LEKARZ WOJSKOWY
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INSTYTUT MEDYCZNY
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Informacje dla autorów

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Letter from the Editor-in-Chief

Welcome!

We are embarking on another publishing year. In December 2022, at a meeting of the Editorial and Scientific Committees of our journal, we decided to increase the membership of both committees with the hope of more extensive collaboration and a broader range of published papers. We have ambitious plans for 2023, as we intend to publish themed issues. They will not, however, be fully dedicated to a single branch of medicine. The plan is to include a legal and historical section, conference reports and interviews in each issue.

In the first issue of the 101st volume of Military Physician we feature a paper discussing the use of the femtosecond laser in ophthalmology, an original ENT paper on a new method of assessing hearing improvement in patients with sudden idiopathic hearing loss, articles on molecular biology and interesting cases from the Nephrology department.

Please also see our interview with the head of the Military Institute of Medicine – National Research Institute, commemorating 20 years of the institute's activity, presenting the institute's development, achievements to date and the strategy for the coming years.

I hope you have an interesting read, and please feel free to submit your new papers.

Prof. Bolesław Kalicki MD, PhD

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HISTORY OF MEDICINE AND THE MILITARY HEALTH SERVICE

REPORTS

INTERVIEW

APPLICATION OF THE FEMTOSECOND LASER IN OPHTHALMOLOGY

Zastosowanie lasera femtosekundowego w okulistyce

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Abstract: The launch of femtosecond laser technology has been an unquestionable milestone in the development of modern medicine. This laser has been successfully implemented in ophthalmology, where it helps during various surgical procedures. Although this technology may still be mainly associated with refractive surgery, it has proved to have dozens of applications in many different branches of ophthalmology. The femtosecond laser has not only been used in laser vision correction procedures, but also in corneal transplantation, keratoconus treatment and cataract surgery. This article discusses the most important applications, effectiveness and safety of femtosecond assisted procedures in modern ophthalmic surgery.

Keywords: femtosecond laser, corneal refractive surgery, presbyopia, cataract surgery, laser in situ keratomileusis.

Streszczenie: Wprowadzenie na rynek lasera femtosekundowego było niewątpliwym kamieniem milowym w rozwoju nowoczesnej medycyny. Laser ten znalazł zastosowanie także w okulistyce, gdzie wykorzystywany jest podczas wielu różnych procedur zabiegowych. Mimo że technologia ta może nadal kojarzyć się głównie z chirurgią refrakcyjną, to okazało się, że ma szereg użytecznych zastosowań także w innych gałęziach okulistyki. Laser femtosekundowy, znany z zabiegów laserowej korekcji wzroku, jest coraz częściej wykorzystywany m.in. podczas transplantacji rogówki, w leczeniu stożka rogówki oraz w trakcie operacji zaćmy. W niniejszym artykule omówiono najważniejsze zastosowania, skuteczność i bezpieczeństwo lasera femtosekundowego w nowoczesnej chirurgii okulistycznej.

Słowa kluczowe: laser femtosekundowy, chirurgia refrakcyjna rogówki, starczowzroczność, chirurgia zaćmy, laser in situ keratomileusis.

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Introduction

Developed in the United States at the end of the 20th century, femtosecond laser technology found its way into medical applications relatively quickly, particularly in ophthalmic surgery [1]. Femtosecond lasers use infrared radiation, which is barely absorbed by the translucent optical media of the eye, such as the cornea and the lens.

The operating principle of the femtosecond laser is based on the emission of light pulses lasting only a few hundred femtoseconds (10-15 s), with a wavelength of 1053 nm and a beam diameter of 0.001 mm. Importantly, the beam does not adversely affect neighbouring tissues [1]. Unlike argon and excimer lasers, which use photocoagulation and photoablation respectively, the operation of femtosecond lasers is based on the photodisruption effect. It is a process involving the release of free electrons and ionised molecules, which, in the form of microcavitational bubbles and acoustic shock waves, can cause the disruption and separation of tissue fragments without traumatising adjacent layers [1]. A wound excised in this way has smooth and even edges, which significantly speeds up the healing process and ensures predictable post-operative results. Thanks to the unique properties of the femtosecond laser, it is possible to make extremely precise and reproducible incisions of various shapes at any depth in many types of tissue.

The practical application of femtosecond laser technology is the result of years of research by the physicists Gerard Mourou and Donna Strickland, who were honoured with the 2018 Nobel Prize in Physics for developing chirped pulse amplification.

This article reviews the current literature on femtosecond laser applications in modern ophthalmology.

Application of the femtosecond laser in refractive surgery

Femtosecond laser technology entered the commercial market with its launch in 2001 under the trade name IntraLase™ and instantly attracted great interest from the medical industry [2]. The process was aimed at producing a corneal flap with a femtosecond laser during LASIK (*laser-assisted in situ keratomileusis*) procedures and replacing the mechanical microkeratomes previously used for this purpose.

With its short rehabilitation period, safety and rapid stabilisation of visual acuity, FemtoLASIK (femtosecond LASIK) shows significant advantages over older refractive procedures [3]. Since its launch, the technology has been significantly developed and refined. The latest equipment of this type achieves frequencies as high as 2 MHz, enabling the corneal flap to be cut out in just a few seconds. The increased operating frequency minimises the amount of energy delivered to the tissues and the negative pressure needed to stabilise the eyeball (much lower than with the microkeratome), which significantly increases the safety of the procedure, enabling a better sense of contrast [4] and ensuring fewer spherical aberrations compared to LASIK.

Corneal flap

In the LASIK method, introduced in 1990, the flap was produced using mechanical devices called microkeratomes. The implementation of the femtosecond laser undoubtedly set a new direction in the development of global ophthalmology and refractive surgery in particular. FemtoLASIK is currently one of the most commonly performed refractive procedures in developed countries. The considerable interest for this method is mainly due to its safety and predictability. The procedure involves a femtosecond laser-generated flap being lifted by the surgeon, and the stroma below is then remodelled with an excimer laser beam. Finally, the flap is placed back in place. With femtosecond laser technology, the created flap can vary in shape, depending on the surgeon's preference and technique. In addition, the angle at which the lateral cut is made, the width, angle and position of the flap hinge can all be changed for better precision and has a positive effect on the stability of the procedure. The femtolaser offers adjustable lateral angle, reducing the risk of potential ingrowth of the epithelium under the flap. Preparing a corneal flap with the femtosecond laser takes no longer than a dozen seconds, and since the flap can be thin (less than 110 microns thick), it is possible to perform the flap procedure on patients with relatively thin corneas as well. This also lowers the risk of a buttonhole or a free cap. The laser method also reduces the occurrence of dry eye symptoms in the postoperative period.

The study by Keziran et al. [5], comparing the results of FemtoLASIK and LASIK procedures on a group of 375 eyes, proved that the FemtoLASIK group achieved more predictable flap thickness, lower postoperative astigmatism values and lower traumatisation of the corneal epithelium. Differences in postoperative uncorrected distance visual acuity (UDVA) between the two groups were not statistically significant. The proportion of patients with full (20/20) UDVA on day 1 after surgery was similar in all groups. After 3 months of follow-up, about 99% of patients had achieved a UDVA of at least 20/40 and about 70% of them 20/20 or better. FemtoLASIK, however, has proved much more predictable in terms of spherical equivalent (SE) values. An SE +/- 0.5 D was achieved by 91% of the patients who underwent surgery, compared to 74% and 73% in the LASIK group, respectively, depending on the microkeratome used. Chen et al. [6] also confirmed the superiority of the flaps produced with the use of the femtolaser over those cut out with manual microkeratomes.

Lenticule

More recent methods of laser vision correction, such as FLEx® (femtosecond lenticule extraction) from 2006 and ReLEx®/SMILE® (refractive lenticule extraction/small incision lenticule extraction) from 2010, completely eliminate the need for an excimer laser, while the ReLEx® method does not require the creation of a corneal flap. This helps to avoid potential complications associated with the flap, including a hole in the flap (buttonhole) and the ingrowth of the epithelium under the flap.

In the FLEx® method, the laser cuts a lenticule in the stroma of the cornea, which is then extracted through the standard circular cut known from the FemtoLASIK technique.

With the ReLEx® technique, on the other hand, the lenticule is extracted through a small 2-4 mm port. The main advantages of the SMILE® method are the high efficiency of the procedure, greater biomechanical protection of the cornea by significantly reducing the size of the vertical incision and a lower risk of dry eye syndrome or corneal ectasia compared to the FemtoLASIK method [7]. Research shows that both FLEx® and SMILE® have good and stable results in refractive parameters, visual acuity, safety profile and predictability [8].

Liu et al. [9] have demonstrated that SMILE®-treated eyes have a lower induction of higher-order aberrations and higher contrast sensitivity than eyes treated with FemtoLASIK-equivalent myopia correction.

The study found that UDVA on day 1 after surgery was better in the FemtoLASIK group, but at the 6-month follow-up there were no statistically significant differences between the FemtoLASIK and SMILE® groups. The UDVA of 20/20 or better was achieved by 99% and 96% of eyes, respectively. The two groups were also not significantly different in terms of postoperative SE values.

Application of the femtosecond laser in presbyopia correction

Presbyopia is an age-related progressive decrease in the ability of the lens of the eye to accommodate, resulting in asthenopic symptoms for near and intermediate distances. Although glasses remain the most popular method of presbyopia correction, the Presbyond® method (FemtoLASIK with simultaneous creation of monovision) allows at least acceptable near visual acuity to be achieved in properly selected patients. It is the preferred procedure for patients over 38 years of age and can be performed in patients with myopia up to -8.0 D, hyperopia up to +3.5 D, with astigmatism up to 2.0 D, and in patients with normopia requiring only an improvement in near vision. An additional prerequisite for this correction method is the positive monovision tolerance test during the qualification examination. The procedure plan is made individually for each patient to produce clear binocular vision at far, near and intermediate distances.

Another method for the correction of presbyopia is the Intracore® procedure, introduced in 2009, based on Bausch&Lomb's FEMTEC® femtosecond laser technology. The technique involves making 5 circular, concentric incisions with the femtolaser in the stroma of the cornea of the non-dominant eye, the widest of which is 3.5 mm in diameter. After the procedure, the cornea becomes multifocal and the central keratometry value gradually changes, stabilising at around 1 D after 12 months. The method has low invasiveness, as it does not involve epithelial disruption or reduction in the central corneal thickness. Significant complications include the halo effect occurring after one year in 36% of patients, and the lack of improvement in near visual acuity reported in 12% of patients [10], making this method rarely used today.

Research is being conducted around the world to develop new methods of correcting presbyopia using the lenticule taken during the SMILE® procedure.

One promising technique is the PEARL (*presbyopic allogenic refractive lenticule*) method, described in 2017 by Jacob et al. [11], which involves implanting an appropriately prepared lenticule fragment obtained from a myopic donor during the SMILE® procedure into a pocket created with a femtosecond laser in the recipient's cornea. The use of the inverted flap method to create a pocket prevents complications and promotes healing. Lenticule implantation modifies the recipient cornea in such a way that it obtains the characteristics of a multifocal plane.

The researchers described a group of several normal-sighted patients with presbyopia who received an implant in the non-dominant eye. Appropriate serological tests to exclude the presence of surface antigens in donors were performed prior to lenticule collection. After surgery, uncorrected near visual acuity improved by 3 to 5 Jaeger rows in the study group. It should be emphasised that the baseline full distance visual acuity did not change. Patients were satisfied with the results of the procedure during the six-month follow-up period. They did not need spectacle correction for near or intermediate distances, and did not report symptoms of dysphotopsia, halo or glare.

Further studies on a larger patient group and with a longer follow-up period are needed to definitively confirm the efficacy of this method in the correction of presbyopia.

Application of the femtosecond laser and lenticule in the treatment of keratoconus

Keratoconus is a degenerative disease characterised by progressive thinning and bulging of parts of the cornea, resulting in irregular astigmatism and lower visual acuity.

Crosslinking (CXL) using riboflavin and UVA light is recognised as an effective and safe treatment option for keratoconus in eyes with a central corneal thickness of at least 400 micrometres [12]. To 'thicken' corneas that are too thin, hypoosmolar riboflavin solutions and contact lenses are used to protect the endothelial layer from radiation. Such modifications are, however, associated with a reduction in the effectiveness of the CXL procedure [13]. The attention of researchers has therefore focused on the possible use of allogeneic lenticula obtained during the SMILE® procedure. In a 2015 study, Sachdev et al. [14] demonstrated that in the Tailored Stromal Expansion CXL procedure the corneal thickness of a keratoconus patient can be increased by directly placing an allogeneic lenticule on the corneal stroma of the recipient. The authors found no serious intraoperative or postoperative complications. Optimum postoperative results and the inhibition of keratoconus progression after 6 months of follow-up are evidenced by stable pachymetry and keratometry values. No significant loss of endothelial cells was observed in this process.

The success of the cross-linking procedure with the use of lenticula was evidenced by the presence of a line of demarcation in each patient's stroma visible on AS-OCT.

Another example of the successful use of lenticula in the treatment of keratoconus was a study published in 2015 by Ganesh et al. [15] which assessed the FILI (*femtosecond intrastromal lenticular implantation*) procedure in combination with CXL in patients with progressive keratoconus and contact lens intolerance.

The study examined, among other things, the effect of the procedure on the shape of the corneal surface, refractive parameters, and the overall safety of the procedure. Six previously cryopreserved lenticules – alloimplants sourced from hyperopia patients operated using the SMILE® method – were implanted to achieve the cone flattening effect. The lenticules were soaked in 0.25% riboflavin and appropriately dissected, obtaining a donut-cake shape to increase the thickness of the recipient's peripheral cornea with a flattened centre (including the top of the cone). The implants were inserted into stromal pockets created with the femtosecond laser and treated with UV light.

After a 6-month follow-up period, there was a significant improvement in both the uncorrected and corrected visual

acuity at far distance, reaching up to several rows on the logMAR scale, and there was a statistically significant improvement in spherical equivalent values. Also described was a generalised flattening of the mean keratometry with a reduction in the higher-order aberrations and a decrease in the asphericity parameters (Q-factor) due to a more regular anterior corneal surface. No significant intra- or postoperative complications were found. Further randomised trials are required to confirm the efficacy of this alternative keratoconus treatment.

Corneal tunnels

The femtosecond laser technology enables the creation of stromal tunnels (pockets) of various sizes and shapes within the central 6-mm corneal zone. An individually selected two-piece ring-shaped implant is inserted to correct myopia up to -3.5 D, keratoconus, or iatrogenic ectasia after keratorefractive surgery [1].

The unquestionable advantages of such rings include the stabilisation or inhibition of keratoconus just a few months after surgery and the option to remove the implants if necessary. The postoperative effect can be modified, such as by replacing the segment with a different one or by changing its position.

Piñero et al [16] demonstrated that the insertion of rings into the tunnels created with a femtosecond laser produced comparable results in visual acuity and refraction to those created with a mechanical expander; moreover, the laser method proved superior in terms of postoperative higher-order aberration.

Hashemi et al. [17], on the other hand, noted that combining this procedure with a tissue enhancement intervention, such as crosslinking, improves UDVA in those keratoconus patients who do not tolerate correction with contact lenses. The risk of severe complications of this procedure is approximately 1%. These include corneal perforation, infectious keratitis and implant protrusion.

Application of the femtosecond laser in cataract surgery

Femtosecond laser-assisted cataract surgery (FLACS) is the most recent technique for cataract surgery.

The first procedure of this type was performed in 2008 in Hungary by Nagy [18]. Due to its precision and reproducibility, this method has gained popularity quite rapidly throughout the world. Femtosecond laser technology reduces the energy required and allows the laser to be applied at a precise depth with minimal damage to adjacent tissue. All commercially available FLACS platforms are equipped with an optical coherence tomography (OCT) imaging system or Scheimpflug camera that precisely guides the laser beam.

To properly direct the laser beam, the operated eye must be properly fixed using a vacuum docking system. The platforms available on the market use different technical solutions. Some have a special applanation lens and a suction system (LenSx®, Alcon and Victus, Bausch& Lomb), others a fluid-filled suction ring (Catalys®, Johnson&Johnson and LensAR) [19].

The main advantages of femtosecond laser cataract surgery are the extreme precision and reproducibility of the location, size and depth of the corneal incisions made, the predictability of the size and location of the capsulotomy, and the reduction of ultrasound energy needed to emulsify the lens nucleus by pre-cutting it into smaller fragments, which reduces the negative impact on the endothelium.

Despite the above advantages, published meta-analyses have not shown a statistically significant benefits of laser-assisted surgery over conventional manual phacoemulsification performed by an experienced surgeon [20]. However, the use of the femtosecond laser in patients with reduced endothelial cell counts has been confirmed to be more beneficial than conventional cataract surgery, one reason being that it reduces corneal traumatisation because less ultrasound is needed to fragment the lens nucleus [21].

The femtofacemulsification procedure is limited by the high cost of the procedure due to the purchase and operation of the femtolaser platform.

Femtosecond laser in penetrating keratoplasty

Modern keratoplasty is another example of the incredible progress that has been made in ophthalmology worldwide since 1905, when E. Zirm performed corneal transplantation for the first time. The femtosecond laser appears to be a promising tool in achieving improved post-operative outcomes, enabling greater precision during trepanation and therefore better alignment and stability of the transplanted cornea.

This increases the contact area between the flap and the recipient tissue, positively influencing the reduction of postoperative astigmatism and the healing process [22]. The use of the femtosecond laser in penetrating keratoplasty builds on a concept proposed in the 1960s by J. Barraquer, with different graft thicknesses in the anterior and posterior cornea [23].

Research has shown that transplantation using one of the two most commonly performed laser trepanation patterns today, i.e., top-hut and zigzag, resulted in better corrected distance visual acuity (CDVA) with less post-operative astigmatism and faster suture removal than in conventional keratoplasty. It should be noted that the failure and rejection rates are similar for both groups.

In a study assessing the top-hut and zigzag laser methods, comparable values were achieved for visual acuity, refractive parameters, endothelial cell count and healing time [24]. On the other hand, laser penetrating keratoplasty using the mushroom technique has proven to be an effective therapeutic option, especially in children. This method achieved optimal refractive values and reduced postoperative astigmatism while at the same time

minimising the risk of rejection due to the relatively smaller posterior portion of the transplanted flap [25].

Femtosecond laser in lamellar keratoplasty

Selective transplantation of corneal layers has numerous advantages, one of the most important being the reduction of the immune response (and risk of rejection) due to the transplantation of a smaller amount of foreign tissue. Thanks to its ability to produce predictable and precise incisions in any configuration and at different depths, the femtosecond laser is also considered a useful tool in lamellar keratoplasty. In deep anterior lamellar keratoplasty (DALK), approximately 95% of the anterior portion of the cornea is removed, with the innermost portions of the cornea and Descemet's membrane and endothelial cell layer left in place [26]. Descemet's membrane can be separated from the anterior layers of the stroma by administering air during the "big-bubble" procedure [27]. The difficulty is in finding a suitable air injection method that can effectively separate the corneal layers without accompanying damage to Descemet's membrane along with the need to convert the surgical procedure to penetrating keratoplasty.

The latest developments using femtosecond laser technology allow the surgeon to create a tunnel in the cornea through which a special cannula is passed until the desired depth is reached. Performing such a high-precision procedure is possible through simultaneous intraoperative visualisation of the corneal layers by optical coherence tomography (OCT) [27].

Buzzonetti et al. [28] compared femtosecond laser-assisted DALK with a conventional procedure of this type in 20 children and reported that in the group of eyes that underwent femtolaser surgery, the alignment of donor and recipient tissues was very precise due to the equal laser-assisted lateral incisions and resulted in a smaller postoperative spherical equivalent and faster healing. The introduction of posterior lamellar keratoplasty has undoubtedly revolutionised corneal surgery, and the technique is now widely used in ophthalmic centres around the world for the treatment of advanced Fuchs' dystrophy or other corneal endothelial diseases. The corneal endothelium is made up of highly specialised cells that play a key role in maintaining corneal translucency, despite not being able to regenerate. Transplantation of these cells, either with a small amount of corneal stroma in Descemet's stripping endothelial keratoplasty (DSEK/DSAEK) or with Descemet's membrane alone as their carrier in Descemet's membrane endothelial keratoplasty (DMEK) in particular, helps to achieve excellent postoperative visual acuity [29]. A key stage of the operation is the removal of the recipient's malfunctioning Descemet's membrane. This is usually done by hand.

Sorkin et al. [30] published a paper in 2019 comparing descemetorexis, i.e., removal of an abnormal Descemet's membrane, manually and using a femtosecond laser. It states that the major advantages of the laser method include, first and foremost, achieving precise centring, as well as the desired shape and size of the membrane area to be removed.

Future applications of the femtosecond laser

Extensive work is taking place to miniaturise and increase the mobility of laser platforms. In the near future, it should be possible to perform femtolaser-assisted primary posterior capsulotomy and to apply appropriate markings to the lens capsule for perfect alignment in toric intraocular lens implantation. Ultimately, there may also be a chance for post-operative modification of the power of artificial intraocular lenses using a femtolaser beam.

Conclusions

Femtosecond laser technology has revolutionised modern ophthalmic surgery. It has created a range of new opportunities and has enabled not only more efficient and safer performance of previously performed procedures but also the development of entirely new, groundbreaking applications.

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DIFFERENCES IN FLUID THERAPY FOR BURNS INVOLVING CHILDREN IN THE PREHOSPITAL SETTINGS AND HOSPITAL EMERGENCY DEPARTMENT

Odrębności w prowadzeniu płynoterapii w oparzeniach u dzieci w praktyce Zespołów Ratownictwa Medycznego oraz Szpitalnego Oddziału Ratunkowego

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Abstract: Thermal trauma is one of the most severe and most frequent injuries, causing significant mortality in the paediatric population. One of the elements of modern burn therapy is proper fluid therapy. The Parkland rule, well-known to doctors, paramedics and nurses, is used to estimate the supply of fluids in a patient injured as a result of burn, and was designed for adults and its use in the paediatric population may lead to serious consequences in the form of excessive fluid overload (known as fluid creep). Underestimating the volume of infused fluids leads to the development of shock and renal failure. Properly conducted fluid therapy should be carried out on the basis of the burn area, the child's weight and factors determining the increase or reduction in fluid supply according to formula used (coexisting injuries, inhalation trauma, etc.).

Keywords: oedema, burns, paediatric population, fluid therapy, crystalloids.

Streszczenie: Uraz termiczny jest jednym z najcięższych i najczęściej występujących urazów powodujących znaczną śmiertelność w populacji pacjentów pediatrycznych. Jednym z elementów współczesnej terapii oparzeń jest prowadzenie odpowiedniej płynoterapii. Doskonale znana lekarzom, ratownikom medycznym i pielęgniarkom reguła Parkland, służąca oszacowaniu podaży płynów u pacjenta poszkodowanego w wyniku oparzenia, została zaprojektowana dla osób dorosłych i jej zastosowanie w populacji pediatrycznej może prowadzić do poważnych następstw w postaci nadmiernego przeciążenia płynami poszkodowanego (tzw. fluid creep). Niedoszacowanie objętości przetaczanych płynów prowadzi do rozwoju wstrząsu oraz niewydolności nerek. Dobrze zastosowana płynoterapia powinna być prowadzona w oparciu o powierzchnię oparzenia, wagę dziecka oraz czynniki warunkujące zwiększenie lub zmniejszenie podaży płynów według odpowiedniej formuły (współistniejące urazy, uraz inhalacyjny, etc.).

Słowa kluczowe: obrzęk, oparzenia, populacja pediatryczna, płynoterapia, krystaloidy.

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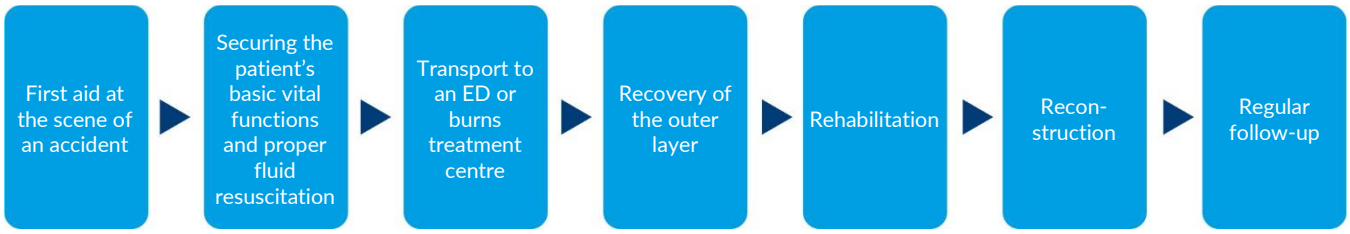
Introduction

Properly managed fluid therapy is crucial to both the recovery and survival of a patient with burns affecting a significant part of the total body surface area (above 20%). Fluid therapy in patients affected by heat, chemicals, electric current or radiation is a component of modern treatment of burn patients and should be started as early as possible after the injury has occurred. Fluid therapy in paediatric burn patients should be based on appropriate fluid therapy formulas so as not to lead to fluid creep. Inadequate fluid therapy can exacerbate the injury, resulting in, for example, an expansion of the ischaemic zone within the burn. Fluid therapy administered to burn patients is a dynamic process that requires continuous evaluation of the approach being implemented.

Systemic response to burns

A burn is defined as damage to the skin and underlying tissues caused by exposure to high temperatures, chemicals, electricity, or radiation. The degree of damage to the skin resulting from thermal trauma depends on the duration of exposure and the temperature of the agent that caused the injury. The physiological response of the body to a burn depends on the total burn surface area (TBSA), its depth, the presence of internal burns, the coexistence of an inhalation burn and/or the inhalation of toxic vapours and the time at which therapy, especially fluid therapy and infection prevention, is implemented. Thermal trauma causes both psychological and physical injury [1, 2].

Figure 1. Care regimen for patients with severe burns.
Rycina 1. Schemat opieki nad pacjentem po ciężkim oparzeniu.



Source: own material.

The systemic response of the child's body to a burn includes cardiovascular, respiratory, metabolic and immunological changes. Changes in the cardiovascular system are caused by an increase in capillary permeability, resulting in the loss of intravascular proteins and the displacement of fluid into the extravascular area, to both injured and healthy tissues. Vascular leakage occurs in soft tissues, such as the skin, muscles, and intestines. The pathophysiology of this phenomenon results from the massive release of inflammatory mediators, i.e., histamine, bradykinin, serotonin, thromboxanes, prostacyclins, prostaglandins and leukotrienes. There is also a secondary release of catecholamines, aldosterone, vasopressin and renin following renal hypoxia. Clinically, this condition is manifested by intravascular volume depletion, soft tissue oedema and a further cascade of catecholamines and cytokinns. As vascular permeability increases, the plasma osmotic response, which is dependent on the escape of proteins into the interstitial space (directly and proportionally), decreases. The massive leakage of fluid into the interstitial space is most strongly expressed in the second hour after the onset of injury. As intravascular plasma fluid leaks out, the volume of the vascular bed also decreases, leading to circulatory decompensation. Locally, oedema exacerbates hypoxia in the surrounding tissues, leading to increased and enlarged necrosis [2, 3].

Initial management of a child suffering from a burn injury

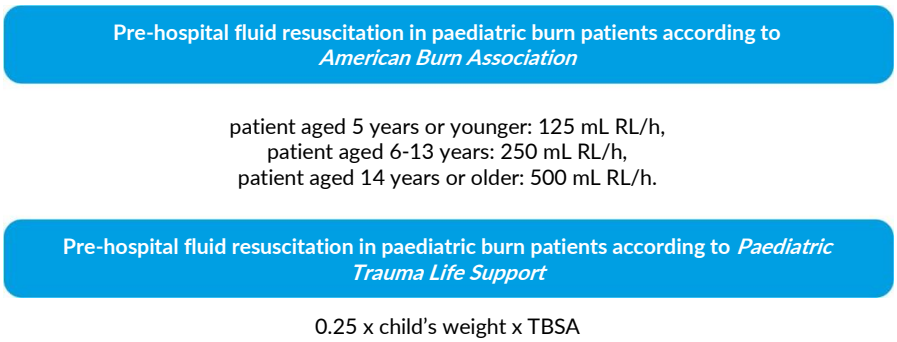
The management of burns is based on the assessment of the capacity of individual systems and the implementation of critical interventions in line with the ABCDE approach:

- Airway - assessment and maintenance of airway patency,
- Breathing and ventilation - assessment of efficiency of respiratory system,
- Circulation and cardiac status - assessment of efficiency of cardiovascular system,
- Disability, neurological deficit and gross deformity - assessment of the state of consciousness and the scale of injuries,
- Exposure, examine and environmental control - trauma examination of the patient and prevention of the development of hypothermia.

When assessing a paediatric patient – using the approach discussed here – we must bear in mind certain anatomical features of the patient, differences in the initial and follow-up examinations, as well as differences resulting from the specific nature of the burn [4, 5] (Fig. 1).

When it comes to assessing airway patency and maintaining it, the main problem that medical staff may encounter is inhalation burn and increasing swelling in the airway, requiring secure protection. A child's airway has a smaller diameter than an adult's, so even a slight increase in swelling significantly impairs airway patency (as airway swelling increases by 1 mm, resistance increases by a factor of 16) [6]. Symptoms indicative of increasing swelling in the child's airway include stridor, increased respiratory effort, tachypnoe, use of accessory respiratory muscles and sternal tightening. The intervention of choice for increasing airway oedema in a paediatric patient should be endotracheal intubation. Once the correct endotracheal intubation procedure has been confirmed, insertion of a gastric tube and gastric decompression is recommended for the intubated child. Children 'swallow' significant amounts of air during crying, which causes the stomach to distend, impairing ventilation. Any child with an inhalation burn should be transported to a burn treatment centre. A procedure to reduce the progression of swelling in the child's airway is to elevate the patient's head by 30 degrees, if there are no contraindications [7]. When assessing the cardiovascular function of a paediatric patient, appropriate fluid therapy should be initiated as soon as possible based on an adequate fluid therapy formula intended for the paediatric patient and a proper assessment of the burned area (i.e., second and third degree burns of the child' body surface calculated as per the Lund and Browder chart or based on the rule of palm for smaller area burns) [8]. When it is not possible to determine the child' weight and TBSA, the American Burn Association recommends starting fluid resuscitation based on the patient's age in the following volumes:

Figure 2. Fluid therapy in the initial management of burn injury in the paediatric patient.
Rycina 2. Płynoterapia we wstępnej fazie postępowania w urazie oparzeniowym u pacjenta pediatrycznego.



Source: own material based on [7].

- patient aged 5 years or younger: 125 mL Ringer lactate (RL)/h,
- patient aged 6-13 years: 250 mL RL/h,
- patient aged 14 years or older: 500 mL RL/h [8].

In the case of burn injury, Paediatric Trauma Life Support (PTLS) recommends starting fluid resuscitation in the initial phase of management calculated using the following formula: 0.25 x child's weight (kg) x TBSA [9] (see Fig. 2. *Fluid therapy in the initial management of burn injury in the patient*).

Formulas proposed by PALS, Luscombe and Owens, the Resuscitation Council UK, or Janus-Młodawska may be useful in estimating the child's weight [10].

Whichever method of pre-hospital fluid supply is chosen, the fluids should be warmed up using available IV fluid warmers; in the absence of dedicated IV fluid warmers, hand warmers can be used, for example. The fluid should be heated to 40-42°C. It is important to remember to properly insulate the container that holds the fluid and the drain [11]. To protect the patient from heat loss, the ambulance compartment should be heated to 28°C [12].

Table 1. Paediatric formulas for estimating fluid requirements after burn injury.
Tabela 1. Pediatryczne formuły służące oszacowaniu zapotrzebowania płynowego po urazie oparzeniowym.

Name	Crystalloid supply	Colloid supply	Glucose supply	Supply method
Cincinnati Rule (younger children)	4 mL/kg/%TBSA + 1500 mL/m2 BSA = Ringer's lactate transfusion volume	12.5g of 25% albumin/L crystalloids in the last eight hours in the first 24 hours of fluid therapy	5% glucose, if needed	Half of the calculated fluid should be transfused within the first eight hours, the other half within the following 16 hours. The composition of the transfused fluids should be changed every 8 hours, i.e. 50 mEq of sodium bicarbonate in the first 8 hours, LR without additions in the following 8 hours, then with the addition of albumin for 8 hours
Cincinnati Rule (older children)	4 mL/kg/%TBSA + 1500 mL/m2 BSA = Ringer's lactate transfusion volume	-	5% glucose, if needed	Half of the calculated fluid should be transfused within the first eight hours, the other half within the following 16 hours.
Eagle's Rule	30 ml/%TBSA + 10% body weight (kg) + 4000 mL/m2 BSA volume of 0.66 sodium chloride transfused	20g albumins/L	5% glucose	The fluid volume calculated using Eagle's Rule should be transfused within 48 hours.
Galveston Rule	5000 mL/m2 TBSA + 2000 mL/m2 BSA = volume of Ringer's lactate transfused	12.5 g 25% albumins/L crystalloids	5% glucose, if needed	Half of the calculated fluid should be transfused within the first eight hours, the other half within the following 16 hours.

Source: own material based on [7].

Fluid resuscitation should be carried out through two large-bore IV accesses. Venous bed cannulation should be attempted as early as possible due to possible swelling. If it is difficult to obtain venous access, placing the access in the area affected by the burn injury is acceptable. Ultrasonography may be a helpful tool in performing cannulation. Intraosseous access is only recommended when IV access is not possible. Improperly performed access in a burn patient may lead to compartment syndrome. Removal of intraosseous access should be attempted as soon as venous access is obtained.

The Emergency Medical Services Team (EMS) should perform an ECG on a paediatric patient with a burn injury resulting from an electric shock. Cardiovascular monitoring should continue for the next 24 hours after the injury due to the risk of cardiac arrhythmias.

Hypoglycaemia and hypoxia may result in impaired consciousness in a child suffering from burns. It is very important to correctly identify the cause of impaired consciousness and treat it properly. In assessing the affected child's consciousness, we must also consider possible alcohol or drug intoxication.

When examining a patient suffering from a burn injury, efforts should be made to stop the burning process, remove clothing, socks, nappies and accurately assess the burn area. All burns must be covered with an appropriate dressing.

During the re-examination, attention should be paid to the following:

- the story behind the occurrence of the injury (circumstances of the incident, type, and duration of exposure to the factor causing the thermal trauma, assistance given at the scene, etc.),
- a full medical history of the patient,
- assessment of the weight of the paediatric patient,
- accurate TBSA determination,
- calculation of the patient's fluid requirements and continued fluid therapy,
- detailed trauma examination,
- securing the thermal trauma with appropriate dressings,
- appropriate analgesia or analgosedation,
- psychological support.

Principles of fluid therapy in paediatric burn patients

Fluid therapy should be started immediately in a paediatric burn patient when the injury covers at least 10% of TBSA. Due to physiological differences, fluid resuscitation in paediatric burn patients should not be based on the rules used for adult patients (e.g., the Parkland rule) [8]. Paediatric patients have a higher surface area to body weight ratio, different fluid requirements and lower glycogen stores. These differences translate into a higher fluid supply in the treatment of burns. Fluid supply according to rules designed for adult patients may be inadequate for paediatric patients. In addition, the rules used in children suffering from burns should include 5% glucose depending on glycaemic falls (two-component rules including glucose supply when needed, i.e., Cincinnati and Galveston rules, intended for ICUs).

Rules for estimating the amount of fluid transfused in paediatric patients aim to determine the amount of fluid to be transfused for estimated fluid resuscitation (EFR) of the child and to cover their maintenance fluids (MFs). The literature is currently emphasising a more individualised approach to the burn patient and adapting fluid therapy to the patient's actual fluid requirements (goal-directed fluid therapy). In this type of fluid therapy, fluid requirements are calculated based not only on the patient's basic vital signs and urine output (UOP) but also on parameters such as systolic blood pressure (SBP) and cardiac output (CA). The principles of goal-directed fluid resuscitation are currently being developed by a number of burn centres and in the future are likely to replace the previously used rules for fluid supply in patients suffering from burn injuries [13]. It should be mentioned that at present there is no formula for fluid supply in a burn patient that accurately represents their fluid requirements. The formulas used for both paediatric and adult patients serve as an auxiliary tool in determining the fluid requirements of the burn patient.

The optimum fluid therapy for a burn patient should proceed in several stages, some of which will be implemented at the scene and some of which will continue at the destination centre.

Fluid therapy stages:

1. accurate TBSA estimation,
2. selection of administration route,
3. initiation of fluid therapy based on the selected rule,
4. monitoring fluid supply and adjusting it to the patient's fluid requirements,

Table 2. Comparison of the composition of Ringer's lactate and Ringer's lactate solution.
Tabela 2. Porównanie składu Mleczanu Ringera i Płynu Ringera.

Name	mmol/1000 mL				Buffer g/1000 mL	pH	Osmolarity mOsmol/L
	Na+	Cl-	K+	Ca2+			
Ringer's lactate	131	112	5.36	1.84	6.34	5.0-7.0	278.5
Ringer's lactate solution	147.2	155.7	4.0	2.25	-	5.0-7.0	309.0

Source: own material.

5. establishing the intended effects of providing fluid therapy to the burn patient,
6. implementing appropriate pharmacological treatment to support the fluid therapy [8].

An important part of providing fluid therapy is choosing the right infusion solution. Most of the fluid therapy regimens used in burn patients are based on the supply of crystalloids or colloids. However, there is no scientific evidence that the use of colloids significantly improves the condition of burn patients [14]. Most of the rules for estimating fluid supply requirements are based on infusion of Ringer's lactate (lactated Ringer's solution, LR). One should note that proper fluid therapy is not always feasible at the pre-hospital stage due to legal reasons. In Poland, paramedics and system nurses may only administer Ringer's solution and not Ringer's lactate, which partly differ in composition, as shown in Table 2.

A different approach to fluid therapy in children with burns is presented by the British Burn Association (BBA) guidelines – they recommend starting fluid therapy when the patient has more than 20% of TBSA. The BBA also recommends the use of an infusion fluid with the highest possible sodium concentration – 0.9% NaCl – under pre-hospital management [15].

As mentioned above, fluid therapy should be initiated as early as possible at the scene, with the amount of fluid transfused estimated on the basis of the patient's weight and, if this is not possible, based on the patient's age.

During further examination, once the medical staff have determined the TBSA and the weight of the child, the fluid requirements should be estimated based on the following formula:

3 mL x BW in kg X %TBSA

As with other formulas for meeting a patient's fluid requirements, the calculated amount of fluids is divided in half, with the first half to be transfused in the first 8 hours and the remaining half within the following 16 hours [8].

When dealing with a child suffering from electric burns, the following formula should be used:

4 mL x BW in kg X %TBSA

Again, the estimated amount of fluids should be divided into two parts and transfused at 8 and 16 hours, respectively. When calculating the amount of transfused fluids, one should note that the 'transfusion clock' starts when the injury occurs, not when help arrives or when the patient reports to the hospital emergency department. According to the American Burn Association, fluid transfusion alone may be delayed if the travel time to hospital is less than 60 minutes and the patient suffers only from a burn with no concurrent injuries. The above fluid transfusion rules are used to estimate the initial fluid therapy in a burn patient. Fluid supply should be adjusted in line with the patient's response to fluid therapy, taking into account the amount of urine excreted [8].

Inadequate fluid therapy can have serious consequences already during the emergency operations at the scene. When fluid therapy is administered to patients with inhalation burns in addition to skin burns in various areas of the body, swelling in the airway will build up more rapidly, necessitating faster airway protection with endotracheal intubation. Endotracheal intubation performed without indications can have serious consequences, including increasing swelling in the patient's airway. To slow down the build-up of swelling inside the airway, the head should be raised by 30 degrees relative to the rest of the patient's body. A patient with coexisting inhalation burns requires proper and accurate monitoring of vital functions. Fluid therapy providing too little or too much fluid may exacerbate the patient's condition, leading, for example, to pulmonary oedema. It should be noted that combinations of skin burns, and inhalation burns may require an increased amount of transfused fluids, with the volume estimated using TBSA-based formulas.

A different approach to fluid therapy in burns is required for children where the burn has been caused by electricity and is followed by myoglobinuria (the characteristic symptom for this condition will be the presence of dark, red-coloured urine). In cases of electric burns, fluid therapy should be administered by titrating hourly volumes of fluid transfused, based on urine output (children weighing up to 30 kg: 1 mL/kg BW/hour and children weighing more than 30 kg, up to 17 years of age: 0.5 mL/kg BW/hour, the hourly urine output should be calculated based on the patient's ideal body weight) and the patient's consciousness. If urine output norms are achieved and consciousness improves, the amount of fluid transfused should be reduced. In the opposite case, the amount of urine passed should not be increased with the use of diuretics. The only way to increase urine output is to increase the amount of fluid transfused. The presence of dark, red-coloured urine, despite properly managed fluid therapy, may indicate the compartment syndrome. If dark coloured urine is present, the UOP should be in the range of 1-2 mL/kg BW/hour, as long as the dark coloured urine persists. Mannitol administration may also be necessary in such cases.

There is a group of patients who may not respond properly to fluid resuscitation:

- patients with coexisting injuries,
- burn patients in whom fluid resuscitation has been significantly delayed,
- patients with severe burns,
- patients injured by explosions,
- patients who were significantly dehydrated before the burn,
- patients under the influence of alcohol,
- patients with specific medical history.

In this group of patients, the management in the pre-hospital and hospital emergency department is the same,

because of the time the patient spends in the care of the medical staff. However, with prolonged extraction and delayed transport to a burn treatment centre, the above considerations should be taken into account while increasing the fluid supply and controlling the patient's urine output.

Conclusion

- The appropriate fluid therapy for burn patients is an important part of modern treatment and should start at the scene.
- Solutions proposed by the ABA, PHTLS and PTLS can be used before estimating a burn patient's fluid requirements using the relevant formulas.
- Further examination should include assessment of the child's exact TBSA and weight and their fluid requirements should be calculated on that basis using the appropriate formula.
- The fluid therapy provided must be monitored, as it may have adverse effects on the patient's health.
- We need to remember that there is a group of patients who may not respond properly to fluid resuscitation.

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CENTRAL PATTERN GENERATOR AND CONTROL OF BREATHING

Ośrodkowy generator wzorca i kontrola oddychania

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Abstract: Fifty years ago, Clark and Euler published a model of the neural control of respiration that stimulated modern studies on the central generator of the rate and depth of breathing. These studies led to new discoveries concerning both anatomical localization and functional characteristics of the respiratory central pattern generator (CPG), and as a result our knowledge has greatly changed. This article describes the history of respiratory CPG research and, more specifically, explains how studies concerning the regulation of breathing parameters affect the creation of new hypotheses and theoretical models of the neural control of respiration. Comparing studies on the generators of cyclic movements of respiration and locomotion helps us to show their significance for clinical research, especially in the field of spinal injury. After partial or total paralysis of muscles, techniques of muscle work assistance are especially important. Therefore, different techniques of assisted locomotion and breathing are presented here, as well as an explanation of how locomotion and breathing can influence each other. The ability to reactivate the spinal neuronal network using either pharmacological or electrical stimulation methods is discussed. Research performed with the application of biologically steered servo-respirators allow for a better understanding of respiratory CPG and show the limits of assisted ventilation in clinical practice. This article presents the role of activity-dependent plasticity in the rehabilitation of locomotor and respiratory muscles after spinal injury.

Keywords: locomotion, plasticity, electrical stimulation, spinal cord, neuronal network.

Streszczenie: Opublikowany 50 lat temu model nerwowej regulacji oddychania Clarka i Eulera był inspiracją dla nowego podejścia do badań ośrodkowego generatora głębokości i rytmu oddychania. Dzięki tym badaniom nasza wiedza dotycząca zarówno anatomicznej lokalizacji, jak i charakterystyki działania generatorów wzorca oddechowego uległa dużej zmianie. W prezentowanym artykule przedstawiono historię badań oddechowego generatora wzorca (CPG), a w szczególności wykazano, jak badania poszczególnych parametrów oddechowych stymulowały rozwój nowych hipotez i teoretycznych modeli ich kontroli ośrodkowej. Dzięki porównaniu badań generatorów ruchów cyklicznych oddychania i lokomocji można zobaczyć ich wzajemny wpływ na rozwój zastosowań klinicznych, szczególnie w przypadkach uszkodzeń rdzenia kręgowego. To właśnie w warunkach całkowitego lub częściowego porażenia wzrasta znaczenie technik wspomagania pracy mięśni. Dlatego też w pracy wymieniono różne techniki wspomagania ruchu i oddychania oraz omówiono ich wzajemne współdziałanie. Przedyskutowano możliwości uaktywnienia rdzeniowej sieci neuronalnej metodami farmakologicznymi lub przy pomocy elektrycznej stymulacji. Badania prowadzone przy zastosowaniu servo-respiratorów sterowanych biologicznie umożliwiły lepsze poznanie CPG oddychania oraz granice użyteczności wentylacji wspomaganej w warunkach klinicznych. W artykule omówiono możliwość zastosowania plastyczności zależnej od aktywności w rehabilitacji pracy mięśni ruchowych i oddechowych po uszkodzeniach rdzenia kręgowego.

Słowa kluczowe: lokomocja, plastyczność, stymulacja elektryczna, rdzeń kręgowy, sieć neuronalna.

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Fifty years have passed since the publication of Clark and Euler's experimental work on the control of breathing rate and depth [1]. Both of these parameters describe the value of lung ventilation, which determines gas exchange in line with the needs of the body. The authors of the study showed that respiratory cycle parameters are related by a hyperbolic relationship. This was the origin of the famous 'Clark and Euler hyperbola', which has been the focus of many studies on the generation of respiratory activity

under different respiratory drive conditions. To date, the work has been cited in more than 900 scientific reports.

Many behaviours and activities of living organisms are underpinned by the rhythmic activity of the neuronal networks that control the motor system. Due to their importance, locomotion and breathing behaviours are investigated the most frequently. Having understood the principles of one generator, we can compare its performance with others. Locomotion is associated with the cyclic movement of the limbs, which can be accelerated or decelerated [2]. Respiration, in turn, is the alternate effort of the inspiratory and expiratory muscles involved in lung ventilation. The value of lung ventilation, i.e., the work of the respiratory muscles, can be described by such parameters as volume (V) and time of inspiration (TI) and expiration (TE) (Fig. 1). The sum of the inhalation and exhalation durations forms the respiratory cycle, which is the inverse of the respiratory rate.

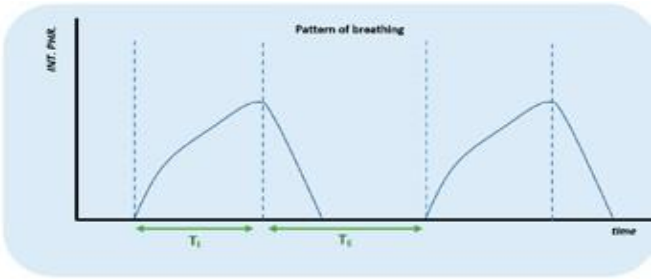

Figure 1. A pattern of breathing presented as an integrated recording of the electrical activity of the phrenic nerve (INT.PHR.), which is proportional to the tidal volume (V).

The inspiration (TI) and expiration (TE) times are marked on the timeline (time).

Rycina 1. Wzorzec oddechowy *(pattern of breathing)* przedstawiony jako zintegrowany zapis elektrycznej aktywności nerwu przeponowego (INT.PHR.), który jest proporcjonalny do objętości oddechowej (V).

Na osi czasu (time) zaznaczono czas wdechu (TI) i wydechu (TE).

The generation of cyclic movements by the nervous system is the topic of research, both in terms of the generation of neural network activity and its interaction with other body functions. For example, increasing the effort associated with movement increases the respiratory drive and lung ventilation [3]. In order to understand how this integration occurs, we need to understand the principles of construction and operation of the central pattern generators (CPGs) for cyclic movements. This paper describes a study of respiratory cycle parameters at rest and during an increase in respiratory drive. For historical reasons, studies of the CPGs of locomotion will be mentioned, as this is what gave rise to the development of studies on the CPGs of respiration. Clark and Euler's work is a good example of the application of knowledge of the CPGs of locomotion to the study on the control of respiration. The importance of testing central pattern generators will be discussed in the clinical cases of spinal cord injury and respiratory failure.

Central generation of respiratory activity

In this chapter, respiration is considered as the cyclical work of the respiratory muscles to provide lung ventilation in accordance with the needs of the body. As respiratory drive increases, the tidal volume and amplitude of airflow through the airways grows. The value of the airflow is related not only to the depth, but also to the rate of respiration, which usually increases with the inspiratory volume. Respiratory drive depends on the body's oxygen demand and the production of carbon dioxide, whose partial pressure can be measured in the exhaled air. Experimental studies on the control of respiration often use air mixtures with added carbon dioxide or altered oxygen content. Rebreathing is sometimes used, with partial pressures of oxygen decreasing and carbon dioxide increasing over the course of the experiment. When rebreathing is carried out under hyperoxia, the response obtained is only a measure of the change in partial pressure of carbon dioxide in the breathing mixture. This helps to investigate the chemical stimulation of respiration under conditions of hypercapnia with either hypoxia or hyperoxia, which has differential effects on peripheral and central chemoreception.

Clark and Euler [1] conducted their fundamental experiments on cats under pentobarbital anaesthesia. Respiratory drive was altered using rebreathing into an oxygen-filled bag; thus, respiration was stimulated with hypercapnia under hyperoxia. It was found that as chemical stimulation increases, the amplitude of inspiration rises and its duration falls. The research found that the inspiratory inhibition threshold, consistent with the Hering-Breuer reflex, decreases with the duration of inspiration. This relationship means that a shorter value of inspiratory time (TI) corresponds to a greater depth of inspiration (Fig. 2). A similar result was obtained with artificial lung inflation conducted at the same level of respiratory drive: deeper inflation corresponded to a greater shortening of TI.

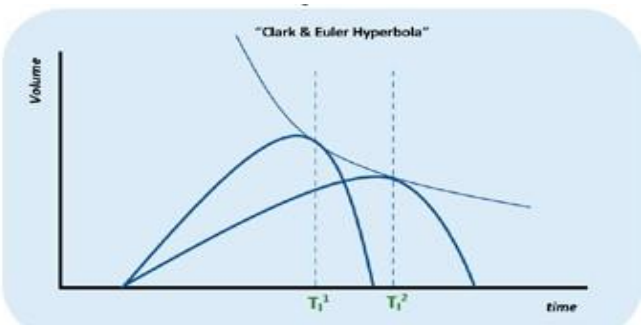

Figure 2. Two examples of tidal volume recordings at different levels of CIA.
The transition points between inspiration and expiration (defined by time TI and volume V of inspiration) are arranged in a hyperbolic relationship.

Rycina 2. Przykładowe dwa zapisy objętości oddechowej na różnych poziomach CIA.

Punkty przełączenia wdechu na wydech (określone przez czas TI i głębokość V-Volume wdechu) układają się w zależność hiperboliczną.

Interestingly, Clark and Euler's work [1] used a respirator that could be triggered by inspiratory activity of the phrenic nerve. This allowed the researchers to administer different volumes of air into the lungs (inflation) with a variable delay from the start of inspiration. It was found that the earlier in inspiration the inflation was delivered, the greater its value had to be in order to inhibit inspiratory activity. This confirmed the previously described observation that the value of the Hering-Breuer reflex threshold decreases with increasing duration of the inspiration. All experiments in which changes in inspiratory duration were obtained showed that expiratory duration (TE) was proportional to TI.

The VxTI hyperbola presented by Clark and Euler (Fig. 2) and the linear relationship between TI and TE constitute the first descriptions of the dependence between the respiratory cycle parameters obtained in an animal experimental model. Since then, research has been carried out in Curt von Euler's laboratory as well as in many others to create a respiratory CPG model consistent with the results. It was recognized that the–CPG is located in the brainstem and is under the control of central inspiratory activity (CIA). The CIA amplitude would depend on the inspiratory off-switch mechanism, which decreases with the time of inspiration and is controlled by the central structures of the bridge and the afferent activity of the vagus nerve from the pulmonary mechanoreceptors. The rate of CIA rise would depend exclusively on the respiratory drive. The study was performed using a variety of experimental models with different techniques for interfering with the operation of the respiratory CPG. Many significant discrepancies were observed between the results obtained and the Clark and Euler model assumptions.

On the whole, criticism of the Euler model after 1972 has focused on the following elements:

- the simplified concept of the inspiratory off-switch mechanism,
- the independence of the rate of CIA rise on vagus nerve activity (vagal activity),
- recognising the respiratory cycle as a finite element, dependent only on the CIA and vagal activity from pulmonary mechanoreceptors within the same breath. The linear dependence of TE on the TI of the preceding inspiration also arose from this assumption. These very features of the CPG of respiration were derived from the locomotion model, in which the parameters of one cycle were a sufficient description of the locomotor CPG.

Subsequent studies have shown that the inspiratory off-switch mechanism not only changes with time but depends on the state and source of CIA arousal at the time. Chemical stimulation, body temperature, central nervous system arousal – these are some of the many conditions that influence the value of the inspiratory off-switch mechanism. For example, during coughing or gasping, the vagal off-switch mechanism can be completely switched off. Then there is the phenomenon of short-term plasticity, or habituation, whereby the threshold value changes over several breaths following a change in stimulus. In such conditions, the parameters of the respiratory cycle change from the onset of the stimulus over more than one cycle. Short-term plasticity has been observed in studies using assisted ventilation [4] and during electrical vagus nerve stimulation [5]. The rate of CIA rise during a single inspiration was also investigated in addition to phenomena lasting longer than one breathing cycle. With the use of assisted ventilation, as will be discussed in later sections of this work, it was observed that vagal activity can alter the CIA during inspiration.

Research using short-term inflation has shown that the rate of CIA rise can be temporarily and reversibly inhibited (graded inhibition) during CIA rise [6]. Thus, the assumption that the CIA depends only on central information was not supported by subsequent models of respiratory CPG [7].

Euler [7] attempted also to determine the location of the neuronal counterparts of the elements of his model. He highlighted the groups of brainstem neurons involved in the inspiratory off-switch mechanism and identified which pathways supply them with peripheral and central information. In his 1983 model, control of expiration, which was divided into two phases with groups of neurons representing them, is already more extensively covered. The division of the respiratory cycle into three phases has long persisted in the modelling of CPG respiration [8]. However, it was not until the research of Feldman's team that the discovery of different inspiratory and expiratory generators was made, which, together with the division of the expiratory phase into two components, led to a model representing the three different generators that make up the CPG of respiration [9]. This is why reference is often made not to one, but to multiple respiratory pattern generators.

Clinical studies

The interest in the structure of the CPG is linked to the practical application of knowledge in clinical settings. In the case of locomotion, for example, we know that motor activity generation occurs at the spinal cord and is modulated by both sensory activity and descending activity from higher central nervous system structures [3]. Spinal injury above the spinal CPG may prevent the generation of motor activity. Experimental studies have revealed that, even when the descending pathways are inactive, stimulation of the spinal locomotor CPG is possible using various techniques for stimulating the spinal neuronal network.

Studies in humans with spinal cord injuries have shown that, with appropriate training and assisting devices, locomotor ability can be restored to a certain extent. This demonstrated that the spinal CPG can 'learn' to generate the rhythm of motor muscle excitation in a changed nervous system configuration. This change in the activity of

the spinal neuronal system is due to its plasticity (long term plasticity) [10].

Enabling physiological activation of motor muscles after spinal cord injury is essential as it counteracts the atrophy of the descending nerve fibres below the injury and the atrophy of the muscles. For this reason, research aimed at understanding the excitation mechanisms of the spinal pattern generators that are found in the motor muscles continues. An additional objective of these efforts is to understand the mechanisms that allow the different CPGs to interact and to identify their interrelationships. In this case, studies on the interaction between locomotor and respiratory central pattern generators are a good example [11].

The respiratory central pattern generator is located in the medulla oblongata and the bridge. In many cases, damage to the cervical spinal cord may completely disrupt the motor connections of the CPG and thus make breathing impossible. In such circumstances, the most common way to keep the organism alive is to use artificial ventilation. Long-term ventilation with a respirator maintenance after spinal cord injury leads to the atrophy of descending nerve fibres and muscles. As respiratory CPGs are located in the medulla oblongata, they are active after injury and could, in theory, control muscle excitation and thus maintain lung ventilation. Attempts to use assisted ventilation, which could reduce respiratory muscle atrophy after spinal cord injury, is discussed below [12].

Artificial ventilation

Artificial ventilation, which delivers air, usually enriched with oxygen, to the lungs at a specified volume, inflation time and expiratory interval, is necessary in the absence of respiratory movements or when the gas exchange is insufficient. The artificial ventilation parameters are set by the respirator operator based on the carbon dioxide content in the exhaled air and selected metabolic parameters. Artificial ventilation is essential to preserve life, but it may cause many problems. During spontaneous breathing, a negative pressure is created in the lungs to promote blood flow, whilst during artificial ventilation, the pressure is higher than the atmospheric pressure and impairs pulmonary circulation. When respiratory movements are maintained, although insufficient, they may interfere with the imposed rhythm of the ventilator and create discomfort. When spontaneous breathing is maintained despite respiratory failure, assisted ventilation, in which the movement of the inspiratory muscles releases a supply of air to the lungs at a constant volume or pressure, appears to be a better method. The operator of the respirator sets the appropriate inflation value, while the frequency of inflation can be adjusted by the patient. With the right cooperation between the patient and the physician, lung ventilation may reach values that are close to the needs of the organism [12, 13].

When writing about ventilation adapted to the patient's respiratory needs, Christer Sinderby [12] mentioned a paper published in 1970 by Andrzej Huszczuk describing a respirator controlled by integrated phrenic nerve activity [14]. Naturally, such a respirator was only intended for experimental animal studies, but it offered the potential to ventilate the lungs in accordance with the actual needs of the body. When the correct control signal gain was set, air was delivered to the lungs in line with the amplitude and duration of the activity recorded from the phrenic nerve. During such assisted ventilation, the volume increases with the rising respiratory drive and the decreasing duration of inflation.

The use of a biologically controlled respirator (servo respirator) brought a fresh impulse to the study of the neural model of respiratory control described by Clark and Euler [4, 15]. Changes in the gain of the signal of the integrated activity of the phrenic nerve allowed gradual changes in tidal volume below and above control values for a period of one or more breathing cycles. In the paper by Bartoli et al. [4], the study was conducted on dogs sedated and ventilated with a respirator controlled by integrated phrenic nerve activity. The respiratory cycle in dogs is longer than in cats, which enabled a more accurate study of the response of phrenic nerve activity to changes in the depth of inflation during a single inspiration. It should be noted that integrated phrenic nerve activity (INT. PHR.) is a measurable representation of centrally generated inspiratory activity, an equivalent of the CIA from Clark and Euler's model [1].

The very first studies, in which the gain of the respirator control signal was altered, showed that changes in tidal volume within a single inspiration were accompanied by a change in the rate of increase of integrated phrenic nerve activity: the higher the inflation volume, the greater the increase in phrenic nerve inspiratory activity (Fig. 3).

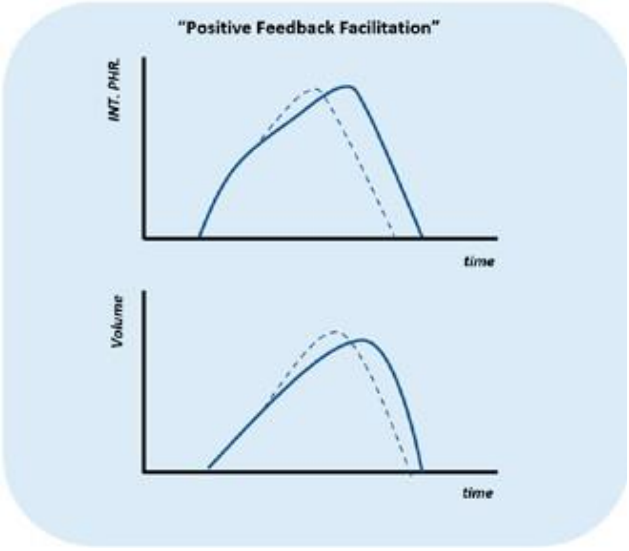

Figure 3. Stimulation of inspiratory activity consistent with positive feedback.
Rycina 3. Pobudzenie aktywności wdechowej zgodne ze sprzężeniem dodatnim.

The solid line represents control inspiration. The dashed line represents the rise in the rate of inspiratory volume

(Volume) and the corresponding rise in integrated phrenic nerve activity (INT. PHR.). The stimulation of inspiration is accompanied by a faster switch from inspiration to expiration.

The phenomenon described by Bartoli et al. [4] is positive feedback, as an increase in inspiratory activity caused a reflex increase in the respirator control signal. This response was inconsistent with the previous understanding of the role of pulmonary mechanoreceptors as a factor inhibiting inspiration.

At the time of Clark and Euler's study, the phenomenon of positive feedback in inspiration was not yet known. It did not occur in earlier studies on cats and rabbits under anaesthesia. Since the paper by Bartoli et al. [4], it is believed that this phenomenon can be suppressed under different anaesthetic conditions and that there are differences between the species in which this reflex is observed. A study on rabbits using a bio-controlled respirator was carried out in the laboratory of Witold A. Karczewski in Warsaw, Poland, and it was found that, although this reflex is not present in measurements of phrenic nerve activity, the activity of the inspiratory intercostal muscles can be stimulated, and this response increases with greater gain of the servo respirator. The difference in the response of diaphragm and intercostal muscle activity may be due to the fact that intercostal muscle activity is more sensitive to vagal stimulation [16].

In addition to the observation of vagal positive feedback within a single inspiration, the study by Bartoli et al. [4] also described several-second changes in the Hering-Breuer reflex threshold after a stepwise change in the control signal gain. The phenomenon is an example of short-term plasticity, whereby habituation of the reflex occurs despite the same stimulation being maintained over several breaths. The amplitude of the observed changes from breath to breath was directly proportional to the set gain of the servo respirator control signal. No similar phenomenon occurred after both vagus nerves were cut [4, 17].

Bartoli et al. (1975) altered the duration of inspiration by changing the gain of the servo respirator. However, the observed changes in TI did not induce proportional changes in expiratory time in line with the linear relationship described by Clark and Euler (1972). Thus, the subsequent characterisation of respiratory CPG proved to be valid only under specific experimental conditions (cats under anaesthesia) and could not be considered universal. To sum up, Bartoli et al. [4] showed that:

- the central off-switch threshold can change over the course of several breaths regardless of the constant value of the vagal information,
- the rate of rise of central inspiratory activity (CIA) depends on the vagal activity within the same inspiration,
- a linear relationship between TI and TE does not occur under all experimental conditions. It is a special case rather than a universal one.

Expiration control

The expiratory phase is often ignored in studies of neural regulation of respiration [10]. This lack of interest is related to the belief that, under resting conditions, exhalation is a passive activity and depends only on the mechanics of breathing. In addition, the first models of respiratory CPGs assumed reciprocal inhibition of the inspiratory and expiratory neuronal networks, suggesting some symmetry in the generation of the two phases of the respiratory cycle.

Contemporary models of respiratory CPG indicate that there are different generators of the inspiratory and expiratory phases [9].

Inspiration time is strongly related by a hyperbolic relationship to the depth of inspiration, while expiration time depends on many conditions. The off-switch (beginning and end of inspiration) is often related to the activity of other CPGs, such as swallowing or locomotion, which affect respiratory rate [18]. This readiness to cooperate with other CPGs may be related to the unpredictability of the TE response to inflation delivered at different moments of expiration: the same stimulus may cause shortening or lengthening of expiration [19]. Studies of neural control of respiration using a biologically controlled servo respirator showed the different regulation of TI and TE long time ago. These studies deserve to be mentioned now that the anatomical substrates of the inspiratory generator and the independent neuronal network of expiratory generation have been found [9].

Experiments on rabbits under halothane anaesthesia investigated changes in respiratory pattern parameters upon administration of oxygen with different CO2 contents in the inspiratory mixture. The study was performed under spontaneous breathing conditions and during assisted ventilation with a respirator controlled by integrated phrenic nerve activity. Assisted ventilation was carried out before and after muscle paralysis and was repeated after both vagus nerves were cut [20].

It was observed in all conditions that tidal volume increased with increasing amplitude of integrated phrenic nerve activity. With assisted ventilation, however, the volume increment for strong respiratory stimulation became increasingly smaller with the same amplitude increment of integrated phrenic nerve activity. This may mean that assisted ventilation (especially after muscle paralysis) has excluded any additional increase in lung ventilation with the use of respiratory muscles other than the diaphragm. In the light of this observation, the experience of those who use assisted ventilation in patients with respiratory failure is worth mentioning. Patients often report the need for an increased tidal volume, even though the respiratory parameters of lung ventilation are set correctly. It is likely that this sensation is due to the release of mechanoreception of the respiratory muscles under assisted ventilation.

In the discussed studies using assisted ventilation in spontaneous breathing conditions, increases in CIA were

accompanied by changes in TE proportional to changes in TI. However, in subsequent stages of the experiment, in which afferent activity from the respiratory muscles and vagus nerves was progressively impaired or suppressed, TE during increased lung ventilation changed the direction of the response: expiration became progressively longer as tidal volume increased. This response was inversely proportional to the TI response. Assuming the original model of respiratory CPG, this type of response would be difficult to justify.

Subsequent studies using a servo respirator applied the same tidal volume delivered by the respiratory pump land triggered inspiration (inflation) by diaphragmatic nerve activity [21]. Under these conditions, the experimental animals could, in theory, regulate the respiratory rate without being able to regulate the depth of breathing. The tidal volume was changed every few minutes to achieve stabilised ventilation conditions at different levels of exhaled CO2. As the set inflation depth decreased, the duration of exhalation and the concentration of exhaled CO2 increased. This demonstrated that experimental animals cannot increase respiratory rate compensatively when respiratory volume decreases [21].

The importance of understanding the respiratory CPG for clinical research

The importance of studying the structure and function of the respiratory CPG is becoming evident in the rehabilitation of vital functions after spinal cord injury. As previously mentioned, knowledge of CPG action helps to plan muscle training that can accommodate locomotor CPG for movement-enhancing activities. Such an accommodation may be called 'activity-dependent plasticity' [10]. In the case of respiration, the neurons involved in CPG are located in supraspinal structures. However, many studies indicate the existence of core networks of interneurons with connections transverse to the spinal cord axis, which are so numerous that even weak descending activity can stimulate them to significant activity [22]. This is for example how researchers attempt to reproduce the generation of expiratory muscle activity sufficient to induce coughing [23]. It should be remembered that there are also inhibitory interactions among the descending pathways and, when damaged, the spinal interneuron network may be more sensitive to stimulation [24].

Electrical stimulation of the spinal cord with high-frequency pulses up to 500 Hz is a new method for studying the spinal neural network [25]. It was found that such stimulation activates the spinal neuronal network and can induce muscle activity in accordance with the physiological pattern of motor fibre discharges depending on their excitation threshold. It is likely that such stimulation activates a network of interneurons, whose large number and organisation of connections at the spinal level may act as a 'feedforward' and simulate descending efferent activity [26, 27]. In the case of spinal cord injuries, where minimal descending connections are preserved, electrical stimulation with high pulse frequency could produce pre-excitation of the spinal network and, through a process of plasticity, develop a new system that meets central motor requirements. For now, high-frequency stimulation can be used to induce coughing [28], similar to the concept by Jefferson et al. [23].

Marder and Bucher [27] and Jefferson et al. [23] cite many papers that suggest that activation of spinal interneuron connectivity networks can lead through activity-dependent plasticity to the generation of motor activity after spinal injury. The authors believe that the known combinations of respiration and locomotion generators at the spinal level [29] may help to reconstruct strong excitation of spinal interneuronal networks. Knowing the structure and interaction of the locomotor and respiratory CPGs, specialised muscle training can be designed to support both locomotor and respiratory activities. There are many directions in the development of such methods: from pharmacological support [30] to the aforementioned electrical spine stimulation [25]. One should also be aware of the limitations that come with the structure of a CPG. We know, for example, that the basic respiratory CPG is unable to compensatively regulate respiratory frequency when inspiratory muscle work is reduced [21]. The rate of spinal respiratory activity could be altered, for example, by stimulating locomotor CPG. Although much of the data published in Clark and Euler's work was later challenged, it was their model that underpinned the impressive growth in respiratory CPG research over the past 50 years.

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MULTI-FREQUENCY HEARING IMPROVEMENT ANALYSIS AS A METHOD TO EVALUATE RECOVERY IN PATIENTS WITH IDIOPATHIC SUDDEN SENSORINEURAL HEARING LOSS

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Abstract:

Introduction and objective.

Hearing improvement assessment in patients with Idiopathic Sudden Sensorineural Hearing Loss (ISSNHL) is complex. Methods used to evaluate the effectiveness of ISSNHL therapy do not include the variety of PTA (Pure Tone Audiometry) curves. The aim of this paper is to assess usefulness of Multi-Frequency Hearing Improvement Analysis (MHIA) as an alternative method employed to evaluate the effectiveness of ISSNHL treatment.

Material and methods.

The medical records of 218 patients with ISSNHL were statistically analysed in a retrospective study with regard to PTA results. Achieved results were compared using the following methods: Siegel's criteria, Wilson's criteria and MHIA.

Results.

The analysis was based on Siegel's criteria, which concerned the effectiveness of the therapy: complete recovery: 94 (43.1%), partial recovery: 20 (9.2%), slight recovery: 17 (7.8%) or no recovery: 87 (39.9%). The MHIA analysis revealed the following weighted arithmetic mean recovery rates: Air Conduction and Bone Conduction respectively – complete recovery (23.5%; 43.14%), partial recovery: (9.12%; 20.51%); slight recovery (6.65%; 7.4%), no recovery (68.36%; 54.98%).

Conclusions.

MHIA corrects the overestimation of complete recovery rate based on Siegel's criteria. Using mean auditory threshold stimulus as a baseline to evaluate hearing improvement in studies could distort the interpretation of the research findings. Clinical features and usability of MHIA in diverse groups of patients require further studies.

Keywords: sudden deafness, hyperbaric medicine, Siegel criteria, hearing assessment, steroid therapy.

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Introduction

Idiopathic Sudden Sensorineural Hearing Loss (ISSNHL) is defined as a sudden, subjective hearing loss in one or both ears. ISSNHL is diagnosed when the vocal threshold stimulus is enlarged by not less than 30 dB in at least 3 frequencies [1] in Pure Tone Audiometry (PTA). PTA involves the use of the two following types of conductions: Bone Conduction (BC) and Air Conduction (AC). The most common methods used to estimate hearing improvement are Wilson's [2] and Siegel's [3] criteria. Wilson's criteria constitute a relative recovery assessment method. Some studies modify this method and describe complete recovery as a 90% hearing recovery. Furthermore, they use reference values as a baseline [4]. This method defines recovery without considering the hearing threshold quotient prior/post to the therapy. Both methods evaluate the successfulness of therapy and are mainly based on the measurement of the lowering the threshold auditory

stimulus. Considering the multiple voice frequencies analysed in PTA, the curves vary as follows: ascending, descending, flat and deep [5]. The current state of research does not clearly define recommendations concerning the ISSNHL treatment. There are studies proving that steroid therapy (ST) is beneficial [1-3, 6-8] and its therapeutic role has an anti-inflammatory effect. The European Committee for Hyperbaric Medicine and the Polish Society of Audiology and Phoniatrics recommend the use of hyperbaric oxygen therapy (HBOT), whereas the Undersea and Hyperbaric Medical Society does not endorse this method [7-12]. The diverse pathophysiology of ISSNHL [13, 14] and various types of PTA curves require an individual approach to the treatment. The available research indicate that benefits may be found in certain types of PTA curves [15]. Moreover, they demonstrate that multi-frequency interpretation is an important step involved in supporting individual ISSNHL therapy. Individual therapy for various types of hearing impairments requires the creation of a more personal type of hearing improvement assessment method.

Aim of the study

The aim of this paper is to assess usefulness of Multi-Frequency Hearing Improvement Analysis (MHIA) as an alternative method used to evaluate the effectiveness of the ISSNHL treatment.

Material and methods

The medical records of 218 patients (117 males, 101 females, mean age 48.8 ±14.5 years old) admitted to the Department of Hyperbaric Medicine of the Military Institute of Medicine – National Research Institute in Warsaw were subjected to retrospective analysis. The admissions of patients took place between 01/2018-12/2019. The authors state that this study was conducted in accordance with the Declaration of Helsinki: Recommendations guiding physicians in biomedical research involving human subjects, Journal of the American Medical Association, 277, 925-926. All the patients were treated with the use of a BAROXHBO hyperbaric oxygen chamber, according to the following hyperbaric medicine procedure:

- compression to 2.5 ATA,
- total compression/decompression time: 10 minutes (1.5 meters/minute),
- oxygenation 3 x 20 minutes with 100% oxygen as the breathing factor,
- oxygen breaks of 2 x 5 minutes, performed routinely to prevent toxic influence on lungs and brain,
- average duration of HBOT: 15.9 days (±4.1),
- average HBOT delay: 8.2 days (±6.6).

Steroid therapy (ST) parameters: initial average dosage of prednisone: 48.5 mg (±15.7), average duration of ST: 15.9 days (±7.8), average ST delay: 5.3 days (±5.7).

Exclusion criteria:

- age <18 years old,
- start of HBOT 30 days after the onset of ISSNHL symptoms,
- coexisting cerebrospinal inflammation,
- neuropsychiatric disorder,
- vascular disorder,
- Meniere's disease,
- hereditary hearing disorder,
- inner ear malformations,
- facial nerve neuroma,
- bilateral ISSNHL,
- subsequent episode of ISSNHL.

Research participants were examined with the help of PTA before and after therapy. The therapy involved the use of an Interacoustics AC40 audiometer in the following frequencies [Hz] (AC – 125, 250, 500, 1000, 1500, 2000, 3000, 4000, 6000, 8000; BC – 125, 250, 500, 1000, 1500, 2000, 3000, 4000). The gathered data were analysed using the following methods: Siegel's criteria, Wilson's criteria and MHIA.

Wilson's criteria [2]

A complete recovery is defined as recovery of hearing to within 10 dB of the pre-hearing loss speech reception score or of the PTA score (if the loss was primarily in the high-frequency range). A partial recovery is defined as recovery of hearing to within 50% or more of the pre-hearing loss speech reception score or of PTA score (if the loss was in the high-frequency range). No recovery is defined as less than 50% hearing recovery [2].

Siegel's criteria [3]

Siegel's criteria as an absolute method used to evaluate hearing improvement are described in the following table (Table 1).

Table 1. Siegel's criteria.

Recovery status	Auditory threshold stimulus after therapy (dB)	Lowering of the auditory threshold stimulus during therapy (dB)
Complete	<25	
Partial	25-44	>15
Slight	45-69	>15
No recovery	>70	<15

According to the current state of scientific data, Siegel's criteria were used to examine differences between mean values of auditory threshold stimulus before and after the therapy.

MHIA

MHIA is based on an absolute comparison method – Siegel's criteria. MHIA is a method used to analyse treatment results in the aforementioned group of patients. Examined frequencies are differentiated between AC and BC, and they are taken into consideration individually regardless of whether they are from a single patient score.

Consecutive lower and higher frequencies are often in reference values in ascending and descending types of PTA curves. In order to avoid overestimation of a complete recovery, PTA results are excluded when the auditory threshold stimulus is lower than 30 dB.

Statistical analysis

Statistical analysis has been performed with use of "Statistica 7.0" software. The T-Student test has been performed in the study group. Normal distribution has been confirmed by the Kolmogorov-Smirnov test.

Results

Average pre-treatment and post-treatment threshold auditory stimuli displayed by 218 patients included in the study were respectively: 54.6 dB (±31.3), 39.6 dB (±32.2). Types of PTA curves were: ascending (24, 11%), descending (86, 39.9%), flat (63, 28.9%), deep (44, 20.2%). The average delay (in days) of implementing ST and HBOT was: 5.3 (±5.7), 12.1 (±6.7).

Wilson's criteria

Wilson's criteria were impossible to apply due to the following reason: pre-hearing loss PTA was not available for any patient and it would be strongly inaccurate to presume that all the patients had impeccable hearing before the onset of ISSNHL. Another key factor needed in order to apply Wilson's criteria was only useful in patients with primarily high-frequency hearing loss, which constituted 87 patients (39.9%). This is not a representative group; hence it is not possible to extrapolate the results to the whole group of patients with ISSNHL.

Siegel's criteria

Regarding Siegel's criteria, the recovery rate was as follows (vide Table 2). Applying Siegel's criteria in this group revealed that there was a significant group (42 patients, 44.7% of complete recovery) that met the complete recovery criteria prior to the therapy.

Table 2. Siegel's criteria in comparison to the other studies.

	Our study	Xie et al. [17]	Sung et al. [23]	Günel et al. [24]2
Complete recovery	43.1%	19.7%	50.9%	11.1%
Partial recovery	9.2%	17.4%	9.8%	22.2%
Slight recovery	7.8%	13.5%	5.9%	40.7%
No recovery	39.9%	49.4%	25.5%	25.9%

MHIA results

(Table 3, 4) Regarding mean PTA results as well as AC and BC, in the spectrum of all frequencies, lowering of the auditory threshold stimulus was detected. Especially visible differences were observed at the frequencies 500-2000 Hz. Slight recovery of hearing was visible at higher frequencies (4000-8000 Hz).

Table 3. Mean values of AC before and after therapy.

Frequency (Hz)	AC					
	Before (dB)	After (dB)	Variation (dB)	Variation (%)	T	p
125	49.6	35.9	13.8	27.8%	4.012	0.000
250	51.4	36.1	15.3	29.8%	4.85	0.000
500	55.9	37.6	18.3	32.7%	5.626	0.000
1000	55.4	38.7	16.7	30.2%	5.010	0.000
1500	68.2	40.0	28.2	41.4%	5.790	0.000
2000	55.7	41.4	14.4	25.8%	4.934	0.000
3000	60.7	46.9	13.8	22.7%	3.949	0.000
4000	61.4	49.3	12.1	19.7%	3.673	0.000
6000	67.1	55.3	11.8	17.6%	3.428	0.001
8000	64.9	57.9	7.0	10.8%	2.063	0.040

Table 4. Mean values of BC before and after therapy.

Frequency (Hz)	BC					
	Before (dB)	After (dB)	Variation (dB)	Variation (%)	t	p
250	42.1	26.5	15.6	37.1%	4.142	0.000
500	49.1	29.2	19.9	40.5%	5.592	0.000
1000	50.1	31.1	19.0	38.0%	5.133	0.000
1500	71.1	33.3	37.8	53.1%	6.647	0.000
2000	53.3	35.5	17.7	33.3%	4.768	0.000
3000	57.8	38.6	19.2	33.2%	4.577	0.000
4000	54.7	39.1	15.5	28.4%	4.173	0.000

Having disqualified initially correct scores (<30 dB), MHIA was used to analyse the remaining data concerning certain frequencies (Table 5, 6). The therapy had a different impact on various frequencies. The highest rate of complete recovery was detected at 4000 Hz in BC. The smallest influence of therapy was detected at 8000 Hz, AC. Recovery in BC was more visible than in AC.

Table 5. Recovery proportions in AC.

Frequency (Hz)	Recovery proportions								
	Incorrect results count (n)	Complete recovery		Partial recovery		Slight recovery		No recovery	
		(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
125	94	16	17.0%	15	16.0%	6	6.4%	57	60.6%
250	135	39	28.9%	14	10.4%	8	5.9%	74	54.8%
500	152	50	32.9%	17	11.2%	14	9.2%	71	46.7%
1000	147	44	29.9%	4	2.7%	5	3.4%	94	63.9%
1500	64	15	23.4%	5	7.8%	3	4.7%	41	64.1%
2000	148	31	20.9%	3	2.0%	12	8.1%	102	68.9%
3000	126	23	18.3%	8	6.3%	10	7.9%	85	67.5%
4000	166	25	15.1%	13	7.8%	7	4.2%	121	72.9%
6000	146	19	13.0%	8	5.5%	9	6.2%	110	75.3%
8000	178	20	11.2%	6	3.4%	3	1.7%	149	83.7%
Weighted arithmetic mean			23.5%		9.12%		6.65%		68.36%

Table 6. Recovery proportions in BC.

Frequency (Hz)	Recovery proportions								
	Incorrect results count (n)	Complete recovery		Partial recovery		Slight recovery		No recovery	
		(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
250	108	41	38.0%	7	17.1%	0	0.0%	60	55.6%
500	128	47	36.7%	9	19.1%	12	9.4%	60	46.9%
1000	126	38	30.2%	10	26.3%	6	4.8%	72	57.1%
1500	57	13	22.8%	3	23.1%	4	7.0%	37	64.9%
2000	135	39	28.9%	7	17.9%	12	8.9%	77	57.0%
3000	114	26	22.8%	8	30.8%	5	4.4%	75	65.8%
4000	144	97	67.4%	7	7.2%	9	6.3%	31	21.5%
Weighted arithmetic mean			43.14%		20.51%		7.4%		54.98%

Discussion

The diverse morphology of PTA curves naturally involves problems with assessing hearing improvement, especially in numerous groups of patients. Amelioration and unification of the assessment methods examining the effectiveness of treatment is crucial to compare the therapy results between various studies.

It was impossible to apply Wilson's criteria [2], primarily due to the erroneous assumptions. Pre-hearing loss speech reception score also could not be considered, because some patients demonstrate their previous PTA results but they were not up-to-date. Therefore, they should not be treated as a baseline to assess ISSNHL recovery. Another key factor needed to apply Wilson's criteria is the hearing impairment present at high frequencies. Such an impairment was present only in patients with descending PTA curves (39.9%). Some studies modify Wilson's criteria and they treat reference values as a baseline to estimate hearing recovery rate [4]. This method can be used only in patients without previous hearing impairments. Nevertheless, it is difficult to prove that a patient had not suffered from any hearing impairment. There are some potential solutions to estimate pre-hearing speech reception score loss, such as comparing the PTA curve with an unaffected ear [16].

In this study, Siegel's criteria [3, 17, 18] revealed an overestimation of complete recovery. This overestimation and the irregular impact on final hearing improvement require a multi-frequency approach to assess the effectiveness of the ISSNHL therapy. Average auditory threshold stimulus can reveal complete recovery and, simultaneously, meet the criteria of ISSNHL in PTA. This limitation has resulted from ascending and descending PTA curves. The recovery rate in comparison to other studies applying Siegel's criteria is presented in table 2. As shown in the table, differences between the results of the studies are significant. Varying outcomes of the implemented therapy might be caused by differences existing in the study groups, PTA curves and therapy protocols. Multi-frequency analysis is a possible solution to improve the prognosis of recovery by not treating differences in PTA curves as an interfering factor. Several studies modify Siegel's criteria in order to assess hearing improvement. Some published studies have attempted to assess recovery with regard to a before-therapy hearing impairment level [19]. This solution allows the assessment of the successfulness of the therapy while considering the ISSNHL level. It can also be used as a method to predict treatment effectiveness before implementing a therapy.

The current state of scientific data presents absolute methods which have limitations comparable to those presented in Siegel's criteria. A method presented by a Korean study [20] simplifies Siegel's criteria, estimating the final hearing level as better than 25 dB. Moreover, it reduces hearing gain by at least 15 dB compared to the pre-treatment level. This study algorithm only included the examination of four frequencies (0.5 kHz, 1 kHz, 2 kHz, 4 kHz). A small number of analysed frequencies can potentially improve Siegel's overestimation. The reductions employed allow us to compare the results between those studies applying Siegel's criteria while taking into account only 4 examined frequencies. Unfortunately, there is still a number of analysed frequencies which do not include any deafness on the boundary frequencies (125, 250, 6000, 8000). These frequencies have usually enlarged the auditory threshold stimuli in ascending and descending PTA curves, which constituted the 111 (50.9%) types included in our study. This makes it a relatively large group. Excluding patients with boundary types of ISSNHL has a potential impact on the final treatment results [18, 21, 22]. Our study revealed that the implemented therapy had a complex effect on the results of the therapy. The numbers of complete recoveries differ from one another at various frequencies (AC: 8000 Hz: 11.2% vs 500 Hz: 32.9%; BC: 1500 Hz: 22.8% vs: 4000Hz: 67.4%). In order to assess how the therapy may influence the final treatment results, a further study is required. Moreover, it is essential to stress the fact that the impact of therapy is nonlinear. A multi-frequency approach to assess the effectiveness of the implemented therapy analysis can potentially be a step into individualizing the ISSNHL therapy. According to the current state of scientific data, there is evidence proving that the implemented therapy may have a different influence on the auditory threshold stimulus, which is important for certain frequencies. An American study [15] proved a selective therapeutical activity of nortriptyline and topiramate, detectable at a hearing impairment at lower frequencies. In comparison to our study, and with regard to the fact that ascending types of PTA curves constituted 11% of the study group, the influence of the therapy on the rest of the group could make this relation potentially insignificant. Supporting the multivariate ISSNHL treatment analysis used in the study [17], together with MHIA, makes it possible to create guidelines for a particular therapy with regard to PTA types, which could consequently improve total outcomes for the ISSNHL therapy. Depending on the examined frequency, the recovery rates presented in table 6 differ from each other by up to 44.6% (complete recovery 4000 Hz vs 3000 Hz). This shows the complexity that the influence which the therapy has on the final hearing improvement in patients with ISSNHL.

Conclusions

MHIA corrects overestimation of complete recovery rate based on Siegel's criteria. Due to the diversity of the PTA curves and rated multiplicity of frequencies, the effectiveness of the ISSNHL therapy assessment is complex. Using mean auditory threshold stimulus as a baseline to evaluate hearing improvement in studies could distort the interpretation of the research findings. Clinical features and usability of MHIA in diverse groups of patients require further studies.

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DIRECTED EVOLUTION OF AAV CAPSIDS FOR IMPROVED EFFICACY AND SPECIFICITY OF DELIVERY TO PRECLINICAL MODELS OF THE HUMAN LIVER

Zwichnięcie stawu kolanowego ze współistniejącym złamaniem bliższego końca kości piszczelowej

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Abstract:

Introduction and objective.

Recombinant vectors derived from adeno-associated viruses (rAAVs) are the leading platform in human gene therapy applications, with high-profile examples targeting diseases of the central nervous system, eye and liver. The liver, quite likely a natural host organ for wild type AAV2, is a particularly attractive target for the development of AAV-mediated gene therapies. Despite the large number of AAV variants current at various stages of development as carriers of liver therapeutics, so far no liver-directed AAV-based therapy has obtained market authorization. Strong preclinical data is the cornerstone of any translational program, and while AAV bioengineering is commonly applied to try to develop novel human-tropic vectors for clinical applications, due to species-to-species differences, dedicated vectors to support preclinical work may need to be developed. Here we applied AAV directed evolution and *in vitro* selection to identify AAV capsids that target human liver cells *in vitro*.

Material and methods.

Using DNA shuffling technology, we have generated a capsid gene library based on natural parental serotypes (AAV1 through to AAV12). A shuffled capsid library was selected in a preclinical model of the human liver.

Results.

The AAV variants were enriched based on how their improved efficiency of transduction of a human hepatocyte cell line were vectorized and subsequently functionally characterized on human cell lines. This directed evolution method enabled us to select a novel AAV variant, AAV-CH4.2. While the selected variant did not exceed the parental serotype in terms of transduction efficiency, it was substantially more efficient at packaging than its closest homolog, serotypes AAV6.

Conclusions.

Based on its strong transduction profile and manufacturability, we believe that AAV-CH4.2 is a strong candidate for further evaluation and as a potential novel gene therapy vector for preclinical studies in human liver applications.

Keywords: gene therapy, AAV, adenovirus-associated viral vector, bioengineering.

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Introduction

Gene therapy brings great hope for people affected by a broad spectrum of currently incurable inherited and acquired diseases. The basic concept behind gene therapy is the delivery of a functional gene into cells or tissues to remediate the consequences of pathology-causing mutations. Although a number of technologies have been developed to facilitate the delivery of genetic payloads to target cells, *in vivo* delivery is limited to a handful of technologies, with methods based on the use of viral vectors gaining in popularity due to promising results in clinical trials. Adeno-associated virus (AAV) vectors seem very promising, due to the lack of pathogenicity and low immunogenicity, wide range of cell tropism and long-term gene expression in non-dividing cells.

AAV is a non-pathogenic human parvovirus, first employed as a gene target vector as early as 1984 [1]. AAV is a small, single-stranded DNA virus, which requires co-infection with a helper virus for efficient replication and viral gene expression. The AAV genome is protected on both ends by 145 base T-shape inverted terminal repeat (ITR) sequences. These structures are implicated in replication, second-strand synthesis, encapsidation, and insertion of the viral genome into an AAVS1 locus on human chromosome 19 [2]. The AAV genome encodes for the four non-structural Rep proteins responsible for viral replication and three capsid proteins (VP1–3), in addition to an Assembly Activating Protein (AAP) encoded on an alternative reading frame within the *cap* gene [3]. A new protein was recently discovered, called "Membrane Associated Accessory Protein" (MAAP), encoded on an alternative ORF in the VP1 region [4].

AAV-derived recombinant vectors (rAAV) represent one of the most powerful gene therapy tools and are currently under investigation in numerous clinical trials, such as phase III studies for haemophilia [5], phase I/II studies for Pompe disease [6] and Parkinson's disease [7]. Three gene therapy products based on natural AAV serotypes have reached the market. These include Glybera (AAV1) for the treatment of inherited metabolic disorder lipoprotein lipase deficiency (LPLD), unfortunately withdrawn from the market in 2017 [8], Luxturna (AAV2) for the treatment of an inherited retinal diseases (IRDs) [9] and, most recently, Zolgensma (AAV9) for the treatment of spinal muscular atrophy type 1 (SMA1) [10]. However, the high cost of large-scale production of clinical grade rAAV, as well as the low efficiency of the transduction of primary human cells, remain the main obstacles for clinical applications of the AAV vector.

Since the AAV capsid is the primary determinant of vector tropism, as well as influences vector packaging efficiency (= yield), AAV capsid bioengineering has the potential to overcome the main limitations of the current AAV vectors. Specifically, AAV bioengineering has been shown that it can help overcome limited tissue tropism, including cell binding, entry, endosomal escape, and trafficking, as well as vector targeting by the immune system [11, 12]. Many different approaches have been developed to engineer AAVs for the transduction of clinically relevant cells and organs [13-16]. Those have been extensively reviewed in Colella et al. [3], [17] and Wang et al. [18]. Capsid diversification techniques can be focused on the entire capsid or can be limited to specific regions, such as the hypervariable regions [19] located within the surface exposed loops. The most frequently utilized capsid modification strategies simulate the processes of natural evolution to create large and highly variable libraries of modified capsid variants with new properties. The capsid libraries can be generated through a number of molecular biology techniques, such as the introduction of point mutations in the capsid gene by error-prone PCR [20], the incorporation of random peptide libraries on the vector surface (AAV display peptide libraries) [21] as well as DNA shuffling. The latter technology is based on the recombination of parental genes, which are first fragmented and subsequently randomly reassembled, resulting in libraries of chimeric genes [12].

Following the generation of a sufficiently diverse pool of AAVs, the directed evolution is applied to facilitate a screen for novel variants with desirable properties. Through submitting the heterologous library of AAVs to an experimentally controlled selective pressure, either *in vitro*, *in vivo* or *ex vivo*, this technique has been successfully applied to engineer novel AAV variants, such as variants capable of more efficient transduction of human pluripotent stem cells (hPSCs), human liver cells, murine and primate retina reviewed in Zinn [22].

The liver in particular is a key target for the development of more efficient AAV vectors, given its involvement in over 500 functions, such as metabolic activities. There are over 100 different liver diseases, both genetic and acquired, which reduce the quality of life and significantly contribute to mortality, resulting in more than 2 million deaths per annum [23]. They also account for an unimaginable healthcare cost worldwide. Several natural serotypes of AAV have been developed as gene therapy vectors, with activities serotypes AAV2, AAV5, AAV8 and AAV9 among the most common choices for liver targeting [24]. Prototypical AAV2 has been the first AAV to be used in a liver-targeted clinical study [25], although the clinical benefit was short lasting and weaker than would have been anticipated based on preclinical studies. UniQure carried out a clinical trial delivering the factor IX in adults with haemophilia B using the AAV5 capsid (AMT-060) [26]. BioMarin Pharmaceutical used AAV5 to deliver the codon-optimized B domain-deleted F8 cDNA (BDD-FVIII) (BMN270) into patients with severe haemophilia A in a phase 1/2 clinical trial, and subsequently expanded the testing of BMN270 into two phase 3 trials (NCT03370913, NCT03392974) [27]. Spark Therapeutics used a modified AAV8 capsid (SPK-9001) to deliver hyperactive FIX variant (R338L) Padua in a clinical trial (NCT03587116) for haemophilia B [28]. AAV8 has been evaluated in multiple liver-targeted clinical studies, with the most prominent being work performed by Nathwani et al towards the development of an AAV-based gene therapy for haemophilia [29]. AAV9 was employed as a vehicle for the administration of the SMN gene, the causative gene in SMA, in a clinical trial conducted by AveXis in SMA patients. The trial demonstrated remarkable improvements in motor milestones and rates of survival in the patients [30].

In addition to the natural variants, the first wave of bioengineered variants has now reached the clinic. Spark Therapeutics used an engineered capsid AAV-LK03 to deliver BDD-FVIII (SPK-8011) in a phase 1/2 clinical trial [31]. LK03 is the first clinically-tested AAV capsid produced by capsid shuffling and library selection, and it was shown to transduce human hepatocytes 100-fold better than AAV8 *in vitro*, along with being more resistant to NAbs than AAV2 [32]. Spark100 is another engineered capsid currently being prepared for phase 3 clinical trials, after demonstrating sustained therapeutic expression of FIX coagulant activity following gene transfer to Haemophilia B patients [33].

However, clinical development would not be possible without the strong support of preclinical data, often obtained from a number of various preclinical model systems. While later stages of preclinical development often utilize *in vivo* models, such as non-human primates, early stage and exploratory studies, such as those developing new therapeutic strategies for hundreds of genetic disorders affecting the liver, often rely on efficient delivery to more robust *in vitro* models, such as those based on immortalized hepatocytes.

Here we present the outcomes of an AAV bioengineering approach used to develop novel AAV variants to support preclinical studies in the area of human liver gene therapy. The study involved the creation of a diverse AAV capsid library, based on twelve natural parental serotypes, and a subsequent selection in a preclinical model of the human liver. The strategy was designed to facilitate the accumulation of modifications within the AAV capsid that can improve the efficiency of transduction of immortalized human liver cells. In addition to giving rise to powerful vector candidates, these studies serve as a proof-of-concept for AAV development using more advanced preclinical models of the human liver and other tissues.

Materials and Methods

Generation of a shuffled capsid library

Shuffled AAV libraries were generated as previously described [12], with some modifications. The capsid genes from wt AAV serotypes 1 through 12 were cloned into a plasmid based on the pGEM-T Easy System (Promega, cat. no A1360) and served as templates for PCR. Gene coding for capsids were amplified in standard PCR reaction, mixes at 1:1 molar ratio, and digested with 0.1 U DNaseI (NEB, cat. no. M030L) for 2-5 min. The pool of fragments was separated on a 1% agarose gel and fragments from 200 to 1000 bp were excited from the gel using a ZymoClean Gel DNA Recovery Kit (Zymogen, cat. no. D4001T).

For primer-less PCR reassembly reaction, 500 ng of gel-extracted fragments was used. Fully reassembled capsids were amplified in a second PCR with primers F: GTACCAGTTCAGTCCAGTTGCA and R: CATGTG-GATCCTGGTACGTGT carrying the overlapping ends to a pLL012_pGEM library rescue vector. A Gibson Assembly reaction was performed by mixing an equal volume of 2 × GA Master Mix (NEB, cat. no. E2611L) with 1 pmol PCR-amplified and DpnI (NEB, cat. no. R0176L) – treated pLL012_pGEM (Forward: 5'-ACTTGTTCACTTTGATGGCGAGG, Reverse: 5'-CTGCACACGACATGACAT-CACG) and 1 pmol of the recovered shuffled capsids. DNA was ethanol precipitated and electroporated into SS320 electro-competent *E. coli* (Lucigen, cat. no. 60512-2). The total number of transformants was calculated by plating 10-fold serial dilutions of the electroporated bacteria. The pool of transformants was grown overnight in 500 ml of Luria-Bertani media supplemented with trimethoprim (10 mg/mL) (Sigma, cat. no. T7883-5G). Ten individual clones were picked, sequenced and analysed using a Sequence Origin Depiction (SOD) plugin (https:// github.com/CMRI-TVG/AAVcodons) created for Geneious (https://www.geneious.com/) [34] and the Xover tool (http://qpmf.rx.umaryland.edu/xover.html).

Libraries were subsequently digested overnight with SwaI (NEB, cat. no. R0604L) and NsiI (cat. no. R0127L), while 1 mg of the insert was ligated using T4 DNA ligase (NEB, cat. no. M0202) at a 1:1 molar ratio into a replication-competent AAV2-based plasmid (pWK5), containing ITR2 and rep2, and unique SwaI and NsiI sites downstream of rep2. Ligation reactions were concentrated using ethanol precipitation, electroporated into SS320 electrocompetent bacteria cells, and grown as described above. Total pWK5 library plasmids were purified with an EndoFreeMaxi prep Kit (QIAGEN, cat. no. 12362).

AAV production and purification

All AAV vectors stocks were prepared by polyethylenimine (PEI) (Polysciences, cat. no. 239662) triple transfection (2:1 PEI:DNA ratio) of adherent HEK293T cells (ATCC, cat. no. CRL-3126). The pAd5 helper plasmid, AAV transfer vector expressing GFP, and an AAV-helper plasmid encoding rep2 and the capsid of interest were transfected at a 2:1:1 molar ratio. For AAV capsid libraries (replication competent), only two plasmids were used: the pAd5 helper plasmid and the pWK5 libraries containing ITR-rep2-CapLibrary-ITR at a 1:1 molar ratio.

Cells were seeded 18 hr prior to transfection into five 15-cm tissue culture dishes to obtain 90% confluency at the time of transfection. Cells were harvested 72 hr post transfection and centrifuged for 15 min at 5,000 g. The media was mixed with a ¼ volume of 40% PEG (Sigma-Aldrich, cat. no. 89510-1KG-F) in 2.5 M NaCl (Sigma-Aldrich, cat. no. 746398-500G) and incubated on ice overnight. After centrifugation at 5,000 g for 30 min at 4°C, the PEG pellet was resuspended in a 1 ml PBS buffer (pH 8.0). The cell pellet was resuspended in a 2 mL PBS buffer (pH 8.5) and subjected to three freeze-thaw cycles. The resuspended PEG pellet solution and the cell lysate were combined. Genomic and free plasmid DNA were removed by incubating with 200 U/mL of Benzonase (EMD Millipore, cat. no. 1016970001) at 37°C for 1 hr. Subsequently 10% sodium deoxycholate (Sigma-Aldrich, cat. no. D6750-25G) and 5M NaCl were added to a final concentration of 0.5% and 1M, respectively. Following incubation for 30 min at 37°C, cellular debris was removed by centrifugation for 30 min at 5,000 g.

Vectors were subsequently purified using iodixanol density gradients as previously described [35]. Amicon Ultra-4 Centrifuge Filter Units with Ultracel-100 kDa membrane

(EMD Millipore, cat. no. UFC810024) were used to perform a buffer exchange and concentration step. Purified AAV particles were stored in a PBS buffer supplemented with 50 mM NaCl, 0.001% Pluronic F68 [v/v] (LifeTech, cat. no. 24040-032) at -80°C.

AAV titration

Vector preparations were titrated by real-time qPCR as described previously [36] using the following primers: GFP-F: 5'-TCAAGATCCGCCACAACATC and GFP-R: 5'-TTCTCGTTGGGGTCTTTGCT for vectors encoding the GFP, and rep2-F: 5'-AAGGATCACGTGGTTGAGGT and rep2-R: 5'-CCCACGTGACGAGAACATTT for replication-competent library preparations.

Library selection and vectorization of evolved AAV capsids

Human hepatoma HuH-7 cells (kindly provided by Dr Jerome Laurence, The University of Sydney) were seeded in complete media (DMEM with 10% [v/v] FBS) at 2 × 10_5 cells per well in 24-well dishes 18hrs prior to infection with the AAV library. Six 10-fold dilutions of the AAV library were added to the media in duplicates. Cells were washed with PBS buffer 6 hrs after infection and wild type human adenovirus 5 (hAd5) (ATCC, cat. no. VR-1516) was added to facilitate AAV library replication. The plate without hAd5 served as a qPCR control. Cells were harvested 72 hrs after hAd5 infection and lysed by three freeze-thaw cycles. After each round of selection, AAV amplification was verified in each well by qPCR. To minimize cross-packaging of multiple vectors in a single cell, the highest library dilution that resulted in no less than a 2-log increase in AAV signal, when compared to the control well without wtAd5, was selected for the next round. Between selection rounds, heat inactivation (65°C for 30min) was used to inactivate the wtAd5 in the samples. After the last, fourth round of selection, AAV capsid sequences were recovered from the wells by PCR using primers flanking the capsid region (Cap-F: 5'-CGATCTGGTCAATGTGGATTTGGATGACTGC, Cap-R: 5' GTAGTTAATGATTAACCCGCCATGCTACTTATCTACATG CAT). PCR-amplified cap genes were cloned into unique SwaI and NsiI sites of recipient helper packaging plasmid (pR2C) (downstream of the *rep2* gene), by digestion of the libraries overnight with SwaI and NsiI, and ligation of the insert and plasmid with T4 DNA ligase at 3:1 molar ratios (1.3 mg of insert and 1 mg of plasmid). Ligation reactions were concentrated using ethanol precipitation, electroporated into SS320 electrocompetent bacteria, and grown as described above. Twenty individual clones were sequenced (Genomed S.A., Warsaw, Poland) to confirm progress of the selection process and to learn the sequence identity of selected capsids.

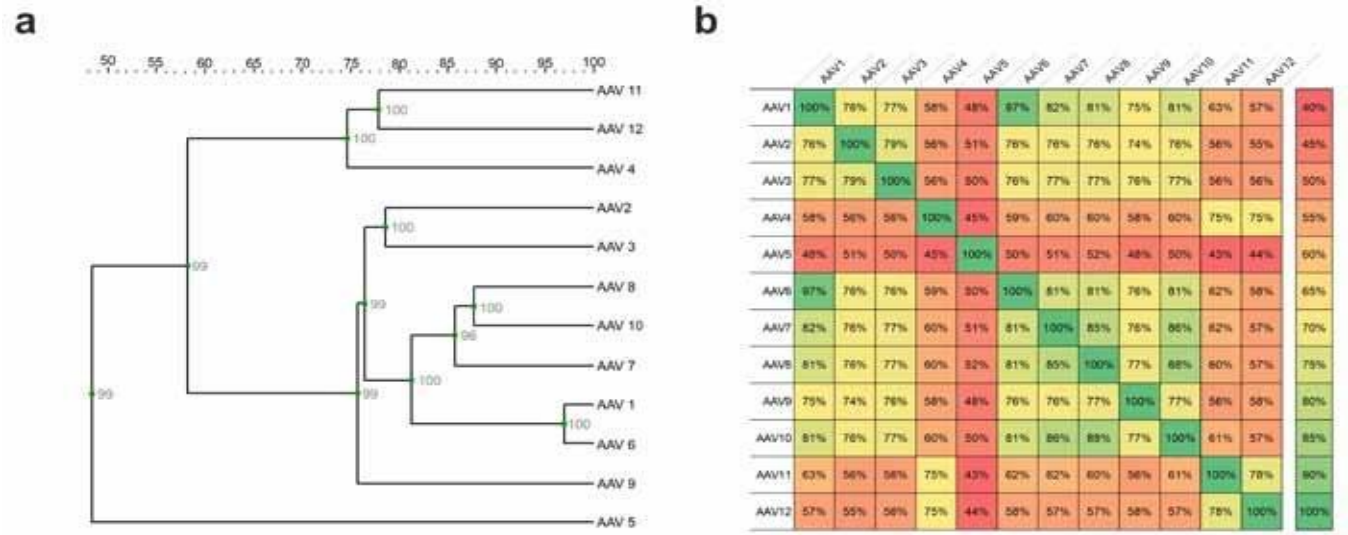
***In vitro* transduction analysis**

For transduction, HuH-7, HEK293 and A549 (ECACC, cat. no. 86012804) cells were plated at 2 × 10_5 cells per well into 24-well plates in complete media. Four hours later, the vector stock was diluted into 0.5 ml of complete media and added to the cells to achieve multiplicities of transduction (MOTs) of 10_5, 10_4 and 10_3. The cells were harvested 72 hrs after transduction using TrypLE Express (Thermo Fisher, cat. no. 12604021), washed three times with PBS buffer and resuspended in 200 µl of cytometer buffer. EGFP expression was quantified in 20,000 cells using a BD FACS Calibur flow cytometer (Becton Dickinson, San José, CA, USA) equipped with a 488-nm argon-ion laser. Analysis of data was performed using Cell Quest Software (Becton Dickinson, San José, CA, USA). The results were shown as a percentage of cells with GFP fluorescence and mean fluorescence intensity (MFI) calculated for each sample (Department of Pathological Anatomy, National Veterinary Research Institute, Puławy, Poland).

Phylogenetic analysis of AAV sequences

Phylogenetic analysis of AAV sequences was performed using BioNumerics 7.6.3 (Applied Maths, Sint-Martens-Latem, Belgium). A dendrogram was computed using multiple alignment with correction Jukes & Cantor and the clustering UPGMA method. The created phylogenetic tree was verified by cophenetic correlation coefficient (green dots).

Figure 1. Parental capsid phylogenetic relationship among 12 AAV variants used in the study.



a) Phylogram tree b) Percent identity.

Results

An AAV capsid library based on DNA shuffling of 12 different AAV parental serotypes was generated to enable selection of novel capsid variants on liver cells *in vitro*. The percent identity between the twelve capsid genes varied from 43% to 97%, with cap 5 being the most distant serotype included in the mix (Fig. 1).

Specifically, DNA shuffling of capsid genes was performed by enzymatic fragmentation of parental AAV serotypes, followed by assembly of a full-length genes to create a diverse library of chimeras (Fig. 2).

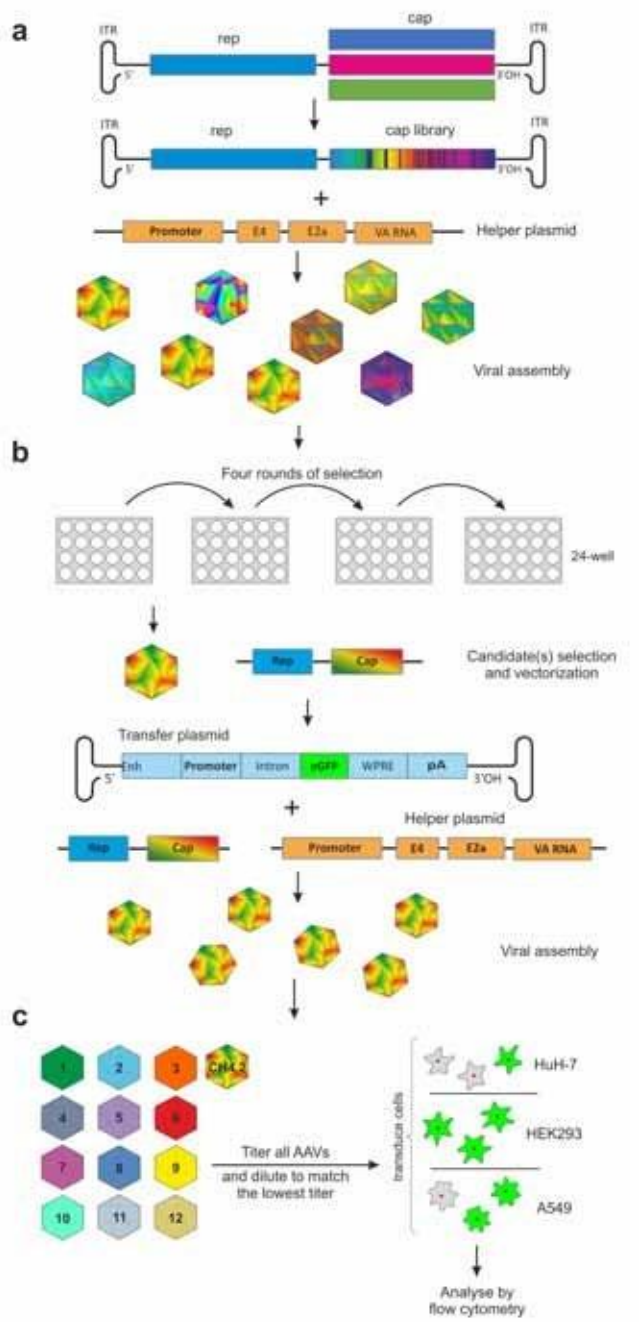
The library was cloned into a rescue vector and the capsid region of ten individual clones from the final plasmid library was Sanger sequenced. Analysis of the capsid gene sequences revealed that all clones were unique and highly diverse, containing fragments from multiple parental donor serotypes. Subsequently, the shuffled library was cloned into a replication-competent recipient construct (Fig. 2a) and an additional ten clones were fully sequenced to confirm the high quality of the final library prior to the vector packaging step (Fig. 3a).

Detailed sequence analysis of the individual clones revealed that the average length of individual fragments from parental donors was 111.1 bp, the average number of parental segments per clone was 19.3 ± 3.4 and the average number of parental variants contributing to each chimera sequence was 8.5 ± 1.2. Shuffling index (percentage of individual clones containing at least one fragment >15bp from a given parental donor) was calculated and the results showed that the contribution of parental sequences from AAV6 and AAV1 was 32% and 29%, respectively. The remaining serotypes with considerable contribution were AAV7, AAV8 and AAV10 (shuffling index of 9.8%, 9.8% and 6.3%, respectively). AAV5, AAV11, AAV12 and AAV4 contribution per single chimera was 1%, 1.1%, 0.3%, and 0.2%, respectively (Fig. 3b).

The results confirmed the extensive genetic diversity and the presence of all twelve parental viruses in the final library pool, with a bias toward AAV6 and AAV1 serotypes.

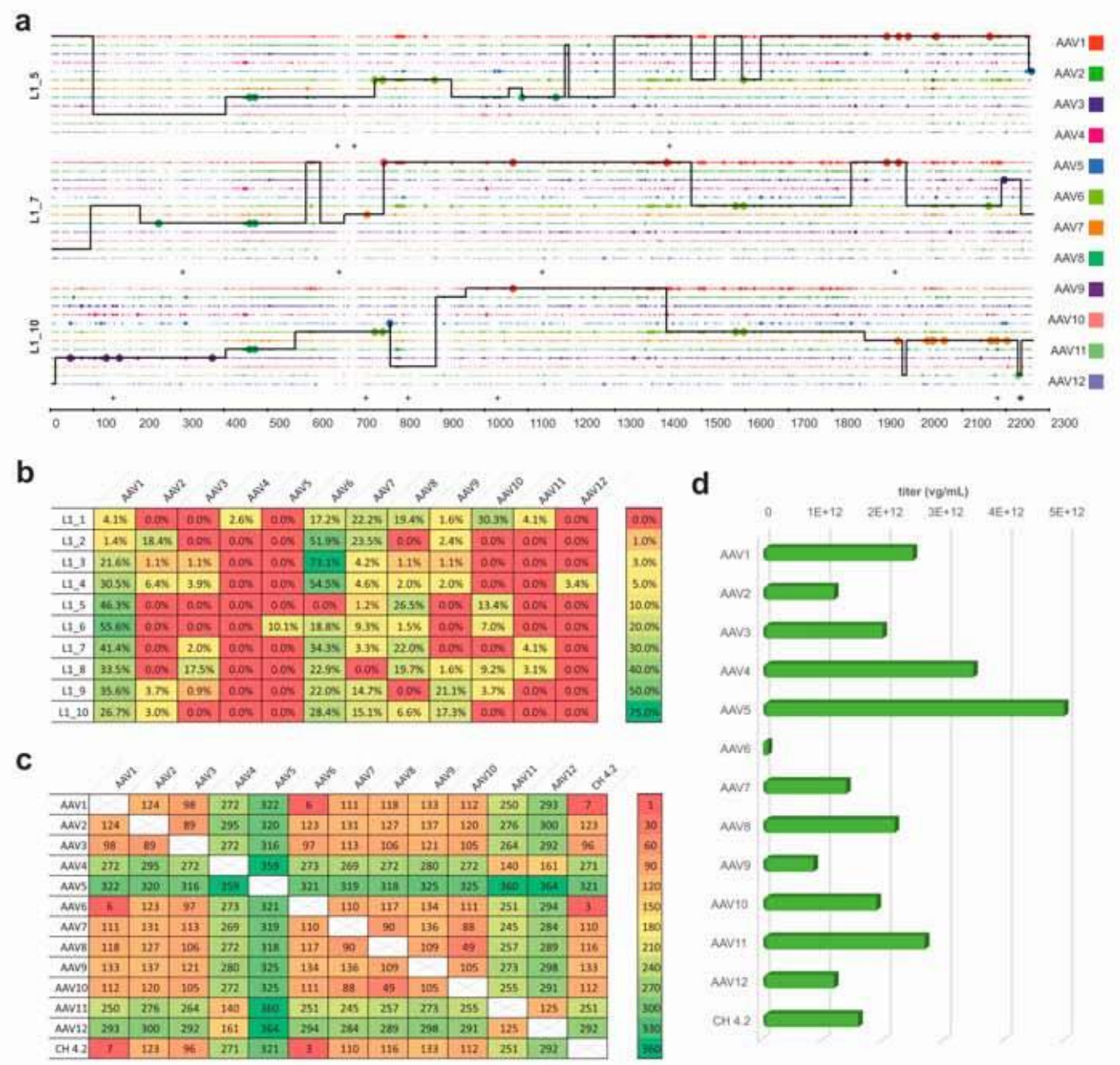
To screen for capsids with enhanced efficiency in liver, the library was packed, resulting in a particle titre of ~$2.9 \times 10_{13}$ genomes/ml in the total volume of 500 µl. The produced AAV library was used to perform four rounds of selection on hepatoma cells (HuH-7) in the presence of wild type human adenovirus type 5 (Ad5) to support AAV replication. A total of 4 rounds of iterative selection were performed and, after the last round of selection, 17 clones were selected and sequenced to verify completion of the selection process. Sequencing revealed successful enrichment of a single chimera (AAV-CH4.2), which represented over 90% of the randomly selected clones. The sequence of AAV-CH4.2 revealed predominant homology to serotypes AAV6 and AAV1. On the nucleotide level, the capsid gene coding for AAV-CH4.2 was 98.55% similar to AAV6 and 97.42% AAV1. On the amino acid level, the sequence identity was 99.59% and 99.05% to AAV6 and AAV1, respectively, or in other words the difference between them was 3 and 7 amino acids, respectively (Fig. 3c).

Figure 2. Schematic representation of the directed evolution of AAV capsids by DNA shuffling and functional validation of selected novel variants.



a) AAV capsid (cap) genes from parental AAV1 to AAV12 were digested with DNase followed by primer-less PCR to reassemble full-length shuffled genes. The shuffled capsid genes were inserted into a plasmid carrying AAV2 ITRs and the AAV2 rep gene. The resulting plasmid library was transiently transfected together with an adenoviral helper plasmid (pAd5, encoding E4, E2a and VA adenoviral genes required for AAV replication), into HEK293 to produce a viral AAV library.
b) The AAV library selection was performed by coinfecting the cultured liver cells with the library and wild-type adenovirus type 5 (wtAd5), which enabled replication of AAV variants that could effectively infect the target cells. Capsid gene from the selected AAV variant was subsequently PCR amplified and cloned into an AAV plasmid carrying the AAV2 rep gene to generate an AAV packaging construct. The AAV packaging plasmid was then transfected together with pAd5 and the transfer plasmid (a plasmid encoding a standard expression cassette consisting of the green fluorescent protein (GFP) transgene, polyadenylation signal (pA), and woodchuck post-transcriptional regulatory element (WPRE)) into HEK293 cells to produce the final AAV-GFP for functional studies.
c) The new vector was used to transduce the target cells, and the GFP expression was quantified. Parental AAVs were included as references.

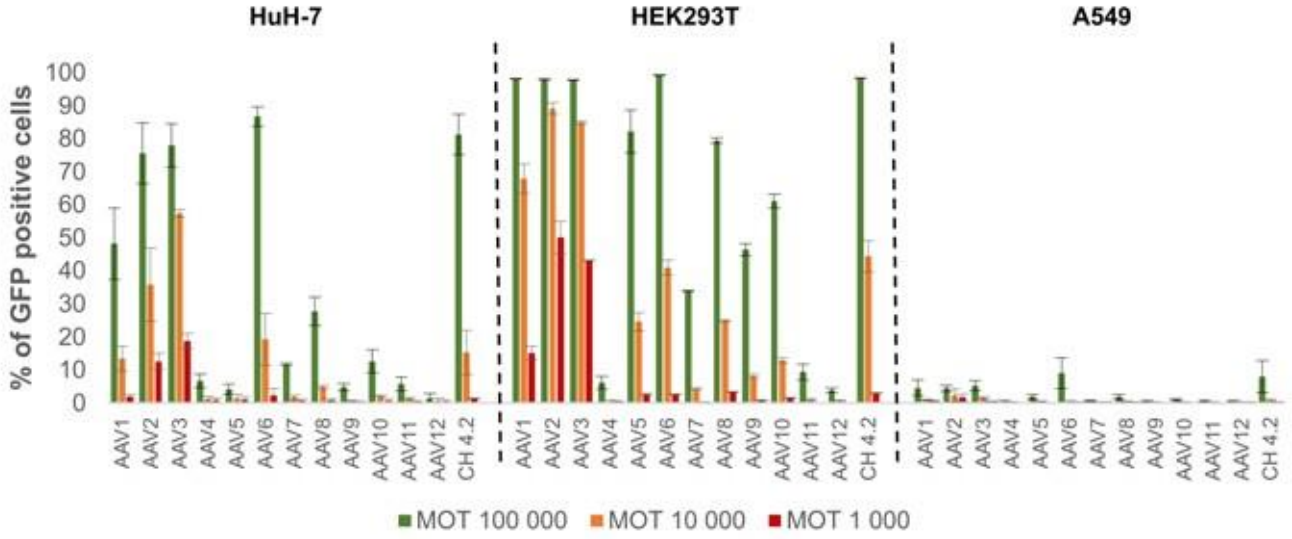
Figure 3. Analysis of the capsid gene sequences and vector yield comparison.



a) Amino acid composition analysis of shuffled capsid clones in the final plasmid library. Parental AAVs are shown in different colours. The size of the dot represents the probability level that a given sequence originated from that particular parental serotype. The solid black line for each chimera (L1_5, L1_7, L1_10) outlines the parental composition of a given variant. A plus sign "+" indicates a de novo mutation that cannot be tracked back to any of the parental donors.
b) Shuffling index, the percentage of individual clones containing at least one fragment <15bp from a given parental donor, between the capsid library, based on a sequence composition of the 10 random clones (L1_1-L1_10), and the parental serotypes, with a green colour indicating a higher and red indicating a lower shuffling index.
c) Amino acid identity score (number of differences at the amino acid level) between individual parental serotypes (AAV1-12) and generated chimera CH4.2, with a red colour indicating higher and a green colour indicating lower identity.
d) Vector yield comparison of the novel selected chimera and parental variants.

Next, the transduction efficiency of the selected variant was assessed on human cell lines of various hepatocytes origin, namely hepato-carcinoma 7 cells (HuH-7), as well as non-liver cell lines, namely embryonic kidney cells (HEK293) and lung carcinoma cells (A549). To do so, the cap AAV-CH4.2 gene coding sequence was recovered by PCR and cloned into a standard helper plasmid downstream of the AAV *rep2* gene. This AAV-CH4.2 packaging plasmid was subsequently used to package AAV vectors encoding the GFP reporter protein under the control of a ubiquitous CAG promoter. The same reporter cassette was also packaged into the twelve parental variants (AAV1-12), which were to be used as reference controls in the transduction study. However, this also allowed for direct vector yield comparison. All vectors were titrated by qPCR, and the titre from the iodixanol production ranged from $5.3\times10_{12}$ (AAV5) to $1\times10_{11}$ vg/ml (AAV6) (Fig. 3d). Although at the sequence level our novel variant AAV-CH4.2 was

Figure 4. Results of flow cytometry analysis for each cell type, individually transduced with parental AAVs and new vector variant (AAV-CH4.2).



very similar to AAV6, AAV-CH4.2 packaged substantially more efficiently than AAV6 with the final titre 10× higher than AAV6. All vectors were diluted to the same concentration and were used to transduce the above-mentioned cell lines at 3 different multiplicities of transduction (MOTs, number of vector particles per target cell): $100\times10_3$, $10\times10_3$ and $1\times10_3$. The cells were harvested 72 hours later, and the efficiency of transduction was analysed by flow cytometry based on the level of GFP protein expression (Fig. 4).

As shown in Figure 4, the levels of transduction differed substantially between cell lines for all AAV tested. Not surprisingly, a higher MOT led to an overall higher level of transduction in all groups. On the HuH-7 cells, AAV2, AAV3, AAV6 and AAV-CH4.2 outperformed all the other vectors, and at MOT $100\times10_3$ functionally transuded 75.7%, 78.1%, 86.7% and 81.4% of HuH-7 cells, respectively. The remaining vectors transduced HuH-7 cells less efficiently and could be grouped into average transducers (AAV1 48.3%, AAV8 27.8%) and poor transducers (AAV10 12.6%, AAV7 11.7%, AAV4 6.7%, AAV11 5.8%, AA9 4.9%, AAV5 4.1% and AAV12 1.5%). The level of transduction of AAV-CH4.2 was comparable with the best vector (AAV6) and more than ten times better than AAV4, AAV11, AAV9, AAV5 or AAV12.

On the HEK293 embryonic kidney cells, AAV1, AAV2, AAV3, AAV6 and AAV-CH4.2 vectors reached a saturation level of transduction (98.3%, 98%, 97.8%, 99.4%, and 98.5% GFP positive cells, respectively) at MOT $100\times10_3$. At MOT $10\times10_3$, AAV1, AAV2, AAV3, AAV6 and AAV-CH4.2 transduced 71.1%, 90.4%, 85.3%, 42.7% and 47.8% cells, respectively. AAV5, AAV8 and AAV10 constituted an average transducer group (26.5%, 25.2%, 12.3%, respectively), while AAV9, AAV7, AAV11, AAV12, and AAV4 were the poorest transducers (7.7%, 4.6%, 1.3%, 0.9%, and 0.8%, respectively). Even though the transduction level of AAV-CH4.2 was lower than AAV1, AAV2 and AAV3, it was still more than forty times better than AAV11, AAV12 and AAV4.

The lung carcinoma line, A549, was the most resistant to AAV transduction, with AAV6 and AAV-CH4.2 leading to the highest level of transduction of 9.1% and 8.1%, respectively. AAV1, AAV2 and AAV3 transduced 4.6%, 4.5% and 5.3% of the cells, respectively, while the remaining vectors transduced only 1% or less of the A549 cells.

Discussion

Currently, most AAV-based vectors used in gene therapy are derived from naturally occurring serotypes (e.g. AAV2, AAV5 or AAV8). Their ability to transduce the target tissues in clinical trials often proves to be much lower than anticipated based on the data from preclinical studies, which is why more functional variants for clinical applications are still in high demand. Strategies that enable shuffling AAV capsids from closely related (e.g. AAV1, 2, 3, 6) as well as evolutionarily distant (e.g. AAV5) serotypes enable the generation of large pools of random variants (AAV libraries), each with a unique sequence and function. Such large libraries form a starting point in directed evolution strategies that enable selection for new variants with the desired properties using preclinical models of human organs. The selection pressure applied can direct the selection process (hence Directed Evolution) towards identification of variants with new critical properties, such as increased transduction efficiency or specificity towards target cells.

However, as the novel bioengineered variants become more efficient at the transduction of primary human cells *in vivo*, they often become less efficient at transgene delivery in preclinical models, such as immortalized cells. In fact, recent data suggests that some capsid properties that help with efficient transduction *in vitro* may differ from those required for the *in vivo* function. For example, strong binding to heparan sulfate proteoglycans (HSPG), which

help with transduction of cells in tissue culture settings, make the vectors inefficient at tissue entry and transduction *in vivo* [37]. This suggests that as the field moved towards more efficient AAV variants for clinical applications, parallel efforts may be required to develop tools in support of the early preclinical work.

To this end, in the present study, the *cap* gene shuffling of 12 AAV serotypes was performed, followed by selection on the human liver cell line, resulting in the identification of a novel variant (AAV-CH4.2). Functional analysis using three-cell lines revealed that AAV-CH4.2 was one of the most efficiently transducing variants when compared to parental AAVs. The *cap* AAV-CH4.2 sequence at the amino acid level is very similar to the *cap* sequence of AAV6 and AAV1, which could be related to the fact that those two serotypes were the most represented ones in the shuffled library used in this study. The overrepresentation of AAV1 and AAV6 may be due to the fact that the high identity of the *cap* DNA sequences directly influences the efficiency of shuffling of the input parental sequences, leading to significant advantage in sequences contributed to by the closely related AAV serotypes [34]. AAV1 and AAV6 have the highest pairwise identity among the parental AAVs (97.1%), and together with AAV7, AAV8 and AAV9 belong to the same clade. More distant parental serotypes, such as AAV5, AAV4, AAV11 and AAV12, did not shuffle efficiently with other variants and were somewhat under-represented in our initial library. The results obtained in our study coincide with the phenomenon described in the literature, that typically evolved capsids differ from a single parent by only up to seven residues (this depends on the library type but usually corresponding to less than 1% of the capsid protein) [12].

It is known that AAVs attach to specific receptors on the cell surface in a serotype dependent manner. The attachment of most of the AAV serotype vectors is first mediated by binding to various cell surfaces or extracellular matrix proteoglycans, which serve as primary receptors. To enter the cells, interactions with additional co-receptors that mediate endocytosis are required [38]. More recently, a type-1 transmembrane protein was shown to be required for transduction by most AAV serotypes and was named the AAV receptor (AAVR) [39]. To compare the transduction efficiency of the selected variant with parental serotypes, cell lines from different human tissues were transduced with AAV-CH4.2 encoding the GFP reporter, using parental AAV1-12 as controls. Studies showed that the newly selected vector, AAV-CH4.2, together with closely related AAV6, were among the best transducing vectors on each of the tested cell lines, which is a very promising result. The second most effective vectors (AAV2 and AAV3) belong to one clade, and the weakest transducers (AAV4, AAV11 and AAV12) also belong to one clade, a fact that can potentially explain the similarity in transduction pattern. Different transduction levels on the studied cell lines, especially the very weak transduction on A549 cells, can point towards the existence of a potential barrier preventing AAVs from cell attachments on cell entry. As described in the literature, the AAV receptor (AAVR) has a specific and restricted expression pattern; for example, it is weakly expressed on airway epithelial cells. For most AAVs, which rely on this receptor for cellular entry, this could impose a strong barrier that holds back the vectors from entry [40].

Gene therapies are currently among the most expensive therapeutics available, which limits access for many patients and thus lowers the overall impact on the patient population. The high cost of gene therapeutics is in part driven by the high cost of vector manufacturing, which also creates a substantial roadblock for many promising translational programs. An efficient method of vector production is essential for successful commercial implementation of a gene therapy. While preclinical studies require substantially lower amounts of vectors, efficient manufacturing is still an important factor when considering the choice of vector. Despite being highly similar to AAV6 at the amino acid sequence and function levels (Fig 4) the selected AAV-CH4.2 can be packaged at substantially higher yield (Fig 3d), increasing its overall value and utility.

The presented data illustrate the utility of AAV bioengineering for the development of AAV vectors for preclinical studies, while the vector packaging and functional data position AAV-CH4.2 as a strong candidate for further evaluation and as a starting point for further bioengineering applications.

Funding

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Declarations. Conflict of Interest

The authors declare no conflicts of interest.

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PREDICTORS FOR SUCCESSFUL LABOUR INDUCTION. THE ROLE OF CERVICAL SCORES

Predyktory skutecznej indukcji porodu. Rola skali dojrzałości szyjki macicy

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Abstract:

Introduction and objective. In obstetrics the induction of labour (IOL) is a common medical intervention. The aim of the study was to evaluate the factors predicting a successful labour induction and to analyse the connection between the components of Bishop score and the result of the induced labour.

Material and methods. This was a retrospective cohort study of 214 labour inductions conducted at the Gynaecology and Obstetrics Ward of Solec Hospital in Warsaw between January and December 2019. The type of delivery, whether vaginal or caesarean, was the outcome.

Results. The prevalence of the failed induction of labour was 28.97%. The main indications for caesarean delivery (CD) were impending foetal asphyxia and failure to progress in labour. Predictors of successful IOL are multiparity (p=0.0015), BMI < 30 of the patient (95% CI: 28.577, 29.756), mature cervix (95% CI: 7.657, 8,237), IOL with oxytocin infusion (p=0.0025) and such indications as gestational diabetes mellitus, premature rupture of membranes, LGA and less foetal movement sensation (p=0.0067). Among all parameters examined with the use of Bishop's score, cervical shortening correlates best with the prediction of successful IOL (Phi Coefficient 0.187)

Conclusions. The strongest predictors for successful IOL are parity and degree of shortening of the cervix.

Keywords: caesarean section, labour induction, parity, cervical score, cervical shortening.

Streszczenie:
Wprowadzenie i cel

Indukcja porodu jest powszechnie stosowaną procedurą w położnictwie. Celem badania było zbadanie czynników predykcyjnych skutecznej indukcji porodu i analiza związku pomiędzy parametrami ocenianymi w skali Bishopa a wynikiem indukcji porodu.

Materiał i metody

Badaniem objęto retrospektywnie 214 indukcji porodu przeprowadzonych w Oddziale Ginekologiczno-Położniczym Szpitala Solec w Warszawie w okresie od stycznia do grudnia 2019 r. Jako wynik indukcji oceniano ukończenie porodu: drogami natury lub cesarskim cięciem.

Wyniki

Nieskuteczna indukcja porodu wystąpiła u 28,97% pacjentek. Najczęstszym wskazaniem do porodu operacyjnego była zagrażająca zamartwica płodu i brak postępu porodu. Wielorództwo (p=0.0015), BMI pacjentki <30 (95% CI: 28,577-29,756), dojrzała szyjka macicy (95% CI: 7,657-8,237), indukcja oksytocyną (p=0,0025) i wskazania do przeprowadzenia indukcji obejmujące cukrzycę w ciąży, przedwczesne odpływanie płynu owodniowego, słabsze odczuwanie ruchów płodu lub szacowaną dużą masę płodu (p=0,0067) są predyktorami skutecznej indukcji porodu. Spośród parametrów ocenianych w skali Bishopa najlepiej koreluje z udaną indukcją porodu stopień skrócenia szyjki macicy (Phi Coefficient 0,187).

Wnioski

Najsilniejszymi predyktorami skutecznej indukcji porodu są rodność i stopień skrócenia szyjki macicy.

Słowa kluczowe: cięcie cesarskie, indukcja porodu, rodność, skala dojrzałości szyjki macicy, skrócenie szyjki macicy.

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Introduction

The development of perinatal care, the methods of monitoring foetal well-being and the increasing age of motherhood contribute to the rising proportion of induced labours.

Indications for induction of labour are divided into maternal and/or foetal. The most common ones include: pregnancy beyond the due date, premature rupture of the amniotic membranes after the 37th week of pregnancy, suspected high foetal weight or hypotrophy, abnormal foetal examination results, and a pregnancy complicated by diabetes, arterial hypertension, or cholestasis.

In 1964, Edward Bishop developed a cervical scoring system for assessing the chances of successful induction of labour (IOL). A score of 8 or more indicates that the cervix is ready for induction, and the chances of a natural childbirth are similar to spontaneous delivery. A score of 6 or less indicates insufficient maturity of the cervix for delivery. The scale has undergone modifications, the most significant being the 1966 Burnett modification and the 2011 simplified scale by Laughon et al.

Aim

The aim of the study was to investigate what determines the success of IOL: what influence the indications, parity, age, BMI, and the sex of the foetus have; and whether the pre-induction or induction method used influences the course of IOL. The analysis examined whether any of the parameters assessed by the Bishop's scale have a higher predictive value for successful IOL, and whether other scales of cervical maturity for labour are useful in daily clinical practice.

Material and methods

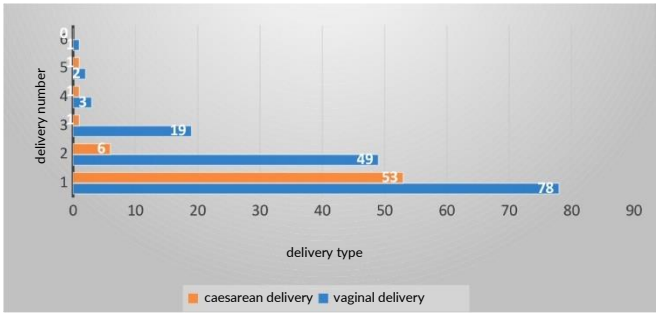
The course of labour induction in 214 patients undergoing this procedure at the Department of Gynaecology and Obstetrics at the Solec Hospital in Warsaw in 2019 was retrospectively analysed. The study included all patients with gestational age ≥ 24 weeks of pregnancy qualified for IOL. In the study group, the most common indications for induction were prolonged pregnancy, premature outflow of amniotic fluid and gestational diabetes. Mean patient age was 30.6 years. Primiparas accounted for the majority, 61.2% of the study group. There were 152 natural deliveries (71.03%) and 62 (28.97%) were completed by caesarean section. The main indications for caesarean delivery (CD) were impending foetal asphyxia and failure to progress in childbirth. The data collected was statistically analysed using the SAS system.

Results

Predictive factors for successful IOL include multiparity, BMI < 30, cervical maturity, oxytocin induction and indications for induction including gestational diabetes, premature outflow of amniotic fluid, reduced foetal movement or estimated high foetal weight. The sex of the foetus or the age of the patient does not influence the type of delivery unless it is not her first delivery.

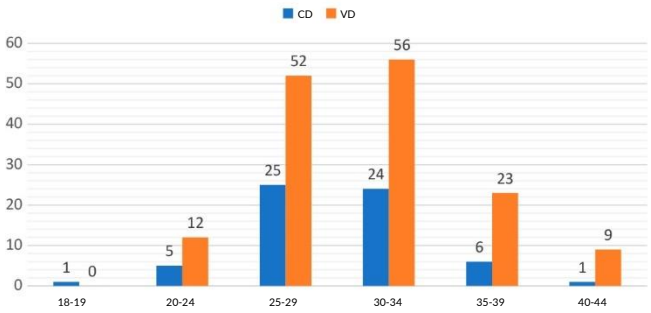
The highest percentage of CDs was among the primiparas and decreased with the number of deliveries the patient had (Fig. 1). In the study group, the percentage of natural deliveries was 59.54% for the primiparas. Among the multiparas, successful IOL was observed in 89.15% ($p < 0.0001$).

Figure 1. Type of delivery depending on parity.
Rycina 1. Droga porodu w zależności od rodności.



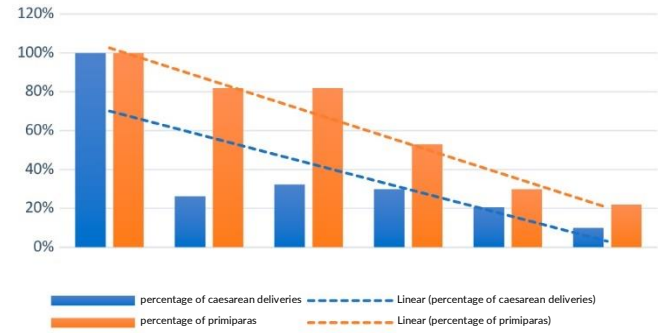
No correlation was observed between patient age and type of delivery. The mean patient age for successful induction was 31.046 (CI 95%: 30.270, 31.822) and 29.516 for unsuccessful induction (CI 95%: 28.472, 30.560). The percentage of caesarean deliveries is similar in all age groups, proportional to the percentage of primiparas, as shown in Figure 2.

Figure 2. Age and delivery type
Rycina 2. Wiek a droga porodu.



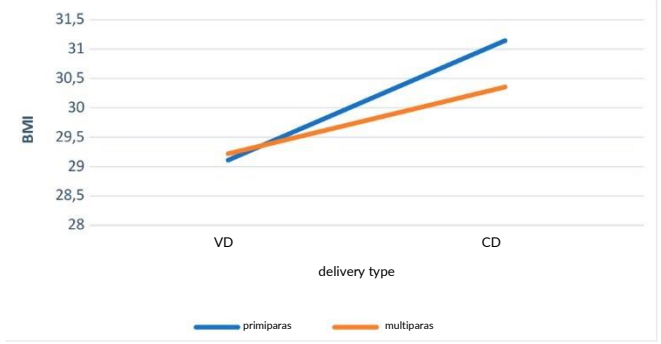
In contrast, a significant relationship can be seen between the age of the mother and the type of delivery in primiparas ($p<0.05$), as shown in Figure 3. As many as 39% of the caesarean deliveries were performed in primiparas aged > 30, 8% for those aged 20-24, 37% for those aged 25-29, and only 15% of the caesarean deliveries were conducted in multiparas.

Figure 3. Age and parity and delivery type
Rycina 3. Wiek i rodność a droga porodu.



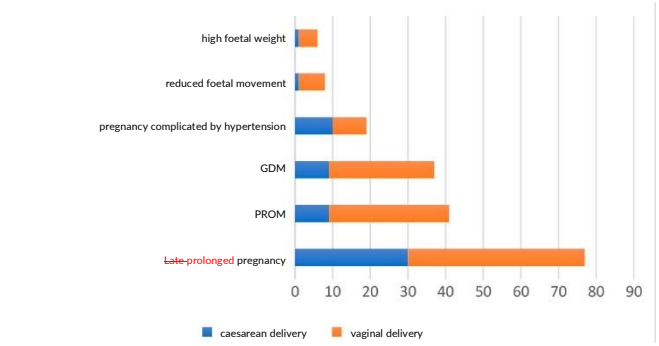
The risk of unsuccessful IOL increases with higher BMI ($p<0.05$), as shown in Figure 4. Among the primiparas whose IOL ended in caesarean delivery, the BMI averaged 31.145 (CI 95%: 30.055, 32.235); in multiparas the value was 30.355 (CI 95%: 29.265, 31.445). Patients who gave birth by vaginal delivery had a BMI of 29.110 – primiparas (CI 95%: 28.521, 29.700) and 29.219 – multiparas (CI 95%; 28.630, 29.809).

Figure 4. BMI and delivery type
Rycina 4. BMI a droga porodu.



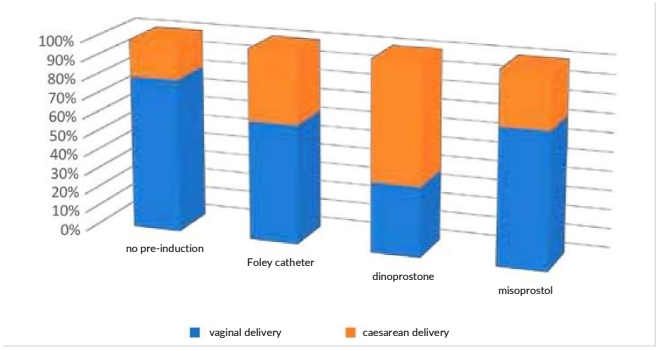
Of the indications for induction analysed, hypertension in the mother was associated with the highest rate of caesarean delivery, as shown in Figure 5. For this group of patients, induction ended in vaginal delivery in only 47.37% ($p=0.0067$). In the study group, among the inductions due to prolonged pregnancy, the rate of caesarean deliveries was also above 30%, at 38.96%.

Figure 5. Indications for IOL and delivery type.
Rycina 5. Wskazania do indukcji porodu a droga porodu.



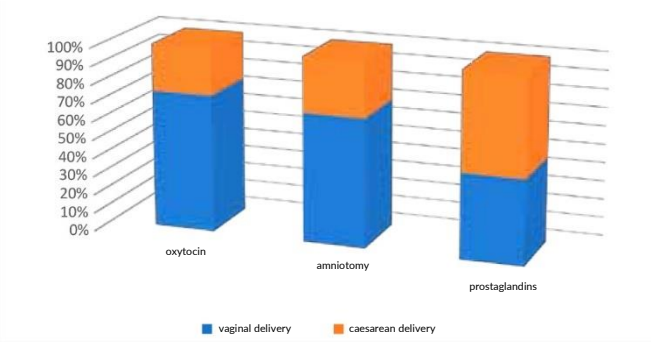
Preinduction with dinoprostone was the least likely to result in a vaginal delivery ($p=0.0005$). When misoprostol was used, the percentage of caesarean deliveries was significantly lower, at 28.57%. Patients with a mature cervix who did not require pre-induction had the highest rate of successful IOL, with vaginal delivery in 80.65% of patients, as shown in Figure 6.

Figure 6. Type of delivery depending on the pre-induction method.
Rycina 6. Droga porodu w zależności od metody preindukcji.



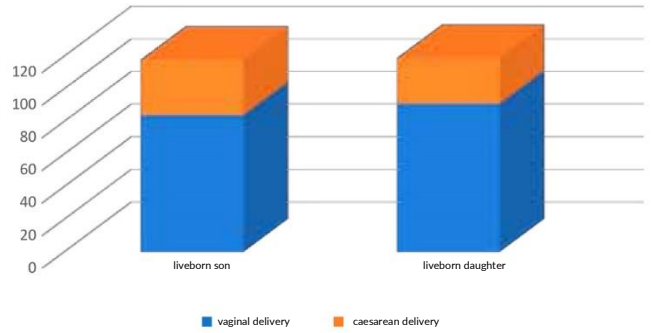
Three IOL methods were analysed: induction with oxytocin, amniotomy, and induction with prostaglandin. Significant differences can be seen in the percentage of successful inductions (Fig. 7): 74.61% after oxytocin, 69.61% after amniotomy and 46.16% after prostaglandin ($p=0.0025$ for oxytocin, $p=0.1127$ for amniotomy; $p=0.0044$ for prostaglandin).

Figure 7. Induction method and delivery type.
Rycina 7. Metoda indukcji a droga porodu.



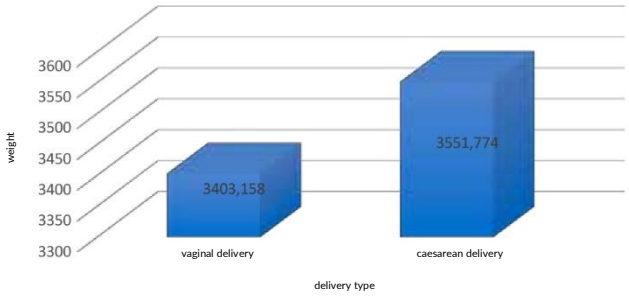
No relationship was observed between delivery type and foetal sex. For both sexes, the proportion of liveborn infants by vaginal delivery was similar. A total of 71% for sons and 69% for daughters (Fig. 8).

Figure 8. Delivery type and foetal sex.
Rycina 8. Droga porodu a płeć płodu.



A relationship was found between foetal weight and successful IOL, as shown in Figure 9. Higher infant weights were observed when IOL was not followed by vaginal delivery: 3551 g (CI 95%: 3448.42 - 3655.12) vs 3403 g (CI 95%: 3300.90 - 3505.40).

Figure 9. Delivery type and foetal weight.
Rycina 9. Droga porodu a masa płodu.



A correlation was found between all parameters assessed by Bishop's score before pre-induction and the outcome of IOL. The strongest correlation was for effacement and consistency of the cervix. The parameter that affected the outcome of IOL the least was dilation (Tab. 1).

Table 1. Correlation of parameters assessed prior to preinduction of labour with successful IOL.
Tabela 1. Korelacja z udaną indukcją porodu parametrów ocenianych przed preindukcją porodu.

Parameter	Correlation coefficient prior to preinduction of labour – phi coefficient
Dilation	0.08
Effacement	0.187
Foetal head progression	0.149
Cervical consistency	0.181
Cervical position	0.128

The strongest correlation between the outcome of IOL and parameters assessed with the Bishop's score prior to induction was found for the degree of cervical effacement. The weakest relationship was observed for the progression of the head in the birth canal (Table 2).

Table 2. Correlation of parameters assessed prior to IOL with successful IOL.
Tabela 2. Korelacja z udaną indukcją porodu parametrów ocenianych przed indukcją porodu.

Parameter	Correlation coefficient prior to IOL – phi coefficient
Dilation	0.108
Effacement	0.139
Foetal head progression	0.005
Cervical consistency	0.107
Cervical position	0.112

Bishop's score parameters assessed prior to preinduction correlate more strongly with successful IOL. If the cervix is mature and does not require preinduction, IOL is successful in the highest percentage. Individual cervical maturity, not requiring preinduction, is a strong predictor of successful IOL.

The relationship between such parameters as cervical dilation and foetal head progression with successful IOL differs significantly prior to preinduction and prior to IOL. Assessment of all cervical maturity parameters is not necessary to estimate the chances of successful induction. Based on the conducted study, there is no need to assess cervical dilation before preinduction of labour. On the other hand, foetal head progression is not important before IOL. However, cervical maturity scores do not differentiate when a cervical assessment is performed. Therefore, the Bishop's score, the modified Bishop's score and the simplified Laughon score are clinically useful.

Discussion

The study found a strong association between unsuccessful IOL and primiparity, hypertension complicating pregnancy, patient obesity and preinduction with dinoprostone. Similar results were obtained by Bjorklund et al. in a cohort study of the Swedish population, identifying parity as the most important risk factor for caesarean delivery during IOL [1].

Tadesse et al. identify primiparity, low Bishop's score, premature outflow of amniotic fluid and pregnancy-induced hypertension as factors for unsuccessful induction [2]. In a study by Górnisiewicz et al., investigating patients who underwent preinduction, primiparity and hypertension were risk factors for caesarean delivery, irrespective of the method used [3].

The study found a significant correlation between age and delivery type in primiparas – the risk of caesarean delivery increases after the age of 30. In their study, Teal et al. found no effect of age on the delivery type in primiparas [4]. Most publications, however, emphasise the relationship between the older age of the patient undergoing induction and the delivery type – Muto et al. found an increase in the rate of emergency caesarean deliveries during IOL in women ≥ 40 to 28.3% vs 18.3% among patients aged 35-39 [5]. Cao et al. compared the delivery type in primiparous, and multiparous women aged 35 years and older: primiparas are almost 1.5 times more likely (OR 1.48 CI 1.33-1.65) to give birth by caesarean delivery [6].

Obeidat et al. reported an adverse effect of high BMI on the course of induction in multiparas [7]. The discussed study shows an increase in caesarean delivery rates with increasing BMI regardless of parity. A study by Taoudi et al. showed that caesarean delivery is performed four times more frequently in obese women than in normal-weight patients [8]. In contrast, Tarimo et al. did not link the increased risk of caesarean delivery to patient obesity [9].

The highest rates of unsuccessful induction of labour in the present study were reported in women undergoing this procedure due to hypertension complicating pregnancy and prolonged pregnancy (≥ 41 weeks), 52.63% and 38.96%, respectively. Similar results were obtained by Awuah et al. – the caesarean delivery rate in patients induced due to hypertension was 56% [10]. When analysing the course of induction of labour in patients aged 35 years or older, Nakano et al. found that patient hypertension and an immature cervix were independent risk factors for emergency caesarean delivery [11]. An increased risk of caesarean delivery in prolonged pregnancy was observed by Lindquist et al. – after the 42nd week of pregnancy up to 96% [12]. Tarimo et al. cited primiparity (RR = 1.46; 95% CI: 1.18-1.81) and prolonged pregnancy (RR = 1.45; 95% CI: 1.09-1.93) as independent factors for caesarean termination of IOL; attention should be drawn to the fact that the relative risks are almost identical, at 1.46 vs 1.45 [9].

In the study group, induction of labour most commonly was attempted due to prolonged pregnancy and premature outflow of amniotic fluid, followed by gestational diabetes and hypertension in pregnancy. A similar pattern of indications for IOL was shown in a meta-analysis by Coates et al. [13]. In research by Kumar et al. [14], the most common indication for IOL was intrauterine foetal growth retardation (18%), followed by premature outflow of amniotic fluid (17%), reduced foetal movements (16%), prolonged pregnancy (15%), and gestational diabetes (13%). According to the report by Kamlungkuea et al., IOL was most commonly due to prolonged pregnancy, gestational diabetes, hypertension, and foetal growth abnormalities [15].

The course of IOL depending on the preinduction method used was examined by Familiari et al. [16]. Based on their analysis, termination of pregnancy by caesarean delivery due to threatened intrauterine foetal asphyxia was necessary in 18.1% of deliveries after the use of dinoprostone, in 9.4% of deliveries after the use of misoprostol and in 8.1% of deliveries after the use of Foley catheters. In the discussed study, the highest percentage of caesarean deliveries was also reached after using dinoprostone as the preinduction method, at 63.16 % ($p=0.0005$). A higher proportion of vaginal deliveries after the use of misoprostol, compared to dinoprostone, is also shown in the paper by Garg et al. [17]. Rossi et al. obtained similar rates of caesarean deliveries irrespective of the preinduction method: 25.6%, 26.3% and 22.0% for misoprostol, dinoprostone and Foley catheter respectively [18].

In the conducted analysis, the highest percentage of vaginal deliveries took place after oxytocin infusion, at 75%, and slightly lower after amniotomy, at 70%. The literature provides a variety of results. Hostinská et al. obtained the highest rate of vaginal deliveries after the use of misoprostol, but the shortest time to delivery after amniotomy [19]. Debele et al. in turn showed in their study that induction with oxytocin alone increased the risk of caesarean delivery (AOR 4.2; 95% CI 2.2-8.1) [20]. Unthanan et al. compared misoprostol and oxytocin, showing a lower caesarean delivery rate after misoprostol, at 13.3% vs 28.8% [21]. In contrast, Branger et al. indicated an increased risk of caesarean delivery after the use of prostaglandins for IOL [22].

In the study, the sex of the foetus did not affect the outcome of induction. Similar results were obtained by Hadar et al. They did not report any impact of foetal sex on the increased risk of caesarean delivery during IOL – for male foetuses, the proportion of caesarean deliveries was 14.4% vs 14.2% for female foetuses [23]. Bademkiran et al., on the other hand, demonstrated a relationship between male sex and foetal heart abnormalities and the risk of emergency caesarean delivery during IOL [24].

Higher infant weights were observed when IOL was not followed by vaginal delivery, at 3551 g vs 3403 g. Kamlungkuea et al. found that a foetal weight < 3500 g is associated with a twofold increase in the chance of successful IOL (AOR 2.193; 95% CI 1.246-3.860) [15]. Meanwhile, Dall'Asta et al. looked at deliveries in patients with estimated high foetal weight; 82.9% of the women had a vaginal delivery and 34.5% met the criteria for foetal weight > 95th percentile [25].

The study found a strong correlation between the outcome of IOL and the effacement. Following a study of a group of 502 patients, Huret et al. showed a correlation between successful IOL due to premature outflow of amniotic fluid and dilation ≥ 2 cm ($p<0.001$) [26]. According to Kamel et al., cervical length is a predictor of successful IOL in primiparas at term (OR 1.08; $p=0.04$) [27]. Diaz's study also showed that cervical length is a predictor of caesarean

delivery during IOL [28]. Kawakita et al. found a relationship between dilation, effacement and foetal head progression, i.e., parameters included in the simplified Bishop's score, and successful IOL in patients with an immature cervix (< 6 points) [29]. Jung et al. compared the predictive value of the modified Bishop's score prior to preinduction and prior to IOL in relation to the duration of labour. They also assessed whether any of the components of the aforementioned score had the highest predictive value. Their results showed a higher pre-induction score, while cervical position was found to have the weakest relationship with the course of labour [30].

Conclusions

Predictors of successful IOL include multiparity, a mature cervix, oxytocin induction and indications for induction including gestational diabetes, premature outflow of amniotic fluid, suspected high foetal weight, and reduced foetal movement. The sex of the foetus does not affect the outcome of IOL. Of the parameters assessed by Bishop's score, the most clinically relevant is the cervical effacement. Therefore, cervical maturity scales that take fewer components into account, including the simplified Bishop's score by Laughon, are useful in clinical practice in addition to the 1964 Bishop's score.

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IgA NEPHROPATHY – ONE POSSIBLE CAUSE OF MALIGNANT HYPERTENSION

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Abstract:

Malignant hypertension (MH) is a state which can rapidly progress to multi-organ failure. It can develop in the course of both primary and secondary types of hypertension. In our study, we present a case of a 36-year-old man diagnosed with renal failure and MH complicated with retinopathy. As the secondary nature of the condition was suspected, extensive diagnostics were conducted. In spite of excluding many pathologies, the underlying cause remained unknown. Eventually, a renal biopsy was performed, leading to a diagnosis of IgA nephropathy. However, the symptoms and course of the disease has not been typical. Nevertheless, IgA nephropathy should be considered as a possible cause of secondary hypertension as well as malignant hypertension. The diagnosis is not easy and a crucial role is played by renal biopsy.

Keywords: IgA nephropathy, malignant hypertension, renal failure, renal biopsy.

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Introduction

Malignant hypertension is the most severe form of hypertension. It is characterized not only by diastolic blood pressure values reaching above 130 mmHg, but also by numerous multi-organ complications, including kidney, brain and heart damage [1], Retinal and vascular impairment can also be often observed [2]. MH occurs with an incidence of approximately 2/100,000 cases per year [3]. It most frequently develops as a result of other problems with health. In young people it almost always indicates that they suffer from some other disease. [4]. What is more, hypertension can be both a cause and a consequence of kidney disease, for example. The presence of malignant hypertension in young people is more likely to be an indication of the secondary form of hypertension. It may be also a result of renal artery stenosis, endocrine disease, obstructive sleep apnea as well as glomerulopathy. This case presents an atypical course of IgA nephropathy and diagnostic difficulties that may arise during the search for the cause of malignant hypertension.

Case

A 36-year-old man had been admitted to the Department of Internal Medicine, Nephrology and Dialysotherapy due to very high blood pressure values and newly diagnosed renal failure. The patient had not been suffering from chronic diseases nor taking any medications. He had noticed a malaise, nocturnal awakenings due to nycturia and daily headaches for three weeks. A week before admission to the hospital, during an ophthalmological appointment, the patient had been diagnosed with retinoschisis. He had received treatment consisting of 25 mg of eplerenone, which he took as advised. The day prior to hospitalisation the patient reported to the regional hospital's Emergency Department due to persistent malaise and an episode of vomiting. The physical examination revealed significantly elevated blood pressure reaching values of up to 205/140 mmHg. Abnormal nitrogen retention indices were noted in biochemical tests. A head CT scan did not show any abnormalities. The patient was administered hypotensive drugs – Captopril, Nitredipine, Nitromint and Furosemide, which caused a decrease in systolic blood pressure by only 10 mmHg. The patient was transferred to a higher-level reference centre. Further tests in the Emergency Department were performed and revealed abnormal nitrogen retention: creatinine 7.1 mg/dl, GFR 9 ml/min/1.73m2, urea 139 g/dl. Metabolic acidosis was observed in the arterial blood test – pH 7.234, pCO2 43.2 mmHg, HCO3 18.3 mmol/l, BE -9.2 mmol/l. In addition, urinalysis showed proteinuria of 300 mg/l, glucose 50 mg/dl and haematuria. Abdominal ultrasound visualised only renal cysts.

The patient was admitted to the Department of Internal Medicine, Nephrology and Dialysotherapy in order to conduct a search to find a cause of the renal insufficiency

and treatment for the arterial hypertension. Upon physical examination, no signs were found of pulmonary congestion, oedema, neurological defects, heart failure or arrhythmia. Based on the clinical picture and the results of the tests, a preliminary diagnosis of malignant hypertension with renal complications was made.

Additional laboratory tests performed in the clinic revealed secondary hyperparathyroidism. However, no autoantibodies: anti-neutrophil cytoplasmic antibodies associated with systemic vasculitis (pANCA and cANCA) or anti-glomerular basement membrane (anti-GBM) were found. The complement system markers were also within the normal range. During hospitalization, a 24-hour urine collection was performed, which allowed the determination of the exact amount of proteinuria, which was 4687 mg/24h. The chest X-ray did not show any abnormalities. Echocardiography was performed to exclude aortic coarctation. This examination also did not show any abnormalities.

While seeking possible causes of the secondary hypertension, hypotensive therapy was implemented and forced diuresis was maintained. The decision on renal replacement therapy was temporarily postponed. Creatinine, urea, ionogram, arterial blood gases and morphology were regularly monitored. The creatinine concentration, which was 7.2 mg/dl on admission, decreased to 5.9 mg/dl. The patient had three ophthalmological consultations during hospitalization. Hypertensive retinopathy of the left eye was diagnosed and anti-VEGF injections were recommended. After appropriate preparation of the patient, an abdominal ultrasound was performed. As in the previous examination, bilateral simple renal cysts of up to 12 mm in size were described. No renal artery stenosis was found in the Doppler ultrasound. Due to proteinuria and haematuria of unclear origin a renal biopsy was then performed. The biopsy revealed a picture that was consistent with IgA nephropathy – the mesangium showed IgA deposits. Fibrotic crescents and segmental sclerosis were observed in the glomeruli.

Due to the lack of significant improvement in the renal function, dialysis therapy options were introduced. According to his preferences and anatomical conditions, the decision about peritoneal dialysis trial was made. The patient was discharged with the recommendation to report urgently to the surgical department for Tenckhoff catheter insertion and subsequent treatment.

Discussion

In this case, a diagnostic challenge occurred during the attempts to determine the cause of malignant hypertension and renal failure. In patients under the age of 40 the presence of malignant hypertension, accompanied by renal failure and retinopathy, requires additional diagnostics for secondary causes of hypertension [5, 6]. However, it is very important to remember that hypertension may be both a cause of kidney damage in the case of poorly controlled primary hypertension, and a symptom of kidney disease in renal hypertension. Clinical symptoms usually do not suffice for a clear differentiation. The duration of hypertension may be helpful for this assessment, as a long-standing course of the disease, especially if poorly controlled, leads to glomerular sclerosis and the development of hypertensive nephropathy. In hypertension secondary to renal disease, the history of hypertension is often much shorter, and may be more rapid and take the form of malignant or resistant hypertension [6].

In the presented case of renal artery stenosis, endocrine disorders, aortic coarctation and systemic diseases that may cause glomerulonephritis, have sequentially been excluded. Renal causes of secondary hypertension include both acute and long-term renal damage. A relatively short history of complaints suggested an acute form of renal failure which, however, is usually associated with oliguria or anuria. In addition, acute or subacute renal damage is also defined by a time criterion, which refers to the progression of changes in the renal function. In the described case it could not be used due to the lack of previous screening examinations. Chronic kidney disease, according to KDIGO 2012, is defined as abnormalities of kidney structure or function, with health implications, lasting for more than 3 months [7]. However, imaging studies performed in this case did not show any renal structural features which could suggest chronic disease. The clinical manifestations of chronic kidney disease depend on its severity. They are initially non-specific – weakness and hypertension, which occurs in up to 90% of patients with chronic kidney disease [8]. Typical abnormalities in laboratory tests are: increased creatinine, urea, uric acid, acid-base imbalance in the form of metabolic acidosis, calcium-phosphate imbalance - hyperphosphatemia, hypo or hypercalcemia, hypernatremia, increased PTH levels, lipid metabolism disorders and anaemia.

The patient, apart from hypertension, did not have the typical clinical symptoms of chronic kidney disease. Additional examinations revealed elevated creatinine and urea, lipid metabolism disorders, hyperphosphatemia, as well as elevated PTH levels. Increased PTH indicates secondary hyperparathyroidism, which is a typical feature of chronic kidney disease. The most common causes of chronic kidney disease are diabetes, hypertension and glomerulopathies [9]. The patient had not previously been diagnosed with diabetes or primary hypertension.

With reference to the described case, worth citing is one of the scientific papers. Chen et al. compared the clinical and histopathological features of primary malignant hypertension and malignant hypertension in the course of IgA nephropathy. They showed that patients with malignant hypertension associated with IgA nephropathy more often have proteinuria and haematuria than patients with primary malignant hypertension [4]. Nevertheless, renal parenchymal biopsy is the test that can clearly determine the cause of kidney damage, which provides an answer to the question whether glomerulopathy is the

cause. The aforementioned study revealed that glomerular changes were more severe in patients with hypertension associated with IgA nephropathy – a higher percentage of glomeruli was affected by sclerotic lesions and formed crescents, due to more intense mesangial proliferation [4]. In the presented case, the histopathological examination of the kidney showed features typical of malignant hyperplasia associated with IgA nephropathy, also showing IgA deposits in the mesangium.

IgA nephropathy could be one of the causes of secondary hypertension as well as malignant hypertension [4]. It is the most frequent form of primary glomerulonephritis worldwide [10] and one of the causes of chronic kidney disease. The incidence is approximately 2.5/100,000 people per year [11]. It is characterized by the deposition of immune complexes in the glomerular mesangium [12]. The clinical picture of IgA nephropathy varies widely, from asymptomatic microscopic haematuria or gross haematuria to nephritic syndrome. However, the most common manifestation in adults is asymptomatic haematuria with diverse proteinuria [13]. Rarely does IgA nephropathy occur as macroscopic haematuria associated with an upper respiratory tract infection. The patient denied similar episodes in the past, and he had not noticed any changes in the appearance of the urine. In rare cases, IgA nephropathy may take the form of rapidly progressive glomerulonephritis. Renal biopsy and finding IgA deposits are required to confirm the diagnosis. During the course of the disease, a gradual deterioration in the kidney function and a decrease in the glomerular filtration rate occurs. In most cases, the progression of the disease is slow. The risk of end-stage renal disease within 20 years of diagnosis is estimated to be between 13% and 39% [14], and depends mainly on the clinical picture and the severity of the histopathological changes. The most important clinical risk factors are persistent proteinuria and hypertension [14, 15]. The histopathological changes considered in the assessment of prognosis are described by the Oxford Classification (MEST score), which evaluates 4 parameters: M –mesangial hypercellularity, E – endocapillary cellularity, S – segmental sclerosis and T – interstitial fibrosis/tubular atrophy [16].

Summary

Malignant hypertension in young patients without previous medical history is an indication to search for secondary causes of the hypertension. If renal failure symptoms coexist, special consideration should be given to renal biopsy. This may be crucial in establishing the proper diagnosis. Although IgA nephropathy usually has a slow, long-term course, its progressive nature may lead to end stage renal failure, which in most cases manifests itself in hypertension, among other symptoms. Chronic kidney disease is associated with an increased risk of death, mainly as a consequence of cardiovascular events. In relation to its progressive nature and the number of complications it can cause, early detection and implementation of treatment are crucial for a good prognosis.

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ACQUIRED HAEMOPHILIA A AND ANTIPHOSPHOLIPID SYNDROME DURING SYSTEMIC LUPUS ERYTHEMATOSUS WITH RENAL MANIFESTATION

Nabyta hemofilia A i zespół antyfosfolipidowy w przebiegu tocznia rumieniowatego układowego z zajęciem nerek

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Abstract: Systemic lupus erythematosus (SLE) is an autoimmune disease whose symptoms result from the deposition of immune complexes in various organs and lead to a chronic inflammatory process. The aetiology of the disease is unknown. It also predisposes to other autoimmune diseases. One of them is antiphospholipid syndrome (APS), associated with the presence of antiphospholipid antibodies and the occurrence of venous thrombosis and obstetric failures. Another example is acquired haemophilia A (AHA), resulting from the presence of antibodies to coagulation factor VIII. Its main symptom is a haemorrhagic diathesis. Due to the variety and multitude of the diseases manifested as a consequence of SLE, the diagnosis and treatment that follows are difficult to navigate. In this article we present a rare case of a patient with SLE, with predominantly renal manifestation and involvement of other organs, who developed both antiphospholipid syndrome and severe, acquired haemophilia A.

Keywords: systemic lupus erythematosus, antiphospholipid syndrome, lupus nephritis, acquired haemophilia A.

Streszczenie: Toczeń rumieniowaty układowy (SLE) to choroba autoimmunologiczna, której objawy wynikają z odkładania się kompleksów immunologicznych w różnych narządach i prowadzą do przewlekłego procesu zapalnego. Nie jest znana etiologia choroby. Predysponuje ona także do wystąpienia innych chorób z autoagresji. Jedną z nich jest zespół antyfosfolipidowy (APS) związany z obecnością przeciwciał antyfosfolipidowych oraz występowaniem zakrzepicy żylnej i niepowodzeń położniczych. Innym przykładem jest nabyta hemofilia A (AHA) wynikająca z obecności przeciwciał przeciwko VIII czynnikowi krzepnięcia. Głównym jej objawem jest skaza krwotoczna. Różnorodność i mnogość schorzeń objawiających się w konsekwencji tocznia sprawia wiele problemów w diagnostyce i leczeniu. W artykule został przedstawiony rzadki przypadek pacjentki chorującej na SLE z manifestacją głównie nerkową, a także z zajęciem innych narządów, która rozwinęła zarówno zespół antyfosfolipidowy, jak i nabytą hemofilię A o ciężkim przebiegu.

Słowa kluczowe: toczeń rumieniowaty układowy, zespół antyfosfolipidowy, nefropatia toczniowa, nabyta hemofilia A.

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease. Its pathogenesis is affected by genetic, hormonal, and environmental factors and complex immunological disorders. There is an increased production of autoantibodies directed against the body's own cells, which form immune complexes and are deposited in various tissues and organs. This leads to generalised inflammation and organ dysfunction [1]. Skin lesions, haematological symptoms and disorders of the musculoskeletal, renal, and nervous systems are particularly characteristic of SLE [1]. As a rule, symptoms from one organ predominate for a long period of time and, depending on the form, the course can vary considerably between patients. Renal involvement occurs in approximately 60% of patients, which is the leading cause of death associated with organ involvement in SLE [2]. The aim of the treatment is to prolong the patient's life and prevent organ damage through the use of symptomatic drugs, including glucocorticosteroids, antimalarials and immunosuppressants. Patients who do not respond to basic treatment require highly specialised treatment, including intravenous immunoglobulin infusions, plasmapheresis procedures or the administration of biologic drugs [3].

Haemophilia A is a condition classified as a plasma haemorrhagic diathesis. Acquired haemophilia A (AHA) is an autoimmune disease caused by the presence of antibodies against coagulation factor VIII. It is very rare and

occurs in both sexes. The idiopathic form occurs in about half of the cases, and the secondary form most frequently appears in the course of other autoimmune diseases, malignancies, allergic diseases and in pregnant women and during the 12 months following childbirth [4]. Approximately 1% of cases of secondary haemophilia A occur in SLE [4]. Symptoms of AHA appear suddenly, in the form of extensive subcutaneous haemorrhages, mucosal or intramuscular bleeding. Invasive procedures are avoided in patients with AHA, as wound haemorrhages after surgery are extremely difficult to control. The treatment of choice in AHA patients is recombinant active FVIII concentrate and immunosuppressive treatment [4].

Case study

A 34-year-old woman with erythema multiforme was admitted to the clinic for suspected SLE with a renal involvement and secondary coagulation disorders. Outpatient examinations showed anaemia, elevated renal parameters: creatinine 2.5 mg/dL, urea 51 mg/dL and an undetectable activated partial thromboplastin time (APTT). The patient had previously presented to her GP due to easy bruising for approximately 2 weeks. The patient's history included the use of naproxen for pain in the right inguinal region, in addition she reported joint pain, night sweats and no menstruation for a year. The patient also had a history of deep vein thrombosis of the left lower leg two years before (without anticoagulant treatment for a year) and a miscarriage.

On physical examination, skin abnormalities included: several haemorrhages, multiple white discolouration on the trunk skin, diffuse erythematous annular lesions on the skin of the thighs and pitting oedema of the lower legs. In addition, there was minor axillary and inguinal lymphadenopathy. The patient was circulatory and respiratory efficient with an elevated blood pressure of 170/100 mmHg. The electrocardiogram showed no abnormalities. Active gastrointestinal bleeding was ruled out and 3 units of red cell concentrate were transfused.

Based on clinical assessment and additional investigations, systemic lupus with renal involvement and secondary coagulation damage was suspected.

In-depth diagnostic evaluation included abdominal ultrasound, which reported a slightly increased echogenicity of the cortical layer of the renal parenchyma and fluid in the pouch of Douglas. Fluid was also detected in the pleural cavities. The chest X-ray, meanwhile, showed streaky thickening in the left lung mid-field. Ultrasound examinations of other areas of the body were also carried out. The axillary lymph nodes were enlarged to 29 mm in length on the right, and to 27 mm in length on the left. Lower limb vein thrombosis and renal vein thrombosis were ruled out. The thyroid gland was not enlarged with normal echogenicity but with increased parenchymal vascularisation.

Echocardiography showed enlarged left heart cavities with mild left ventricular diastolic dysfunction and an ejection fraction of 60%, in addition to fibrosis of the mitral valve leaflets with a small regurgitation. High-resolution tomography without contrast showed slight splenomegaly, the presence of a peripherally located nodule with a smooth contour and a length of 6.5 mm in the first segment of the right lung, in addition to low-grade fibrotic changes at the base of the lower lobes of both lungs.

Laboratory results showed severe nephrotic syndrome with protein excretion of 10 g/d, hypoalbuminaemia (1.9 g/dL), hypoproteinaemia (6.0 g/dL) and hypercholesterolaemia (total cholesterol 237 mg/dL). Furthermore, there were abnormal renal function markers (creatinine 2.1 mg/dL, eGFR 29 mL/min). Phase-contrast microscopy showed glomerular haematuria (90% dysmorphic erythrocytes). Inflammatory markers were normal (CRP 0.4 mg/dL). Additional tests showed elevated levels of anti-dsDNA antibodies (high level of 293 IU/mL) and anti-beta-2 glycoprotein 1 antibodies (19 U/mL), as well as a significant deficiency of the C3 and C4 of the complement system (C3: 49 mg/cL, C4: 4 mg/dL). ANAs, ANCAs, anticardiolipin antibodies and lupus anticoagulants were not present.

After analysing the results of the blood clotting parameters, which indicated a severe disorder: APTT > 180 s, blood clotting factors VIII and XII activity < 1%, and von Willebrand factor 236%, a diagnosis of haemophilia secondary to connective tissue disease was made. Lupus activity was assessed at 16 points using both the SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) and the EULAR (European League Against Rheumatism) classification criteria for systemic lupus erythematosus. Due to the coagulation disorder, a renal biopsy was not performed.

Treatment with intravenous infusions of methylprednisolone 500 mg/day for 5 days was initiated, and 10 days later 250 mg/day for 3 days was given, followed by the inclusion of oral prednisone 40 mg/d. After ophthalmic contraindications were ruled out, 2 x 200 mg hydroxychloroquine was included.

Hypotensive and diuretic treatment was started, resulting in optimisation of blood pressure and reduction in peripheral oedema. Due to signs of urinary tract infection and a positive urine culture test (*E. faecalis*), antibiotic therapy with amoxicillin was initiated according to the antibiogram.

Given the patient's poor dental condition (no dental appointments for 20 years), an oral and maxillofacial surgeon was consulted before immunosuppressive treatment was included, who recommended extraction of nine teeth with gangrenous roots. However, the procedure could not be performed due to the diagnosed acquired haemophilia.

Three weeks later, the patient presented to the department for elective hospitalisation to assess disease activity.

Laboratory tests revealed moderate anaemia (Hb 9.2 g/dL), proteinuria (6.7 g protein in daily urine collection), hypoalbuminaemia (2.6 g/dL), hypercholesterolaemia (total cholesterol 286 mg/dL) and creatinine at 1.6 mg/dL. The dose of allopurinol was increased because of an elevated uric acid level (7.6 mg/dL). On the other hand, APTT (58.6 s) and complement C3 (82 mg/dL) improved, and there was a reduction in anti-dsDNA antibodies (46 IU/mL).

Symptoms of an *E.coli* urinary tract infection were noted, and antibiotic therapy was implemented.

Three 500 mg methylprednisolone infusions were then given, and the prednisone dose was reduced to 30 mg/day.

The patient's next elective hospitalisation (July 2020) was preceded by a stay in the Department of Haemostasis Disorders and Internal Medicine at the Institute of Haematology and Transfusiology due to severe allergic eczema, most likely as a result of a drug reaction. For this reason, hydroxychloroquine therapy was temporarily suspended. The procedure involving the extraction of gangrenous teeth was successfully carried out there.

At follow-up, FVIII activity remained in the lower limit of normal (55%), and no lupus anticoagulant was found in the blood. Other results included prolonged APTT up to 36 s, protein excretion 1.5 g/day, albumin 2.8 g/dL with 4.7 g/dL total protein. The anti-dsDNA antibody result was negative (0.6I U/mL), but the amount of complement C3 was reduced (69 mg/dL). Due to the continuous fluctuation of renal function parameters, a decision was made to intensify pharmacotherapy, mycophenolate mofetil (MMF) 2 x 1 g was included, the dose of prednisone was reduced to 20 mg, and hydroxychlorchine was maintained. Following another urinary tract infection, sulfamethoxazole with trimethoprim was included. After a positive urine culture test *(E. coli* ESBL+), the antibiotic therapy was switched to meropenem.

Two months later (September 2020), the patient was admitted to the department due to an exacerbation of nephrotic syndrome. Immunosuppressive treatment was intensified, with the dose of MMF increased to 3x1g/d and no change in the dose of prednisone.

Another stay in the clinic occurred seven months later (April 2021) and was due to another relapse and an *E. coli* ESBL+ refractory urinary tract infection. Once the infection was cured, cyclophosphamide (CYC) therapy was planned.

A total of 3 doses of cyclophosphamide (3 x 500 mg) were given with mesna as protection. Initially, the tolerance of the treatment was good. After receiving the third dose, the patient developed acute drug-induced liver damage, which was confirmed by laboratory (AspAT 1520 U/L, ALT 2289 U/L, bilirubin 2.1 mg/dL) and imaging tests (liver damage in abdominal ultrasound). Hepatic venous thrombosis, portal vein thrombosis, and infection with potentially hepatotoxic viruses were ruled out. When hepatoprotective treatment was introduced, liver parameters improved.

A decision was made to discontinue cyclophosphamide, MMF 2 x 1 g was restarted, and prednisone 20 mg/day was maintained and then the dose was reduced to 10 mg/day, hydroxychloroquine was maintained. Antithrombotic treatment was included.

Only partial remission of the nephrotic syndrome was achieved as a result of the treatment.

Selected results of the patient's laboratory tests are included in the Table to enable analysis of the progression of the disease and changes in the associated parameters.

Discussion

SLE is an autoimmune disease whose presentation depends on the organs involved. The most common symptoms include erythematous skin lesions, headache and joint pain, urinary symptoms (proteinuria, haematuria) and central nervous system involvement [5]. Furthermore, SLE may predispose patients to other conditions, particularly autoimmune diseases. In addition to the typical symptoms, the patient presented with a rare manifestation of SLE in the form of AHA. The disease is caused by antibodies against coagulation factor VIII, resulting in impaired coagulation factor VIII function and an increased tendency to bleed. Unlike in the more common form of the disease, congenital haemophilia, in this case FVIII levels do not always correlate with the severity of the haemorrhagic diathesis and spontaneous bleeding into joints is very rarely observed. The most common symptom of AHA is extensive subcutaneous haemorrhage and mucosal and wound bleeding after surgery or extraction of teeth [4]. The diagnosis is made based on a prolonged aPTT with normal other haemostasis parameters. In half of AHA cases, the cause of the disease cannot be established. Approximately 10% of AHA is a complication of malignant tumours, and 8% of patients develop AHA during pregnancy or in the 12 months after delivery. Autoimmune diseases also predispose to AHA, with an estimated 10% of patients affected and SLE accounting for 1% of cases [4]. The patient in question met the criteria necessary for the diagnosis of AHA. Additionally, the patient's CBC showed significant anaemia, which, due to the lack of clinical symptoms, had probably been developing for a long time and the patient was well adapted to it. Due to the risk of bleeding from the wounds, extraction of the decayed teeth was delayed until haemostasis parameters had stabilised, and this in turn delayed the implementation of immunosuppressive treatment. Another difficulty resulting from the coagulation disorder was the inability to perform a renal biopsy, a test that is the gold standard for disease stage assessment, prognosis, and treatment selection [6].

year	month	protein in urine [0-10 mg/dL]	total protein [6.4-8.3 g/dL]	complement C3 [90-180 mg/dL]	complement C4 [10-40 mg/dL]	anti-dsDNA antibodies IgG [IU/mL]	ANAs	creatinine [0.5-0.9 mg/dL]	eGFR [mL/min/1.73m^2]	ammonia [15-43 mg/dL]	Hb [11-18 g/dL]	Hct [35-55%]	RBC [3.5-5.5 x10^12/L]	APTT [23-35 s]	factor VIII (activity) [50-150%]
2020	March	100	6.3	53	3			2.3	26	50	8.0	23	2.89		
	April	705.7	6.0	49	4	293 pos.		2.3	29	139	8.7	25	3.14	>180	<1
		100	5.6					2.0	30	195	8.9	25	3.23	104.1	
								3.8	14	172	9.2	27	3.3	92.2	
								1.9	32	168					
	May	300	4.7	82	15	46 pos.		1.1	39	89	9.2	27	3.14	58.6	
								1.9	32	114	10.3	29	3.56		
	June	100	5.08					1.39	43.4	92	11.2	32	3.79	32.22	45.81
								1.53	38.85	98	11.6	34.1	3.92	30.8	62.3
	July	27.46	4.38					1.27	47.89	90	12.7	37.1	4.41		66.84
		1000	4.7	69	13			1.4	45	60	10.7	30	3.56	36	55.6
		67				0.6 neg.	0.1 neg.	2.3	26	79					
	September	1000	5	103	21			1.3	50	78	11.1	31	3.83	30.7	172
		98.7				36 pos.	0.1 neg.								
2021	April	300	5	150	30			1.7	36	56	12.2	36	4.67	30.1	
		143.5				19 pos.	3.4 pos.				12.7	39	4.97	34.5	
	May	300	5.4	110	21			1.6	39	60	12.2	37	4.66	30.4	
	June	100	6.1					1.4	45	71	11.7	35	4.25	31	
											11.7	35	4.32	30.4	
	July	68	6.1					1.7	36	74	12.6	37	4.58		

Antiphospholipid syndrome (APS) is yet another autoimmune disease characterised by the presence of antiphospholipid antibodies. It features vascular thrombosis and obstetric failures. A minimum of one clinical criterion and a minimum of one laboratory criterion must be met to diagnose APS. The clinical criteria include at least one episode of vascular thrombosis or obstetric failure. Laboratory criteria, on the other hand, include finding lupus anticoagulant or anticardiolipin antibodies or anti-β2-glycoprotein 1 antibodies in plasma at least twice at intervals of no less than 12 weeks. APS often co-occurs with SLE [7]. The risk of developing chronic kidney disease or a cardiovascular disease is then increased. Moreover, APS may develop as a result of infection, vaccination, cancer or as a side effect of the administered drugs. Approximately 1-3% of healthy individuals may have antiphospholipid antibodies despite the absence of clinical symptoms [8]. Our patient suffered a deep vein thrombosis of the left lower limb in 2018 and had a history of miscarriages. She also suffers from hypertension. The presence of lupus anticoagulant and beta2-glycoprotein 1 antibodies in the serum, together with the clinical picture, led to the diagnosis of APS. Prevention of APS symptoms involves chronic use of vitamin K antagonists [7]. In this case, due to haemophilia, anticoagulant therapy was initiated with a delay, after APTT and clotting factors had stabilised.

Renal involvement due to immune complexes and inflammatory infiltration leading to organ damage is observed in approximately 60% of SLE cases [9]. It may range from lesions exclusively in the mesangium, through segmental and diffuse proliferative inflammation to advanced glomerular sclerosis [10]. Nephrotic proteinuria is present when the amount of protein in the daily urine collection is more than 3500 mg/24 h. If, in addition, the albumin concentration falls below the lower limit of normal and oedema is present, nephrotic syndrome is diagnosed. Antibodies to native DNA (anti-dsDNA) are most characteristic of patients with renal complications in SLE [2]. There is often active urinary sediment (leached and dysmorphic erythrocytes, erythrocyte casts) indicative of proliferative lupus nephropathy. Proteinuria occurs in all SLE patients with renal involvement, and 40-60% have nephrotic proteinuria [11], which was what our patient presented with. Approximately 50% of patients not responding to treatment will require dialysis. In the absence of nephropathy remission, 5-year and 10-year survival estimates are 70% and 60%, respectively [11].

The patient's test results (Table 1) suggest a correlation between high anti-dsDNA antibody titres and low serum complement components and the presence of proteinuria. Such parameters indicate a high immunological activity of the disease and point to an unfavourable course. The numerous hospitalisations resulted in improvement, but no permanent remission of the nephrotic syndrome could be achieved. Fluctuations in GFR values were between 14 and 51 mL/min/1.73 m2. In addition, the patient suffered from recurrent urinary tract infections, treated in line with the antibiogram.

Steroids are the primary treatment for SLE. Hydroxychloroquine is used as an adjunct, but because of the risk of retinopathy, it can only be incorporated into treatment once ophthalmic contraindications have been ruled out [12]. Other options include methotrexate, azathioprine, and mycophenolate mofetil. The drug is selected based on the location and severity of the lesions, the patient's procreative plans, and side effects [11]. Scales such as the SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) or classification according to ACR/EULAR (American College of Rheumatology/European League Against Rheumatism) are helpful in diagnosis and management decisions. In severe, refractory cases, cyclophosphamide – a cytostatic used mainly in oncology and rheumatology – is used. It is administered parenterally, in monthly pulses, following the EUROLUPUS 2019 regimen. The cumulative dose of 0.3 g/kg body weight should not be exceeded.

In the patient described above, concomitant use of hydroxychloroquine and prednisone resolved the skin symptoms, joint pain, and pleural effusion, but full remission of nephrotic syndrome was not achieved. The patient's treatment was intensified only after removal of the teeth, which were active foci of infection. Treatment was then extended to include MMF, but insufficient improvement was observed. After the implementation of cyclophosphamide therapy, as per the EUROLUPUS 2019 regimen, there was a gradual decrease in proteinuria, although a positive anti-dsDNA antibody titre persisted. Due to the drug-induced liver damage, conversion to treatment with the less toxic mycophenolate mofetil was required. Recovery of normal liver function is observed in the majority of patients after discontinuation of cyclophosphamide; thymonacide can be used as an adjunct.

The patient described exemplifies the difficulties posed by the co-existence of multiple conditions, multi-drug therapy and its side effects. Some diseases prevented diagnosis and treatment of others, whereas the choice of effective treatment involved a number of dangerous consequences.

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MY 70 YEARS WITH NEUROLOGY
Moje 70 lat z neurologią

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Abstract: The article is a description of the professional career of a Polish neurologist, scientist, and teacher, who for about 60 years was associated with the Military Institute of Medicine – Professor Teofan Domżał. Writing in the first person, I present my decades-long contribution to the development of Polish and world neurology. The background for the described events is also the characteristics of the changes in the organization of the military health service and the Military Institute of Medicine over the decades. The work also illustrates the great progress that has been made in the field of neurology during my professional life.

Keywords: neurology, history, development of neurology.

Streszczenie: Artykuł jest opisem kariery zawodowej polskiego lekarza neurologa, naukowca, nauczyciela, od około 60 lat związanego z Wojskowym Instytutem Medycznym – profesora Teofana Domżała. Pisząc w pierwszej osobie, przedstawiam swój kilkudziesięcioletni wkład w rozwój neurologii polskiej i światowej. Tłem dla opisywanych wydarzeń jest także charakterystyka zmian w organizacji wojskowej służby zdrowia i Wojskowego Instytutu Medycznego na przestrzeni dziesięcioleci. Praca ta obrazuje również wielki postęp, jaki dokonał się w dziedzinie neurologii w ciągu mojego zawodowego życia.

Słowa kluczowe: neurologia, historia, rozwój neurologii.

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I joined the Department of Neurology at the Medical Academy in Łódź in 1952, under the supervision of Prof. Eufemiusz Herman, and then in 2022 I celebrated my 70th year with neurology. The decision to make neurology my future medical speciality was something that built up gradually. After my second year, I started working as a demonstrator in the Department of Descriptive Anatomy (Fig. 1). There I found my interest in the structure and function of the brain, and the brain – that most important human organ – is, after all, the subject of neurology. In the fourth year, I was enchanted by the lectures on neurology conducted masterfully by Prof. Eufemiusz Józef Herman, who always included a demonstration of a patient suffering from the discussed disease. At that point, I decided that neurology was the most interesting and important medical speciality. It is the 'queen of medicine', as David Wechsler – a psychologist and the creator of the well-known intelligence tests – said of neurology at one of congresses. I also took note of *The Story of San Michele* by Axel Munthe for its remarkable description of the work at the Paris School of Neurology, which was set up in 1862 and headed by the famous Jean Martin Charcot, considered the father of the speciality.

Prof. Herman, author of many textbooks and scientific papers and co-founder of the World Federation of Neurology, was my first teacher (Fig. 2).

Figure 1. Assistant in the Department of Anatomy at work in the dissecting room.
Rycina 1. Asystent w Zakładzie Anatomii w prosektorium przy pracy.

Figure 2. Prof. Eufemiusz Józef Herman (1892-1985).
Rycina 2. Prof. Eufemiusz Józef Herman (1892-1985).

He was also the most gifted student of the world-famous neurologist, psychiatrist and neuropathologist, Edward Flatau, who in 1904 established the Polish school of neurology, as head of the Department of Neurology at the Jewish Hospital in Warsaw's Czyste district (17 Dworska Street, now Kasprzaka Street). His famous rounds were attended by all doctors interested in the new speciality. After the war, Herman became professor and head of the Department of Neurology at the newly established University of Łódź. In his department I learned the basics of neurology, semiology, patient examination and 'neurological reasoning', which today has been replaced by computers. Semiology is the science of neurological symptoms, the building blocks for classifying a syndrome, such as focal, disseminated, diffuse or systemic, while the 'reasoning' indicates the site of damage and its presumed cause.

The personality in a uniform

I graduated in 1951. I delayed taking the final exam for fear of being called up to the army, which was a terror in those days. I did not last long – I passed the exam in 1952 and received my red diploma as the top student. In July of that year, I was called up to the Officer Training Course in Śrem, after which, with the rank of lieutenant, one was usually assigned to a military unit somewhere in the country.

The recruit period was a disaster for me. We were humiliated and deprived of our dignity and personal freedom. From the morning to the evening bugle call, all that could be heard was a barrage of inarticulate words from the NCOs and the officers emphasising their superiority by their roars. We felt like we were in a prison camp. Just before promotion, I managed to get out of that hell and went home and back to work at the department. I was called up again the following year. I thought it was a mistake, but my explanations that I had already received similar training a year before did not help. After a month of torment, we were released and, full of hope that the army business was over, I returned home and to my work. I was wrong – in September 1954 I was called up for the third time and had to undergo the same recruit training yet again. It was a nightmare. I was sure that someone deliberately wanted to break me mentally and physically. That was actually true. I met that man after many years. When I asked him why he had done that, he replied cynically that he had wanted to help me with my military and academic career. This is how I became a lieutenant in the Polish People's Army assigned to military neurology at the Hospital of the Military Medical Training Centre (WCWMed) at 113 Żeromskiego Street in Łódź.

The Department of Neurology there was headed by Col. Władysław Stein (Fig. 3), 14 years Herman's junior. Before the war, he worked as an assistant under Sterling – also a student and successor of Flatau at the Jewish Hospital in Warsaw. He was therefore from the same school of neurology as Herman. Drafted into the army in 1939, he was wounded in the Battle of Kutno and taken prisoner by the Germans. Imprisoned in a prisoner of war camp, he survived the war and had his life saved from the Holocaust. The camp commandant was a good German who protected the prisoners, including Jews. He even let them study and learn foreign languages.

Figure 3. Col. Asst. Prof. Władysław Stein (1906-1960).
Rycina 3. Płk doc. Władysław Stein (1906-1960).

As I began the military chapter of my life, I stood at attention in front of Col. Stein, reporting my arrival in accordance with the regulations using the words: *'Colonel, Lieutenant Domżał...'*, when Stein interrupted my report, patted me on the shoulder in a friendly manner and said: *'Give the reporting a rest, grab your coat and let's do the rounds'*. I was surprised and deeply moved by Stein's unpretentiousness and openness, by this friendly, non-military and non-regulatory greeting. I took an instant liking to the man. In the army, which I got to experience at its worst, such treatment of subordinates and such a personality in a uniform was an exception. Stein had the soul of a civilian and did not fit into the military drill, which was evident at first sight. His passion was neurology, but he had to practise it in the army. He was erudite, spoke

German, French, Russian and English, and read medical news in foreign-language journals daily.

There were two more doctors working on the 60-bed ward in addition to Stein: Mieczysław Strzałko – captain at the time, Stein's deputy – and Capt. Jerzy Bruzda. We went to work in uniform and before our duties started there was a general briefing given by the hospital's commandant, Col. Rudolf Duda, a neurologist from the pre-war Lviv school of Henryk Halban. Most of the medical officers, like me, found themselves in the army against their will, by conscription, and were not members of the Polish United Workers' Party (PZPR). The numerous political and military training courses held throughout our service often ended with the question as to when we would be discharged from the army. However, after 1957, when we could finally leave the military ranks, none of the questioners left. This was because the Military Medical Academy (WAM) was being organised and we were encouraged to stay, tempted by the prospect of a higher salary and an academic position, as well as the prospect of educational research work and private practice. I was promised an assistant professor position because by 1956 I already had a second degree with a specialisation in neurology, and the WAM provided the option of obtaining doctoral and associate professor degrees.

The files in the patient rooms

In 1958, the Military Medical Academy was established, and our responsibilities changed. In addition to working with patients, we also had to teach students during clinical exercises and lectures. Stein had coronary artery disease and had suffered several heart attacks, so we spared him and often replaced him in his lectures. We also participated in the examinations. New colleagues were recruited to the department: Zdzisław Cywiński, Klemens Słowicki, Czesław Rymczonek, and Tadeusz Wranicz. The neurosurgical subdepartment was turned into a separate department, which was taken over by Prof. Jan Bromowicz from Kraków, Prof. Kunicki's student, who had been called up to the army before that time.

The department was given more responsibilities but no extra space. The medical staff had two small offices connected to each other by a door. Stein worked in one of them, and assistants with assistant professors in the other tiny one, where there was space neither for a wardrobe nor for any desks. We changed our clothes in the Head's office, hung our uniforms in a small wardrobe, and worked in the patient rooms, with folders of the patient histories laid out on the tables so that we could enter the daily observations of their condition and the results of the examinations carried out. In the morning before rounds, we would sit in the Head's office and listen to the news he had learned from the magazines read the previous day. That was our professional training.

Our scientific work was less successful. Every Saturday, the Head gave everyone a topic to work on. He did not get the job done and often changed the topics, so we could start specific studies ourselves if we wanted to, which positively affected our creativity. The Head did not particularly like to read our papers, so I would go and present them to Prof. Herman – he was, after all, my teacher and Master. When I showed Herman my first paper, prepared for publication, describing a family with cerebellar atrophy, the professor corrected it, leaving almost none of my original sentences. I felt terribly crestfallen and vowed never to pick up a pen again. I was later grateful to him for this lesson. I became friends with Herman and his wife through chess, as Róża, the professor's wife, was a passionate chess player and, before the war, the chess champion of Poland. I met her while playing in the chess championship in Łódź and I was often invited to her house for a game, and later for the professor's or Róża's name day parties.

Herman was once visited by his friend from London – Prof. MacDonald Critchley, president of the World Federation of Neurology. His presentation at the department was a theatrical demonstration of a neurological examination and made a great impression. Critchley was a music fan and an admirer of Chopin, so Prof. Herman asked me to take them to Żelazowa Wola to attend a concert, The English guest, I was happy, and I was honoured.

Stein would come to work early and walk around the patient rooms, listen to the nurses' reports, and knew what was going on in the department even before we arrived. This was unbearable for the diligent Strzałko, who decided to clock in before him. This, in turn, upset the Head, who started coming in even earlier. This silent competition ended in an outburst of displeasure, with the frustrated Stein shouting: *'Strzałkides, do you want to kill me? My wife won't let me get up any earlier!'* The Head often jokingly twisted our names: Strzałkides, Domżałkiewicz, etc.

The fruits of modernity

In 1957, the facility bought a Kayser electroencephalograph for us. Mr Kayser and his wife brought the apparatus in parts in their car from Copenhagen and assembled it on site at the department for a week, while teaching us how to use it and how to perform the examinations. I was appointed by Stein to learn its operation in order to perform tests and report the results. I put the electrodes on patients' heads, performed the procedure and reported the findings all alone (Fig. 4). It was not until a year later that I got a lab technician to help me.

Figure 4. The author attaching electrodes before an EEG test.
Rycina 4. Autor zakłada elektrody przed badaniem EEG.

The first fruit of the EEG was my publication on changes in bioelectrical brain function in migraine. Later on, I studied the influence of various external factors on the recording of activity during EEG. I also explored sleep and sleep disorders. Based on the recording of brain function, I demonstrated the immaturity of the cerebral cortex as a cause of such parasomnias in children and adolescents. This was also the subject of my doctoral dissertation. I also studied the effects of drugs on the bioelectrical function of the brain. For pathological and experimental pain, I found an elevation of the sedation threshold after barbiturates (the so-called Shagass threshold), which was an objective confirmation of the pain sensation. Sleep and pain became the focus of my research when I started working in Warsaw. In 1972, I went with Prof. Juliusz Narębski from Toruń to Basel for the 1st Sleep Research Congress, and we became founding members of the European Sleep Research Society.

In those days, night-time incontinence was a common disorder in the military and a serious jurisprudential problem. Col. Stein was instructed to solve this challenging issue scientifically. Crowds of bed-wetting soldiers from all over Poland flocked to the department in the hope of being discharged from the army – mainly recruits, among whom many were malingerers. Stein did not solve the problem, but he was appointed an associate professor (there was no post-doctoral degree yet) for his research. The issue cleared up on its own as civilisation developed and the political system changed, but at that time we were having a lot of trouble with it and no methods were effective in combating that military scourge. Col. Doc. Władysław Stein died in 1966, shortly after retiring. The department was succeeded by Mieczysław Strzałko, who obtained his post-doctoral degree and became a professor, while I was his deputy.

Pioneers and Galenics

One major development, introduced into brain diagnosis by a Portuguese neurologist, Egaz Moniz, was arteriography. Arteriography was not made available for routine clinical examination until after World War II. Radiologist Jerzy Zajgner, later professor, and head of the Department of Radiology at WAM, and I were the first to introduce this examination method in our department. I undertook to inject contrast into the common carotid artery and Zajgner pulled out cassettes with the obtained images at appropriate intervals. The three consecutive cassettes showed an image of the cerebral arteries, then the capillaries and, finally, an image of the veins. The procedure took place within the range of X-rays, and we were protected by heavy lead aprons and gloves. Since that time, arteriography became a routine examination in our hospital, although initially only in neurology, where the diagnosis was established through consultation with neurosurgeons and radiologists. Before arteriography, the only available contrast examination was pneumoencephalography, which involves the introduction of air into the cerebral ventricles via a lumbar puncture. The procedure was very painful. If a brain tumour was suspected, ventriculography was performed by introducing air directly into the ventricles through a hole in the skull. This was done to prevent the cerebellum from wedging into the spinal canal due to a sudden drop in intracranial pressure after a lumbar puncture. Both methods of contrast brain examination were introduced in 1919 by an American neurosurgeon, Walter Dandy. These procedures were only discontinued after the implementation of computer-based testing in the 1970s or 1980s.

The examination of cerebrospinal fluid collected by lumbar or suboccipital puncture was a routine diagnostic procedure in neurology. The suboccipital puncture was used to inject contrast into the spinal canal. The contrast was a lipiodol that was heavier than cerebrospinal fluid and sank to reveal the permeability of the spinal canal in cases of suspected spinal cord compression. Persistent low-pressure headaches were common after lumbar punctures.

Treatment of neurological diseases was limited to the dozen or so products available, the same ones that were used before the war. Neuropharmacology was based on alkaloids: morphine, strychnine, atropine, scopolamine, caffeine, etc. The most popular remedy for all neuroses was a mixture of bromine salts with luminal and valerian. Luminal was used for epilepsy, only later to be followed by the introduction of phenytoin and, in 1960, by carbamazepine, whose efficacy increased to 60%. Various vitamins were widely used, especially B1 in all neuropathies. A 40% glucose solution with vitamin C was administered intravenously in a cerebral oedema and euphylline was given to stroke patients. Most medicine mixtures were prepared by the pharmacy in the form of a wafer, pill, solution, extract, or infusion, following the doctor's prescription, just as in Galen's time. Such drugs were called 'Galenics' and they continued to be used until mid-20th century, as each doctor had a favourite tried-and-tested recipe and a wide range of composition options.

Life turned upside down

As a result of the EEG examinations I conducted, I published several papers and a doctoral thesis in which I presented my own theory of cortical immaturity based on a common cause, i.e., parasomnia. I defended my thesis in 1960 and was awarded the degree of PhD in Medicine. This event boosted my ambitions and became the driving force behind further scientific work.

In October 1964, I was promoted to Doctor of Science. The topic of my post-doctoral thesis was the development of a new method for quantitative testing of pressure and pain sensory loss. Pain was also the subject of my lecture and later the main focus for the department in Warsaw.

After my post-doctoral thesis, the rector of the WAM, Gen. Prof. Marian Garlicki, announced that I was being considered for the post of head of department at the newly opened 2nd Central Teaching Hospital of the WAM in Warsaw. The news caught me off guard because I had never thought of moving out of Łódź, where my whole family lived. I was living a comfortable life working as an associate professor and deputy head of the department and had my own flat with a garage and a fairly popular private practice. My wife was a senior assistant at the Department

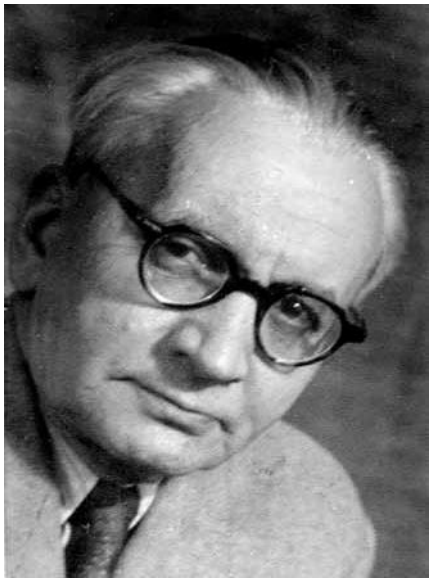

Figure 5. Col. Stefan Bogusławski MD, PhD, Head of the Department of Neurology at the Hospital of the Ministry of Defence.
Rycina 5. Płk dr Stefan Bogusławski, ordynator neurologii w Szpitalu MON.

of Ophthalmology at the Medical Academy and my daughter attended a nearby school. Moving to Warsaw would turn our whole lives upside down. In the army, however, refusal was not an option, especially since it was a considerable promotion after all.

An order from the Minister of National Defence with the nomination ended all discussions and on 22 February 1965 I became the head of the Department of Neurology at the 2nd Central Hospital of the WAM in Warsaw. Thus began the Warsaw phase of my life – which continues to this day.

I had to build the department in Warsaw from scratch. The personnel consisted of employees from two dissolved

Figure 6. Col. Felicjan Roguski MD, PhD, Head of the Department of Neurology at the Regional Hospital in Warsaw.
Rycina 6. Płk dr Felicjan Roguski, ordynator neurologii w Warszawskim Szpitalu Okręgowym.

departments of Warsaw's military hospitals: the Hospital of the Ministry of National Defence at Koszykowa Street and the Regional Hospital at Nowowiejska Street. Commander of the Hospital of the Ministry of Defence and head of the neurology department, Col. Stefan Bogusławski MD, PhD (Fig. 5), and the head of department at the Regional Hospital, Col. Felicjan Roguski (Fig. 6), were my assistant professors, and all the assistants were older than me. It was an extremely difficult and awkward experience for me, as I had taken my Level II neurology exam with Col. Bogusławski only a few years earlier. Both gentlemen were extremely tactful and did not want to embarrass me, so they decided to work in neurological outpatient clinics and retired a few years later.

Initially, the department was located on two floors, one for neurosurgery. The Department of Neurosurgery was to be managed by Prof. Jan Bromowicz, but he left the WAM and returned to Kraków, where he lived, and took over the practice from Prof. Adam Kunicki, who had returned to Warsaw. After a year, two neurosurgeons from the Warsaw Department of Neurosurgery joined the department by a decision of the authorities – Stanisław Rudnicki as assistant professor and Apolinary Kępski as senior assistant. Soon, a neurosurgery sub-department was also opened, and a few years later, after the post-doctoral thesis of Dr. Rudnicki, it became the Department of Neurosurgery.

An electroencephalography and electromyography laboratory, as well as a biochemistry and cerebrospinal fluid examination laboratory were established in our department. A psychiatric outpatient clinic with a psychiatrist and psychologist as well as a pain research and treatment clinic were also created, as pain was our main research topic. For several years I fought for the establishment of a psychiatric department, considering how large the central teaching hospital was, but the head psychiatrist at WAM resisted my efforts, arguing that the one WAM department in Łódź was enough for the army. Later, however, my aspirations were fulfilled and the psychiatrist Stanisław Ilnicki (first my assistant, then a professor) became head of the Department of Psychiatry at our hospital.

I implemented a new work regime and habits. The physicians were required to attend autopsies of the deceased at the department. I performed brain dissections personally as part of my training for assistants. The neuropathological examination was a verification of our clinical diagnoses and also a lesson in brain anatomy.

The daily briefing included a report from the on-call doctor and the nurses on the latest news from the department. On-call admissions of new patients were also discussed, as were the diagnostic and treatment plans. On selected days of the week, we read the abstracts of some of the most interesting articles from the scientific literature, and once a week, on Wednesdays, we held radiology meetings. There was also a daily thorough round of the patient rooms in a set order, and once a week a general round with all the doctors and nurses.

At the steering wheel

During one of my first rounds, I was presented with an unprecedented case. It was a soldier who had been transferred from neurosurgery following a ventriculography with the results ruling out the presence of a brain tumour. I was moved by his story, which began with his glasses breaking during a recruit training exercise. He went for a prescription for a new pair to an ophthalmologist, who found a suspected slight swelling of the optic discs, which could indicate an increase in pressure, so he referred the patient to a neurologist. The neurologist asked the patient if he had had any headaches – of course, anyone would say 'yes' to such a question in the hope that they could be discharged from the army. The neurologist, therefore, referred the soldier to neurosurgery with a suspected brain tumour. At the time, there was no other method of ruling out a tumour than ventriculography, and this was done. I had him discharged from military service for the suffering caused by his justified lying and for the holes in his head that had been made in accordance with the diagnostic procedures. Blurred fundus optic nerve disc margin is not an oedema, and it happens very often in healthy people, to the confusion of inexperienced doctors.

One important task of the department was the postgraduate training of doctors taking a three-month pre-examination course. Such courses were held at each department twice a year – in the spring and autumn – and culminated in a board examination for Level I and Level II of the specialisation. During my tenure, more than 500 doctors completed the course and passed the specialisation exam. Failed exams were a rare occurrence, as during the course I explored the level of knowledge mastered by the physicians and sent home those who did not show progress.

The Szaserów Hospital, as our facility was often called, provided medical services not only to the military but also to civilians whenever places were available. The Head of Health Services was subordinate to the chief quartermaster, who changed the name and rank of the hospital several times as he saw fit. It was the 2nd Central Teaching Hospital of the WAM – Postgraduate Education Institute, and eventually became the Military Institute of Medicine after the transformation of the political system and the dissolution of the Military Medical Academy. The quartermaster used his position of authority and issued various orders to the Head of Health Services, who passed them on to the hospital commandant in accordance with the hierarchy. There was often confusion along the way between the protégés' names or neurology and urology, resulting in quite a few conflicts, often leading to various personal repercussions, including removal from office. Medical services were provided to unauthorised persons, as well as party and government dignitaries. Orders were given to examine a friend or to admit a patient without indications or chronically ill patients in an extreme condition from other hospitals 'for the time being', etc. Even professors were recalled from leave for consultations or to perform a trivial procedure if the higher authorities had to be accommodated. There were three attempts to remove me from office. Once for refusing to consult a dignitary on the day I was leaving on holiday, the second time when my brothers stayed abroad. The third attempt was related to the 1987 Congress of Neurologists in Gdańsk, on the eve of John Paul II's visit. At the time, I was president of the General Board of the Polish Neurological Society and some agent reported that anti-government speeches had taken place at the general meeting and that I was given a steering wheel made personally by Lech Wałęsa. The denunciation went to all members of the PZPR Politburo and I was interrogated by the secret service. The chief prosecutor of the Polish Army found no crime, and I received the steering wheel from the organisers to mark my election to a second term as president.

On pain with wisdom and gravity

Good relations with the quartermaster's office and the management of the Health Service secured my permission to travel abroad and attend congresses, various conferences, and training courses. Each stay abroad or domestic meeting with a foreign national required a detailed report listing the names and giving the content of the conversations. We quickly got used to this style of working in the army and did not really care too much about these requirements, nor did we show excessive enthusiasm in meeting them. Most of the department heads in the hospital did not belong to the PZPR, including me, but there was little pressure to join the party or any related discrimination.

Being in charge of the department was very challenging, as we had teaching and research responsibilities in addition to our service work. Topics related to military service were given priority in research, but we were free to conduct studies in any field. The teaching activities, on the other hand, required relevant expertise, as well as experience and knowledge of the current scientific literature. I had already gained some insight in this respect in Łódź. Professional training of staff took place each day at the morning briefing and during rounds. The assistants and I also delivered lectures once a week on a selected topic. Most of the assistants did not have degrees appropriate to their roles in the department. Additionally, they had neither any experience in conducting such research nor the motivation to do so.

The main topics of research (apart from those related to military service) included pain, pain testing methods, reasonably objective assessment, and treatment. I had already developed an interest in this topic and devoted my post-doctoral lecture to it. We developed various methods and scales for objective pain assessment. Electromyographic studies of the muscular response to pain were conducted to measure the sensation of pain, distinguishing muscular defence independent of the will as an increase in muscle tone, and a reaction in the facial muscles invisible to the naked eye as an expression of suffering. We also looked at the psychological response responsible for the magnitude of the intensity of suffering. The research focused on the pain reflex as an objective symptom of pain that can be quantified. We also created a 5-point intensity scale for non-specific sacral pain.

Other investigated areas involved the mechanisms by which classical acupuncture affects pain and concluded that meridians, fixed puncture points, and special needles are of no practical use. The same effect is produced by

Figure 7. General Board of the Polish Neurological Society, Wrocław 1970, the author is ninth, left to right.
Rycina 7. Zarząd Główny PTN, Wrocław 1970 r. autor dziewiąty od lewej.

injecting an irritant into the pain points with safe disposable needles. Our method of stimulation was called 'needling'.

I formed the Section for the Study of Pain in the Polish Neurological Society (PTN) and the Section for the Pathophysiology of Pain in the Committee of Neurological Sciences of the Polish Academy of Sciences. My friend and Prof. Herman's successor at the Łódź Department of Neurology, Prof. Antoni Prusiński, with whom I worked closely in both sections, specialised in headaches. In addition to neurologists, members of these sections included psychiatrists, psychologists, anaesthetists, and neurosurgeons interested in the surgical treatment of pain. This was the foundation of what later became the Association for the Study of Pain. We had joint scientific conferences, usually held in Radziejowice, where we presented and discussed the results of our research and new developments in international literature. I also wrote three books about pain.

Another issue studied by the department was the treatment of nervous system diseases. We were the first in Poland to treat Parkinson's disease with levodopa. The paper was published in the Neurologia i Neurochirurgia Polska (NNP) journal in 1972. In the 1990s, we introduced botulinum toxin treatment for torticollis, eyelid, and laryngeal spasm and hemifacial spasm. To this day, our department provides outpatient treatment of various dystonias with the injection of this toxin. I taught special courses on botulinum toxin treatment organised by the manufacturers in Austria. The lectures from the courses were published in a special NNP supplement. I wrote a textbook on botulinum toxin treatment (two editions). The treatment of nervous system diseases was the subject of one book and many articles and chapters in neurology textbooks.

With our own methods we also attempted to treat Huntington's disease, sacral pain, headaches, and circadian rhythm sleep-wake disorders after strokes. I formed the Therapy Section of the PTN and the symposia it organised each year at the Staszic Palace featured clinical assistants and invited guests presenting advances in the treatment of diseases of the nervous system. The Therapy Section was the largest of all at some point, with more than 500 members, and existed for 30 years. It was dissolved at my request because drugs and treatment are now on the agenda of every neurology conference.

There were also many case-study and review publications on various topics. I wrote more than 300 papers published in various national and international scientific journals. My colleagues contributed around 100 publications and dozens of posters and lectures at neurology conferences in Poland and abroad. I was the supervisor of 35 PhD thesis and six DSc thesis. I wrote nine books and dozens of chapters for various textbooks.

Neurological heir

The year 1973 was marked by the sudden death of my colleague and friend, Stein's successor, Col. Prof. Mieczysław Strzałko. I was appointed chief neurologist of the Polish Army in his place. This meant new duties and responsibility for service, teaching and research in military neurology departments in central and provincial hospitals. It was my duty to visit such departments and check the professional training and the quality of the services provided by the medical staff.

As the years went by, my military promotions and titles also advanced: in 1970 I was made colonel, 1971 an associate professor and in 1982 a full professor. In addition to my position as head of the department and chief neurologist, I have held many positions in the Polish Neurological Society. In 1970, I was elected to the General Board of the Polish Neurological Society (Fig. 7), and 2 years later I became the vice-president. In 1984, at the congress in Szczecin, I was elected President of the General Board of the PTN and held this position for two terms – until 1990. I became a member of the General Board and remain the Honorary President of the PTN to this day. I have been taking an active part in Board meetings for more than half a century. I served one term as the chairman of the Committee on Neurological Sciences of the Polish Academy of Sciences and directed the work of the Section on the Pathophysiology of Pain. I am an honorary member of many Polish and foreign scientific societies. In 2007, the Senate of the Medical University of Łódź awarded me the honourable title of *doctor honoris causa* (Fig. 8).

Figure 9. Prof. Herman visiting the department, left to right, sitting: Teofan Domżał, Eufemiusz Herman, Mirosława Hołyst, Barbara Radzikowska; left to right, standing: Jolanta Gierczak, Włodzimierz Szepielow, Henryk Szymański, Bożena Zaleska, Eugeniusz Jaszczuk, Stanisław Zalejski, two students, Elżbieta Orłowska, Waldemar Pakszys, two students, Danuta Zajkowska, Halina Milczarek, student, Janusz Kwaucki.
Rycina 9. Odwiedziny prof. Hermana w klinice, od lewej siedzą: Teofan Domżał, Eufemiusz Herman, Mirosława Hołyst, Barbara Radzikowska; od lewej stoją: Jolanta Gierczak, Włodzimierz Szepielow, Henryk Szymański, Bożena Zaleska, Eugeniusz Jaszczuk, Stanisław Zalejski, dwoje kursantów, Elżbieta Orłowska, Waldemar Pakszys, dwaj kursanci, Danuta Zajkowska, Halina Milczarek, kursant, Janusz Kwaucki.

Figure 8. *Doctor honoris causa*, Łódź 2007
Rycina 8. *Doctor honoris causa*, Łódź 2007 r.

Figure 10. The author – Head of the Department of Neurology of the WAM in Warsaw, 1985.
Rycina 10. Autor – kierownik Kliniki Neurologicznej WAM w Warszawie 1985 r.

I retired in 1997 but remain active in the department as a consultant. The department is now headed by my student, Docent Jerzy Kotowicz. I am invited to different scientific conferences with lectures on a variety of topics. Since 2000, I have given 116 talks, most of them opening lectures.

I am currently studying the history of neurology and I feel I am the neurological heir of Flatau's school – his neurological grandson, one might say. I cultivated the traditions of this school throughout my clinical work in Łódź and Warsaw. My teacher, Prof. Eufemiusz Józef Herman, was invited to see his student at the Warsaw department in 1979 and gave a talk about military neurologists in the past (Fig. 9). He passed away in 1985. I attended his funeral at the Old Cemetery in Łódź on behalf of the PTN General Board, of which I was president at the time. The department is currently headed by Prof. Adam Stępień, my student.

In my 70 years of working with neurology, there have been great advances that have completely changed medicine and our speciality. The neurology of today has moved far beyond the one that I learned. Neurological diagnosis started with a hammer and pin, but it has since acquired wonderful diagnostic equipment and a huge arsenal of medicines. Still, technological advances in diagnosis are outpacing advances in therapy. Symptomatic treatment still significantly prevails over causal treatment. However, there are doubts as to whether the directions of research and progress are relevant to the potential of our brains and whether they meet the needs of the patients who require our help. If as much money was spent on progress in saving people as is spent on killing them, the progress would be far greater. The curse cast on mankind since the time of Cain and Abel is still at work and remains more powerful than reason. Nature continues to spawn psychopaths capable of controlling the masses with deranged ideologies and propaganda machines. 'People become foolish in bulk but wise at retail', the Polish Nobel Prize winner Wisława Szymborska once said in an interview.

Galileo said that God gave man reason to use it. Scientists say that only 10% of our brain is used in everyday life. I hope that there will be people who can use the full potential of their brain to save our planet from annihilation.

REPORT OF THE 20TH INTERNATIONAL VASCULITIS AND ANCA WORKSHOP 2022

Sprawozdanie z 20. edycji International Vasculitis and Anca Workshop 2022

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Abstract: The 20th International Vasculitis and ANCA Workshop took place in Dublin on 3-6 April 2022. It was the largest international congress focused on systemic vasculitis. During this event the results of the newest clinical trials were presented by the representatives of major specialized international centres. The main event was preceded by a series of lectures dedicated to the patients who were represented by the patient's associations, such as Vasculitis International and Vasculitis Foundation. The main lectures focused on new biologic therapies of vasculitis of various calibres. The results presented the clinical trials regarding the agents related to the mode of action associated with the C5a blockade. The summary of all parts of the MAINRITSAN study, which concerned maintenance therapy with rituximab in ANCA associated vasculitis (AAV), was also discussed during the congress. The remaining lectures concentrated on monitoring biologic therapies, complications of AAV therapy in elderly patients and the clinical course of ANCA negative vasculitides.

Keywords: 20th International Vasculitis and ANCA Workshop, vasculitis.

Streszczenie: 20. edycja International Vasculitis and ANCA Workshop odbyła się w dniach 3-6 kwietnia 2022 r. w Dublinie. To największy międzynarodowy kongres dotyczący układowych zapaleń naczyń, na którym prezentowane były wyniki najnowszych badań klinicznych dotyczących tej grupy chorób, a wykłady wygłosili prelegenci z kluczowych ośrodków zajmujących się ich leczeniem. W ramach tego wydarzenia odbyły się również sesje z wykładami dla pacjentów, których reprezentowały stowarzyszenia pacjentów Vasculitis International oraz Vasculitis Foundation. Główne tematy wystąpień koncentrowały się na nowych, biologicznych terapiach zapaleń naczyń różnego kalibru. Prezentowane były wyniki badań dotyczących preparatów, których mechanizm działania związany jest z blokadą cząsteczki C5a dopełniacza. Podsumowano również wyniki wszystkich części badania MAINRITSAN dotyczącego stosowania rituximabu w terapii podtrzymującej zapaleń naczyń związanych z przeciwciałami ANCA. Pozostałe zagadnienia dotyczyły między innymi: monitorowania terapii biologicznych, odmienności leczenia osób starszych, przebiegu klinicznego ANCA negatywnych zapaleń naczyń.

Słowa kluczowe: International Vasculitis and ANCA Workshop, zapalenia naczyń.

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The 20th International Vasculitis and ANCA Workshop took place in Dublin between 3 and 6 April 2022. The chairpersons were Prof. Mark Little (Trinity Health Kidney Centre, Nephrology, St. James Hospital, Dublin) and Dr. Michael Clarkson (Cork University Hospital). It was the largest international congress focused on systemic vasculitis. During this event the results of the newest clinical trials were presented by the representatives of major, specialised international centres from all over the world. Due to the specific nature of vasculitis and the variety of clinical manifestations, the treatment of this group of patients is handled by physicians from different specialities. Accordingly, the guests at the congress included nephrologists, rheumatologists, and immunologists.

Prof. Benjamin Terrier (Hôpital Cochin, Paris) summarised the results of all three arms of the milestone MAINRITSAN trial investigating maintenance therapy with rituximab in patients with ANCA associated vasculitis (AAV). Rituximab, a monoclonal antibody targeted against the CD20 molecule present on B lymphocytes, is an option for AAV maintenance therapy. The most important conclusion from all arms of the aforementioned study is that rituximab should be the gold standard in maintenance therapy for ANCA associated vasculitis.

Figure 1. 20th International Vasculitis and ANCA Workshop 2022.
Rycina 1. Konferencja 20-th International Vasculitis and ANCA Workshop 2022.

The next series of lectures covered drugs affecting the complement system. Prof. Peter Merkel (University of Pennsylvania) presented the results of a study involving vilobelimab in the treatment of AAV. Vilobelimab, a monoclonal antibody, blocks the biological action of C5a by binding to it. The results of the study seem promising in terms of the possible replacement of chronic

Figure 2. Trinity College, Dublin.
Rycina 2. Trinity College, Dublin.

Figure 3. Trinity College, Dublin.
Rycina 3. Trinity College, Dublin.

glucocorticosteroid therapy with the new drug. Another lecture on complement blocking therapy was given by Prof. Annette Bruchfeld (Karolinska Institute, Stockholm). It concerned avacopan, whose mechanism of action involves blocking the C5a receptor. The product is administered orally, which is an additional advantage. The results of the ADVOCATE study addressed the beneficial effect of the drug on improving renal function and reducing proteinuria in patients with renal involvement in AAV. Prof. David Jane

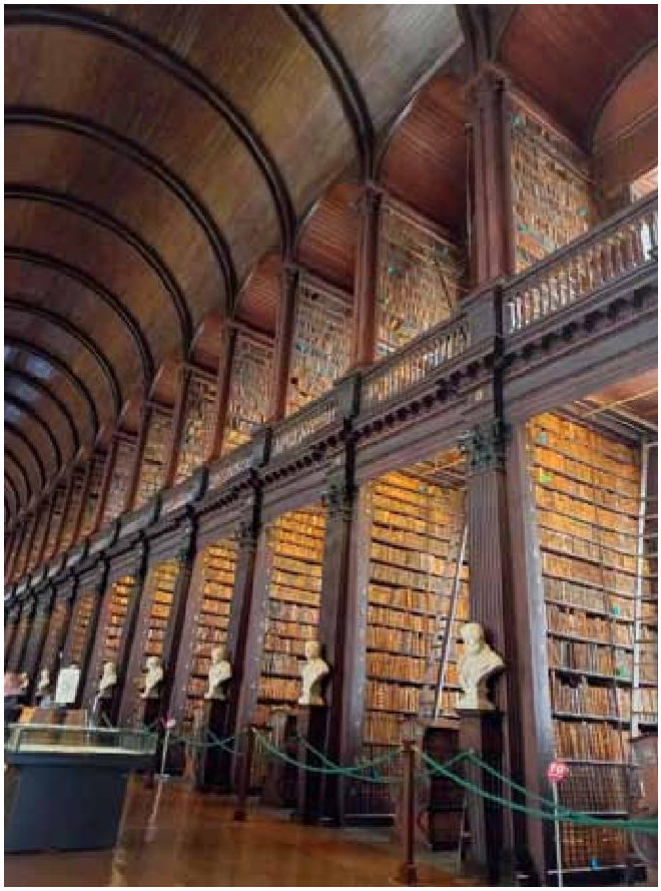

Figure 4. Trinity College, Long Room.
Rycina 4. Trinity College, Long Room.

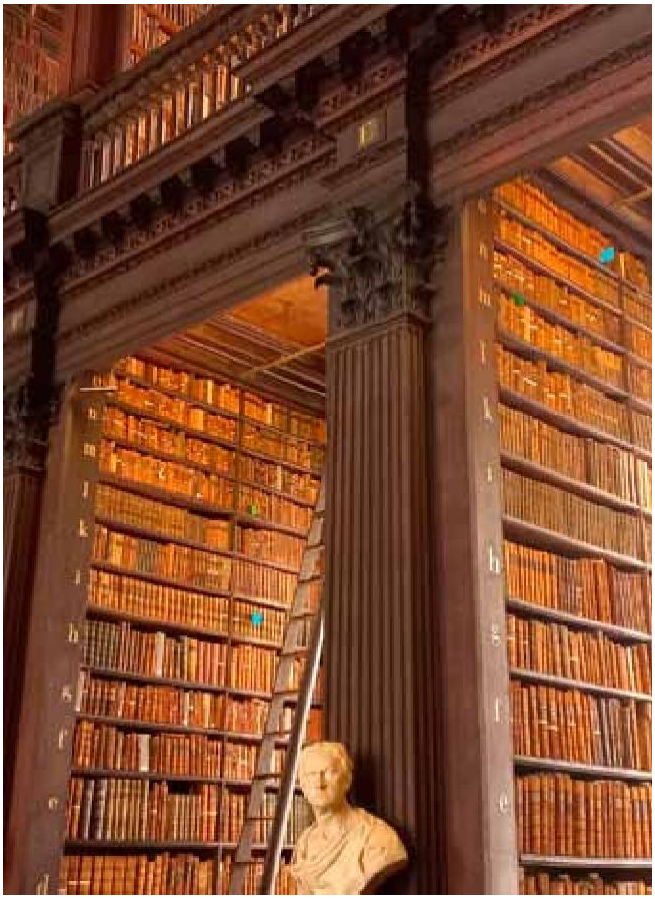

Figure 5. Trinity College, Long Room.
Rycina 5. Trinity College, Long Room.

(University of Cambridge) provided similar promising data on the effect of avacopan on renal function.

Dr. Reza Zonozi (Massachusetts General Hospital) presented the results of a study discussing prolonged maintenance therapy (over 2 years) with rituximab in AAV patients. Drug administration depended on an increase in ANCA antibodies or an increase in B lymphocyte counts. Dr Mark McClure (University of Cambridge) presented the results of an analysis of the effects of treating elderly (over 75) AAV patients.

Before the congress officially commenced, lectures for patients were also held onsite and online. International associations of systemic vasculitis patients, such as Vasculitis International and the Vasculitis Foundation, were also present at the congress.

As the congress was held in Dublin, there was the added benefit of visiting the famous 16th-century Trinity College and its impressive library holding 700,000 volumes, including the Book of Kells, one of the most valuable illuminated medieval manuscripts, at the forefront.

WE ARE READY FOR THE CHALLENGES OF THE MODERN ERA

Jesteśmy gotowi na wyzwania współczesności

Grzegorz Gielerak[1], Zuzanna Chodzeńska[2]

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Abstract: On 27 November 2022, the Military Institute of Medicine celebrated its 20th anniversary. In an interview, the director of the institute, General Professor Grzegorz Gielerak, presented the achievements to date and the challenges facing the Military Institute of Medicine – National Research Institute in the coming years.

Keywords: Military Institute of Medicine – National Research Institute, medical troops.

Streszczenie: 27 listopada 2022 roku Wojskowy Instytut Medyczny obchodził 20. rocznicę powstania. Dyrektor Instytutu gen. prof. Grzegorz Gielerak przybliża w wywiadzie dotychczasowe dokonania oraz wyzwania stojące w najbliższych latach przed Wojskowym Instytutem Medycznym – Państwowym Instytutem Badawczym.

Słowa kluczowe: Wojskowy Instytut Medyczny – Państwowy Instytut Badawczy, wojska medyczne.

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Interview with Lt. Gen. Prof. Grzegorz Gielerak MD, PhD, Head of the Military Institute of Medicine – National Research Institute (WIM-PIB).

During the previous autumn, WIM-PIB celebrated its 20th anniversary. How has the Institute changed in that time?

On 27 November 2002, a regulation of the Minister of Defence established a new research and development unit – the Military Institute of Medicine (WIM). The WIM became a public health facility. Since then, its main activity, in addition to treatment, has been to carry out research and development work in the field of medical sciences, taking into account the needs of the Polish Armed Forces. The Military Institute of Medicine has become one of the largest, multi-profile accredited healthcare institutions in Poland, with the highest referral level. It is the central clinical, teaching, consulting, and research centre of the military health service. It is accredited by the Minister of Health and has ISO- and AQAP-compliant quality management systems.

WIM researchers aim to find solutions to problems considered to be priorities for the development of science and the economy. The strategic research directions of the WIM in the field of health risks include diseases of civilisation, new medicines, and regenerative medicine. Another important focus of the research activity is the development work related to protection and survival on the battlefield, within the framework of national security and defence.

Figure. Lt. Gen. Prof. Grzegorz Gielerak MD, PhD
Rycina. Gen. broni prof. dr hab. n. med. Grzegorz Gielerak.

In your opnion, which area of WIM's activity has changed the most
The past 20 years have been marked by intensive technological development of the Institute in a variety of fields – from the branches of medicine counteracting diseases of civilisation to battlefield medicine, regenerative medicine, telemedicine, and robotic surgery. The Centre for Robotic Surgery, equipped with two state-of-the-art da Vinci Xi surgical robots, has been operating at the WIM for two years, enabling extremely complex surgical procedures to be carried out using minimally invasive techniques. Today, we are the first hospital in Poland to have two surgical units that have been used by WIM specialists to perform more than 500 procedures in as many as seven fields of medicine.

The WIM also has its own basic research laboratories. The Laboratory of Molecular Oncology and Innovative Therapies investigates the mechanisms of diseases of civilisation, such as cancer, heart disease or diabetes and works on developing innovative treatments.

We are a leader in the use of telemedicine, for example in cardiology. An example of such activities is the Amulet platform for the acquisition, collection, and analysis of medical data, which uses models to support the diagnosis and treatment of patients with heart failure, latest advancements in telediagnosis tools and cardiac telenursing.

Huge progress has also been made in digitalisation. Initially the Institute relied on one and then several servers. The WIM of today consists of more than 300 running servers and resources counted in petabytes of data, kept in continuous operation 24 hours a day.

The last year has been exceptionally eventful for WIM. Which achievements do you consider to be of the most value?
In the past year, thanks to the support of the Ministry of Defence, we continued a multi-year programme for the expansion and modernisation of the institute-hospital, which has enabled further clinics and departments to acquire modern diagnostic and therapy equipment. Further improvements have been made in the delivery of healthcare services, as well as in work quality, comfort, and safety. Thanks to the consistent large-scale investments, the institute's equity capital exceeded one billion PLN last year, making it the best-equipped healthcare institution in Poland for yet another year.

We inaugurated the year 2022 with the opening of the hospital in Legionowo, which is a branch of the Military Institute of Medicine. The new healthcare provider in the Mazowsze region has the capacity to hospitalise 90 patients at the same time in the specialities of cardiology, surgery, gynaecology, ophthalmology, and intensive care, which are key to the health needs of the population. It has an excellent intensive care unit, accounting for 30% of the total number of inpatient beds provided.

It was also a period in which we obtained a formal confirmation of the institute's outstanding scientific position, once again reflected in the award of category A in the parametric evaluation of scientific units.

The capital and capabilities accumulated over the years, which are particularly important for the planning and implementation of state policy required to ensure national defence and public security, have been confirmed by the WIM becoming a National Research Institute. This significantly increases the possibilities for carrying out activities that are particularly important for the planning and implementation of state security policy. At the same time, it opens a new period in the operation of the Military Institute of Medicine.

During the ceremony marking the 20th anniversary of the establishment of the Military Institute of Medicine, President Andrzej Duda announced that the Ministry of National Defence, with the support of the National Security Bureau and the participation of WIM, is working on a new type of military unit – the Medical Forces Component. Can you give us an overview of this project?
The main task envisaged for the Medical Forces Component is to secure the operational readiness of the Armed Forces through medical support for the other types of troops, medical education, and training, including the creation and transfer of knowledge improving the preparation and survival of the soldier on the battlefield. To fulfil these tasks in an efficient and orderly manner, the structure of the military health service should be re-organised and set on a foundation of process and product innovations that overcome the organisational standards used until now, which were often abstract constructs disregarding reality. The organisation of a network of hospitals within the country, whose distribution, number, competence, and capacity corresponds to the projected size of sanitary losses as a result of a potential armed conflict on the national territory, should be seen as the most advisable. The profile of the units forming the national network of state security system hospitals should include entities ready to provide comprehensive and multispecialist medical assistance, with particular focus on battlefield injury treatment.

The next task is to provide conditions that enable intensive and up-to-date training of the professional personnel of the Armed Forces and, at the same time, to retain the most competent, creative, and dedicated individuals. A logical and coherent career path for a military physician consistent with military pragmatics should be the product of personal aspirations and the needs of the Armed Forces fostered from the very beginning of service through substantial professional training from leading national and foreign military institutions.

Have the last 20 years of development of the Military Institute of Medicine prepared the facility to engage in such a task?
Definitely. I can say with full responsibility that we are ready for such demanding modern challenges.

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