI scale: 9-12). The strength of adductor muscles of thigh increased from 1 to 2 according to Lovett scale, quadriceps muscles of thigh from 1-2 to 3 according to Lovett scale. The physical efficiency got improved. The patient was satisfied with therapy as well as the high sense of security. Body weight support and rehabilitation of gait simulated on treadmill bring measurable profits to patients after spinal cord injury.

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9. Tabele i ryciny należy ograniczyć do niezbędnego minimum. Tabele numerujemy cyframi rzymskimi. Tytuł tabeli w jęz. polskim i angielskim umieszczamy nad tabelą. Opisy wewnątrz tabeli zamieszczamy w języku polskim i angielskim.
10. Ryciny (fotografie, rysunki, wykresy itp.) numerujemy cyframi arabskimi. Tytuł ryciny w jęz. polskim i angielskim umieszczamy pod ryciną. Opisy wewnątrz rycin zamieszczamy w języku polskim i angielskim.
11. Odnośniki do piśmiennictwa zaznaczamy w tekście cyframi arabskimi i umieszczamy w nawiasie kwadratowym.
12. Streszczenie powinno mieć charakter strukturalny, tzn. zachować podział na części, jak tekst główny. Objętość streszczenia zarówno w języku polskim jak i angielskim – ok. 250 wyrazów.
13. Autor dostarcza pracę na dyskietce oraz 3 egzemplarze, w tym 1 kompletny, zgodny z dyskietką, zawierający nazwiska autorów i nazwę instytucji, z której praca pochodzi (patrz pkt. 5 i 9) oraz 2 egz. przeznaczone dla recenzentów bez nazwisk autorów, nazwy instytucji i innych danych umożliwiających identyfikację.
14. Na dyskietce w odrębnych plikach powinny być umieszczone:
   a) tekst pracy,
   b) tabele,
   c) ryciny (fotografie w formacie BMP, TIF, JPG lub PCX; ryciny w formacie WMF, EPS lub CGM),
   d) podpisy pod ryciny i tabele w formacie MS Word lub RTF.
15. Fotografie powinny mieć postać kontrastowych zdjęć czarno-białych na błyszczącym (ewentualnie matowym) papierze. Na odwrocie należy podać imię i nazwisko autora, tytuł pracy, numer oraz oznaczyć górę i dół.
16. Należy zaznaczyć w tekście miejsca, w których mają być zamieszczone ryciny. Wielkość ryciny: podstawa nie powinna przekraczać 120 mm (z opisami).
17. Piśmiennictwo – tylko prace cytowane w tekście (maksymalnie 30 pozycji) – powinno być ponumerowane i ułożone wg kolejności cytowania, każdy tytuł od nowego wiersza. Pozycja piśmiennictwa dotycząca czasopisma musi zawierać kolejno: nazwisko, inicjał imienia autora (ów) – maksymalnie trzech – tytuł pracy, tytuł czasopisma wg skrótów stosowanych w „Index Medicus", rok, numer tomu i stron. Przy cytowaniu pozycji książkowej (monografii, podręczników) należy podać nazwisko i inicjały imion autorów, tytuł dzieła, wydawcę, miejsce i rok wydania.

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