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YES MEETING
12th YOUNG EUROPEAN SCIENTIST MEETING

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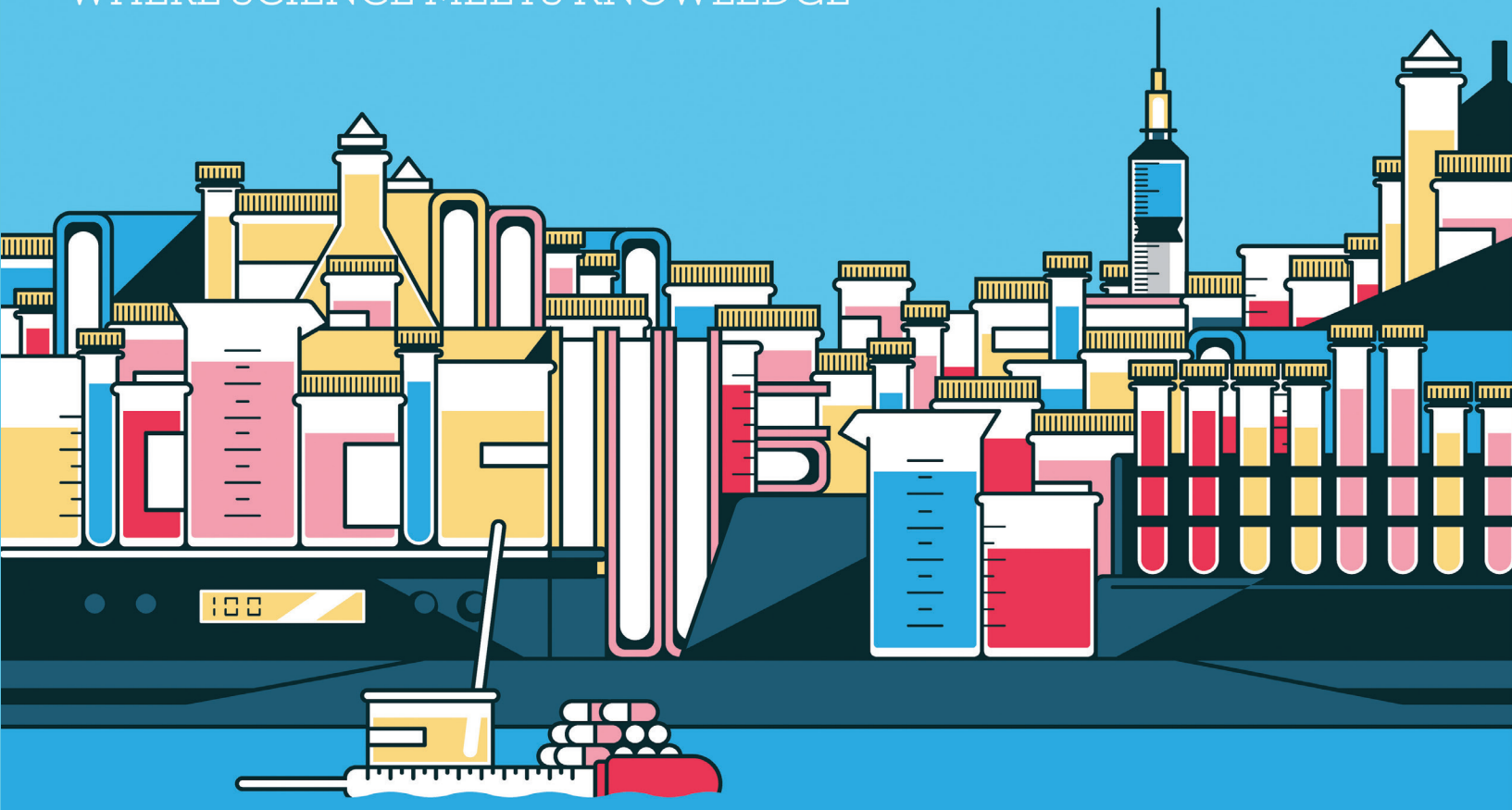

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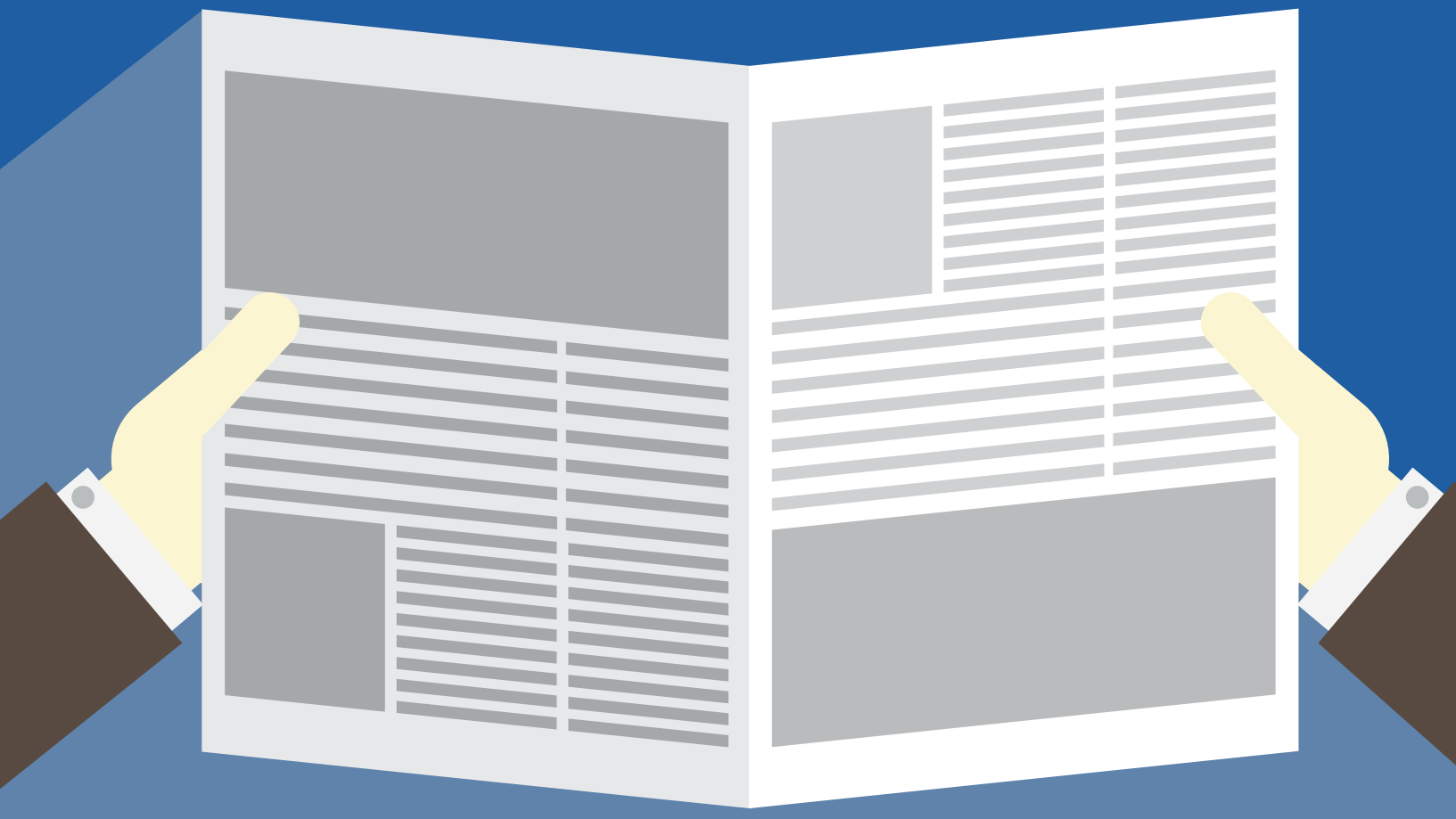

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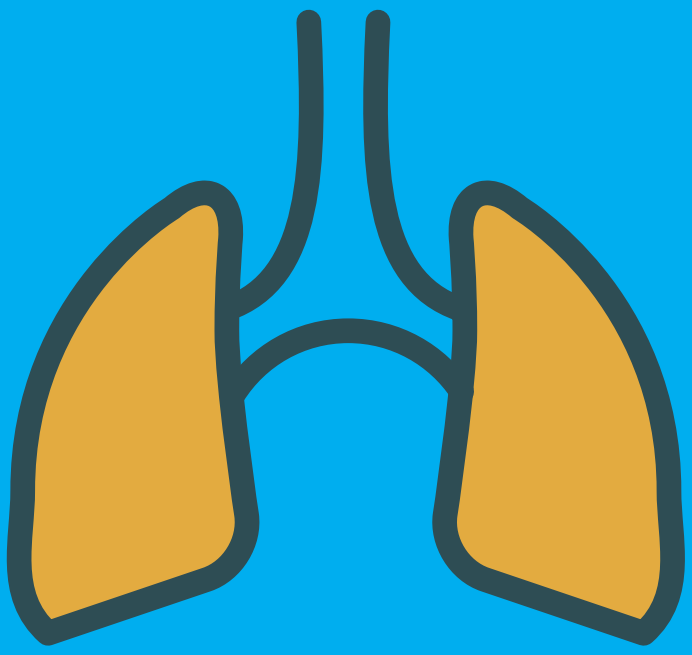

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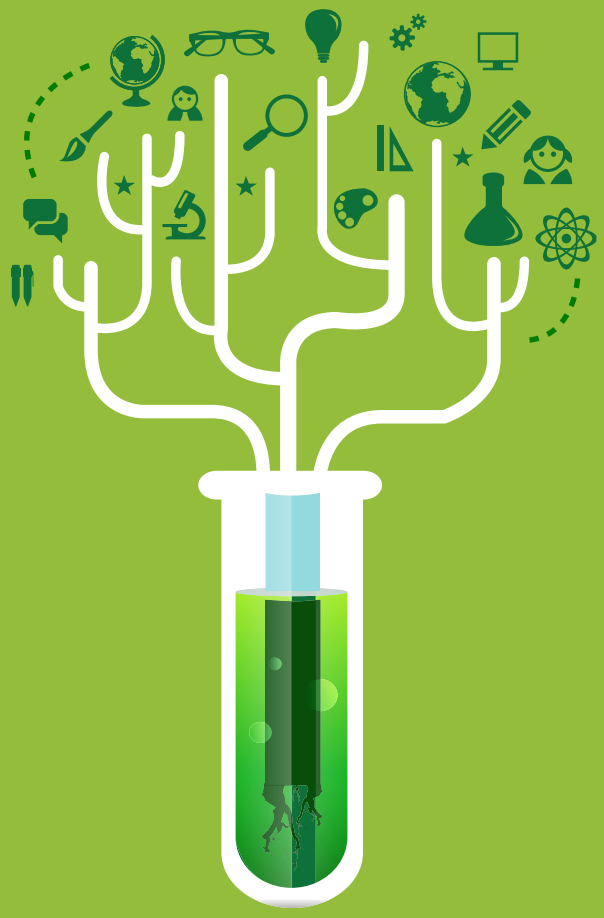

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Porto Biomedical Journal (abv. PBJ) is an online, open-access and free-to-submit journal devoted to publishing high quality, original biomedical research.

Porto is where the idea originated, but the name implies more. In Portuguese, 'porto' means 'harbour,' which relates to our vision: a gateway to a scientific hub governed by principles of communication and sharing. Inspired by the historic background of the Discoveries that shapes our cultural and social heritage, as well as by the multitude of knowledge-seeking journeys that started out in the tortuous contours of our coastal lines, we intend to connect the islands of scientific communities currently in existence, thus increasing the value of Science in all its forms of expression.

To ensure the quality and scientific relevance of PBJ, the journal counts with a diversified and international editorial board, gathering members from nearly 20 countries around the world, and only accepts original research and review articles that undergo a strict revision process in a double-blind refereeing system supervised by Elsevier, a procedure that safeguards the fairness of the article selection process.

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Editorial

Welcome to the 12th YES Meeting!

Catarina Metelo-Coimbra

Faculty of Medicine of the University of Porto, Portugal

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The 2017 Young European Scientist Meeting's edition will gather nearly 500 biomedical students, coming from 30 different countries, in the *Antiga*, *Mui Nobre*, *Sempre Leal e Invicta cidade do Porto* (the Old, Most Noble, Always Loyal and Undefeated city of Porto).

YES Meeting was born in 2006 as a result of a pioneer idea of a young student from the Faculty of Medicine of the University of Porto (FMUP). His goal was to gather together students from several biomedical areas, alongside with researchers from cutting-edge centers, giving them the opportunity to present and discuss their work.

Despite being considered by many a very ambitious concept, the value of this idea started to take shape with the support of the Faculty, our dear Professors and some firms that believed and shared the value of the initiative, which is exclusively organized by FMUP medical students. This was a completely original project in Portugal and attracted speakers from some of the major research centers in the world since the very beginning.

Throughout the 11 editions of the congress, we were able to count on the presence of speakers coming from the Universities of Zurich, Basel, Oxford, Cambridge, Leiden, Lausanne, Johns Hopkins, Harvard, Texas, Duke, Yale, San Diego, and the Karolinska, Howard Hughes and Max Planck Institutes, to mention just a few. Our invited speakers have enjoyed themselves in Porto, and ever since helped spreading the word about this conference, allowing it to cross the European borders and reach Asia, America, and Africa!

In 2017, the YES Meeting will bring to its participants the subjects of autophagy (the theme related to the 2016 Nobel Prize in Medicine), Huntington's disease, psychopathy, *ex vivo* surgery, the "human-on-a-chip" technology, myoelectric controled prosthetics, memory formation, and spinal cord injury repair through neuroprosthetic systems. As our special guests, we welcome Professor Nicholas Lydon, recipient of the 2009 Lasker Award for the discovery of Imatinib, a revolutionary molecule for the treatment of chronic myeloid leukemia, and Professor Ada Yonath, 2009 Nobel Prize in Chemistry laureate, for her work concerning the ribosome.

For the 12th Edition, we established a partnership with *Porto Biomedical Journal* (PBJ), the scientific journal that was brought to life from a common effort between FMUP and Centro Hospitalar São João (CHSJ), the largest Portuguese teaching hospital, which is deeply tied to the Faculty. Some of the most prestigious members of both institutions take active part in PBJ's Editorial Board, as well as the YES Meeting Scientific Committee.

It is our belief that in the present context of globalization and increasing velocity of changes regarding distinct forms of

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knowledge, students must be encouraged to exchange experiences concerning scientific matters and technological development. Furthermore, junior research should be faced as a complement to the biomedical curriculum, instead of an alternative. An "AND" rather than an "OR"! Both components are connected and, in a synergic manner, have the potential to enable the raise of more complete professionals.

Therefore, in this September/October issue of PBJ, you may find our Presenting Students' Abstracts, selected out of 238 submissions in the fields of Neurosciences, Oncology & Molecular Biology, Public Health & Medical Informatics, Internal Medicine, Immunology & Physiology, and Surgery. Their research work is published alongside with articles written by renowned scientific authorities, who have previously been at the YES Meeting or will be speaking at the 12th edition of the congress. On behalf of the Organizing Committee, I would like to thank the speakers from our past and current edition for having accepted to contribute to this project and uniquely enrich it.

It is my pleasure to invite you all to leaf through this issue's pages, aimed at breaking boundaries, in a remarkable platform where Science meets Knowledge.

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Editorial

The very first year of PBJ

Raquel Soares (Associate Editor)[a,b,c]

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[c] *Member of Executive Commission, i3S, Portugal*

As described in its homepage, the purpose of PBJ is to promote science and health research without barriers. This purpose was well addressed by the editor-in-chief, in the first editorial. PBJ was expected to: (1) produce a full spectrum of top-quality papers in Biomedicine, (2) strengthen reputation in scientific and scholarly community, and (3) expand to other databases.

In just one year, around 50 papers were published in PBJ, gathering knowledge in biomedicine domains as distinct as biology, biochemistry, bioengineering, genetics, immunology, nutrition, medical education, psychology, sociology, psychiatry, medical informatics, epidemiology, neurosciences, cancer, respiratory disease, cardiology, radiology, surgery, and pediatrics. This panoply of domains is authored by researchers with very distinct backgrounds that have at least one thing in common: to provide high quality scientific advances in health research to the PBJ readers.

The majority of papers published within this year in PBJ are accomplished by effective collaborations held between Hospitals, Universities and Research & Development institutions. That is one of the first lessons PBJ provides for students – Science is a collaborative issue! You cannot do science alone!

Yet, we can say: "No big deal!", since PBJ actually arises from the commitment of FMUP and the S. Joao Hospital Center consortium. And it is located at the Porto health sciences pole, one of the best environments for favoring scientific collaborations, gathering together hospitals, faculties and R&D units. But location is not enough! A full commitment of researchers and students in publishing their work was needed to successfully achieve this task. And PBJ made it possible!

Another crucial point is the fact that science does not have frontiers. Again PBJ fulfilled this accomplishment, since twenty-five percent of the papers published within this year are international ones, either from partnerships with worldwide teams, or entirely published by international research groups. This lesson was also endorsed by PBJ.

Finally, researchers, and particularly students already welcomed PBJ. Nearly 20% of the papers published at PBJ this year were performed by students, particularly PhD and master students, but also undergraduate MD students, who recognized this journal to spread their studies. Thus, the scientific and scholar community is already paying attention to PBJ as well.

The first year of a scientific journal is always a difficult task, requiring hard-work of the whole team and lots of patience. Looking back to the very beginning, PBJ achieved high standards concerning the number and quality of publications, authorships, and projection in research, clinical and academic community. Keeping this in mind, to expand to new databases is the next challenge and it is not far away. Full success is ahead!

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Editorial

The inter-dependence of basic and applied biomedical sciences: Lessons from kidney development and tissue-engineering

Jamie A. Davies

University of Edinburgh Medical School Edinburgh, Scotland, UK

Introduction

The sciences are often divided into the 'basic' and the 'applied'. The usual view is that applied sciences depend on basic sciences for essential knowledge, but that the converse is not the case: this might explain why, at least in the author's country, applied scientists seem often to be viewed as 'below', in some social sense, their basic-science colleagues. As a hybrid scientist/technologist who has worked for many years in both the basic science side of developmental biology (embryology) and its applied side (tissue engineering), I would argue that each depends very much on the other. This short article will illustrate the point with the story of kidney development–how scientists came to understand how natural kidneys develop and how tissue engineers have learned to engineer realistic kidney organoids in culture.

Foundations

Attempts to engineer renal tissue would be futile without some understanding of how natural kidneys form in the embryo. The foundations for this basic knowledge were laid by a small number of anatomists working in the 19th and early 20th centuries.[1–6] Between them, they established that the metanephric (permanent) kidneys are the third pair of kidneys formed in development, the first two pairs (pronephros and mesonephros) regressing in females or being adapted for reproductive functions in males. The drainage ducts of the temporary kidneys, the Wolffian ducts, send a branch out from their caudal ends: this branch is called the ureteric bud (UB) (Fig. 1a). The UB crosses the peri-Wolffian mesenchyme to invade a specific area of the caudal intermediate mesoderm, called the metanephrogenic mesenchyme (MM: also called by some authors the 'metanephric mesenchyme' although this term is ambiguous, applying equally to the adult, so should be avoided). Once in the MM, the UB begins to branch giving rise, eventually, to the tree-like urine collecting duct system of the kidney (Fig. 1b). As the UB branches, the tips of its branches become capped with a population of mesenchymal stem cells, the 'cap mesenchyme'. Each cap mesenchyme maintains itself throughout kidney development and it divides with the underlying ureteric bud tip so that new branches are capped. As well as maintaining itself, the cap mesenchyme sheds cells from its distal zone (i.e. the part furthest from the tip), and these cells aggregate, epithelialize and become excretory nephrons, which connect at their distal ends to their nearby UB branches (Fig. 1c). Blood vessel progenitors invade the proximal pole of the new nephron to make a glomerulus, and the glomeruli later connect to the systemic circulation (the timing being revealed by the observation that injecting tracer into systemic circulation fails to dye all glomeruli of the early kidney[2]). It should be noted that, although contemporary authors tend to cite 21st century research papers for almost all of the above, it was all known before 1930 and even terms like 'cap mesenchyme' and 'stem cell' were in use by then.[4,5,7]

The first steps towards mechanistic understanding

The first steps towards a mechanistic understanding of kidney development, in terms of establishing how particular events depend on other events, came from early attempts to build embryonic kidney components in isolation from the rest of the kidney. The work was made possible by the development of techniques for culturing kidneys in the lab, at first in clotted serum or hanging drops and, later, on filters in relatively simple media.[5,8,9] An attempt to produce nephrons outside the body by culturing MM without its UB failed: the mesenchyme produced no nephrons and just rounded up and died.[8] Isolation of MM and recombining it with UB, however, resulted in the formation of a kidney rudiment with nephrons, indicating that nephrons depend for their formation on an inductive signal from the ureteric bud. The observation that other tissues such as dorsal spinal cord could substitute for ureteric bud in inducing nephrons[10] indicated, in the language of the era, that the induction was permissive (telling cells they could go ahead and follow a predetermined path) rather than instructive (specifying to naive cells exactly what they should do: as we have understood more about the nature of signalling, the concept of instructive induction has largely disappeared from embryological thinking).

Attempts to construct renal tissues and, in particular, failure to do so, therefore had the effect of exposing very important features of normal kidney development. In the molecular era, the pathway mediating the induction of nephron formation has been

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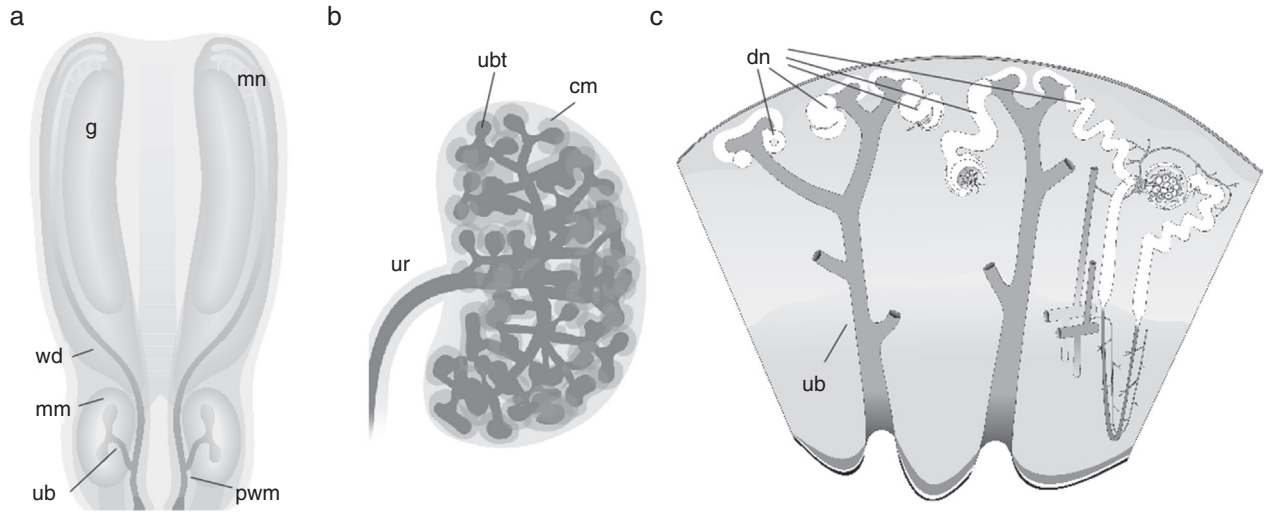

Fig. 1. Normal development of the metanephric kidney. (a) Depicts the early embryo, in which the Wolffian ducts (wd) run caudally from the mesonephros (mn) and associated gonad (g) towards the cloaca: a ureteric bud (ub) crosses the peri-Wolffian mesenchyme (pwm) to enter the metanephrogenic mesenchyme (mm). (b) Shows the unbranched portion of the utereric bud becoming a ureter (ur) and he branching portion becoming a collecting duct tree: cap mesenchyme (cm) forms around the ureteric bud tips (ubt). (c) Depicts a time series of stages of nephron development: developing nephrons (dn) forr from cells left behind by the cap mesenchyme and undergo a stereotyped sequence of morphogenetic events to become a complicated, elgongated tube: the excretory nephron. Images have been obtained with permission from the GUDMAP database (http://www.gudmap.org/Schematics).

identified by a combination of pharmacological,[11] gene expression, and knockout studies. A reciprocal induction of UB branching by MM was also identified, led by the implications of a knockout phenotype that abolished branching.[12,13] In recent years, many more signalling interactions have been identified (see McMahon[14] for a recent review), leading to the view that the developing kidney organizes its anatomy through the exchange of signals mediating rich feedback (feeding back information about what has been built to the processes that will build the next stage). This feedback makes the system highly adaptive and tolerant of error.

Making renal organoids by reaggregation of stem cells

The discovery that kidney development is regulated by a web of cell interactions that give it self-organizing character, capable of adapting to create realistic anatomy even under unusual constraints such as developing on a flat surface, raised an interesting question: could a random mix of renogenic stem cells (UB and MM types) organize itself into realistic kidney tissue? An early attempt to do this, by disaggregating the UB and MM of a young kidney rudiment into a cell suspension and then reaggregating it met with failure: too many cells died by apoptosis.[15] This was not surprising, perhaps, for it is known that mammalian cells depend on normal cell–cell and cell–matrix contacts for their survival and in the absence of these contacts they die: the process is called anoikis.[16]

In the course of a quite different project, centred on the basic-science question of the role of actin reorganizion in the morphogenesis of renal tubules,[17] it had been observed that inhibition of Rho-dependent kinase (ROCK), the focus of attention for the study because it is a regulator of actin organization, greatly reduced apoptosis in developing kidneys. The finding was incidental, not considered in the paper that came out of the work, but it was mentioned in an internal lab meeting. Remembering it, Unbekandt and Davies[15] used ROCK inhibition to give re-aggregating renogenic stem cells temporary 'life support' as they re-established connections with one another. This prevented excessive apoptosis and allowed the cells to undergo significant self-organization. The result was an oragnoid in which UB cells formed distinct cysts that matured into collecting duct treelets. Around these, the MM formed nephrons which divided into segments (proximal tubule, distal tubule etc.) and connected to nearby collecting ducts (Fig. 2a).

Generating large-scale anatomy

Organoids formed by self-organization, as described above, have realistic micro-scale anatomy but completely lack organ-scale features, such as being organized around a single collecting duct tree, with a distinct cortex and medulla. This apparent boundary between fine and large-scale features is a typical feature of self-organizing organoid systems. It suggests that critical information, missing from self-organizing organoids, must be present in the embryo. Searching for it, to improve organoids, raises new questions about natural embryogenesis. The presence of multiple collecting duct treelets in simple renal organoids formed by reaggregation, for example, draws attention to the critical importance of a natural feature of normal kidney development: the collecting duct progenitor (UB) enters the MM as one single tubule that never loses its integrity. This creates an asymmetry in the system that ensures that everything is arranged around the single tree. Extracting one ureteric bud treelet from a re-aggregate and combining it, intact, with reaggregated MM cells, so that there is now only one source of UB cells in the system, results in a more realistic renal anatomy with everything arranged around a single collecting duct tree (Fig. 2b).[18] This improved organoid even develops a distinct cortex and medulla in a suitable culture system.[19] This observation, made in the tissue engineering arena, informs embryological knowledge by confirming the critical role of the collecting duct tree in organizing large-scale anatomy.

The collecting duct system of an organoid made as above does not, however, have an exit tube: all branches of the tree are collecting ducts, with no ureter. Gene expression studies conducted for basic embryology have shown that the peri-Wolffian mesenchyme, through which the ureter passes before entering the MM, expresses signalling molecules distinct from those of the MM itself. Cut-and-paste experiments that result in the (immature) ureter stalk being surrounded by MM, or the branching part of the UB being surrounded by peri-Wolffian mesenchyme, suggest that UB cells' decision about whether to make a branching collecting duct tree or a non-branching, uroplakin-expressing ureter, is governed by

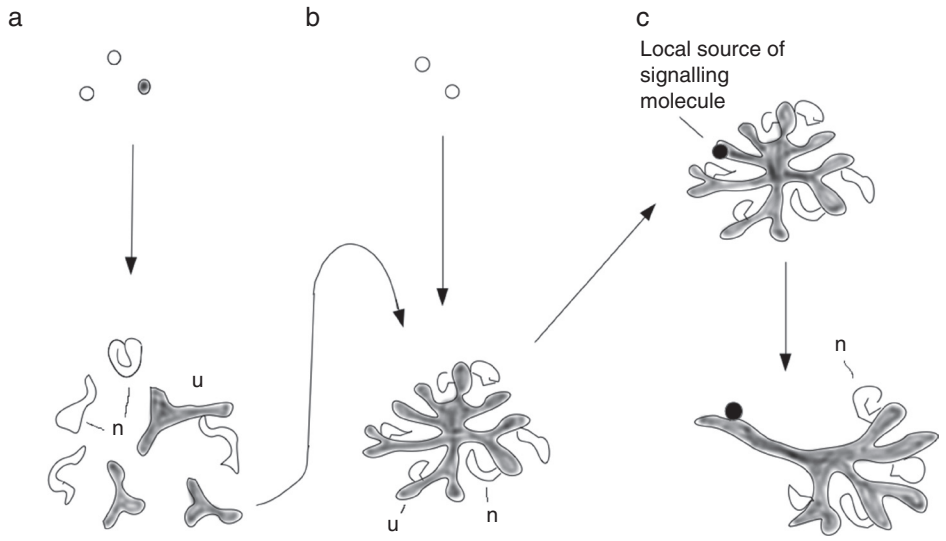

Fig. 2. Methods of renal tissue engineering. (a) Shows a basic reaggregation method (Unbekandt & Davies, 2010), in which mixed renogenic stem cells (UB and MM cells) are reaggregated and form small UB treelets and young nephrons near and connected to them. (b) Shows a serial-aggregation method, in which just one UB treelet is isolated from a primary aggregate and aggregated with fresh aggregated MM cells: now the tissue organizes itself around a single, large UB/collecting duct tree (Ganeva et al., 2011). (c) Shows the effect of applying local growth factors, characteristic of peri-Wolffian mesenchyme, near one developing collecting duct in the Ganeva system. This duct ceases to branch and nephrons do not develop around it, polarizing the whole organ so that this branch is ureter-like, and even expresses uroplakin.

signals from the mesenchyme that surrounds it. This knowledge can be used to add further realism to the organoid system: surrounding just one young collecting duct of an organoid based on a single collecting duct tree with signals characteristic of peri-Wolffian mesenchyme causes that duct to branch no more, to induce no nephrons to form near it, but instead to thicken and express uroplakin (Fig. 2c). This manipulation, effectively a further symmetry-breaking step, increases the realism of the organoid considerably. It also enriches basic embryological knowledge by positively identifying the cause of the UB stalk becoming ureter not collecting duct.

Kidneys from pluripotent stem cells

The tissue-engineering experiments described above all used *ex fetu* cells that were already committed to a renogenic fate and came from the area about to form a kidney. Clearly the routine construction of human kidneys from human *ex fetu* sources would be neither practical nor, to many people, ethical. Producing them from pluripotent human cells, for example hiPS cells,[20] is the obvious alternative, but this depends on the development of a method to 'program' pluripotent stem cells to the renogenic fate. Designing such a method is usually done by 'walking' cell through the sequence of signalling environments that would be experienced in the life of a cell that reached a renogenic fate in a real embryo (reviewed by Little and Takasato[21]). Establishing this sequence depends on two types of basic embryological information: lineage and signalling. The lineage information, from epiblast to intermediate mesoderm to nephrogenic zone, was established long ago, mainly by careful observation with no need for genetic cell marking (reviewed by Saxen[7]). The sequence of signalling events was worked out by a combination of observation of expression patterns of signalling molecules, and assessing the effects of defined signalling factors on cells at various points of the lineage.[22,23] The first people to apply this information to the problem of engineering renal tissue from pluripotent stem cells (in this case, mouse ES cells) were Kim and Dressler,[24] who treated ES cells with retinoic acid, activin and BMP7 to make them into intermediate mesoderm cells that would differentiate into kidney tubules when placed into an embryonic kidney or combined with embryonic spinal cord. The cells did not, however, make kidneys on their own, suggesting that they may have represented MM but not UB.

Several subsequent attempts to make kidneys from ES or iPS cells met with similar frustrations in generating either MM or UB but not making both in a form that would generate a renal organoid.[25–28] This led Taguchi and colleagues[29] to return to basic developmental biology, to examine more carefully the origins of, and the environments experienced by, the cells that make the UB and the MM. The conclusion from this careful analysis is that cells that give rise to the rostral end of the intermediate mesoderm, and thus eventually the UB, experience retinoic acid earlier and for longer than those in the caudal end of the intermediate mesoderm that make the MM. Using this new basic embryological information, discovery of which was prompted by frustrations on the applied side, Takasato and colleagues[30,31] returned to the problem of programming iPS cells to make self-organizing kidneys on their own. They showed that they could, by varying the length of exposure to a particular growth factor during programming, choose whether the cells become almost all MM, almost all UB, or a mixture. The mixture, after a short induction by a Wnt agonist, produced organoids very similar to those made by reaggregation of *ex fetu* murine renogenic stem cells described above.[15] The important difference was that Takasato's organoids[31] were made from human cells taken all the way from the pluripotent state. It will no doubt only be a matter of time before the realism of these organoids is improved using the symmetry-breaking tricks described above for the murine system.

Concluding remarks

Along the path to iPS-derived renal organoids, described above, applied science has both drawn on and informed basic science, and basic science has in its turn drawn on the results of tissue engineering work. The interchanges of information, some of which are summarized in Fig. 3, emphasizes the falseness of the dichotomy between basic embryology and tissue engineering. It also suggests the foolishness of the way we tend to organize research communities. Embryologists and tissue engineers tend to publish in different journals (though with some overlap, for example in *Organogenesis* and *Development*) and go to different conferences. How much

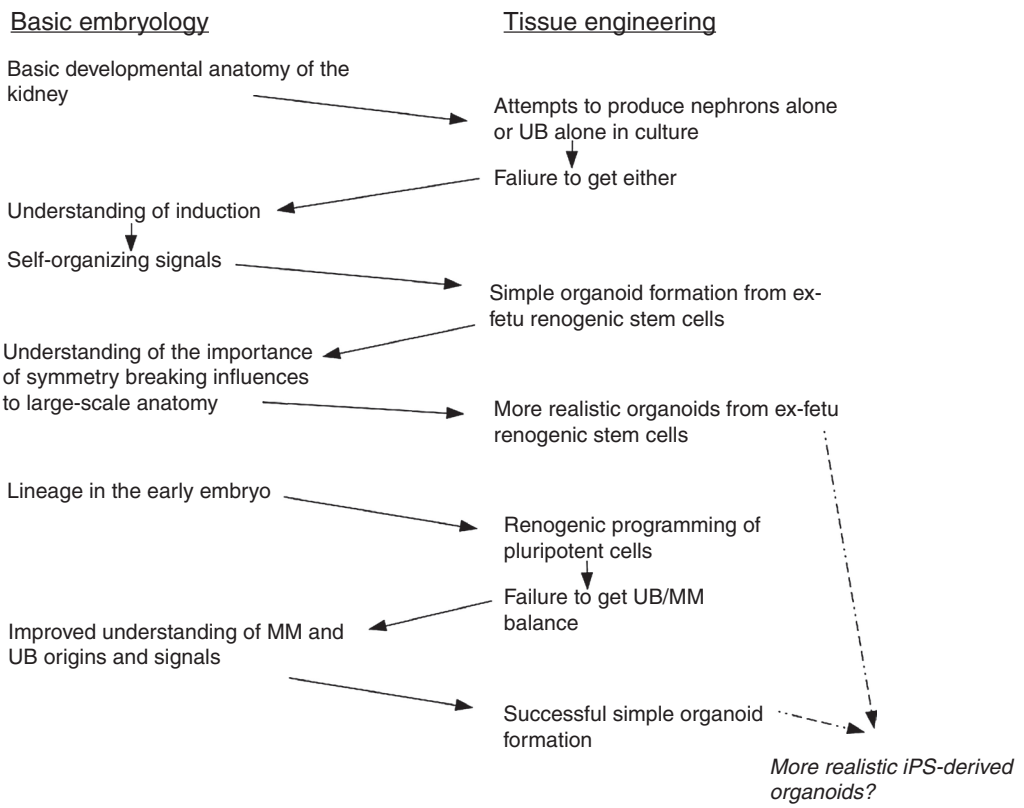

Fig. 3. Examples of the cross-talk between research in basic renal embryology and research in renal tissue engineering, showing how advances in each have provided foundations for advances in the other.

faster might both fields progress if the communities mixed more, and how much faster might other fields of biomedical science and technology advance if they did the same?

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Ending infectious diseases in the era of the Sustainable Development Goals

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ABSTRACT

Concentrated global attention is needed on the long-term epidemics of infectious diseases, such as HIV, tuberculosis (TB), malaria, hepatitis and neglected tropical diseases (NTDs), which require a magnified response sustained over a long period to bring them to an end. Building on the progress made towards the Millennium Development Goals (MDGs), the World Health Organization (WHO) has developed a joint global public health approach to accelerate progress and meet the ambitious global targets set for 2030 for HIV, TB, malaria, hepatitis and NTDs in the era of the Sustainable Development Goals (SDGs). Drawing on the common elements of the individual disease strategies, the new approach provides opportunities for joint and synergistic efforts. The framework emphasizes key actions including expansion of universal health coverage (UHC), ensuring equity and respect for human rights, establishment of a new approach to strategic information within and beyond health, strengthening of health systems with integrated delivery of interventions, pursue of sustainable financing, and promotion of research across the spectrum from product development to implementation research.

Introduction

In the new millennium, epidemics of SARS, influenza, Ebola and Zika have concentrated global attention on infectious disease outbreaks and the acute response needed to bring them to an end. However, much more focused attention is also needed on the long-term endemic infectious diseases such as tuberculosis (TB), HIV, malaria, hepatitis and neglected tropical diseases (NTDs). These diseases are estimated to have caused, together, about 4.3 million deaths in 2015, i.e., nearly 12,000 deaths every day. This represents 8% of deaths worldwide.[1] They remain a wide-reaching concern in global health on account of the mortality burden and economic impact. Many of these infectious diseases, often concentrated among the poorest populations in the world, including in high-income settings, also cause chronic illness, disability, sequelae, stigma and exclusion from society. Bringing them to an end requires a magnified and sustained decade-long response.

A new global public health approach in the Sustainable Development Goals (SDGs) era

The United Nations (UN) Sustainable Development Goals (SDGs),[2] established by all nations to pursue development in all countries of the world, represent a unique opportunity to reconsider approaches to health, including to endemic, long-standing infectious diseases. The principles of the SDGs incorporate a universal, globally applicable, quest of peace, prosperity, economic progress, and equity and social justice for all people. They foresee a much increased investments on development through both domestic and aid-related financing. Its 17 goals (and 169 targets) are considered integrated and indivisible, therefore promoting a multisectoral approach towards solutions. This multidisciplinary approach applies well to health, which is determined by multiple factors that often are not addressed within the health sector alone and require necessarily collaboration and partnership with different sectors. SDG3, "Ensure health lives and promote well-being for all at all ages", includes target 3.3 calling to "End the epidemics of AIDS, tuberculosis, malaria, and neglected tropical diseases and combat hepatitis, water-borne and other communicable diseases". This target, set for 2030, is very ambitious, but the hope is that it may stimulate more rapid investments and efforts towards the

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control and, ultimately, the elimination of the human scourge represented by those infectious diseases.

Since many features are common in the response to the endemic infectious diseases, the World Health Organization (WHO) promotes a coherent and joint public health approach to accelerate progress towards meeting the ambitious 2030 global targets.[1] This approach has important policy implications in relation to expanding universal health care coverage (UHC), ensuring equity, ethics and respect for human rights, enhancing and broadening strategic information, strengthening health systems with integrated delivery of interventions, pursuing sustainable, long-term financing eventually focusing on domestic resources where possible, overcoming technical barriers such as antimicrobial resistance, and promoting research across the spectrum from product development to implementation research.[1]

The WHO approach draws on the common elements of the individual global public health strategies to end the epidemics of TB,[3] HIV,[4] malaria,[5] and NTDs[6] and to combat hepatitis[7] by 2030. The approach embraces a bold aspiration and vision of a world free of these diseases and provides opportunities for synergies and accelerated progress. A common goal, indicators (incidence and mortality reduction) with associated targets, and often similar categories of interventions along the continuum of health services have been established (Panel). These reflect a strong shared support to Universal Health Coverage (UHC) that is bound to become the top priority of the World Health Organization,[8,9] with the targets serving as milestones on the path towards it by 2030. In line with increasing recognition of the interdependence of the health care and public health fields,[10] a public health approach provides the framework for the delivery through the public and private sector of effective interventions for improved care and prevention on a massive scale.

The joint approach to end the long-term epidemics of infectious diseases is underpinned by a common set of principles and key elements (Table 1). The principles include maximizing the efficient and cost-effective use of resources through good governance and stewardship, with adequate monitoring and evaluation ensuring accountability; engaging with a coalition of communities and civil society as part of the extended health system; delivering on a commitment to equity, ethics and human rights to ensure "no-one is left behind", i.e. everyone shares equally in progress; and accelerating efforts of local adaptation of global strategies. The key elements include patient-centred care (which is at the heart of synergies between clinical medicine and public health); strong political commitment to ensure UHC and social protection; strengthening the enabling environment through multisectoral approaches and implementing health policies conducive to disease control with regulatory support; and harnessing innovation and expanding research as the drivers of improved health care.

Table 1
The main principles of a joint global public health approach towards ending the epidemics of TB, HIV, malaria and NTDs and combating hepatitis by 2030.[1]

Vision: a world free of endemic infectious diseases
Sustainable Development Goal: "By 2030, end the epidemics of AIDS, TB, malaria and NTDs and combat hepatitis . . ."

Targets and indicators

Indicator	Global targets for disease burden reduction
Incidence reduction	**TB** 80% reduction in TB incidence rate by 2030 (compared with 2015) **HIV** Reduce new HIV infections to less than 500,000 by 2020 (compared with 2.1 million new HIV infections in 2010), and by 2030 reduce the annual number of new infections by 90% **Malaria** At least 90% reduction in malaria case incidence by 2030 (compared with 2015) and malaria eliminated from at least 35 countries **Hepatitis** Reduce 6–10 million HBV and HCV infections (in 2015) to 900,000 infections in 2030 **NTDs** (note that reaching the individual NTD targets should result in at least a 90% reduction in the number of people requiring interventions against NTDs; this is the combined NTD indicator to be monitored under the SDGs) Eradication of guinea worm disease (2015) and yaws (2020) Global elimination of leprosy (2020), lymphatic filariasis (2020), trachoma (2020), onchocerciasis (2025) and human African trypanosomiasis (2020, with zero incidence in 2030) Regional elimination of schistosomiasis (2020), rabies (2020) and visceral leishmaniasis (2020) Regional interruption of intra-domiciliary transmission of Chagas disease (2020) 25% reduction in the number of cases of dengue (2020, compared with 2010)
Mortality reduction	**TB** A 90% reduction in TB deaths by 2030 (compared with 2015) **HIV** Reduce global AIDS deaths annually to below 500,000 by 2020, and by 80% by 2030 (compared to 2010) **Malaria** At least 90% reduction in malaria mortality rate by 2030 (compared with 2015) **Hepatitis** Reduce 1.4 million HBV and HCV deaths (in 2015) to under 500,000 deaths by 2030 **NTDs** 50% reduction in number of deaths due to dengue by 2020 (compared with 2010)

Main categories of interventions along the continuum of health services

Main category of intervention	Relevant diseases
Prevention	
Social and behavioural change	HIV, TB, malaria, viral hepatitis, NTDs
Injection and blood safety and universal precautions	HIV, viral hepatitis
Harm reduction for people who use drugs	HIV, TB, viral hepatitis
Immunization	TB, viral hepatitis (HAV, HBV, HEV)
Preventive drug treatment	HIV, TB, malaria, NTDs
Testing, diagnosis and case-finding, and treatment	HIV, TB, malaria, viral hepatitis, NTDs
Care	
Morbidity management and disability prevention	HIV, TB, viral hepatitis, NTDs
Vulnerability and risk reduction	
Vector ecology and management	Malaria, NTDs
Water, sanitation and hygiene (WASH)	Viral hepatitis (HAV and HEV), NTDs
Veterinary public health	TB, viral hepatitis (HEV), zoonotic NTDs

Actions within the health sector

The relationship between individual health programmes and the overall health system operations is at the heart of a joint approach to this range of diseases: strengthened and performing health systems are crucial in creating a solid platform for sustainable action. This is exemplified by the End TB Strategy,[3] the second pillar of which foresees a number of policy changes that go beyond the normal realm of national TB-dedicated programmes. These includes, for instance, the need of a general policy of universal coverage and social protection to prevent people affected by TB to fall below the poverty line incurring catastrophic costs given their disease, the existence of sound essential medicine standards guaranteeing quality and proper use, of the implementation in health facilities of proper infection control practices. Likewise, both the

Table 2
Examples of interconnection of the relevant disease strategies with broader development strategies.

Disease	Relevant interconnected sustainable development strategy
TB	Nutrition (SDG2), gender equality (SDG5), energy (SDG7), reduced inequalities (SDG10), sustainable cities and communities (SDG11)
HIV	Nutrition (SDG2), education (SDG4), gender equality (SDG5), reduced inequalities (SDG10), sustainable cities and communities (SDG11)
Malaria	Education (SDG4), water and sanitation (SDG6), sustainable cities and communities (SDG11), climate change (SDG13)
NTDs	Nutrition (SDG2), education (SDG4), water and sanitation (SDG6), climate change (SDG13)
Hepatitis	Education (SDG4), sustainable cities and communities (SDG11), reduced inequalities (SDG10)

HIV and viral hepatitis strategies stress that achieving targets will require robust and flexible health systems characterized by strong health-information systems, efficient service delivery models, a sufficient and well-trained workforce, reliable access to essential medical products and technologies, adequate health financing, and strong leadership and governance.[4,7] Conversely, disease control programmes have an integral and supporting role in contributing to development of health systems, since the health benefits of these interventions go beyond the containment of specific infections.[11]

Interactions beyond the health sector

The achievements of sectors other than health have a fundamental role in ensuring benefits for the health of all citizens. For example, among SDGs, progress in those dealing with poverty alleviation, nutrition, education, gender equality, clean water and sanitation, affordable and clean energy, reduction of inequalities, sustainable cities and communities, climate action is crucial for a positive impact on health.[2] Similarly, action on the transport sector, industrial and foreign policy, and agriculture have a profound impact on health and in particular on the infectious disease burden. In keeping with a key feature of the SDGs that development sectors are integrated and indivisible, the global public health strategies to end the epidemics of TB, HIV, malaria and NTDs and to combat hepatitis by 2030 are interconnected with broader development strategies (Table 2). Above all, the elimination of poverty (SDG1) underpins progress against the wide range of communicable diseases, with other development strategies being closely interconnected with the communicable diseases shown in the table.

Conclusion

The new SDGs framework calls for a shift from individual disease control strategies for TB, HIV, malaria, hepatitis and NTDs to a more coherent and global public health approach that also reflects the key feature of the SDGs that development sectors are integrated and indivisible. This shift is crucial in accelerating progress and meeting the ambitious global targets set for 2030. Key strategic objectives – including accelerating the expansion of universal health coverage and social protection, reaching vulnerable populations which have so far been underserved, and bolstering research capacity and monitoring and surveillance – ultimately all depend on a strengthened focus on action in countries supported by a new global policy framework.

Role of the funding source

This paper reflects the contributions of WHO staff in developing the WHO report "Accelerating progress on HIV, tuberculosis, malaria, hepatitis and neglected tropical diseases.

Authors' contributions

The two authors contributed equally to the development of this paper.

Conflicts of interest

The authors declare no conflicts of interest.

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Rostrum

Pediatric Surgery remains the only true General Surgery

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ABSTRACT

This article states that Pediatric Surgery remains probably the only remaining General Surgery because it is not about organs and systems but rather the whole Surgery from fetal life until completion of growth and maturation.

Pediatric surgeons are currently involved in prenatal treatments for fetal diseases, they take in charge the surgery of congenital malformations, acquired neonatal diseases, common conditions like hernias, undescended testes and appendicitis, but also of the more complex gastrointestinal, broncho-pulmonary or genitourinary conditions, tumors, trauma and solid organ transplantation. For this, like other surgical specialists, they use open, endoscopic and minimally invasive techniques. The broad spectrum of diseases, many of them scarcely prevalent, makes training long and hard, but this challenge accounts for the greatness of this specialty. Pediatric surgeons also carry out research work in their field because they are aware that understanding of why the conditions treated by them occur is mandatory.

In summary, Pediatric Surgery is a lively, exciting, difficult specialty that offers an attractive alternative to young doctors interested in surgery.

While the tremendous increase of knowledge and the growing complexity of techniques shattered Surgery into several subspecialties, Pediatric Surgery remains true General Surgery.

Why is this? Is it due to the relative youth of this specialty? Is it because of less knowledge or complexity? No. The reason is that Pediatric Surgery is not a subspecialty but rather the Surgery of a period of life. It is not defined by organ or organs treated but like Pediatrics, by the age of the patients until completion of growth and maturation.

This confers to Pediatric Surgery a unique identity since it entails the treatment of surgical diseases of fetus, newborns, infants, children and adolescents altogether. It is true that the bulk of cases is smaller and that the spectrum of conditions has to be larger in order to acquire and maintain expertise.

Pediatric surgeons, like all other specialists, treat a large number of common conditions like hernias, testicular maldescents, appendicitis or phimosis that are the "bread and butter" of our everyday work. However, what is specific of the specialty are congenital malformations. Most of them are relatively rare conditions that may involve every organ of the body and require a deep knowledge of embryology and fetal biology and a top-level expertise. Esophageal atresia,[1] congenital diaphragmatic hernia,[2] anterior abdominal wall defects like omphalocele or gastroschisis,[3] duodenal[4] and jejuno-ileal atresias,[5] malrotation or anorectal malformations[6] as well as broncho-pulmonary[7] or genitourinary conditions require prompt treatment, delicate surgery and sometimes, additional operations later on in life.

Many malformations are nowadays diagnosed in utero and pediatric surgeons are involved in prenatal counseling and, in a few cases, in prenatal instrumentation or operations. This is true for some neck tumors, massive pulmonary malformations and particularly for spina bifida that clearly benefits of prenatal repair.[8]

But, of course, these malformations are more often managed immediately after birth, when defensive mechanisms, maturity and neonatal biology are different from those of older patients. Until the blooming of this specialty in the middle of the past century, survival and functional results in these particular conditions were very discouraging. Nowadays, survival after repair of esophageal atresia approaches 90%[9,10] and similar figures are possible for most of the other conditions cited, except congenital diaphragmatic hernia in which pulmonary hypoplasia with persistent pulmonary hypertension limits the progress. Nevertheless, this has been immense in the last two decades.[11]

A very rare field that summarizes all these activities and requires the higher levels of expertise is the separation of conjoined twins mainly done during infancy and childhood. Shared organs, complex anatomy and huge technical challenges make these operations a sort of orchestral work in which multiple organ specialists (general,

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urologic, plastic, orthopedic and cardiovascular pediatric surgeons) work together seeking the best endowment of each member of the set for a separate life of the best possible quality. Fortunately, these complex malformations are rare but its managements illustrate better than any other the complexity of this area of General Surgery.[12,13]

Pediatric surgeons also manage gastrointestinal conditions at all ages including gastro-esophageal reflux, Hirschsprung's disease, inflammatory bowel disease, duplications or vascular malformations of the intestine. And they take care also of thoracic conditions like pulmonary sequestrations, bronchiectasis, spontaneous pneumothorax or parasitic lung cysts.

In many countries, the field of action of Pediatric Surgery also covers all genito-urinary conditions like vesico-ureteral reflux, hydronephrosis, megaureter, urethral valves or reno-ureteral duplications that represent a large share of the case mix.

Trauma is the first cause of mortality below 18 years in developed countries and this explains why Pediatric Surgeons are also involved in this field. Although the trend in Europe and in the US has been to leave fractures into the hands of trauma or orthopedic specialists, in some countries these are treated within our departments and complex thoraco-abdominal trauma involving the respiratory tract and solid and hollow viscera is taken care of in them.[14–16]

Cancer is the second cause of mortality at this age and pediatric surgical departments develop active oncologic activity. Benign soft tissue tumors like teratomas,[17] lymphangiomas, arterial or venous malformations and others are treated by surgery. Malignant tumors of the kidney (Wilms' tumors),[18,19] the neural system (neuroblastoma, ganglioneuroma),[20,21] the germ cells (endodermal sinus tumors) or the soft tissues (sarcomas)[18,22] are also treated by pediatric surgeons. Their participation in pediatric oncologic teams is one of the reasons for the encouraging results obtained in this field in the last decades in which cure rates reach more than 90% for some of them.

Some pediatric surgeons felt that their input was also necessary in the field of organ transplantation and their contribution was crucial to develop some techniques now routinely used in this field.[23] Several teams, including our own developed very active programs of transplantation including all solid organs[24–26] that are fully taken care of by pediatric surgeons with results that match those of the best adult programs in the world. After 700 liver, 300 kidney and 100 small bowel transplantations in our own department, we believe that our specialty has done as well in this field as its adult counterparts.

This broad spectrum of diseases, many of them scarcely prevalent, that require open, endoscopic and minimally invasive surgical techniques makes training a long and hard process. The limited exposure to some of these rare conditions, and the challenge of maintaining top-level expertise accounts for the greatness of this specialty.

And finally, like other specialties, Pediatric Surgery is committed to carry out research work in their field. Understanding why and how the conditions treated by us occur is mandatory to optimize their management. And if we do not interest other scientists in these conditions, many issues will remain unresolved.

Separation of several subspecialties within Pediatric Surgery is a probably unavoidable trend in the immediate future, but those tempted by this should keep in mind that there is some risk in restricting the field of application of their skills to a progressively smaller number of individuals with the ensuing difficulties to maintain and teach expertise.

In summary, Pediatric Surgery is a lively, exciting, difficult specialty that offers an attractive alternative to young doctors interested in truly General Surgery.

Conflicts of interest

The author declares no conflicts of interest.

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Optogenerapy: When bio-electronic implant enters the modern syringe era

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ABSTRACT

Resort to medications dates back million years ago with the use of medicinal plants. In the nineteenth century, significant contributions in medicine appeared in different domains, among which the invention of a specific drug delivery device; the syringe. Nowadays, injection therapy of bio-manufactured drugs is routine practice for chronic diseases but remains constraining and painful. New emerging advanced therapies invest in genetic, electronics and cell-based therapy for addressing unmet needs for the caregivers and the patient. As digital process in health (eHealth) gains momentum, connected advanced bio-electronic devices now offer new strategies for personalized injection therapies. In this review, we take a journey along the genesis path of a new drug delivery system: the Optogenerapy, a synergy between optogenetic and gene therapy. Inside a bio-electronic implant, electronics and optogenetics are interfaced by light as a traceless inducer signal. By controlling a synthetic optogenetic pathway in the cell, therapeutics delivery can be fine-tuned with a precise spatiotemporal control. The technology holds promise of a new modern syringe era capable of producing a drug of interest at will directly inside the patient.

Introduction

The success of modern medicine results from a synergy between the development of drug delivery devices and the ever-growing pharmacopoeias. The pioneering work of Francis Rynd in 1844, on a syringe-based infusion of fluids into human body led not only to the development of the hypodermic syringe but also revealed the systemic mode of action of an analgesic drug through the circulatory system. The discovery of a precise administration route for opiates will be soon associated with patient repeatedly self-injecting drugs. The first prophylactic targeted use of syringes was not limited to chronic pain. Due to the limited action of the early insulin preparations, diabetic patients had to be injected several times per day. Later, intravenous delivery of penicillin G during World War II popularized the use of a life saving injection. From the 18th century hand-made glass model to the plastic disposable insulin syringe introduced in 1970, today's syringes are mass-produced by billions. The increase in the lifestyle associated chronic diseases (obesity, heart disease, stroke, cancer, type 2 diabetes, and arthritis) generates a worldwide growth of self-administered medications. In 2020, the apprehension is that chronic illnesses will be responsible for almost three-quarters of all deaths on earth.[1] As an alternative option to injection therapy, cell transplantation and gene therapy promises may hold true but are not yet delivered. The risk associated with new emerging cell therapies often requires lifetime patient survey. The development of modern medical devices is closely related to innovation in the fields of electronics and photonics. The maturation of the medical technology for the pacemaker and the electroencephalogram (EEG) has set the stage for a bionic heart[2] and brain-computer interface breakthrough innovations.[3] The exploration and mapping of the brain set new frontiers in genetic engineering. To decipher the neuronal maps, synthetic biology researchers develop a portfolio of optogenetic tools. The genetic reprogramming introduces photo-activable molecular actuator[4,5] in the genome of a neuron to shed light on neural network structures. This technology has not yet found its translation path to the patient bedside. In 2016, 17% of new molecular entities (NME) approved by the FDA are personalized medicines.[6] New drugs serving unmet medical needs will be soon available. Genetically-engineered therapeutic proteins (antibodies, interleukins, peptides) represent a unique class of drug called biologics. In one hand biologics are difficult to manufacture requiring complex good manufacturing practice (GMP) bioprocessing installations, on the other hand, they offer higher target-specificity and

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reduced incidence of off-target effects that helps to qualify the drug in preclinical stages.

A challenge of tomorrow medicine is to develop a device that would replace the syringe therapy by a new implanted drug delivery device. Optogenerapy system associates the optically controlled bio-manufacturing of the therapeutic protein and its perfusion within the circulatory system. The technology takes its name from the contraction of optogenetics and gene therapy. The optogenerapy multidisciplinary device by combining advances in cell encapsulation, optogenetic and electronic engineering could find its space in a globalized futuristic biocybernetic eHealth scenario. The cybernetic implies a closed loop control of the human by controlling chronic states in concert with a chain of connected biosensors to remote telemedicine. In this review, we will take a journey along the development path of what may be seen as "biocybernetic syringe."

Encapsulated cell technology

A first significant step in the development of the cellular-based drug delivery device of tomorrow is the constant development of cell-transplantation therapy, first applied to diabetes and now translated to other types of disease.

In 1921, the hormone insulin was detected as a treatment for diabetes, but soon the need for long-term therapy appears.[7,8] Considering that insulin cannot be taken in a pill form, it is usually injected. Despite considerable research efforts from large medical device manufacturing company like Medtronic with the development of advanced personalized strategy with closed loop system[9]; alternative options to injection therapy are still today limited.

At first, the pancreas was transplanted as a whole with the constant need for immunosuppression treatment (Fig. 1). Unfortunately full immunosuppression did not offer long-term solution due to the increased risks of incoming infections and the potential causes of cancer. Instead of the whole pancreas the therapy targeted the isolation of islets of Langerhans from the pancreas (Fig. 1). One solution to transplant the islets was to use immune privileged sites, i.e. protected from immune destruction. For example, transplantation of pancreatic pieces in the anterior chamber of the eye was favoured for islets injection.[10] These sites were shown to allow the islets engraftments to stay longer before immune-rejection from the patient. In 1933, Bisceglie was the first to propose a treatment of diabetes with encapsulated insulinoma.[11,12] Later, by studying immune rejection, Algire et al.[13,14] evaluated the possibility to encapsulate the islets in a membrane with different pore sizes. The idea was to prevent immune rejection by circulating cells. As a result of their study creating a chamber with small pores diameters (<0.45 μm) offers immune protection to the islets. This semi-permeable membrane confinement strategy offers a double advantage; on one side, the immune cells cannot penetrate the pores of the membrane, on the other side oxygen and nutrients can pass and supply the cells.

A large literature reports the successful transplantation of islets in diverse animal models and patients. The current techniques achieve selective permeability with intra- or extra-vascular macro-devices surrounding islets, micro-devices containing fewer encapsulated islets, coatings with permeability selective material and finally nano-encapsulation to protect each islet[8] (Fig. 1).

Micro-devices enclose small number of islets into a hydrogel. Multiple materials for semi-permeable encapsulation are used to isolate the implanted cells from the host. Alginate is one of the most popular due its excellent biocompatibility and facility of utilization.[12] Besides alginate beads, agarose, cellulose, chitosan and others materials are reviewed for cell encapsulation by De Vos et al.[15]

This review will focus on macro encapsulation device. In contrast to the alginate encapsulation and the hydrogels that cannot be explanted, membrane confinement offers the greatest safety for the patient as the genetically modified cells do not circulate in the vascular system and the device could be removed at any time.

Commercialization of macro-devices started at the end of the 1990s by Baxter Healthcare who designed the TheraCyte implant. The device is composed of Teflon membranes and a polyester mesh to allow neovascularization[8] after implantation. Studies in rodents were promising but failed to translate into clinical applications in humans. Concomitantly, a small biotechnology company Islet Sheet Device capitalized on alginate sheet to encapsulate islets. A principal difficulty during beta-cell transplantation is ischaemia. As inadequate oxygenation due to a lack of vascularization remains one of the leading cause of implant failure. Beta-O2 devices concentrate their efforts on designing methods to improve the oxygenation of the device by injection or production of oxygen.[8]

After the success of cell-based therapy from islets transplantation, the field of cell-based implant emerged as an interesting therapeutic option to treat multiple types of diseases. In 2002, Broadhead et al. encapsulated PC-12 cells, in hollow-fibre membrane allowing neurotransmitter release to quantify dopamine level in the culture. While changing the permeability of the membrane, they show that they could fine-tune the neurotransmitter release.[16] They further improved the design of their technology by implanting a refillable cell encapsulation device and tested it in rat brains as a treatment of Parkinson's disease.[17] As for the first prophylactic target of a syringe, encapsulation was also used to relieve chronic pain. Bovine chromaffin cells were isolated and implanted into the sheep for six weeks. The cells were able to release norepinephrine and met-enkephalin.[18]

Biologics manufacturing has set up the basis of the empirical selection technology of super producer cell lines. Recent advances in genetic engineering are further refining the technology with specific genome editing tools.[19] Synthetic biology applied to encapsulated cell technology emerged as an innovative therapeutic platform to produce biologics directly at their delivery site. This way, the host encounters no risk associated with direct genetic modifications or have genetically modified cell perfused in the body. The genetically modified cells are isolated from the host in a secure manner. As a treatment for Alzheimer's disease, retinal pigment epithelial cells were engineered to produce nerve growth factor and their implantations were evaluated for 12 months in minipig animal model.[20] The technology was further improved by the development of particular cell scaffold that supports the growth of tissue like structure in the implant chamber. The cell-based device was successfully tested in Alzheimer's patients. Four patients were implanted for six months with a device containing cells capable of releasing nerve growth factor.[21] The acceptance and the safety of the technology were proven after the devices were retrieved from the patient for analysis. The group of prof. Aebischer at the EPFL valued the efficacy of monoclonal antibodies against amyloid to act as a therapy for Alzheimer's disease.[22] Their macroencapsulation device successfully secreted anti-amyloid-antibodies in rodent animal model.[22] Many therapeutic applications could derive from this technology; non-limiting examples of target disease area include neurological disease: multiple sclerosis, stroke, epilepsy, Huntington's disease, Parkinson's disease.[23,24]

As cells can be engineered to produce and deliver therapeutic drugs of choice, cell encapsulation offers an excellent method to achieve drug production and delivery directly in the patient itself. Compared to the "traditional syringe", there is no need for drug manufacturing or galenic formulation before implantation. As the cells may continuously grow, there is assumed unlimited drug availability.

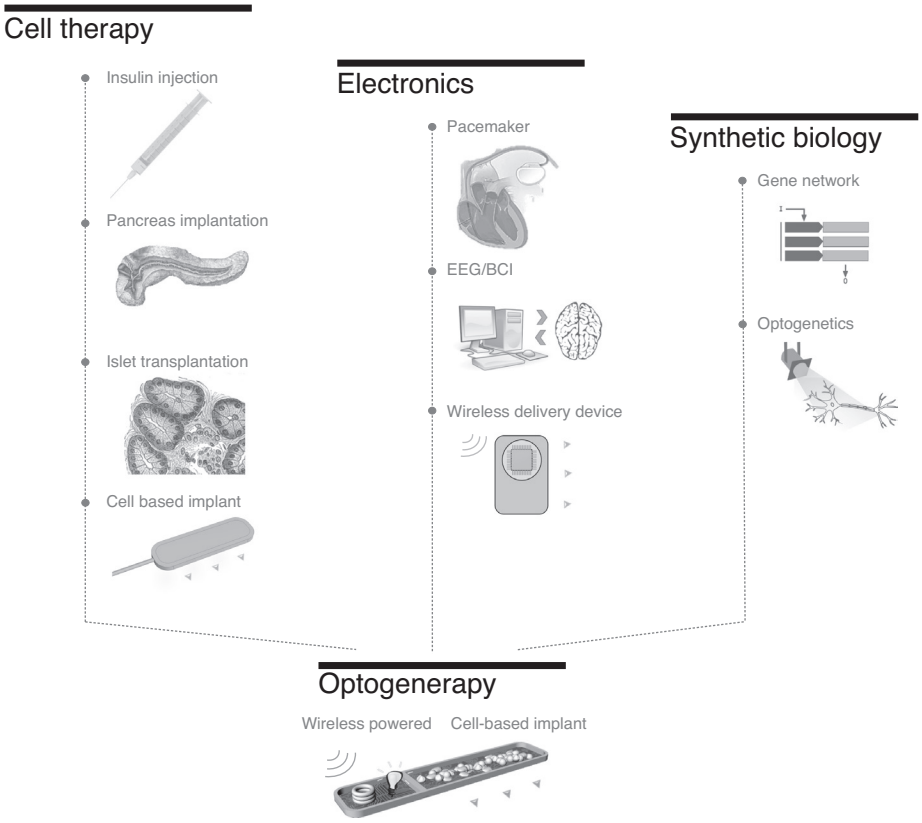

Fig. 1. Genesis of optogenerapy. The concept of optogenerapy emerged subsequent to the development of the domains of cell therapy, electronics and synthetic biology. Cell therapy, guided by the progress in encapsulated cells for diabetes therapy, opens the path of cell-based implant. Electronic medical devices leaded by the development of pacemakers abled the release of therapeutics wirelessly (Microchip). Finally synthetic biology and optogenetic permit the control of cells protein production capacity simply by light. Optogenerapy, as a multidisciplinary approach, consists of a wireless powered bio-electronic cell based implant to create an embarked optoelectronic circuit. It triggers the bio-manufacture and release of a therapeutic protein by the engineered cells.

Electronic medical devices

Electronic medical devices such as pacemakers exist for about 50 years (Fig. 1). Before being implantable, pacemakers consisted of large portable devices able to deliver electric pulses. Then the co-founder of Medtronic, Earl Bakken, developed the first wearable pacemaker operated by a battery in 1957.[25] Innovations in medical devices are continuously progressing, researchers at Harvard University developed a soft robot surrounding the heart and capable of compressing functions to help the beating.[26] The Carmat bioprosthetic heart is now moving forward with phase III clinical trials.[2]

Most of the implanted electronic devices deliver electricity as therapeutic action (pacemaker, deep brain stimulators, gastric stimulators). Using electronics précision to programme the drug delivery would bring another control on the corrective action and a better patient acceptance. Indeed repeated injections of therapeutics are painful for the patients and result in poor adherence to treatment. Farra et al. achieved the control of a wireless drug delivery implant[27] (Fig. 1). The MicroChip consists of multiple doses of drugs divided into reservoirs that can open at will by electrothermal ablation of the enclosing membrane. Eight women were implanted with the device for a period of four months and demonstrated the efficient release of the human parathyroid hormone as a treatment for osteoporosis.[27] Another drug delivery device from Medtronic, integrates a closed loop system where the insulin pump is able to release insulin from a catheter programmed by a wearable glucose biosensor.[9] In the future, this machine biology interface could be integrated into a web of connected biosensor that will ultimately be linked to the Internet of Things (IoT) (Fig. 1). It corresponds to the growing network of connected objects able to communicate and to coordinate monitored parameters.

Innovations in electronics are continuing their progresses and overcome the current problems preventing the translation towards effective medical devices. One of the primary challenges in electronic medical devices is the constant need for powering. In the case of miniaturized devices, the utilization of batteries inflicts difficulties due to their short lifetime and the large space required for implementing this component.[28] An old engineering principle, discovered by Nicola Tesla is the transmission of energy through air. The solution consists of transferring the power generator to an external source wirelessly. The emitter-coil synchronizes the electricity generation in the implant energy-harvesting antenna. Wireless power transmission relies on an electromagnetic field to transmit energy to a miniaturized implant.[29,30] The technology enables not only the electrical powering of the device but also a possible communication route between the emitter and the receiver.

The field of electrical engineering offers new possibilities and tools to engineer implantable miniaturized devices capable of operating as transceivers. For example, advanced Brain Computer Interfaces (BCI) are connected to an articulated prosthesis (Fig. 1). Paralyzed patients by focusing their attention on a brain task can take control of a prosthetic arm.[31] The electrical activity of the brain is de-convoluted with the help of sophisticated algorithms and used to programme a computer interface.

Synthetic biology and optogenetics

Synthetic biology engineering approach often employs an electrical engineering vocabulary to describe gene network behavior[19]

and bio-calculators.[32] Designer cells can be a programme to perform simple switch to more complex tasks like logic gate calculation.[32] Recent modelling assisted gene networks support the assembly of an ADC converter[33]; an oscillator;[34] a double pole double throw (DPDT) switch[35] and boolean logic gates[32]; essential modules to assemble more complexes calculator networks.[19] Genetic engineering offers the possibility to construct robust regulatory pathways for bioprocess[36] but also to programme cell fate[37] (Fig. 1).

For therapeutic applications, the cells can be programmed to sense a disease marker level and respond accordingly by secreting a therapeutic protein.[38,39] Proof of concept of this theranostic gene network was performed for Gout arthritis,[39] thyroid disorder,[40] diabetes,[41] psoriasis.[42]

Well-characterized light sensor proteins can serve as a genetically-encoded optically-controlled switch[43] (Fig. 1). Light irradiation activates a synthetic pathway to trigger a cell potential but also to control the expression of a gene via second messenger signalling pathway.[44,45] Light can be use to trigger the expression of one protein but it can also be used to control an organ[46,47] or synchronize cellular behaviour's.[48–50] Cellular implants responding to blue-light were designed based on hollow fibres containing blue light sensitive designer cells secreting Glucagon peptide GLP-1.[51] Blue light cytotoxic properties and the difficulties to accurately dose the gene response with transdermal light-illumination led to the investigation of other synthetic light controlled gene network system. Near infrared (NIR) biolumination source implanted in the brain is showing a promising result for new therapies to prevent neuro-degeneration.[52] Using NIR as a traceless inducer of gene network is also an attractive strategy to control a bacteriophytochrome associated nucleotide cyclase domains.[4,45] By tapping into second-messenger pathways, prokaryotic phytochrome can control eukaryotic innate immune response pathways or talk to specific chimeric transactivator proteins.[45,51,53]

An "optogenerapy" concept emerges as an innovative cell-based implant to orchestrate the administration of biologics. It consists of a synergy of mature technology in the domains of macroencapsulation, electronics, and optogenetics (Fig. 1). In contrast to the previous approaches, an electronic module is embarked within the device. It is a first of its kind, as it associates genetically modified cells protected from the immune system thanks to semi-permeable membranes and an optoelectronic interface to control cellular behaviour confined within the implant.[53] All implanted therapeutic devices so far were either composed of only electronic or just encapsulated cells, but not a combination of these two areas. The electronic module controlling a light source is used as a trigger for the light sensitive designer cells.

An energy harvesting antenna is powering the wirelessly powered cell-based implant. Its remote-controlled action offers the practician a full control over the infusion therapy. The device can play a fundamental role in many therapeutic applications. Integrating the optogenerapy implanted module to an electronic biosensors network will set the scene for new digital therapeutic processes. Miniaturized wearable biosensors probing patient parameters are already available into clothes and will foster the development of future eHealth platforms.

The engineering field of the wearable biosensor is now developing the algorithms that will enable to integrate patient commands captured with EEG devices. It is now possible with a wearable BCI to control the lightning of a switch or driving a wheelchair simply by thinking.[3] As proof of concept for the optogenerapy device, a BCI interface was used to programme the secretion of a reporter protein marker in the bloodstream of a rodent animal model placed on the wireless transmitter.[53] A human user wearing an EEG headset was performing a mental task to wirelessly command the illumination time of the implanted cell-based device in mice. The experiment confirmed that it was possible to "mind-controlled" a wirelessly piloted micro-bioprocessor implanted using a biosensor-derived signal.[53]

The biosensor can directly measure a disease marker, like glucose for type 2 diabetes patients. Ye and co-workers gained advantage from the optogenerapy technology to secrete glucagon like peptide 1 (GLP-1) in a rodent model of diabetes.[54] In the experimental set up the glucose monitoring data is integrated via a mobile phone platform in a similar closed loop system as the Medtronic insulin pump except that the wearable biosensor trigger the wireless powered implanted LED.

The translation of the optogenerapy technology from the laboratory to the bedside requires the development of small-scale GMP manufacture of generic designer cells and an ambulatory procedure to implant the device. Each cell type will be designed according to the disease targeted and to the patient needs. The external wearable device controlling the implant would help the patient and the practician to fine-tune the infusion therapy.

Conclusion

The continuous improvements in cellular therapy have set the knowledge to a possible path of cell-based engraftment using macro encapsulation devices. In the case of diabetes, the difficulties in implanting the islets reside in re-creating a particular niche for the cells. Even after implantation the disease is still present and actively target the beta cells. Recent progresses in genetic engineering may help in the development of alternative strategies to provide diabetes with therapeutics.[37] The advances in electronic medical device and the development of optogenetic tools open perspectives for engineering an optogenerapy implant. Inside a bioelectronic implant, electronic components wirelessly connected switch on a LED to activate engineered cells rendered capable to respond to light and to trigger the release of therapeutics. Optogenerapy proof of concepts demonstrated how a wireless cell based implant could be controlled by; (i) a brain computer interface to have mind controlled drug delivery; (ii) a connected glucose biosensor for controlling diabetes therapeutic. Further cell engineering research is still required to define the best engraftment and neo-vascularisation parameters that are essential for the success of implanted bio-electronic devices. The ultimate goal is to achieve a closed-loop regulatory system sensing a disease marker and reacting in consequence by releasing the therapeutic drug in a precise and controlled amount. The premises of optogenerapy implants aspire to act as future bio-cybernetic syringes regulating our organisms independently.

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Conflicts of interest

The authors declare no conflicts of interest.

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ABSTRACT

HIV is responsible for millions of deaths around the world and in the absence of available treatment capable of a cure, only the vaccine can offer protection against this virus. However, and after three decades of research, such a vaccine remains elusive. Here, I attempt to explain the major challenges on the development of an anti-HIV immunogen, and how the three-dimensional pictures of antibodies interacting with this virus can guide us to the design of a successful vaccine.

Background

According to the World Health Organization (WHO), the Human Immunodeficiency Virus (HIV), has taken 39 million lives since the beginning of the epidemic to 2015; and with over 2 million new cases of HIV-1 infection reported in 2015 (http://www.unaids.org/en/resources/fact-sheet), this global epidemic does not show signs of remission. The HIV-1 virus replicates by infecting T cells, which are an essential component of our immune system.[1] HIV-1 infection, therefore, compromises the host's immune system eventually leading to its absolute dysfunction. Individuals suffering from untreated HIV-1 infection will ultimately develop the acquired immune deficiency syndrome (AIDS)[2] and will see their survival threatened by otherwise harmless viral or bacterial pathogens. Although there are groups in the population under a higher risk of HIV-1 infection, the epidemic affects people of all ethnic groups, genders and conditions. Current treatments limit viral replication and progression to AIDS in infected patients, however no curative treatment or vaccine is available to date. Thus, finding a cure or designing an efficacious vaccine against HIV-1 infection is undoubtedly a question of great public interest.

The need for a vaccine

Shortly after infection, HIV-1 integrates its genetic material in the host's DNA, an event currently impossible to revert. Therefore, the design of a vaccine against HIV-1 is paramount in order to prepare the immune system to act promptly and neutralize this pathogen before the establishment of a permanent infection.[1] Indeed, the scientific community understood the challenge early on and has dedicated tremendous efforts to obtain immunogens capable of triggering a protective immune response against HIV-1. However, after over three decades of intensive research and 6 unsuccessful HIV-1 vaccine efficacy trials,[1] we still lack an efficacious vaccine. Surprisingly, 20% of the HIV-1 infected individuals develop antibodies (Abs) of extraordinarily broad and potent neutralization activity against nearly all of the 4000 different HIV-1 strains represents that the diversity of circulating viral isolates.[3] Also, some of these Abs isolated from HIV-1 infected patients show protective effects in non-human primates when passively administered before viral challenge. Understanding the conditions in which humans can develop this type of immune response could pave the way for the design of a successful and universal HIV-1 vaccine.

Why have we failed?

The only viral component capable of inducing a protective antibody immune response in humans is the envelope glycoprotein (Env) located on the exposed viral surface.[1] This protein is comprised of two subunits, the surface unit gp120 and the transmembrane subunit gp41, that associate non-covalently to form a trimer of hetero-dimers. Env plays a key role in the viral life cycle by mediating viral entry into the host cell through the interaction with the CD4 and CCR5/CXCR4 receptors on the surface of the target T cells. Such an infection mechanism requires Env conformational changes between two main Env states: the pre-fusion and post-fusion, with remarkable structural differences between

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these two. Naturally, for its immunogenic properties, the structure of the pre-fusion state is of major importance for the scientists because neutralizing Abs recognize this Env state. However, a clear picture of the three-dimensional structure of HIV-1 Env protein remained elusive for decades. In the past 5 years we have achieved notable breakthroughs in the field of HIV structural biology leading to the determination of several high-resolution crystal and cryo-EM (electron microscopy) structures of the HIV pre-fusion Env.[4–7] The laboratory of Professor Ian Wilson, at the Scripps Research Institute, spearheaded most of the effort that lead to the successful determination of the first crystal structure for an HIV-1 Env protein. Wilson and colleagues engineered a soluble HIV-1 trimer platform where the conformation of this extremely flexible and unstable protein is locked in the pre-fusion state, enhancing the conditions to grow Env protein crystals.[8–10] The outcome of this outstanding research is reported in top scientific journals and unveils the atomic details of this long sought-after protein structure, offering a plausible explanation as to why previous attempts to produce a vaccine may have failed.

New path to vaccine: structure-guided immunogen design

These high-resolution Env structures not only shed light on the molecular details of the HIV-1 fusion mechanism but also, and perhaps most importantly, provided us with the clear mapping of the neutralizing antibody epitopes (regions of antibody recognition) of those broadly neutralizing antibodies (bNAbs) isolated from HIV-1 infected patients.[1] Such knowledge is of paramount importance since the immunogens to be used in a potential vaccine regimen must contain all the components required for antibody recognition and binding. Notably, Wilson and colleagues also discovered that the same epitope recognized by a mature anti-HIV-1 antibody is not necessarily the same epitope that a precursor antibody will bind to. Indeed, work published in Cell by Garces, Wilson and colleagues,[11] identified structures within the epitope (i.e. glycans) that partially block the binding of germline Abs, which are the precursors to the bNAbs isolated from infected HIV-1 humans. These observations had a profound impact in the understanding of the many hurdles involved in triggering the right B cells to initiate antibody affinity maturation leading to a bNAb. These observations also led to the design of a sequential set of immunogens to be administrated in a controlled fashion in order to teach the immune system, step by step, how to generate the desired bNAb.[12]

Proof of concept

Although the vast structural information provided us with valuable knowledge of the intimate relationship between the HIV-1 virus and the human immune system, the direct test of a vaccine in humans is beyond consideration. Fortunately, the availability of new animal models (humanized mice) allowed scientists to prove that immunization strategies, using a series of modified Env proteins, can lead to the development of bNAbs. These mice have cells (B cells) that produce antibodies known to be the precursors of broadly neutralizing antibodies against HIV-1.[12–14] The series of Env proteins used to immunize these mice, guided the maturation of the precursor antibodies to become antibodies that could efficiently neutralize HIV-1 viruses.

Is the HIV-1 vaccine within reach?

This billion-dollar question does not have a simple answer. The structure-guided rationale presented above shows promising results in animal models, demonstrating that this approach is indeed capable of eliciting Abs similar to those bNAbs isolated from HIV-1 infected individuals.[1] Similarly, neutralizing antibodies isolated from Ebola patients were used to successfully treat many patients from the latest Ebola outbreak responsible for 11 thousand deaths in West Africa. So does all of this scientific progress indicate that we will, in time, produce a successful vaccine against the HIV-1 virus? Let's go step by step. The HIV-1 virus exhibits an extraordinary mutational rate, higher than the Ebola, Hepatitis-C and Influenza viruses. To better illustrate this difference we could say that the genetic diversity within a single HIV-1 individual is equivalent to the diversity for the Influenza virus globally, and still the flu vaccine does not always show 100% efficacy. Because the HIV infected individual can live for many years as a virus carrier, it would be important to develop an HIV vaccine that is easily administered and available to everyone in the world to maximize protection coverage, since just one infected individual can spread the disease over such a long period of time. Although we do not have an efficacious vaccine today, the research on the HIV vaccine is also helping to overcome some limitations in our understanding of the immune system in humans. How B cells first recognize the antigens and the role of germinal centers is still largely unknown but may be of tremendous importance to develop a vaccine capable of developing bNAbs.[15] It is also possible that in the pursuit of an HIV-1 vaccine, we may well advance our understanding of the Immune system to a point where other vaccines may be developed. An example of this, is the structure-based vaccine recently developed to prevent Respiratory Syncytial Virus (RSV) infection in infants, where high resolution envelop glycoprotein crystal structures were used to generate an immunogen that improved the current RSV vaccine.[16,17]

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Rostrum

Buying time or arresting development? The dilemma of administering hormone blockers in trans children and adolescents

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ABSTRACT

In recent years, the use of gonadotropin-releasing hormone (GnRH) analogues in adolescents with gender dysphoria (GD) to suppress puberty has been adopted by an increasing number of gender clinics, generating controversial debate. This short essay provides an overview of the difficulties associated with this heterogeneous group of adolescents and discusses arguments for and against the suspension of puberty. Further, it reviews the main follow-up studies conducted in some of the world's largest clinical centres for gender-variant children and adolescents.

How long have I been here, what a question, I've often wondered. And often I could answer, An hour, a month, a year, a century, depending on what I meant by here, and me, and being.

-Samuel Beckett

Gender-variant children and adolescents compose a heterogeneous group of persons who present an incongruence between their perceived gender identity and the gender to which they were assigned at birth. This incongruence can cause significant distress (gender dysphoria) and may require clinical intervention. The complex phenomenon of gender dysphoria (GD) is described in detail in the 5th edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*).[1]

Over the last 20 years, youth referrals to gender clinics have dramatically increased. In Europe, the two largest gender clinics for children and adolescents are the Gender Identity Development Service (GIDS) in London and the VU University Medical Center in Amsterdam. Both centres have witnessed a significant increase in referrals over the past 10 years (e.g. in London, referrals increased from 97 to more than 2000 between 2009/2010 and 2016/2017[a]), along with an impressive decrease in the mean age of referred clients and an inversion in the sex ratio of referrals to favour natal females.[2,3] Research on children and adolescents with GD or gender variance (GV) is sparse. However, some findings are emerging.[4,5] It is now acknowledged, for instance, that children's GD/GV persists after puberty in only 10–30 per cent of all cases; when it does not, the children are referred to as 'desisters'.[1,5] At present, there is no way to predict which individuals will or will not suffer from GD into adolescence or adulthood. However, 'persisters', whose GD continues into adolescence, are more likely to experience GD in adulthood (to a degree of almost 80 per cent).[6,7]

Whether or not GD persists, gender-variant children are at risk of suffering many psychological adversities, mostly linked to body dissatisfaction (e.g.[8]) and a lack of acceptance within the family and social environment (e.g.[9]). Children with GD have been shown to be more psychologically vulnerable in comparison to the general population (e.g.[10–12]). Their psychological problems seem to be of a more internalised nature (e.g. depression, anxiety, eating disorders) than an externalising nature.[10,12,13] However, there is considerable variability across studies (for an overview, see[14]). For these children, family and peer relations are generally poorer than for non-referred children (e.g.[10,15]). As Bandini and colleagues[16] point out, it has been demonstrated that children showing gender variance are at higher risk for maltreatment and abuse.[17,18] Moreover, some studies have reported a high frequency in trans persons of childhood sexual and physical abuse, perpetrated by parents and caregivers (e.g.[19–21]). Finally, research has shown that trans youth are at higher risk of self-harm, suicidal ideation and suicidal attempts (e.g.[22–24]).

To address the clinical needs of such a complex population and to reduce their risk, specialised centres have developed various models of intervention. One of those, on which this short essay focuses, is the use of hormone blockers to suppress puberty.

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[a] Data presented by Bernadette Wren at the conference 'Hot Topics in Child Health: Transgender and Gender Diverse Children and Adolescents' held at the Royal College of Paediatrics and Child Health, London, June 2017.

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This methodology is becoming increasingly common in several specialised centres. The intervention was developed by Dutch clinicians in the framework of a combined approach, including medical therapies as well as psychotherapy, social intervention and family work.[25–28] It consists of a fully reversible medical therapy that suspends pubertal development. Individuals who have reached Tanner stage 2 or 3[29] and are considered eligible for treatment[b] are administered gonadotropin-releasing hormone (GnRH) analogues, which temporarily suspend pubertal development. These analogues act on the pituitary gland, inhibiting hormone secretion and temporarily suppressing the endogenous production of oestrogen in girls and testosterone in boys. These hormones are sometimes called 'blockers', because they prevent the development of secondary sex characteristics. During this stage of treatment, in the absence of pubertal physical changes, the child is guided through an exploration of other gender roles, in order to experience congruence with the presumed innate gender identity. As Steensma and colleagues[30] point out, the suspension provides adolescents with GD 'time and rest before making definite decisions on gender reassignment without the distress of developing secondary sex characteristics'. Cohen-Kettenis and colleagues[27] consider it an extended diagnostic phase, in which the distress that the physical feminisation or masculinisation was producing is significantly reduced. For these authors, the early suppression entails great advantages for transitioning to the desired role throughout one's life, and thus minimises the harm to youth and maximises their opportunity for good social and sexual relationships. The process of passing to the other gender is made significantly easier.

The child normally also receives psychological assistance in determining whether or not to proceed to hormone therapy – specifically, the administration of cross-sex hormones, which is the first step in irreversible gender reassignment. Alternatively, she or he may interrupt therapy and revert to the assigned gender. Once endogenous sex hormone production is resumed, the pubertal development is thought to restart normally.[31,32]

Although the use of puberty suppressants is described in international guidelines, there is no consensus in the Endocrine Society Guidelines and the Standards of Care of the World Professional Association of Transgender Health.[33,34] The primary risks of pubertal suppression include adverse effects on bone mineralisation (which can theoretically be reversed with cross-sex hormone treatment) and compromised fertility; data on the effects on brain development are still limited.[26,35]

Several studies have proven the effectiveness of early medical interventions and the safety of these interventions with regard to physical and psychological harm. Overall, research has shown improved psychological functioning during suppression, no change of mind in terms of gender identity and the reduction or disappearance of distress related to GD; in addition, several studies have reported an increase in GD and harmful behaviour when blockers are not used.[34,36]

In their longitudinal study on the first 70 adolescents to receive puberty blockers, de Vries and colleagues[37] reported an improvement in general functioning after two years, along with a decrease in depression and behavioural and emotional difficulties. Fifty-five of these 70 individuals were assessed later in early adulthood, after cross-sex hormones had been administered and gender reassignment surgery had been performed. Depressive symptoms had decreased, general mental health functioning had improved and no regret about transitioning was found. Many (about 70 per cent) reported that their social transition had been 'easy'. Cohen-Kettenis and colleagues,[38] in a 22-year follow-up of the first described adolescent treated with GnRH analogues and cross-sex hormones, reported overall improved psychological well-being and no clinical signs of adverse effects on the brain. An improvement in global functioning following puberty suppression was also found in the UK study of Costa and colleagues[39] in their follow-up of adolescents at the GIDS centre in London.

Consistent with the Dutch and British studies was Spack and colleagues' report[40] about their sample of 97 patients at a clinic in Boston, MA, in which no adolescents showed regrets regarding puberty blocking or subsequent cross-sex hormone use.

However, use of this intervention has only recently begun, so no other follow-up studies are available and many questions are still unanswered. Thus, many professionals remain critical about the puberty-blocking treatment (e.g.[25,41,42]). The primary counter-arguments are as follows:

1. At Tanner stage 2 or 3, the individual is not sufficiently mature or authentically free to take such a decision.[25,41]
2. It is not possible to make a certain diagnosis of GD in adolescence, because in this phase, gender identity is still fluctuating.[25,41,42]
3. Moreover, puberty suppression may inhibit a 'spontaneous formation of a consistent gender identity, which sometimes develops through the "crisis of gender"' (p. 375).[43]
4. Considering the high percentage of desisters, early somatic treatment may be premature and inappropriate.[25]
5. Research about the effects of early interventions on the development of bone mass and growth – typical events of hormonal puberty – and on brain development is still limited,[7] so we cannot know the long-term effects on a large number of cases.
6. Although current research suggests that there are no effects on social, emotional and school functioning, 'potential effects may be too subtle to observe during the follow-up sessions by clinical assessment alone' (p. 1895).[25]
7. The impact on sexuality has not yet been studied, but the restriction of sexual appetite brought about by blockers may prevent the adolescent from having age-appropriate socio-sexual experiences.[41]
8. In light of this fact, early interventions may interfere with the patient's development of a free sexuality and may limit her or his exploration of sexual orientation.[41,42]
9. Finally, for trans girls (natal boys with a female gender identification), the blockage of phallic growth may result in less genital tissue available for an optimal vaginoplasty.[44]

Vrouenraets and colleagues[45] conducted a remarkable study interviewing various professionals of 17 treatment teams of children and adolescents worldwide, finding that the majority of professionals recognised the distress of teens with GD/GV and felt that early intervention was urgently needed. At the same time, though many teams embraced the so-called 'Dutch approach', a general feeling of unease was expressed, due to the lack of long-term physical and psychological outcome studies. One of the main arguments in support of early intervention was limiting suicidal risk. Subsequently, the same group[46] interviewed trans adolescents. Surprisingly, the adolescents also seemed cautious. Many had doubts about the ability of a person so young to make such a significant decision, but they also emphasised the capital importance of preventing the development of secondary sexual characteristics. The adolescents seriously weighed the short- and long-term consequences of treatment, but this awareness did not stop them from wanting to suspend puberty.

[b] Eligibility criteria for hormone blockers are: '(i) a presence of gender dysphoria from early childhood on; (ii) an increase of the gender dysphoria after the first pubertal changes; (iii) an absence of psychiatric comorbidity that interferes with the diagnostic work-up or treatment; (iv) adequate psychological and social support during treatment; and (v) a demonstration of knowledge and understanding of the effects of GnRH, cross-sex hormone treatment, surgery, and the social consequences of sex reassignment' (Cohen-Kettenis et al., 2008, p. 1894).

Finally, a warning comes from Alessandra Lemma, a psychoanalytic author who has contributed important and innovative insights into transsexualism.[47,48] In a recent paper, she worryingly suggests that in some instances puberty suppression can "result in a marked distortion in the young person's relationship to time" (p. 361)[49] that will negatively impact the adaptation and integration of identity following gender transition.

Conclusion

I hope that this brief excursus has clarified the supporting and opposing arguments with respect to the use of hormone blockers to suppress puberty. On the one hand, the treatment might impede experiences that are seriously traumatic for individuals with professionally and accurately diagnosed GD, limiting suicidal risk and preventing other adverse psychological consequences. On the other hand, the treatment risks hindering the individual's development of a free personality, sexuality and identity, thus disconnecting the young person from the typical experiences of her or his age, with no certainty of the long-term effects on physical health. Suppression of puberty may suggest that the person is deprived of adolescence – the crucial time to deal with identity issues, experiment and pursue unstable convictions regarding the self. However, as Bernadette Wren suggests, there is no evidence that "young people's conviction about their gender identity is, typically, as unstable as other value-laden convictions" (p. 224).[50] From a psychological perspective, the main dilemma is to understand whether buying time at such a precocious age truly enables children to explore deep personal meanings, or whether it freezes youngsters in a prolonged childhood, secluding them from certain aspects of reality and isolating them from peer groups. This is a rather difficult issue to confront in quantitative follow-up studies (which of course are crucial for monitoring physical and psychological outcomes). Thus, qualitative and clinical studies may have a great deal to offer, especially when conducted by expert clinicians who know these children very well. In any case, as for many other aspects of gender identity development, it is crucial that a person-by-person approach is adopted (as performed by the abovementioned gender clinics) to tailor effective and appropriate interventions according to individual needs.

Conflicts of interest

The author declares no conflicts of interest.

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Rostrum

The importance of outdoor play for young children's healthy development

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ABSTRACT

Changes in current societies are affecting childhood experiences. Time for outdoor play is diminishing, contributing to more sedentary lifestyles, disconnected from the natural world. Recognizing the importance of outdoor play for young children's healthy growth, a project focused on the exploration of the outdoor environment was developed with a group of young children in an early childhood education setting in Portugal. The project aimed to transform educational practices, moving from frequent indoor activities to a regular use of the outdoor environment. In this paper, we present the main dimensions related to outdoor play that emerged during the project (contact with natural elements, importance of risk, socialization opportunities) and highlight the role of professionals and families in creating quality outdoor play opportunities.

Introduction

The importance of play for children's healthy development is grounded in a strong body of research.[1–3] As a natural and compelling activity, play promotes cognitive, physical, social, and emotional well-being, offering the necessary conditions for children to thrive and learn. Through play, the child can experiment, solve problems, think creatively, cooperate with others, etc., gaining a deeper knowledge about his/herself and the world. From an early age, the possibility to experience several opportunities for unstructured play, in which the child can decide what to do, with whom and how, promotes positive self-esteem, autonomy, and confidence.

Acknowledging the influence of time and space in play experiences, in this article we address the special contribution of the outdoor environment to fulfil children's needs for free play, reporting into an outdoor educational project developed with a group of 14 children, between 15 and 36 months old, in a Portuguese early childhood setting. The work presented aims to identify important dimensions of outdoor play that contribute to effective learning and healthy development, and highlight the role of professionals and families in the provision of such experiences in early childhood education settings.

Outdoor play in current society and education settings

The specific features and stimulus of the outdoor environment provide for different play opportunities that can hardly be replicated inside.[4] The outdoors can be described as an open and constantly changing environment, where it is possible to experience freedom, gross and boisterous movements, and contact with natural elements.[5] While playing outside, children benefit from being exposed to sunlight, natural elements, and open air, which contributes to bones development, stronger immune system and physical activity.[6,7] The need to be physically active from an early age is particularly relevant if we consider the concerning growth of children's obesity and overweight. According to the World Health Organization,[8,9] Portugal is the second European country with the highest values of overweight among children with 11 years old (32%), being preceded by Greece (33%) and proceeded by Ireland (30%). Also, play in green outdoor environments promotes higher levels of attention and well-being.[10–12]

However, the growing evidences about the importance of outdoor play does not seem to have an impact in the type of experiences that children have access to. Contrary to what would be expected, opportunities for outdoor play are diminishing, in consequence of globalization, technology expansion and urban growth.[13]

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A growing culture of fear about the possible accidents that might happen affect parent's and professionals attitude towards outdoor play, so children tend to be kept inside, occupied with structured activities, and controlled by adults.[3,14,15] Possible hazards, interactions with strangers and car traffic are the most frequent factors mentioned by parents for not letting their children play outside, even though they acknowledge the importance of such experiences.[13,16] According to Gill,[14] these fears are often brought by misinterpretations of reality, without having a real expression in society. For example, the fear about child's abduction is not linked to an increase in these type of crimes, although a greater emphasis is given to these situations by social media.

Adding to this, there is a concern to occupy children during the day, considering that most parents work long hours and want to guarantee the best opportunities for children to acquire different skills and knowledge. Academic activities and sports often occupy children's time to play freely. Going from one activity to another, children tend to be transported by car, without experiencing the outdoor environment through the interaction with the community.[17,18]

In this scenario, there is a need to raise general awareness regarding children's right to play outdoors, as well as its potential in supporting children's well-being, learning and development. Acknowledging the time children spend in educational settings, concerns about the time and space to play outside should be integrated in education planning and intervention, starting in day-care and kindergarten. In Portugal, research shows that early childhood education is too centred in what happens inside the activity room, wrongly considering that the outdoor environment serves merely as recess time, during which children can stretch their legs and expend their energy.[19–23] A recent study focused on the use of outdoor spaces in four Portuguese kindergartens showed that the number of times children go outside is very reduced, especially in the winter months. Children tend to spend long periods in closed environments, more exposed to disease contamination and saturated air.[24] Also, the time spent outside was often insufficient, varying between 16 and 30 min. This is a very short period for children to take advantage of the benefits related to outdoor play, being recommended a minimum of 40 min, per day.[7]

The outdoor education project

With the desire of offering a different educational response to young children and acknowledging the importance of the outdoors for learning and development, a Portuguese early childhood a Portuguese early childhood centre took the initiative of implementing an innovative outdoor education project, going against the tendency of keeping children inside. During a period of three years, the setting located in a rural area in the centre of Portugal, catering for children from 4 months to 10 years of age (from 6 to 10 years old children attend an after school service), introduced gradual changes in pedagogical practices, in order to create quality outdoor play opportunities for children.

Despite having a large and well equipped outdoor area, with natural elements and different type of structures to promote play, in the beginning of the project the children did not used the outdoors regularly. The professionals perceived the indoor environment has being more secure and comfortable, so they avoided going outside for long periods. They were also afraid about possible accidents or diseases that might affected children, fearing for negative reactions from the families.

To overcome these obstacles, a methodology close to action-research was adopted to facilitate practices' transformation, which included teachers' training in outdoor play, regular team meetings and observations of children's play. In this way, problems that emerged were interpreted as mile stones in the process of improving educational practice and specific strategies were experimented to achieve a solution.

This outdoor project directly involved all the early childhood teachers of the institution (5 women) and, indirectly, the board of the setting, other professionals, families, community members and, of course, the children. In this paper, the experience of one of the groups, with 14 children between 15 and 36 months old will be shared.

Through qualitative data collection techniques, such as observations, written records, videos and photographs focused on children's outdoor play, three dimensions were identified as key to promote learning and development: contact with natural elements; importance of risk; socialization opportunities. The analysis of each dimension will take in consideration current international literature. Also, these three components of outdoor play can only be fully developed if children are accompanied by attentive and responsive adults, concerned with their needs and interests.[25,26] The role of professionals and families in this project is also presented, considering that cooperation between adults is an important aspect for the success of outdoor play experiences.

Promoting learning and development outside

Contact with natural elements

The outdoor environment offers unique stimulus that capture children's attention and interest. Sticks, rocks, flowers, soil, water, etc., are explored with curiosity and drive to learn, as they offer countless possibilities for play. As White[27] states, natural elements are open-ended materials, that can respond to children's imagination and needs. In this process of reinvention and assigning new meaning to objects (e.g. a stick can be a gun, a boat or a pen), it is possible to mobilize skills related to divergent thinking, creativity, problem solving, among others. The use of natural elements in children's play also creates a more sustainable strategy in what concerns resources provision. Natural elements are easy to find, cheap and they do not offer the limited options that commercial toys do.[28]

The exploration of natural elements is also important to capture children's attention to the richness and diversity of Nature. The sense of discovery and fascination influences meaningful learning and allows for the development of an emotional connection towards the environment. If we assume that attitudes of respect and care are more likely to emerge regarding something that is dear to us, than it is crucial to promote a sense of belonging and familiarity towards Nature from an early age to facilitate ecological and sustainable behaviours along life.

Through outdoor play and the exploration of natural elements, it is possible to promote education in its broadest sense. Activities related to playing with soil and water can serve as examples of learning opportunities in which concepts related to mathematics, science or language were promoted in an integrated way. As children filled and emptied containers, several times, they could explore notions related to weight, volume and time, and as they talked about what they were experiencing, new vocabulary was being acquired. Similar findings were found in other researches, showing, for example, children's ability to learn and employ mathematical products and procedures during outdoor play, using their body as a learning tool.[29,30]

It what concerns health, the interaction with natural elements such as the soil helps build immunity. Growing research has been showing the importance of experiences that promote the contact with "harmless microbes", that provide protection against diseases.[31] Among the group we worked with, some children had respiratory and skin problems (e.g. asthma and eczemas), and going

outside often helped them deal with periods of aggravated symptoms.

Importance of risk

Today's society often neglects the importance of risk for children's learning and development. A culture of fear lead us to underestimate what children are capable to do, creating an even more "dangerous" learning environment, where children do not have the possibility to learn, by experience, how to stay safe.[14,32] It is essential to adopt a wider vision of risk, going beyond the possibility of accidents to consider the positive implications related to the feelings of success and happiness when a challenge or a new skill is mastered.[33,34]

In the outdoor environment, opportunities to exceed personal limits often emerge in situations like climbing up a tree or using a tool. In risky play, the adult should interpret the signs of the child, giving the necessary support or space that he or she needs. From our experience and following other studies in this area, it is possible to state that risky play promotes important skills related to persistence, entrepreneurship, self-knowledge and problem solving.[35,36]

During outdoor play, children should have the opportunity to experiment moments of failure and success, learning by trial and error. If we try to prevent all risky situations, children will not know how to deal with unpredictable environments and will lack the necessary confidence to overcome challenges in an autonomous way. During the project, we had different situations in which risk emerge, for example when wild mushrooms appeared in the garden, after a period of rain, and children were interested by that phenomena. In that situation, we could either prohibit the exploration or help children understand what was happening in the safest way possible. Choosing the second option, we told the children that it was very dangerous to eat the mushrooms and we gave them some tools to facilitate observation (e.g. magnifying glass and clamps). We always remain close to them, helping, and answering to the questions that emerged. If we had avoid going out because of the mushrooms or if we had ignored that situation, an important learning opportunity would have been missed.

Socialization opportunities

The environment created outside can offer interesting conditions for children and adults to show different aspects of their personality, which normally do not emerge during the time indoors. Following the findings of Maynard, Waters and Clement,[37] we have realized that outdoor play allows for a deeper knowledge about children, facilitating a more adequate educational intervention from the adult. Likewise, less conflicts occur during outdoor play and children tend to cooperate more with each other.[28,38] The characteristics of the space (open and unpredictable) enable the development of joint goals between children, leading to experiences of companionship among peers. During outdoor play, children become teachers and learners, sharing their knowledge and skills to accomplish different tasks or challenges. In this process of cooperation, it is possible to develop empathy, as children begin to understand other's people feelings and needs. The crucial difference about socialization in the outdoor environment is that opportunities for interaction happen in a gradual way, giving children the possibility to choose the moments to connect with others or to play individually, without having to continually run into each other as it so often happens in close and exiguous rooms.

The interaction with adults also seem to be facilitated in the outdoor area. In different moments along the project, adults recognized that they felt more available to support children outside, where they felt relaxed and calm. This statement suggests that the outdoor environment is not only a healthy environment for children, but also for adults, where the levels of stress and anxiety seem to diminish. Other studies found evidences that support different models of interaction between adult and child during outdoor play, being more child-led, flexible and based on dialogue about children's discoveries and interests.[4,39,40]

The role of professionals and families in the provision of outdoor play experiences

To develop quality outdoor practices, that can have a positive impact in children's health and development, it is fundamental to promote conditions for adults to feel comfortable and motivated during the time spent outside. Adult's involvement will influence the type of experiences that children have access to and how they incorporate new knowledge. From the experience acquired during the project it is possible to state that teamwork is a crucial component for quality planning and intervention, facilitating the need for constant evaluation and reflection upon children's well-being and involvement.

Besides from collaboration among professionals, families should participate as much as possible in outdoor play. If professionals explain to the parents why it is important to play outside and make an effective effort to get them involved and satisfied, possible negative reactions related to fears about children getting sick, dirty or injured will be progressively solved. It is important to never forget that most families just want the best for their children and it is the job of professionals to help them achieve this goal. Desirably, the valorization of outdoor time from parents will also promote the integration of these type of experiences in family routines, creating conditions for stronger and more positive effects in children's development.

To overcome parents' anxieties and to promote quality outdoor play experiences, it was very important to assure that all children had proper equipment to play outside in different weather conditions (e.g. waterproof suits and rubber boots for winter). Having the adequate clothes is an essential dimension to assure children's safety and health. Also, we encouraged the parents to talk to the children's paediatrician about outdoor play, especially regarding children's respiratory and skin problems. This effort of articulation between health and education professionals was very important to earn parents' confidence in this learning approach.

Finally, the cooperation between family and school allowed for a progressive improvement of structures and play resources available outside. Often, parents offered their skills and time to the setting, working afterhours to build or recover play structures (e.g. trees houses, benches and tables for children) or collecting daily objects for children to play with (e.g. kitchen supplies to play with soil and water).

During the development of the project we always good lines of communication with families, trying to find solutions and strategies that satisfied everybody's needs.

Final thoughts

The need to guarantee that children have the possibility to play outside, facing adventures and challenges, without being constantly engaged in activities controlled by adults is a recent concern for most western societies. We have evolved to a more modern, technological, and globalized world but, in the process, we lost habits and experiences that influence our quality of life. One of the major challenges of present and future generations may be the need to find a balance between an increasingly "busy" society and the preservation of experiences of well-being and connection to the world. The educational settings have an important role in this process, guarantying that during the first years of life children have the means and opportunities to develop a positive self-esteem, curiosity and motivation about learning and good socialization

skills. The quality experienced in education services may help the child to overcome vulnerabilities related to other contexts (e.g. poverty, low levels of parents' education). Opportunities to contact with Nature, deal with risks, and socialize with peers and adults in a responsive and caring environment will contribute to quality educational experiences, influencing children's motivation and enthusiasm about learning and school.

The valorization of early years and outdoor play can be understood as a mean to promote healthier lifestyles, acknowledging that today's children will be the adults of tomorrow. Parents, educators, and policy makers should work to promote better childhood experiences, guarantying that children's interests are considered in urban and school planning. Without ignoring the slow rhythm of practices transformation, it is important to instigate educational settings to promote outdoor play, considering the amount of time that children spent in school and the impact of those experiences for learning and development.

With these ideas in mind, this testimony aims to highlight the importance of outdoor play in natural environments for children's learning and development and to inspire and challenge others to take advantage of the opportunities that the outdoor environment can offer.

Conflicts of interest

The authors declare no conflicts of interest.

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Original article

Complications of coracoid transfer procedures for the treatment of recurrent shoulder dislocation

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ABSTRACT

Background: Different surgical procedures have been described for the treatment of the recurrent anterior dislocation of the shoulder. Despite the documented success of the open procedures, some studies suggest that the arthroscopic technique leads to more favorable results. However, there still seems to be some disagreement concerning the incidence of complications, when comparing open and arthroscopic techniques.

Objective and methods: As an attempt to clarify these doubts about the incidence of complications associated with the different techniques, this study contains a free literature review along with a retrospective case series of the patients who underwent these procedures in an University hospital in the past 10 years.

Discussion and conclusion: There are various techniques for the treatment of the recurrent dislocation of the shoulder, all of them with known success when it comes to prevention of recurrence. However, all of them are invariably associated with high complication rates.

Despite being associated with a slightly higher re-operation rate, in the literature, the arthroscopic technique was found to have an overall lower rate of complications when compared to the open procedures. Centro Hospitalar São João (CHSJ) presented a higher rate of *screw related complications* and *revision surgery* than the literature. However, concerning other complications and when assessing the procedures individually, no tendency was verified. One can therefore conclude that, despite being scarce, the Centro Hospitalar São João CHSJ data roughly overlap the literature.

Introduction

Different surgical procedures have been described for treatment of the recurrent anterior dislocation of the shoulder.[1] Currently, efforts are being made to determine parameters that can be widely used to decide what procedure to perform. Balg and Boileau[2] have created an instability score (ISIS) to determine pre-operative risk factors in patients with recurrent instability. This score intends to help the surgeon decide whether to perform a soft tissue procedure or a bone graft procedure. So, in patients with high recurrence risk, coracoid transfer procedures that place the coracoid process on the anteroinferior border of the glenoid cavity are an alternative to the soft tissue procedures (Bankart). The first coracoid transfer procedure was described by Latarjet[3] in 1954 and by Helfet (who named the procedure after Bristow) in 1958, having suffered some modifications since then.

The difference between both (Latarjet and Bristow) lies in the coracoid graft position. The Bristow procedure places the longer axis of the graft perpendicularly,[4] whereas the Latarjet procedure places it parallel to the glenoid cavity.[5] In both, the final effect is a bone block that reinforces the anteroinferior border of the glenoid cavity and a stabilizing sling effect achieved by the transfer of the coracoid and conjoint tendon through the subscapular muscle.[6]

Despite the documented success of the open procedures, some studies suggested that the arthroscopic technique is associated with a cosmetically more favorable result, as well as with a lower post-operative morbidity and a faster recovery. And, as far as the procedure is concerned, studies claim that this minimally invasive technique allows a more accurate positioning of the graft, theoretically lowering the complications associated with its dislocation.[7] However, there still seems to be some disagreement concerning the incidence of complications, when comparing open and arthroscopic techniques.[8]

Abbreviation: CHSJ, Centro Hospitalar São João.

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This study focuses exclusively on coracoid transfer procedures, which, despite being effective on patients with a high risk of recurrence, are also associated with certain complications that must be taken into consideration before, during and after the surgery.

Thus, as an attempt to clarify these doubts about the incidence of complications associated with the different techniques, this study contains a free literature review alongside a retrospective *case series* of the patients who underwent these procedures in an university hospital in the past 10 years.

Methods

A literature review was performed, using the PubMed database. The keywords were Complications; Bristow; Latarjet; Open; Coracoid Transfer; Bone-block; Arthroscopic; Shoulder; Glenohumeral; Instability; Dislocation.

Complication was defined as an adverse event or morbidity caused by the surgery and the complications included in this study were recurrent instability (dislocation, subluxation and positive apprehension test), pseudarthrosis, graft dislocation, graft fracture, osteolysis/graft reabsorption, arthrosis, screw related complications (loose, migration, fracture), pain, hematoma, infection (deep or superficial), neuromuscular/vascular complications, revision surgery and functional restrictions.

The inclusion criteria were: English or Portuguese language studies published after 2005; Case series with human participants; studies reporting the complications of the original or modifications of the Bristow/Latarjet procedures for the treatment of the recurrent dislocation of the shoulder.

The exclusion criteria were: studies on any language other than Portuguese or English; studies published before 2005; studies in animals; level of evidence V, opinion articles, anatomic studies, biomechanical studies, or studies referring only to the surgical or image techniques. Case reports, abstract only publications and revision articles with no original data were also excluded, as well as studies reporting only the outcomes of revision surgeries and isolated soft tissue stabilization procedures (Bankart). The studies reporting the results of more than one technique were only included if a clear distinction of the outcomes of each procedure was possible.

The title, abstract or both of each article were reviewed. The full texts were reviewed when inclusion was anticipated, when there was no abstract available or when a decision regarding inclusion or exclusion could not be made from the title and/or abstract alone. The references of the included studies were also reviewed for potential inclusion, for any additional articles not identified through the database search. A total of 19 articles were included,[1,5,8–24] the data were organized and descriptive statistics were calculated and analyzed.

A retrospective review of the patients submitted to coracoid transfer procedures in the Orthopedics and Traumatology department of the *Centro Hospitalar São João* (CHSJ), an University hospital in Porto was also performed. The data were extracted from computer records. The inclusion criteria were: surgeries performed between January 2006 and December 2015. The exclusion criteria were: soft tissue stabilization procedures and revision surgeries.

From the 69 patients submitted to stabilization procedures for the recurrent dislocation of the shoulder, only 34 were submitted to coracoid transfer procedures. The others underwent soft tissue procedures. Thus, 34 patients were included and such as in the literature review, the data were organized and descriptive statistics were calculated and analyzed.

In the statistical analysis, the chi-square test was used to evaluate the differences in the incidence of complications among the different procedures. When the sample was too small, the Fisher's Exact test was used. A similar way was used to assess the qualitative differences of the functional scores results and the t student test was used to analyze continuous variables. $p < 0.05$ was deemed statistically significant.

Table 1
General data from the literature and CHSJ, the University hospital. Since many articles do not refer to some of the variables, the total number may not coincide in all of them. The data from each included study is discriminated in Table 2.

	Literature	CHSJ
Analyzed studies	19	–
Total of shoulders	962	34
Gender		
Male	713	27
Female	185	7
Mean age at the time of the surgery	27.6 years old	28 years old (15–57)
Operated shoulder		
Dominant	365	–
Non-dominant	212	–
Left	–	15
Right	–	19
Average follow up period	8 years (3 months–35 years)	8 months (1 month–5 years)
Technique		
Bristow	429	19
Latarjet	307	10
Arthroscopic Latarjet	226	5

Results

Literature review

In the literature review, a total of 898 patients (962 operated shoulders) were included, of which 713 (79%) were male and 185 (21%) were female.

The mean age at the time of the procedure was 27.6 years old. The dominant side was involved in 365 (63%) cases whereas the non-dominant side was involved in 212 (37%). The average follow-up period was 8 years (ranging from 3 months to 35 years) (Table 1). From all the operated shoulders, 429 underwent the Bristow procedure, 307 the Latarjet procedure and 226 the arthroscopic Latarjet procedure (Table 1). The data from each included study is discriminated in Table 2.

Range of motion limitation refers to movements in every direction, but the most significant one was the restriction in external rotation, found in 82% of the shoulders with *range of motion limitation*. Screw related complications include screw fracture, migrations and the presence of loose or prominent screws. *Neuromuscular complications* include intraoperative alerts (26), axillary nerve damage (5), musculocutaneous nerve damage (1) and deltoid muscle atrophy (3). Besides intraoperative nerve alerts, no other intraoperative complications were reported in the literature.

As presented in Table 4, and although the open techniques were generally associated to a higher rate of complications, the arthroscopic technique is associated with a significantly higher number of *hematoma* and *revision surgery*.

Relative to the functional scores, graphics A and B compare the qualitative functional results ("Excellent", "Good", "Fair" and "Poor") reported in the literature, associated with the Bristow and Latarjet techniques (Graphic A) and with open and arthroscopic techniques (Graphic B). There is a significantly higher percentage of "Excellent" results with the arthroscopic techniques when compared to the open procedures, while these were associated with higher "Good" and "Fair" results (Fig. 1).

Table 2
Collected general data for each included study.

Article	Number of patients	Number of shoulders	Gender		Mean age at the time of surgery (years)	Operated shoulder		Follow up period (years)	Technique
			Male	Female		Dominant	Non-dominant		
da Silva LA et al., 2015[22]	51	52	42	9	31	29	23	1.8	Latarjet
Mahirogullari M et al., 2006[10]	–	30	27	3	23	27	3	2.3	Bristow
Balestro JC et al., 2015[21]	11	12	7	4	28.6	6	6	2	Latarjet
Mizuno N et al., 2014[19]	60	68	49	11	29.4	39	29	20	Latarjet
Atalar aC. 2013[15]	35	35	–	–	35	28	7	2	Latarjet
Matthes G et al., 2007[13]	29	29	16	13	–	16	13	3.16	Bristow
Hovelius L et al., 2006[9]	113	115	95	23	29.25	54	64	15	Bristow
Bouju Y et al., 2014[16]	68	70	48	20	26.7	44	26	13	Latarjet
Emami MJ et al., 2011[1]	30	30	30	–	30.56	28	2	5	Bristow
Schroder DT et al., 2006[11]	54	57	53	1	22	23	19	26.4	Bristow
Bajracharya AR et al., 2007[12]	21	21	12	9	23.4	19	2	2.5	Bristow
Cunningham G et al.,	36	36	34	2	26	–	–	0.52	Latarjet
2016[8]	28	28	24	4	25	–	–	0.58	Arthroscopy
Lafosse L et al., 2010[5]	98	98	56	42	27.5	–	–	1.5	Arthroscopy
Ebrahimzadeh MH et al., 2015[23]	36	36	35	1	24.6	34	2	3	Arthroscopy
Dumont GD et al., 2014[18]	62	64	55	9	29.4	–	–	6.3	Arthroscopy
Gordins V et al., 2015[24]	31	31	23	8	26.7	–	–	35	Bristow
Hovelius L et al., 2011[14]	97	97	60	20	28	–	–	17	Bristow
Zarezade A et al., 2014[20]	19	19	19	–	30.4	–	–	–	Bristow
Delaney RA et al., 2014[17]	34	34	28	6	28.4	18	16	0.25	Latarjet

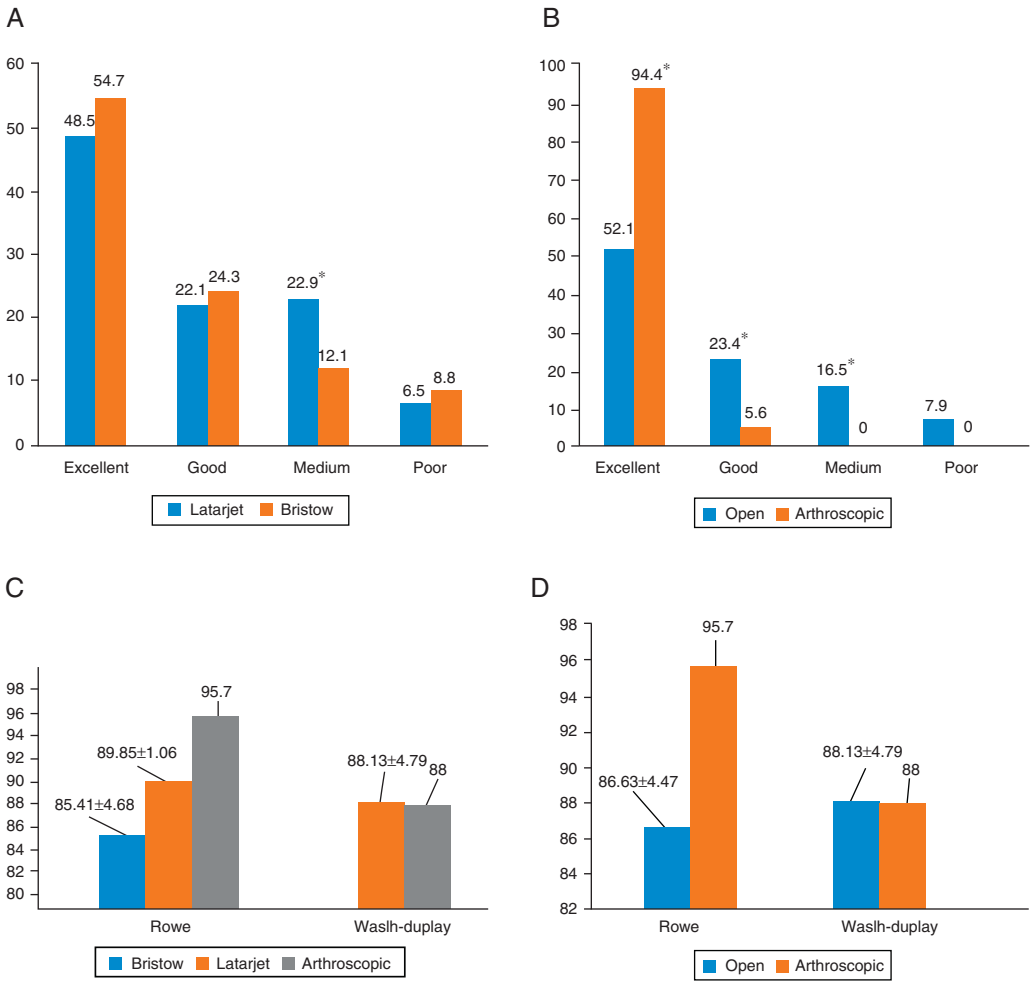

Fig. 1. Graphics describing and comparing the percentages of qualitative evaluation and quantitative means of the functional scores. (A) Percentage of each functional result associated with Bristow and Latarjet procedures. (B) Percentage of each functional result associated with open and arthroscopic procedures. (C) Quantitative mean of the functional scores associated with the Bristow, Latarjet and arthroscopic techniques. (D) Quantitative mean of the functional scores associated with the open and arthroscopic techniques.

Table 3
Total of complications found in the literature and in CHSJ, the University hospital. The data are presented as number and percentage of individuals that suffered that complication.

	Literature (962) *n* (%)	CHSJ (34) *n* (%)
Instability		
Redislocation	25 (2.6%)	1 (2.9%)
Subluxation	18 (1.9%)	–
Positive apprehension test	39 (4%)	1 (2.9%)
Radiographic complications		
Pseudarthrosis/nounion/fibrous union	45 (4.7%)	2 (3.2%)
Graft dislocation	20 (2%)	–
Graft fracture	5 (0.5%)	–
Osteolysis/graft reabsorption	36 (3.7%)	2 (5.8%)
Arthrosis	122 (12.7%)	1 (2.9%)
Screw related complications	29 (3%)	6 (16.1%)*
Intraoperative complications	–	2 (5.8%)
Functional complications		
Range of motion limitation	389 (40.4%)	15 (44.1%)
Loss of strength	20 (2%)	
Pain	118 (12.3%)	4 (117%)
Hematoma	9 (0.9%)	1 (2.9%)
Infection		
Superficial	11 (1.1%)	–
Deep	1 (0.1%)	–
Neuromuscular/vascular complications	35 (3.6%)	5 (14.7%)
Revision surgery	34 (3.5%)	4 (11.7%)*

* = $p < 0.05$.

Graphics C and D represent the comparison between the quantitative means of the Rowe and Walsh Duplay scores associated with Bristow, Latarjet and Arthroscopic (Graphic C) and with the open and arthroscopic procedures (Graphic D). The differences between the procedures were not statistically significant despite the overall better evaluation of the arthroscopic technique with the Rowe score. To be noted that only 2 studies relating to the arthroscopic procedure reported functional scores results: one of them used the Rowe Score and the other used the Walsh-Duplay score, thus, the mean and standard deviation was not calculated for this technique in graphics C and D.

CHSJ

Relative to the CHSJ data, a total of 34 patients were included, of which 27 (79%) were male and 7 (21%) were female. The mean age at the time of the procedure was 28 years old. Although the dominance of the operated shoulder was not registered, 15 of them were right shoulders and 19 were left. The average follow-up period was 8 months (ranging from 1 month to 5 years) (Table 1).

Out of all the operated shoulders, 19 underwent the Bristow procedure, 10 the Latarjet procedure and 5 the arthroscopic Latarjet procedure (Table 1) and 47% of all the surgeries were performed by the same surgeon.

The total number and percentage of complications (from all the techniques) is discriminated in Table 3. The *intraoperative complications* include graft fracture and loss of fixation. Revision surgery was performed due to screw related complications (3) and graft reabsorption (1). As found in the literature results, the most significant movement affected by the *range of motion limitation* was external rotation, found in 67% of the shoulders with *range of motion limitation* and neuromuscular complications included axillary nerve damage (1), atrophy of the deltoid muscle (2), atrophy of the triceps muscle (1) and chronic neurogenic atrophy (1).

Table 5 describes and allows the comparison between the complications found associated with Bristow and Latarjet, as well as the comparison between open (Bristow and Latarjet) and all arthroscopic techniques. The statistically significant results are marked in Table: * = $p < 0.05$. There was a significantly higher number of pseudarthrosis associated with the arthroscopic technique when compared with the open procedures. There were no other statistically significant differences relating to the other complications.

No functional scores were registered in this hospital.

Literature vs CHSJ

Relative to the total of complications, the CHSJ had a higher percentage of *screw related complications* and *revision surgery* when compared to the data reported in the literature (Table 2) and considering only the Bristow procedure, there is a significantly higher proportion of *arthrosis* and *range of motion limitation* in the literature when compared to the CHSJ. Among all the open procedures, there is a significantly higher proportion of *arthrosis* in the literature when compared to the CHSJ. On the other hand, there is a significantly higher proportion of *screw related complications* and *revision surgery* in the CHSJ data when compared to the literature and relative to the arthroscopic procedure, there was a significantly higher proportion of *pseudarthrosis* and *screw related complications* in the CHSJ data.

Discussion

This study aimed to compare the incidence of complications associated to the different coracoid transfer procedures, open and arthroscopic, and simultaneously assess whether the data from the CHSJ differ significantly, or not, from the literature. Despite being effective and widely used techniques for the treatment of recurrent dislocation of the shoulder, the Bristow–Latarjet procedures are generally associated with a substantial complication and reintervention rates, both in the literature and the CHSJ.

In the literature, the arthroscopic technique had a lower proportion of complications, being, however, associated with a higher reintervention rate when compared to the open techniques. Note that due to the relatively low number of arthroscopic surgeries performed in the CHSJ so far and, possibly, due to the shorter follow-up period, the low number of complications registered in this hospital precludes a good comparative analysis between the different techniques. The fact that the arthroscopic procedure is relatively recent and the surgeons are still in an early stage of the learning curve allows a greater room for progression so that better results can be achieved. Another factor to consider is the duration of the surgery. Theoretically, this is longer for arthroscopic procedures, what could translate in a higher postoperative functional limitation rate. This tendency was verified in the literature but not in the CHSJ.

Although *Infection* was a complication rarely found in the literature, it is still important to state that no case of infection was observed in the CHSJ data. Functional complications such as *range of motion limitation* were found in a high percentage of patients both in the literature (40.4%) and the CHSJ (44.1%) and there were no significant differences between techniques. The restriction in external rotation (the most common functional complication), for instance, may have important implications for athletes and should be one of the topics taken into consideration when making the therapeutic decision. Another complication widely mentioned in the literature was arthrosis (12.7%); however, it was only present in 2.9% of the CHSJ patients. This discrepancy can be explained by the large difference between the follow up periods.

The percentages associated with each complication may not relate to their real incidence since not all the studies focus on the

Table 4
Complications associated with open (Bristow and Latarjet) and arthroscopic techniques found in the literature review. The data are presented as number and percentage of individuals that suffered that complication.

	Bristow (429) *n* (%)	Latarjet (307) *n* (%)	Open (Bristow + Latarjet) (736) *n* (%)	Arthroscopic (226) *n* (%)
Instability				
Redislocation	19 (4.4%)*	5 (1.6%)	24 (3.2%)*	1 (0.4%)
Subluxation	15 (3.5%)*	2 (0.6%)	17 (2.3%)	1 (0.4%)
Positive apprehension test	15 (3.5%)	20 (6.5%)	35 (4.7%)	4 (1.8%)
Radiographic complications				
Pseudarthrosis/nounion/fibrous union	28 (6.5%)	11 (3.6%)	39 (5.3%)	6 (2.6%)
Graft dislocation	12 (2.8%)	7 (2.3%)	19 (2.6%)	1 (0.4%)
Graft fracture	4 (0.9%)	–	4 (0.5%)	1 (0.4%)
Osteolysis/graft reabsorption	16 (3.7%)	17 (5.5%)	33 (4.5%)	3 (1.3%)
Arthrosis	89 (20.7%)*	21 (6.8%)	110 (14.9%)*	12 (5.3%)
Screw related complications	9 (2%)	9 (2.9%)	18 (2.4%)	11 (4.9%)
Pseudarthrosis/nounion/fibrous union	28 (6.5%)	11 (3.6%)	39 (5.3%)	6 (2.6%)
Functional complications				
Range of motion loss	229 (53.4%)*	62 (20.2%)	291 (39.5%)	98 (43.4%)
Loss of strength	20 (4.7%)*	–	20 (2.7%)*	–
Pain	59 (13.7%)	59 (19.2%)	118 (16%)*	–
Hematoma	1 (0.2%)	2 (0.6%)	3 (0.4%)	6 (2.6%)*
Infection				
Superficial	6 (1.4%)	3 (0.9%)	9 (1.2%)	2 (0.8%)
Deep	1 (0.2%)	–	1 (0.1%)	–
Neuromuscular/vascular complications		34 (11%)*	34 (4.6%)*	1 (0.4%)
Revision surgery	13 (3%)	7 (2.3%)	20 (2.7%)	14 (6.2%)*

* = $p < 0.05$.

Table 5
Complications associated with open (Bristow and Latarjet) and arthroscopic techniques found in the CHSJ the University hospital. The data are presented as number and percentage of individuals that suffered that complication.

	Latarjet (10) *n (%)*	Bristow (19) *n (%)*	Open (Bristow + Latarjet) (29) *n (%)*	Arthroscopic (5) *n (%)*
Instability				
Redislocation	–	1 (5.3%)	1 (3.4%)	–
Positive apprehension test	–	1 (5.3%)	1 (3.4%)	–
Radiographic complications				
Pseudarthrosis	–	–	–	2 (40%)*
Graft reabsorption	1 (10%)	–	1 (3.4%)	1 (20%)
Arthrosis	–	–	–	1 (20%)
Screw related complications	–	4 (21%)	4 (13.8%)	2 (40%)
Intraoperative complications	1 (10%)	1 (5.3%)	2 (6.9%)	–
Functional complications				
Range of motion limitation	3 (30%)	2 (10.5%)	14 (48.2%)	1 (40%)
Pain	1 (10%)	1 (5.3%)	3 (10.3%)	1 (40%)
Hematoma	1 (10%)		1 (3.4%)	–
Neuromuscular/vascular complications	–	5 (26.3%)	5 (17.2%)	–
Revision surgery	1 (10%)	3 (15.8%)	4 (13.8%)	1 (40%)

* = $p < 0.05$.

same outcomes. For example, only 2 studies referred to *neuromuscular/vascular complications*, one of them focusing solely on these. Therefore, the short number of studies referring to *neuromuscular/vascular complications* limits the comparative analysis between techniques as far as this item is concerned. However, in theory, nerve and vascular damage during arthroscopic coracoid transfer would be of major concern because of the proximity of the brachial plexus and the axillary vessels.

There are several differences between the studies included in the literature review and the one performed with the CHSJ, namely, the sample size and the follow up period, which limit the comparison between literature and CHSJ. The mean age and the gender distribution were similar. Another limitation of this study lies in the scarcity of the hospital records and in the lack of standardized radiological assessment protocols in the CHSJ. The complications found in the CHSJ were obtained exclusively from retrospective data, which could possibly underestimate their incidence, since the records may not include minor complications.

To sum up, there are various techniques for the treatment of the recurrent dislocation of the shoulder, all of them with known success when it comes to prevention of recurrence. However, all of them are invariably associated with high complication rates, which reinforces the need to discuss them with each patient pre-operatively. Despite being associated with a slightly higher re-operation rate, in the literature, the arthroscopic technique was found to have an overall lower rate of complications when compared to the open procedures.

CHSJ presented a higher rate of *screw related complications* and *revision surgery* than the literature. However, concerning other complications and when assessing the procedures individually, no particular tendency was verified. One can therefore conclude that, despite being scarce, the CHSJ data roughly overlap the literature.

An active search for complications as well as a functional evaluation through standardized scores, in a long term prospective study, would be a way to overcome this study's limitations and clarify which of the techniques would guarantee better long term

outcomes, concerning stability, motion, functional scores and prevention of arthrosis.

Conflicts of interests

The authors declare no conflicts of interest.

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ABSTRACT

This study was undertaken to assess the antifertility effect of hydroalcoholic leaves extract of *Pandanus odoratissimus* Linn. which is traditionally used by the woman in Rajasthan state of India to regulate the fertility. The antifertility activity of the extract at dose levels (200 and 400 mg/kg, orally) was evaluated in two experimental animal models. The extract was found to be safe up to a dose of 4000 mg/kg of the extract when administered orally. A good antiimplantation (37.13%) activity in female rats was observed at the tested dose level (400 mg/kg). The extract, when administered alone at 200 mg/kg dose to immature female albino rats, enhanced the estrogen level in the serum whereas significantly decreased the estrogen level at 400 mg/kg dose. The extract along with estradiol at dose level of 400 mg/kg significantly ($p < 0.01$) decreased the level of estrogen, in comparison to standard group rats indicating the antiestrogenic nature of the extract. The antiestrogenic effect of the extract at higher dose (400 mg/kg) might be due to negative feed-back inhibition on anterior pituitary. Preliminary phytochemical screening has revealed the presence of alkaloids, carbohydrates, flavonoids and saponins in the hydroalcoholic leaf extract of the plant. The antifertility effect of the plant might be due to antiimplantation as well as antiestrogenic effect of the extract which in turn might be due to some of the chemical constituents present in the extract. The results shows that hydroalcoholic extract of *P. odoratissimus* L. leaves possess significant antifertility activity at 400 mg/kg, thus, justifying the traditional use of this plant in fertility regulation in females.

Introduction

The increasing population is one of the biggest problems faced by most of the countries, with its inevitable consequences on all aspects of development, especially employment, education, housing, health care, sanitation and environment.[1] The whole world is now recognizing the need for fertility planning. Fertility regulation with plants or plant preparations and medicaments has been mentioned in the ancient texts of indigenous systems of medicine of many countries.[2–4] The use of plants as abortifacients, emmenagogues, and local contraceptives in different countries of the world has been comprehensively summarized recently.[5] The synthetic contraceptive agents currently available produce the side effects like hormonal imbalance, hypertension, increased risk of cancer and weight gain.[6] Therefore, the search for safe, effective and orally active plant based alternative is highly desired for fertility regulation.

Pandanus odoratissimus L. synonym *P. tectorius* belonging to family Pandanaceae, is commonly known as '*Umbrella tree*' in English and '*Kewra*' in Hindi and is widely distributed along Indo-Malayan coasts from India and Sri Lanka throughout Southeast Asia to Taiwan, the Ryukyu Islands, Malaysian islands and Australia.[7] In Ayurveda, Unani, and Siddha systems of medicine, the leaves are used for treating backache, rheumatic diseases, epilepsy, wound healing, nervous disorders, loss of appetite, indigestion, constipation, diabetes, infertility, skin diseases, urinary disorders, and fever.[8]

The flowers of *P. odoratissimus* L. are powdered and included in medicines, which are either sniffed like snuff or smoked for asthma and other bronchial infections.[9] The leaves are thought to be useful in leprosy, smallpox, scabies and diseases of the heart and brain.[10] The plant has been traditionally used as abortifacient by the tribal women in Rajasthan state of India.[11] To the best of our knowledge, no study on the *P. odoratissimus* in the fertility regulation has been reported till date. Based on this evidence, we investigated the effect of the hydroalcoholic leaf extract of *P. odoratissimus* on fertility in female rats.

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Material and methods

Procurement and identification of plant material

The leaves of plant were collected from the botanical garden of Kurukshetra University, Kurukshetra during October 2009 and identified as *P. odoratissimus* L. (Family: Pandanaceae) by Dr. H.B. Singh, Scientist Incharge, Raw Materials and Museum, National Institute of Science Communication And Information Resources, New Delhi where a voucher specimen (NISCAIR/RHMD/Consult/-2009-2010/1381/183) has been deposited for further reference.

Preparation of extract

About 620 g of shade dried leaves were powdered, sieved and extracted hydro-alcohol (Ethanol: Water; 30:70) using Soxhlet at a temperature of 50 °C for 72 h) using soxhlet apparatus. The extract was concentrated to semisolid mass using rotary evaporator (Heidolph 4011, USA) and then lyophilized. The yield of the lyophilized extract was approximately 22.69% w/w and preserved in refrigerator at 4 °C for further use.

Phytochemical screening

Preliminary phytochemical screening of the hydroalchoholic leaf extract was performed as per the reported methods to reveal the presence of major class of phytochemical constituents.[12]

Animals

Colony-bred healthy fertile male and female albino rats in the weight range of 150–200 g were selected for the antiimplantation study whereas immature female rats in the weight range of 25–40 g were used for *estrogenic/antiestrogenic* effect. The animals were obtained from animal house of Kurukshetra University and maintained under laboratory conditions of temperature (21.5 ± 22 °C), humidity (60 ± 1%) and 12-h light and dark cycle. They were allowed free access to feed and water *ad libitum*. Experimental protocol and procedure used in the study were approved by Institutional Animal Ethical Committee (Regn. No. 562/GO/02/a/CPCSEA) of Kurukshetra University, Kurukshetra and according to the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi.

Preparation of test samples and dosing

The dose of leaf extract was selected based on the previous report[13] and was administered at 200 and 400 mg/kg doses in the present study. The dose of extract was reconstituted by suspending the required quantity of HAEPO in Tween 80 (2% v/v in saline) freshly before use and was injected per orally (p.o.). Vehicle control groups received equal volume of Tween 80 (2% v/v in saline).

Antifertility studies

Anti-implantation activity

The fertile female rats were kept with male rats of proven fertility in the ratio of 2:1 during the proestrous or estrous phase and examined for the evidence of copulation next day morning. The rats showing the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and that day was marked as 1st day of pregnancy, and such rats were divided into three groups. Group I served as control and received only vehicle (Tween 80, 2% w/w in saline). Group II and III rats received the extract orally at the dose level of 200 and 400 mg/kg from day 1 to day 7 of pregnancy. On 10th day, laparotomy was performed under light ether anesthesia using sterile conditions and uteri were examined to count the number of implantation sites.[14]

Table 1
Effect of HAEPO on number of implantation in female rats.

Treatment	Dose (mg/kg)	No. of implantation sites (mean ± SEM)	% Inhibition of implantation on day 10
Control (Tween 80, 5% v/v, p.o.)	–	11.66 ± 0.33	Nil
EE (1 μm/rat/day; s.c)	–	–	–
HAEMC (p.o.)	200	9.10 ± 0.33	19.98
	400	7.33 ± 0.33**	37.13
HAEMC (p.o.) + EE (s.c)	200	–	–
	400	–	–

N = 6; Nil = zero.
** Significant with respect to control: $p < 0.01$.

Esteogenic/antiesterogenic activity

Immature female rats (25–45 g) were divided into six groups consisting of 5 animals each. Group I (Control) was administered with vehicle (Tween 80, 2%, v/v) only. Group II (Standard) received standard drug 17α-ethinylestradiol (EE; 1 μm/rat/day) suspended in olive oil subcutaneously. Group III and IV received the extract HAEPO alone at doses of 200 mg/kg and 400 mg/kg p.o., respectively. Group V and VI received the extract HAEPO orally along with EE (1 μm/rat/day) subcutaneously for 7 consecutive days. On 8th day, all rats were sacrificed under light anesthesia. The blood serum was processed for the estimation of important biochemical parameter, i.e. estrogen level.[15]

Statistical analysis

All the values were expressed as mean ± S.E.M. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. The levels of significance were taken at $p < 0.01$ in relation to control and standard.

Results

Phytochemical screening

Preliminary phytochemical studies of the extract revealed the presence of alkaloids, carbohydrates, flavonoids and saponins.

Antifertility study

Anti-implantation activity

A dose dependant antiimplantation effect was observed (Table 1). With increase in the dose of the HAEPO, the percentage of implantation inhibition increased and was significant ($p < 0.01$) at higher dose, i.e. 400 mg/kg. In this study, the extract showed 19.98 and 37.13% anti-implantation effect at the doses of 200 and 400 mg/kg body weight, respectively. The extract showed maximum inhibition of implants at higher dose, i.e. 400 mg/kg.

Estrogenic/anti-estrogenic activity

Effect of HAEPO on biochemical parameters

The effect of extract on various biochemical parameter has been shown in Table 2. The HAEPO extract, when administered alone at 200 mg/kg dose to immature female albino rats, enhanced the estrogen level in the serum whereas decreased the estrogen level at 400 mg/kg dose. The extract along with estradiol at dose level of 400 mg/kg significantly ($p < 0.01$) decreased the level of estrogen,

Table 2
Effect of HAEMC on biochemical parameter in immature ovariectomized female rats.

Treatment	Estrogen (pg/ml)
Control (Tween 80, 2% v/v)	122.83 ± 0.70
EE (1 μm/rat/day, s.c.)	441.11 ± 0.59
HAEMC (200 mg/kg, p.o.)	$427.52 \pm 5.43^{nc,ns}$
HAEMC (400 mg/kg, p.o.)	$133.03 \pm 0.90^{*,\Delta\Delta}$
HAEMC (200 mg/kg, p.o.) + EE (1 μg/rat, s.c.)	138.91 ± 2.196^{nc}
HAEMC (400 mg/kg, p.o.) + EE (1 μg/rat, s.c.)	$156.13 \pm 2.192^{*,\Delta\Delta}$

$N = 6$; nc – not significant with respect to control: $p > 0.05$; ns – not significant with respect to standard; * – significant with respect to control: $p < 0.01$; ΔΔ – significant with respect to standard: $p < 0.01$.

in comparison to standard group rats indicating the antiestrogenic nature of the extract.

Discussion

In spite of the widespread use of herbal medicines, few scientific studies have been undertaken to ascertain the possible mechanism of action and efficacy of traditional remedies.[16] Since *P. odoratissimus* L. has been traditionally used to induce abortion among the women in Rajasthan, India[11] and no scientific evidence in this regard is present till date, the present study was performed to evaluate the anti-fertility effect of hydroalcholic leaves extract (HAEPO) of this plant in female rats. The extractive values of hydroalcoholic leaves extracts obtained as 22.692% (w/w), respectively, which gives an idea about the solubility pattern of the phytoconstituents present in the drug.

In anti-implantation study, a dose dependant anti-implantation effect was observed (Table 1). With increase in the dose of the HAEPO, the percentage of implantation inhibition increased and was significant ($p < 0.01$) at higher dose, i.e. 400 mg/kg. In this study, the extract showed 19.98 and 37.13% anti-implantation effect at the doses of 200 and 400 mg/kg body weight, respectively. The extract showed maximum inhibition of implants at higher dose, i.e. 400 mg/kg.

The HAEPO extract, when administered alone at 200 mg/kg dose to immature female albino rats, enhanced the estrogen level in the serum whereas decreased the estrogen level at 400 mg/kg dose. The extract along with estradiol at dose level of 400 mg/kg significantly ($p < 0.01$) decreased the level of estrogen, in comparison to standard group rats indicating the antiestrogenic nature of the extract.

The antiestrogenic effect of the HAEPO extract at higher doses (400 mg/kg) might be due to negative feed-back inhibition on anterior pituitary. Both estrogenic and anti-estrogenic properties have also been shown by the other plants in previous studies.[17–20] For the implantation and sustenance of pregnancy, exact equilibrium of secretion of estrogen and progesterone is necessary. Any imbalance in these hormones may cause anti-implantation or induce abortion.[21] The compounds of hormonal value usually disturb the hormonal milieu in the uterus and provoke an anti-fertility effect.[22] It has already been reported that steroids,[23] flavonoids (flavones, flavonones and isoflavones) alkaloids and phenolics occurring in variety of plants[24,25] are reported to have shown anti-fertility activity in laboratory animals. Preliminary phytochemical screening has revealed the presence of alkaloids, carbohydrates, flavonoids and saponins in the hydroalcoholic leaf extract of *P. odoratissimus* L. Therefore, the presence of one of these phytoconstituents might be responsible for the anti-fertility activity of hydroalcoholic leaves extract of *P. odoratissimus*.

Conclusion

From the present study, it concluded that hydroalcoholic leaves extract of *P. odoratissimus* leaves possesses significant antifertility effect which might be due to the inhibition of implantation and antiestrogenic effect which in turn might be due to the presence some phytoconstituents in the plant. Further studies are required to elucidate exact mechanism of antifertility action and isolation of the active components responsible for antifertility effect.

Conflicts of interest

The authors declare no conflicts of interest.

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Original article

Neoadjuvant endocrine therapy in breast cancer patients

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ABSTRACT

Background: The aim of this study is to evaluate if the extension of neoadjuvant endocrine therapy (NET), beyond the conventional time, allows additional downstage of the tumour, in order to perform a breast conservative surgery (BCS), and to analyze if it is a good option for long-term control in patients who refuse or are unfit for surgery.
Patients and methods: We retrospectively reviewed a database containing all patients treated in our institution with NET. All included patients were post-menopausal with primary local disease. The type of response obtained was assessed using modified RECIST criteria.
Results: Thirty-three patients were included. Two patients had tumours with 90% expression of oestrogen receptors and all the others had 100%. The tumour size in the largest diameter was 6.51 cm before treatment and 5.18 cm after. Eighteen patients achieved a partial response after 10.28 months of therapy. Patients that were proposed to downstage the tumour performed 9.71 months of therapy until surgery and all were submitted to BCS. Progression occurred after 27.5 months.
Conclusion: Endocrine therapy is a feasible option for a longer time to allow additional downstage of the tumour and is a good solution in patients who refuse or are unfit for surgery.

Introduction

Breast cancer (BC) is the most common type of cancer in women.[1,2] Its incidence is expected to increase with ageing of the population.[1,2] As estrogens have a major impact in BC development and progression, endocrine therapy is an increasingly used treatment option both as adjuvant (after surgery) or neoadjuvant (alone or before surgery).[3–5] Currently used drugs are tamoxifen and aromatase inhibitors (AIs): letrozole, anastrozole and exemestane.

In what concerns pre-menopausal women, neoadjuvant endocrine therapy (NET) is contra-indicated[3,6,7] because studies are lacking to take conclusions, and for the moment, the ones that exist are contradictory.[8,9] On the other hand, regarding post-menopausal patients, in the most important international Guidelines[3,6,7,10] there are no precise orientations related to NET.

For many years tamoxifen was validated as initial sole treatment for frail elderly women, with overall response rates achieving 73%.[11] A study from Nottingham randomized women with estrogen receptor (ER)-positive cancers for mastectomy plus 5 years of tamoxifen versus tamoxifen alone for 5 years.[12] The update of the phase III GRETA trial did the same but there were no data concerning ER expression.[13] Some results of the studies mentioned are shown in Table 1.

Nevertheless, the group in which there is more controversy is that of young and fit postmenopausal women with inoperable locally advanced tumour in whom breast conservative surgery is not possible.[3,9,14–18] In luminal cancers, the pathologic complete response (pCR) rate to chemotherapy is much lower and, consequently, neoadjuvant chemotherapy is of limited benefit.[3,9,14–17] Actually, there are some studies that compare hormonal therapy to chemotherapy before surgical treatment in strong ER expression tumours.[9,15,19] The major results of those studies are shown in Table 2.

Besides the controversy over the effectiveness of NET, there is also no consensus on how long the treatment should last. The conventional treatment period is 3–4 months.[16,17,20,21] However, recent trials have reported better results with the extension of the treatment's duration.[17,18]

Dixon et al. conducted a study in which patients with locally advanced ER-positive cancers were submitted to neoadjuvant letrozole.[21] More recently Llombart-Cussac et al. published a small phase II trial with letrozole and the goal was to establish mean time to maximum response.[22] Until the 4th month those who progressed or had stable disease were excluded, 37.1% women improved after

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Table 1
Results of the Nottingham study and the update of the phase III GRETA trial.

	Nottingham[12]		Update of phase II GRETA trial[13]	
	BC-specific survival (%)	Local progression (%)	Death related to BC (%)	Local progression (%)
Tamoxifen + mastectomy	66	2	36.3	11.2
Tamoxifen alone	64	43	39.4	47.6

Table 2
Results of the studies of Alba, Palmieri and Semiglazov.

	Neoadjuvant hormonal therapy (%)	Neoadjuvant chemotherapy (%)
Overall response rates		
Alba[9], Palmieri[15], Semiglazov[19]	58–77.3	60–90.6
Breast conservation Semiglazov[19]	43	24
Breast conservation Alba[9]	56	47

Table 3
Results of the studies of Dixon, Llombart-Cussac, Allevi and Carpenter.

	Dixon[21]	Llombart-Cussac[22]	Allevi[18]	Carpenter[17]
Overall response rate (%)				
3rd month	69.8			
4th month			49.6	55
8th month			85.3	
12th month		76	95.0	72
24th month	83.5			
Breast conservation rate (%)				
3rd month	60			
12th month				69
24th month	72			
Median time to response (months)				
Median time to maximum response (months)	3.9			
Median time to enough response for breast conservation (months)	4.2			7.5
Progression (patients)	7 out of 63			

the 6th month but none after the 8th month.[22] In 2013, Allevi et al. created three cohorts with 40 patients each, that took letrozole for 4, 8 and 12 months.[18] All women were older than 65 years old, with luminal BC and unfit for chemotherapy.[18] Carpenter et al., in 2014, conducted a study with post-menopausal women not eligible for breast conservation, that took letrozole for 12 months.[17] The most important results of the studies mentioned above are described in Table 3.

Given that there are no standardized guidelines on how long NET should be performed, there is a real need for more studies focusing on this matter. Therefore, the aim of this study is to evaluate whether the extension of NET, beyond the conventional time of[3–4] months, allows additional downstage of the tumour, in order to perform a breast conservative surgery (BCS), and to analyze if it is a good option for long-term control in patients who refuse or are unfit for surgery.

Patients and methods

We retrospectively reviewed a database containing all patients with BC, from January 2007 until October 2015, who were treated with NET (with or without surgery) in our institution. All included patients were post-menopausal women with primary local disease, who had been submitted to NET with tamoxifen or AIs. Patients excluded were those with metastatic disease, inflammatory BC, previous radiation directed to the chest or chemotherapy for other concurrent cancer during the period of study.

We collected data concerning age; indication to endocrine therapy; multifocality; tumour size (T) and status of axillary nodes (N), according to TNM staging system for BC; tumour histologic subtype and grade; status and percentage of expression of ER and progesterone receptors (PR); HER2Neu amplification; drug used; response to treatment; time to response and surgery. Positive ER expression is defined as >1% of expression and positive PR expression as >20% of expression.

Tumour size before treatment was assessed through mammography and ultrasound in all patients. Post-treatment size was measured in the surgical specimen in one patient and obtained through clinical observation in two patients; all the others were measured by mammography and ultrasound. The efficacy of the treatment was evaluated using modified RECIST criteria: (1) total response: the tumour is no longer palpable or visible in images; (2) partial response: sustained reduction of at least 30% of the tumour size; (3) progression: an increase of 20% or a new lesion, axillary node or metastasis; and (4) partial response followed by progression: reduction of at least 30% of the tumour size followed by an increase of 20% or a new lesion, axillary node or metastasis.[2,23]

Immunohistochemistry was carried out to assess: HER2 status, using the anti-Her-2/neu (4B5) rabbit monoclonal antibody; ER, with the anti-estrogen receptor (SP1); PR, with the anti-progesterone receptor (1E2); using the Bench Mark ULTRA. Number of copies of the HER2/neu gene was obtained through Silver-enhanced in situ hybridization (SISH), using the BenchMark XT and the probe INFORM TM. The equipment is commercialized by Ventana® Medical Systems (Tucson, EUA).

Statistical analysis was performed using the SPSS® software version 23.0 (SPSS Inc., Chicago, IL). For categorical variables data was presented as absolute frequencies and percentages. For continuous variables, data was presented as mean ± standard deviation. Categorical variables were compared using chi-squared test while continuous variables were compared using independent samples *t*-test. The size of the tumour before and after treatment was analyzed using a Wilcoxon Test. Differences were considered statistically significant if the corresponding *p*-value was ≤ 0.05.

The study was approved by the Institutional Review Board and thus meets the standards of the Declaration of Helsinki.

Results

Thirty-three patients were included. The mean age was 81.94 ± 6.86 years old, with a maximum of 97 years old. Twelve (36.4%) of our patients had tumours ulcerating the skin. Two patients had tumours with 90% expression of ER and all the others had 100%. In our institution, HER2 expression/amplification is not routinely determined in the elderly. The baseline characteristics of our population are shown in Table 4.

Patients were submitted to NET due to comorbidities in 13 patients (39.4%) and refusal to undergo surgery in 12 (36.4%). Eight patients (24.2%) had the referred treatment to downstage the tumour prior to surgery.

Thirteen surgeries were performed: 8 BCS that represent all the patients in the group that was doing NET to downstage the tumour; 4 surgeries after progression on endocrine therapy (2 mastectomies and 2 BCS); 1 mastectomy in 1 patient unable to undergo surgery from the start for psychological reasons.

The tumour size in the largest diameter was 6.51 ± 11.00 cm before and 5.18 ± 10.28 cm after treatment ($p = 0.008$), which represents a statistically significant reduction in tumour size.

Eighteen patients (54.5%) achieved a partial response; 4 (12.1%) showed partial response followed by progression. Stable disease was observed in 7 (21.2%) of the patients and 4 (12.1%) suffered progression. No patient showed complete clinical response nor pCR.

Table 4
Baseline characteristics of the population included in this study.

Parameters	Global series ($n = 33$)
Mean age ± standard deviation	81.94 ± 6.86 years
Multifocality	
Yes	7 (21.2%)
No	26 (78.8%)
T	
T1b	2 (6.1%)
T1c	4 (12.1%)
T2	15 (45.5%)
T4b	12 (36.4%)
N	
Positive	12 (37.5%)
Negative	20 (62.5%)
Tumour histologic subtype	
No special type	28 (84.8%)
Lobular invasive carcinoma	2 (6.1%)
Other	3 (9.1%)
Tumour histologic grade	
1	12 (36.4%)
2	17 (51.5%)
3	4 (12.1%)
ER status	
Positive	33 (100%)
Negative	0 (0%)
PR status	
Positive	25 (78.1%)
Negative	7 (21.9%)
HER2 status	
Positive	1 (4.5%)
Negative	21 (95.5%)
Drug used	
Tamoxifen	9 (27.3%)
Anastrozole	23 (69.7%)
Letrozole	1 (3%)

The mean time to obtain response in the group with partial response was 10.28 ± 6.85 months, with a minimum of 3 and a maximum of 24 months. 11.11% of the patients (2 in 18) achieved response at the 4th month or earlier and 38.89% (7 in 18) achieved response in the 6th month or earlier. In the group with stable disease, the time elapsed from the beginning of therapy until last visit was 11.57 ± 7.70 months.

In the patients with partial response followed by progression, the time during which response was documented was 13.50 ± 3.42 months; after that time, the women remained stable until progression occurred in the 22nd month or later and, in average, after 35.25 ± 13.37 months of therapy, which means that after a reduction in tumour size have ceased to be observed, 21.75 months of stability went by until the disease progressed. Patients who suffered progression without any previous response, did so only after 19.75 ± 8.01 months of treatment. The overall mean time to progression (with or without initial partial response) was 27.5 ± 13.47 months.

Analyzing those patients that refused or had co-morbidities to undergo surgery, we found that overall progression occurred in 32% (8 in 25) of the cases but only one women who progressed did it before the first year of treatment (8th month); actually, four patients progressed between the 18th and 24th month and another three between the 40th and 58th month, which was the maximum. The patients proposed to downstage the tumour had performed 9.71 ± 6.84 months of therapy until surgery.

There were no statistically significant differences when comparing patients' and tumours' characteristics and the type of response using modified RECIST criteria.

Discussion

Nowadays, endocrine therapy as sole treatment or before surgery is generally reserved for frail elderly women, patients with comorbidities regardless of age and women who refuse surgery. Not surprisingly, mean age was 81.94 years old and the indication for endocrine therapy in 76% of our patients was co-morbidities or surgery refusal.

Incoming with previous studies, our results also demonstrate that endocrine therapy is effective – tumour size in the largest diameter was 6.51 cm before and 5.18 cm after treatment, representing a statistically significant reduction in tumour size. Therefore, this reinforces that NET is a good alternative to chemotherapy for Luminal tumours.[9,15,19]

In our literature review, the overall response rate to NET (with or without surgery), regarding women with strong ER expression, ranged from 58 to 83.5%.[8,9,15,18,19,22] Since our patients did not achieve complete clinical response, our overall response rate equals the rate of partial response, which was 54.55%. We believe this slightly difference is related to two facts: 9 patients (27.3%) were on tamoxifen, that has been shown to perform worse than aromatase inhibitors,[8,18,24,25] and not all of our patients are Luminal A breast cancers – HER2 expression/amplification was determined only in 22 patients (66.7%), 4 tumours (12.1%) were grade 3 and 7 (21.9%) did not express progesterone receptors.

In our study, we did not determine the pre-treatment eligibility to BCS. However, all the eight patients proposed to tumour's downstage, after 9.71 months of NET therapy, preserved their breast, which supports recent trials that suggested that the extension of endocrine therapy would improve maximum reduction in tumour volume, sufficient for BCS.[17,20,21]

Llombart-Cussac et al. suggested endocrine resistance if the disease is stable or if it is progressed by the 4th month.[22] On the other hand, if at this time there is any response, treatment duration should be individualized.[22] The median time to achieve clinical

response, in the 18 patients who had partial response was 10.28 months. Moreover, on that group, only 11.11% of patients (2 in 18) achieved response at the 4th month or earlier and 38.89% (7 in 18) achieved response at the 6th month or earlier. Therefore, in our study, the required time of therapy to suggest endocrine resistance is longer than the 4 months suggested by Llombart-Cussac et al.[22]

Some tumours, despite having ER expression, fail to respond.[26] 45.4% of the patients (the groups of stable disease, progression and partial response followed by progression) exhibited resistance to endocrine therapy. According to Carpenter et al., the neoadjuvant approach allows early selection of endocrine resistant ER-positive tumours.[17]

In previous studies, pCR rate ranges from 0% to 3%,[8,9,15,19,22] except in one study by Allevi et al. that reaches 17.5% by 12 months, although we did not find any explanation for this result.[18] In our study, the pCR rate was 0%. However, unlike neoadjuvant chemotherapy, pCR in the context of NET is not considered prognostic.[16]

In GRETA trial, even in those patients that took tamoxifen alone, median time for progression was 19.3 months and if, while waiting for maximum response, progression occurred, this delay did not worsened prognosis.[13] In fact, our results show that patients who suffered progression (with or without partial response), progressed after a mean time of 27.5 months. During that time those patients remained with stable disease or had partial response. That represents that even patients who had the worst outcome only began to suffer latter in the course of therapy. In the case of patients with a low life expectancy and who refused to or cannot undergo surgery, it is a long time of disease control.

Our study has some limitations: being retrospective we have not programmed follow-up visits, therefore we were not able to determine maximal time to response; also 9.1% (3 in 33) of our patients were not measured by ultrasound or mammography after treatment, as it is recommended by the RECIST criteria. Thirty-three is a small number of patients, however, it represents the elderly patients treated with NET, in our institution, in the last 9 years. Finally, we did not evaluate functional status of our patients.

In conclusion, our study support that NET can be done beyond the conventional[3–4] months to allow additional downstage of the tumour and, therefore, preserve the breast,[17,18] because all patients proposed to downstage, after 9.71 months of NET, were submitted to BCS. Besides that, in the group which achieved response, 10.28 months was the mean time to accomplish it, also a longer time than the conventional. Our results showed that even patients who had the worst outcome, this is, who progressed, only began to suffer latter in the course of therapy, after a mean time of 27.5 months, which in the case of patients with a low life expectancy and who refused to or cannot undergo surgery, represents a long time of disease control.

Conflicts of interest

The authors declare no conflicts of interest.

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Case Report

Secondary peritonitis by *Actinomyces odontolyticus*

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ABSTRACT

Abdominal actinomycosis is a rare infection and the non-recognition of this particular microorganism may led to a prolonged septic process and recurrent disease. We hereby present a case report of 53 years-old woman with a secondary peritonitis due to this microorganism and our option to perform a long course of penicillin derived antibiotics, after suture of a perforated gastric ulcer caused by a foreign body.

Introduction

Actinomycosis are chronic and slowly developing infections, caused by bacteria from the genus *Actinomyces*, which are facultative anaerobic or strictly anaerobic gram-positive rods. *Actinomyces* spp. colonize the endogenous mucosa, such as the upper respiratory, gastrointestinal, and female genital tracts, causing disease when injury to the mucosal barrier is present – usually by trauma, surgery or infection.[1]

Numerous species have been described, being *Actinomyces israelii* the most frequent in human infections.[1]

Similarly to other abdominal actinomycosis, infections by *Actinomyces odontolyticus* usually arise from mucous membranes.[2]

The usual sites of invasive disease by this microorganism are the heart, lung and mediastinum. Bacteremia has been described, mainly in immunosuppressed patients.[3]

Abdominal infections caused by *Actinomyces* spp. most commonly occur in patients who have experienced gastrointestinal surgery or have suffered trauma to the bowel.[1] The abdominal cavity as a site of infection accounts for nearly 20% of all actinomycosis sites. There are no recent estimates of the prevalence of this disease, with the estimated population prevalence being one case per 40–119,000 people.[4]

Case report

A 53-year-old woman, with a medical history of depression, medicated with fluoxetine, trazodone chlorhydrate and clonazepam, came to the Emergency Department due to a 5 days long epigastric pain. Any other symptoms were absent.

During physical examination, the patient was afebrile, vital signs were normal and pain was felt in the upper abdomen on deep palpation – without any signs of peritonitis.

Blood tests were taken and were unremarkable, apart from 14,100 leucocytes/μL (86,6% neutrophils) and an increase in the Reactive C-Protein (3392 mg/L).

A Computerized Tomography (CT) scan was performed, and it revealed a circumferential thickening of the distal gastric wall, with adjacent fat densification and a hypodense collection on the gastric wall, with 3 × 2.3 cm, which suggested the diagnosis of a contained gastric perforation.

The patient was admitted. Conservative treatment was decided, with intravenous fluid therapy, analgesia and broad-spectrum antibiotics – Piperacillin/Tazobactam.

After 48 h as an in-hospital patient, she started to be febrile, which was interpreted as failure of medical conservative treatment, and was submitted to surgery.

Intraoperative findings consisted of abundant fibrosis between the posterior wall of the stomach and the body of the pancreas. Purulent abdominal fluid was collected from the abdomen and sent to microbiological analysis. A 5-cm-long fishbone was extracted from an intramural gastric abscess and a simple suture of the defect was performed.

The patients postoperative course was unremarkable and she was discharged 4 days after the surgery.

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The microbiological analysis of the abdominal fluid identified a single bacterium – *A. odontolyticus*. Hemocultures were negative.

We chose to perform a further 3-week course of parenteral penicillin followed by 8 weeks of oral amoxicillin. Follow-up of this patient, with clinical, analytical and image reevaluation took place three months after surgery – the patient remained asymptomatic, blood tests values were within normal range and the CT scan showed complete resolution of the inflammatory process. The patient was considered to be cured, and antibiotics were suspended. Due to the possibility of recurrence, the patient remains in follow-up.

Discussion

Abdominal infections caused by *Actinomyces* spp. are uncommon. As for its origin in gastric perforation, around 20 cases have been reported in the literature.[4] Appendicitis is the most common cause (65%).[5]

The preoperative diagnosis of abdominal actinomycosis was not considered in this case. This is consistent with the literature description that only 10% of the cases are diagnosed prior to surgery.[4]

The possibility of a perforated gastric malignancy was considered, as digestive tract actinomycosis is known to mimic malignancy.[6]

The probable cause for infection by this pathogen in this patient was the disruption of the gastric wall due to a foreign body. *Actinomyces odontolytica* is one of the most predominant *Actinomyces* species in biofilms on tooth surfaces. It is also common in the pharynx and in the distal esophagus. This last site has a relatively stable environment for bacterial colonization by the agent.[7]

Little is known about the virulence factors of *Actinomyces* spp., but they are able to evade clearance by the host immune system and, thus, cause a chronic invasion.[6] While the vast majority of actinomyces-related infections are polymicrobial (up to 95%),[7] in this particularly clinical case, *Actinomyces odontolytica* was the only identified pathogen.

Abscess drainage and long course of antibiotics with penicillin derived antibiotics is the recommended treatment for infections caused by *Actinomyces* spp.[8]

Long-term antibiotics (up to a year) may be required.[4] This concept is changing, as when optimal surgical resection of infected tissues is achieved, a 3-month course of antibiotics is possible.[6] The actual role of surgery usually takes place in cases of extensive necrotic lesions or when antimicrobial therapy fails.[7]

Actinomyces infections can recur years after initial treatment or may linger asymptomatically if primary treatment is not curative.[4]

CT-scan or Magnetic Resonance Imaging are used to perform the follow-up on patients with medical treatment.[5] We opted to performed a CT-scan three months after surgery, despite her being asymptomatic, to ensure the complete resolution of the collection, which could otherwise lead to recurrence of the infection.

Conflicts of interest

The authors declare no conflicts of interest.

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PS004

Why novel methods are not always the best? – Multifactorial analysis of hyperandrogenism in women

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Aim: The aim of the work was to compare different methods of hormones evaluation, including blood and saliva samples and the realiability of those methods in diagnosing hyperandrogenism among women caused by various reasons.

Introduction: Hyperandrogenism among women is a common problem. There are different hormones that can be evaluated with various methods to diagnose and monitor patients. Less invasive and quicker methods of screening, like salivary samples, more and more are used in medicine. However, they may be not as accurate as expected.

Methods: 39 women with clinical or biochemical hyperandrogenism and 29 healthy individuals in control group were enrolled. The diagnosis of hyperandrogenic syndrome covered: 13 patients with polycystic ovary syndrome (PCOS), 23 with idiopathic hyperandrogenism, 2 with congenital adrenal hyperplasia and 1 adrenal cortical carcinoma. Assessed hormones included: serum total testosterone (T) measured with liquid chromatography-tandem mass spectrometry LC-MS and immunoassay, salivary T by Salimetrics test; DHEA-S, androstendione and 17-OH Progesterone by LC-MS and immunoassay.

Results: In 9 out of 38 patients' results of salivary testosterone showed normal levels, while with LC-MS method increased levels were depicted in the same women. Similarly, 41% women with hyperandrogenism had elevated testosterone with ELISA method, whilst having Salimetrics test results within normal range. In 28% normal testosterone levels measured by LC-MS method, DHES-S was elevated. All patients with elevated androstendione presented with elevated concentration of either testosterone or DHEA-S. Elevated DHEA-S was observed in 56.5% patients with FHS and 15.4% with PCOS.

Conclusion: Salivary testosterone is not a sufficient method in diagnosing biochemical hyperandrogenism. Measurement of serum testosterone by LC-MS itself is not enough to diagnose biochemical hyperandrogenism. DHEA-S should also be evaluated when hyperandrogenism is suspected. Androstendione measurement is not obligatory in diagnosis. This is the first study analyzing numerous hormones with various methods in patients with hyperandrogenism caused by different diseases.[1–4]

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PS151

Comparison between effects of antibiotics, NSAIDs and their mixture on the growth of microorganisms

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Aim: To compare the effects of antibiotics, NSAIDs and their mixture on the growth of microorganisms.

Introduction: Commonly, when a patient has an infection, doctors prescribe NSAIDs for pain and inflammation that may be caused by infection as a part of symptomatic treatment. And antibiotics are also prescribed as an etiological treatment. Our experiment that was performed last year came to a conclusion

that NSAIDs, notably: Aspirin, Ibuprofen and Diclofenac could inhibit the growth of some microorganisms including *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. These results, although performed in vitro were promising especially with the growing rate of bacterial resistance towards antimicrobial agents.

Methods: We used antibiotics: Penicillin, Gentamicin and Ceftriaxone; NSAIDs-non-selective: Aspirin, Diclofenac and Ibuprofen and COX-2 selective: Celecoxib. Samples were taken from the oral cavity of patients with liver diseases. Cultures were made of the samples taken and they were inoculated onto an agar plate. Then three well were made in the agar plate: in the first well we put an NSAIDs, second well with an antibiotic and in the third we put the mixture of both NSAID and antibiotic. The agar plates were placed into an incubator for 24 h at a temperature of 37 °C. The experiment was done twice to get accurate results.

Results: The analysis of the obtained results shows that in group 1 (antibiotics) was the highest inhibition 39.3 ± 3.6 mm, in the group 2 in which there were NSAIDs gave the results as shown 31.7 ± 4.1 mm, and last investigative group 3 with mixture was 27.3 ± 1.8.

Conclusion: From the obtained results we can conclude that a mixture of NSAIDs and antibiotics does not improve antibacterial effect of antibiotics. In fact, NSAIDs seem to even lower the efficacy of antimicrobial drugs. Special attention should be paid while administering NSAIDs to patients who are on antibiotic therapy since the combination of these two groups of drugs lower the antimicrobial effect.

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PS003

Comparison of metabolic syndrome rates in living-donor and deceased-donor kidney recipients – A three-year follow-up

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Aim: Comparison of MS rates in kidney recipients.

Introduction: Metabolic syndrome (MS) is characterized by coexistent pro-atherogenic disorders and insulin resistance. MS also increases cardiovascular risk.

Methods: A total of 112 living-donor ($n = 54$) and deceased-donor ($n = 58$) kidney transplant recipients were evaluated for metabolic syndrome (MS) in months 6, 12, and 36. The National Cholesterol Education Program – Adult Treatment Panel III (NCEP-ATP III) criteria were used. Both groups were compared in terms of MS rates. Moreover, correlations between MS and other parameters (age, gender, dialysis type and duration, donor type, immunosuppressant drugs, acute rejection episodes, smoking, levels of triglycerides, uric acid, creatinine, eGFR, and proteinuria) were evaluated. The chi-square, McNemar's test, Student's *t* test, Welch's *t* test, Mann–Whitney *U* test, Fisher's test, and Shapiro–Wilk test were used in the statistical analysis.

Results: MS rates following living-donor (LD) and deceased-donor (DD) kidney transplantation (KTx) in months 6, 12, and 36 were 0.148 vs. 0.276; 0.173 vs. 0.316; 0.235 vs. 0.182, respectively. MS rates in LD KTx recipients were lower than those in DD KTx recipients in months 6 and 12, especially in males (0.14 vs. 0.379; $p = 0.0251$), but they increased systematically in subsequent months of follow-up. MS was more commonly diagnosed in older recipients ($p = 0.019$), with lower MDRD eGFR values ($p = 0.009$), who received more anti-hypertensive drugs ($p = 0.046$). The dialysis type, donor type and the number of transplantations had no effect. The logistic regression model indicated that the factors contributing to MS were elevated uric acid levels and proteinuria.[1,2]

Conclusion:

1. MS rates in LD KTx recipients in month 6 and 12 following transplantation are lower than those in DD KTx recipients.
2. MS rates in LD KTx recipients tended to progressively increase during follow-up.
3. MS was more common in older patients with poorer kidney function, higher uric acid levels and proteinuria.

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PS073

Association between body composition and magnesium level in midlle aged women

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Aim: Aim of the study was to compare total magnesium serum concentration between subjects with increased fat mass in the body composition and subjects with normal body composition as to determine the association between total magnesium serum level and parameters of the body composition and glucose metabolism.

Introduction: Metabolic disorders and chronic diseases may associate alterations in body composition and could be related with disturbances of the magnesium blood level. Obesity is a chronic disease characterized by disturbances of the body composition, commonly associated with disorders of carbohydrate metabolism.

Methods: The study included 40 women with body composition disturbances (increased percentage of the total fat mass) and 20 age matched women with normal percentage of the total fat mass. All subjects underwent analysis of the components of body composition [bioelectrical impedance analysis, fat mass percentage (FAT%), fat free mass percentage (FFM%)], laboratory analysis of blood samples (automated analyzer systems) with determining the parameters of glucose metabolism and total magnesium serum concentration. Insulin resistance index (HOMA-IR) was calculated using equation involving fasting insulin and glucose concentration.

Results: Women with increased percentage of the total fat mass had signifficantly lower total magnesium serum concentration comapared to control group (0.83 ± 0.07 vs. 0.9 ± 0.06 mmol/l, $p = 0.00$). Moderate correlation was found between serum concentrations of total magnesium and FAT% ($r = -0.47$, $p = 0.00$), FFM% ($r = 0.44$, $p = 0.00$), fasting insulin levels ($r = -0.43$, $p = 0.00$) and HOMA-IR ($r = -0.44$, $p = 0.00$).

Conclusion: Women with increased total fat mass in the body composition have significantly lower total magnesium serum concentration, compared to women with normal body composition.

Additionally to increased fat mass, insulin resistance is associated with total magnesium level in middle aged women.

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PS137

Increased paraoxonase and arylesterase activity in thyroiditis patients compared to healthy individuals

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Aim: The aim of this study was to assess whether there is a significant difference in paraoxonase and arylesterase activities and distribution of phenotypes in thyroiditis patients compared to healthy volunteers.

Introduction: Human serum paraoxonase 1 (PON1; EC3.1.1.2) is an antioxidant enzyme showing both paraoxonase and arylesterase activities. The PON1-192 polymorphism has two isoforms, namely PON1 Q and PON1 R. PON1 Q contains a glutamine at position 192. PON1 R contains an arginine at position 192. It shows a 6 fold higher activity towards paraoxon hydrolysis compared to Q isoform. Arylesterase activity is similar in both isozymes. The R allozyme shows a greater degree of stimulation of its paraoxon-hydrolyzing activity by 1 M NaCl than does the Q allozyme. The ratio of Salt stimulated PON 1 activity/Arylesterase activity (P/A ratio) is trimodally distributed. The three modes correspond to paraoxonase phenotypes, QQ, QR and RR.

Methods: Fifty thyroiditis patients and one hundred and thirty seven apparently healthy individuals were enrolled in this study. Serum samples of both groups were analysed for basal paraoxonase activity, salt stimulated paraoxonase activity (with 1 M NaCl) and arylesterase activity (spectrophotometrically). P/A ratio was used to assess the phenotypes (dual substrate method).

Results: Basal PON 1 activity (205.27 ± 115.00 U/l vs. 251.1 ± 129.6 U/l, $p = 0.002$) and arylesterase activity (159.53 ± 37.11 vs. 177.59 ± 46.90, $p = 0.024$) was significantly higher in thyroiditis patients compared to healthy volunteers. Percentage of QQ phenotype was significantly higher in thyroiditis patients compared to healthy individuals. Percentage of QR phenotype was significantly lower in thyroiditis patients compared to healthy individuals. There was no difference in percentage of RR phenotype in thyroiditis patients and healthy individuals.

Conclusion: Serum PON 1 activity and arylesterase activity was significantly higher in thyroiditis patients compared to healthy individuals. Percentage distribution of phenotypes in thyroiditis patients was significantly different from healthy individuals.

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PS111

The relationship between dyslipidemia and disease activity in Iranian population with systemic lupus erythematosus

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Aim: This study was designed for evaluating the relationship between dyslipidemia and diseases activity in systemic lupus erythematosus (SLE) patients.

Introduction: In spite of high prevalence of dyslipidemia in SLE patients and its role in patients' cardiovascular events, there was scant study about the relation between dyslipidemia and disease activity in SLE patients in Iran.

Methods: This analytical cross-sectional study was conducted during 2014–2016 on SLE patients who referred to the Hasheminejad hospital (Tehran – Iran). The serum levels of triglyceride, cholesterol, LDL, and HDL were measured, then dyslipidemia and correlated factors were evaluated. The activity of disease was determined by SLE disease activity index (SLEDAI).

Results: 62 out of 72 patients (87%) were female and the mean age was 34 years. The median disease duration was 1 year and 49% of patients had active disease (SLEDAI ≥ 6). Proteinuria and nephritis were observed in 18% and 24%, respectively. 62% of patients had at-least one abnormality in their lipid profile. High cholesterol (>200 mg/dL), high triglyceride (>150md/dL), high LDL (>130 mg/dL) and low HDL (<50 mg/dL in females and <40md/dL in males) levels were observed in 25%, 42%, 20% and 49% of patients, respectively. Patients with active disease had lower age and disease duration in comparison of others ($P < 0.05$), while there were no differences in terms of sex and weight between patients in active and inactive phases ($P > 0.05$). The frequency of proteinuria, nephritis and decreased level of complements were higher in active SLE patients, too. Patients with active disease had also higher levels of serum cholesterol, triglyceride and LDL and lower level of serum HDL. In logistic regression, the odds ratios of patients with high cholesterol, using more than 10 mg/day prednisolone and with low serum HDL level for having active disease were 6.6, 5.6 and 3.4, respectively ($P < 0.05$).

Conclusion: Our findings showed that dyslipidemia is prevalent in SLE patients especially in patients with active SLE disease. In addition, patients with high cholesterol, using more than 10 mg/day prednisolone and with low HDL had higher chance for having active disease. Hence, it seems that there is a relation between disease activity and lipid profile abnormalities in SLE patients.

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Neurosciences Paralell Oral Session
Friday, September 15th, 14h00

PS168

Astrocytic A2A receptors: Novel targets to manage brain disorders

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Aim: To validate astrocytic adenosine A2A receptors (A2AR) as a novel target to prevent abnormal glutamate overexcitation.

Introduction: Astrocytes are responsible for clearance of extracellular glutamate, a process controlled by A2AR, extolling

their key role as regulators of synaptic transmission and of the abnormal glutamate overexcitation implicated in both acute and chronic brain diseases. We have previously showed that activation of astrocytic A2AR reduce astrocytic glutamate uptake under physiological and pathological conditions,[1–3] and that A2AR are aberrantly up-regulated upon multiple brain insults.[4–6]

Methods: We incorporated EGFP reporter either alone or combined with either a small hairpin to down-regulate A2AR (shA2AR) or a control sequence (shCTR) into Mokola Lyssavirus (Mok-G) and Vesicular Stomatitis Virus (VSV-G) lentivectors and tested whether Mok-G-coated lentivirus selectively and efficiently transduced astrocytes in primary culture or in mouse brain through stereotaxic administration of lentivectors into striatum [STR], hippocampus [HIPP] and prefrontal cortex [PFC] (compared to neurotropic VSV-G-coated lentivirus as controls). Herein, we evaluated viral spreading and cell-type transduction through immunofluorescent colocalization of EGFP with glial (GFAP and vimentin) and neuronal (NeuN) markers.

Results: After 25 days post-infection, Mok-G_EGFP transduced 68% of cultured astrocytes (EGFP- and DAPI-positive, $n = 1$); 100% of GFAP-positive cells colocalized with EGFP as well as 86% cells expressing Vimentin only and 47% expressing both Vimentin and GFAP. Mok-G shA2AR lentiviruses robustly reduced A2AR immunoreactivity compared to Mok-G shCTR in cultured astrocytes. At 4 weeks post-brain administration, Mok-G_EGFP was expressed mainly in astrocytes (GFAP-positive cells) in both STR and HIPP, and to a lower extent in the PFC, whereas VSV-G-coated lentivirus colocalized with NeuN marker and not with GFAP in any tested brain areas.

Conclusion: These data supports the ability of Mok-G lentivectors to efficiently transduce astrocytes to control A2AR density, paving the way for their application to control pathophysiological processes involving astrocytes.

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PS077

Adenosine A1 receptor antagonism prevents DSI in hippocampal CA1 pyramidal cells

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Aim: How adenosine interfere with a short-term form of neuronal plasticity dependent on endocannabinoid, the depolarization-induced suppression of inhibition (DSI).

Introduction: The widely consumed psychoactive drug cannabis, containing cannabinoid compounds, and/or caffeine, with adenosinergic antagonizing proprieties, exert their central actions by affecting cognitive operations such as learning and memory. Indeed, endogenous adenosine and endocannabinoids (eCB) are known to interfere with physiological synaptic plasticity phenomena that represent the neuronal substrate of memory formation.

Methods: Whole-cell voltage-clamp recordings (Vh = −70 mV) were performed on hippocampal CA1 pyramidal cells of 3 to 5 weeks-old C57BL/6 mice. Slices (350 μm thick) were perfused with artificial cerebrospinal fluid (aCSF) supplemented with glutamate receptor antagonists (CNQX, 25 μM and DL-APV, 50 μM) to block glutamatergic transmission and isolate GABA-mediated responses. Inhibitory postsynaptic currents (IPSCs) were evoked every 3 s through a stimulation electrode placed in stratum radiatum. The recording electrode was filled with a CsCl-based intracellular solution and DSI was evoked through a 5 s voltage step of +80 mV. The magnitude of DSI was measured 9 s after the depolarizing step and DSI recovery was evaluated between 30 and 60 s after depolarization.

Results: When recording eCB-mediated DSI we observed a decrease in electrical-evoked IPSC amplitudes to 81.0 ± 5.4% of baseline ($p < 0.01$, $n = 14$) that fully recovered to 90.2 ± 5.4% after 30–60 s. The adenosine A1 receptor antagonist, DPCPX (100 nM), prevented DSI, recordings showing a non-significant change in IPSCs amplitude to 95.1 ± 12.0% of baseline ($p = 0.3473$, $n = 10$) that was maintained throughout the recovery period (87.1 ± 12.0%).

Conclusion: These results suggest that tonic adenosine A1 receptor activation is necessary for the occurrence of DSI. The mechanisms involved in this process remain unclear and need further investigation.[1–4]

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PS087

High-sucrose diet effects on the dendritic trees of developing neurons of the adolescent rat

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Aim: In the present study, we aimed to explore the effect of high-sucrose diets on the dendritic trees of immature granule cells of the adolescent male rats.

Introduction: Adolescence is a period of high susceptibility to exogenous factors as the rat brain is still developing. Evidence shows that high-sucrose diets may be more detrimental to adolescent rats, therefore we intended to study immature granule cells in the hippocampal formation of these animals. For that, we used

doublecortin (DCX), a microtubule-associated protein expressed by neuronal precursor cells and immature neurons, which is used as a marker for neurogenesis.

Methods: At 4 weeks of age, adolescent male Wistar rats were randomly allocated to control group ($n = 7$) and to an high-sucrose (30% sucrose) diet group ($n = 4$; HS) during 4 weeks. After this period, rats were sacrificed and DCX immunocytochemistry was performed. The dendritic trees of the DCX-immunostained cells were drawn with the aid of a camera lucida. A metric analysis of the dendritic trees was performed, and the following parameters were quantified: total dendritic length, the total number of terminal segments, the total number of intermediate segments, mean length of terminal segments and mean length of intermediate segments.

Results: Our results show that the total dendritic length of HS adolescent rats was significantly reduced when compared with controls ($p < 0.03$). There were no other differences in the others parameters quantified.

Conclusion: In conclusion, the dendritic trees of immature neurons of the dentate gyrus of HS adolescent rats appear to be disturbed after the exposition of this diet. This data confirms previous evidence reporting adolescence as a susceptible period of the brain development with likely consequences in cognition. If that is so, and if the reported results can be extrapolated to man, public health interventions are necessary to advise adolescents concerning their diet.

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PS109

Looking for modulatory brain areas in the visual circuit related to freezing behaviour

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Aim: I have been studying a visual circuit that is known to trigger freezing: the connection between the Retina, the Superior Colliculus and the Parabigeminal Grey. The aim of this investigation is to look for cerebral nuclei that could be inputs of the SC and, therefore, regulate this behaviour. In other words, it is a search for modulatory brain areas in the circuit: Retina → SC → PBg.

Introduction: Information supplied by the retina initiates interactions in the brain that eventually lead to conscious perception of the visual scene, conventional reflexes such as adjusting the size of the pupil or triggering certain behaviours. Innate defensive behaviours evoked by threatening stimuli are essential to survival. When a danger suddenly appears, a mouse can either scape or freeze. I am interested in how the visual world cause freezing and why.

Methods: The tracing strategy used is based on two injections (stereotaxic surgery) with two different retrograde viruses. The first injection is in the PBg with a modified HSV (Herpes Simplex Virus) and the second one, 21 days later, with RVdG (Rabies Virus G-deleted) in the SC. The combined characteristics of these viruses allowed me to specifically follow the circuit. After perfusing the animals, slicing the brains and staining with specific antibodies attached to fluorochromes, I took images with a fluorescent confocal microscope.

Results: With a pertinent image processing and comparison with the brain atlas, I was able to identify which brain areas were mostly labelled: zona incerta, substantia nigra and L5 in V1 (visual cortex).

Conclusion: It is known that these three nuclei are involved in other visual pathways but this finding suggest that they also could have a role in freezing response to a visual stimulus. The current work is now focused on finding out how each one participates in modulating the behaviour.

Acknowledgements: This thesis is going to be evaluated by the University of Barcelona and it is supported by the KU Leuven, the experiments were performed at NERF (Neuro-Electronics Research Flanders) in the Karl Farrow's Laboratory.

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PS178

Cafeteria-diet effects on learning and memory, anxiety and fear response of the adolescent rat

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Aim: We aimed to explore the effect of high caloric diets on adolescent male rats to mimick the feeding behavior of human adolescents in the Western world.

Introduction: Age of high-caloric diet exposure is an important factor for the cognitive and anxiety outcomes as key processes of brain development and maturation occur during adolescence. Evidence shows high-caloric diets to affect differently learning and memory performance in an age-dependent way, being more detrimental to adolescent rats.

Methods: At 4 weeks of age, 30 adolescent male Wistar rats were randomly allocated to control, high-sugar (HS) and high-fat high-sugar (HFHS) diet groups during 4 weeks. After this period, behavioral tests were performed to study: (1) anxiety behavior in the elevated plus-maze (EPM) and open field tests, (2) learning and memory processes in the Morris water maze (MWM) and novel object recognition test, (3) fear response in fear conditioning tests and (4) depression state in forced swim test.

Results: Our results show that only HFHS-treated rats presented more anxiety than control rats, spending more time in the closed arms and less time in open arms of EPM. Moreover, HFHS-treated animals presented an impairment of spatial learning in the final phase of acquisition and an impairment of spatial memory, since these rats spend less time in the target quadrant of MWM and cross less times the former position of the platform. There were no differences between groups regarding locomotor activity, fear acquisition and memory, object novelty detection and exploration, and depression state.

Conclusion: In conclusion, anxiety behavior and spatial learning and memory are particularly affected by a cafeteria-type diet in young rats. This data confirms previous evidence reporting adolescence as a susceptible period of brain development to neural insults. Furthermore, the results show that there are different cognitive

and emotional behavioral consequences between HS and HFHS diets.

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Oncology & Molecular Biology Paralell Oral Session
Friday, September 15th, 14h00

PS081

GE11 positive exosomes as a potential RNAi delivery system in clear cell Renal Cell Carcinoma

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Aim: Use GE11 positive (GE11+) exosomes as a targeted delivery system to EGFR overexpressing cells for the treatment of clear cell Renal Cell Carcinoma (ccRCC).

Introduction: ccRCC is the most prevalent subtype of renal cancer and the most lethal urologic tumor. Generally, it is radio-chemotherapy resistance, and frequently associated with relapse after 5–11 months upon targeted therapy treatment, which highlight the need to develop new therapeutic strategies. Exosomes, extracellular vesicles of 40–150 nm that mediate intercellular communication, have emerged as promising therapeutic tools due to their engineering potential and ability to evade the immune system.

Methods: EGFR is known to be overexpressed in ccRCC thus, the expression of GE11, a peptide that binds to EGFR, in exosomes membrane enable a targeted delivery of therapeutic molecules to EGFR overexpressing cells. Exosomes derived from HEK293T were engineered to express the GE11 peptide on their surface and incubated with normal or tumor renal cell lines.

Results: Our results revealed EGFR overexpression at the mRNA and protein levels in a ccRCC cell line, compared to a normal renal cell line. Furthermore, tumor cells presented increased protein levels of phosphorylated EGFR when compared to normal cells. These results support the hypothesis of using an EGFR-based exosomes delivery model, the GE11+ exosomes. A higher percentage of tumor cells internalized GE11+ exosomes compared to exosomes derived from HEK293T cells transfected with control condition. Additionally, tumor cells exhibited an increased mean of fluorescence intensity compared to the control, suggesting that each cell uptakes more GE11+ exosomes in an EGFR-dependent manner. Importantly, GE11+ exosomes were internalized in a greater proportion by tumor cells rather than normal renal cell lines.

Conclusion: Overall, the use of GE11+ exosomes as a new delivery system is a promising therapeutic strategy for ccRCC treatment. Ultimately, these exosomes can be loaded with RNAi-based drugs to target deregulated genes in ccRCC.

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PS145

Evaluation of combined cytoplasmic AR in tumour cells expression and tumour CD3 T-cells infiltrate as a prognostic score for patients with prostate cancer

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Aim: We aimed to assess the prognostic value of using a cumulative score evaluating the expression of Androgen Receptor (AR) and the presence tumour inflammatory infiltrate as a prognostic marker for prostate cancer (PCa).

Introduction: PCa is the most common male cancer, in Europe. Currently, at diagnosis, only tumour-based factors, including clinical stage, tumour grade and circulating concentrations of Prostate-Specific Antigen (PSA) are used to predict PCa outcome. However, this can vary within patients sharing the same clinical conditions, leading to patient's over/under treatment. It is now recognized that cancer progression is also dependent on tumour's interaction with its microenvironment, specifically with immune cells. Therefore, the development of predictive biomarkers, capable of combining these two factors should be considered.

Methods: Immunohistochemistry for AR expression and CD3 T-cells was performed on biopsies from a cohort of 361 patients diagnosed with PCa. Semi-quantitative weighted histoscore and quantitative assessments were used.

Results: High cytoplasmic AR expression in tumour cells and high CD3-T cells presence were associated with reduced overall survival ($p = 0.000055$, and $p = 0.004$, respectively), with strong association ($p = 0.001$) on X2 analysis. When patients were grouped as having: both markers low or one low and low/moderate and one high, and both high, this cumulative prognostic score was strongly associated with overall survival ($p = 0.000001$), being the mean overall survivals: 7.1 years (95% CI 6.5–7.6), 6.0 years (95% CI 5.4–6.6) and 3.8 years (95% CI 2.4–5.0), respectively. Moreover, on multivariate analysis, it was considered a significant independent predictor of overall survival (HR 1.982, 95% CI 1.018–3.859, $p = 0.044$).

Conclusion: These results confirm the clinical utility of assessing both tumour and microenvironment characteristics when predicting patients' outcome, and suggest that the presence

of high cytoplasmic AR expression in tumour cells and CD3 T-cells predicts poor outcome for patients diagnosed with PCa.

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PS165

ALDHs as potential biomarkers in myeloid neoplasms – Preliminary study

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Aim: The aim of the study is to evaluate the expression of aldehyde dehydrogenase (ALDH) in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) to verify their potential as a marker for the diagnosis and/or prognosis of these diseases.

Introduction: ALDH superfamily is a group of 19 enzymes critical to the protection against toxic aldehydes, and have been associated with multiple diseases, namely in cancer. MDS are characterized by ineffective hematopoiesis associated with progressive peripheral blood cytopenias, and a predisposition toward leukemic transformation. MDS pathophysiology is a complex multistep process that involves genetic and epigenetic abnormalities in genes associated with differentiation, cellular proliferation, and apoptosis. Since ALDHs are involved in some of these biological processes, the deregulation of these enzymes may influence MDS and AML development.

Methods: To this end, we analyzed the expression levels of 8 ALDH isoforms, ALDH1A1, ALDH1A2 ALDH1B1, ALDH1L1, ALDH1L2, ALDH3A2, ALDH4A1, and ALDH16A1, in 31 patients (16 MDS and 15 LMA) and 19 healthy controls. ALDH expression levels were analyzed using RT-PCR and differentially expressed genes were quantified by qPCR. The statistical analysis was carried out by variance analysis and χ^2 test. Survival were analyzed by Kaplan Meier curves ($p < 0.05$).

Results: Preliminary results indicate that all MDS patients express ALDH16A1 isoform whereas only 67% of controls ($p < 0.05$) show expression of this isoform. Moreover, AML patients have lower ALDH1A2 expression levels than MDS and controls and only 20% of AML patients express this isoform (MDS = 54% and controls = 55%). The ALDH1L2 is only expressed in chronic myelomonocytic leukemia subtype of MDS. Furthermore, the expression of ALDH isoforms does not appear to influence patient overall survival.

Conclusion: According to these results, ALDH isoforms have differential expression patterns in MDS and AML patients when compared with controls and each other. Further studies are needed to prove their potential as a diagnostic/prognostic biomarkers.

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PS155

Discovery of novel mechanisms of centrosome amplification and their therapeutic value in cancer

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Aim: To understand the mechanisms of centrosome amplification and their therapeutic value in cancer.

Introduction: Centrosomes are the major microtubule-organising centres of animal cells. Centrosome amplification (CA) – the presence of more than two centrosomes in a cell – is a common feature in cancer[1] and was recently shown to be sufficient to drive tumourigenesis.[2] Recent work from the Bettencourt-Dias Lab has identified a new recurrent feature of cancer cells: centriole over-elongation, which also promotes CA. However, origins of those abnormalities and their therapeutic value remain poorly understood.

Methods: We have screened the NCI-60 panel of human cancer cell lines[3] for centriole number and individual length to test their frequency and interdependence. We have thereby also generated a metric capturing each abnormality level per cell line that we then correlated with the publicly available molecular (e.g. genomic, transcriptomic and proteomic) and drug-sensitivity quantitative profiles for that panel.

Results: Our single-centriole analyses showed that longer centrioles are more common in cells with CA and that cells do not control their overall centriolar mass when the centriole number increases. Moreover, cancer cell lines with longer centrioles proliferate slower due to an accumulation of cells in G1 phase, suggesting that centriole length defects could lead to a cell cycle delay in G1. In addition, our original genome-wide approach highlighted putative mechanisms associated with susceptibility to both abnormalities, such as the proteasome protecting cells from CA. Correlation with drug activity identified some compounds as potential therapeutic options to selectively target cells with higher incidence of centriole abnormalities.

Conclusion: This work provides the first single-centriole-level portrait of centriole abnormalities in cancer and contributes to the understanding of their molecular origins, namely by revealing novel molecular mechanisms in cell cycle biology. Given the cancer-specificity of these abnormalities, the identified compounds will inspire the development of drugs to selectively target cancer cells.

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PS164

Cytotoxic effects of parthenolide on lymphoid malignancies' cell lines

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Aim: The aim of this study was to evaluate the therapeutic potential of parthenolide (PRT), an NF-kB inhibitor, on acute lymphoblastic leukemia (ALL) and Burkitt Lymphoma (BL) cell lines and characterize the type of cell death induced and its molecular mechanisms.

Introduction: Playing an important role in the regulation of diverse biological processes such as cell proliferation and survival, nuclear factor kappa B (NF-kB) is closely associated with various human malignancies. Deregulated NF-kB signaling has been appointed as one important player in all stages of tumorigenesis. PRT has a dual anti-tumor effect – NF-kB pathway inhibition and oxidative stress induction – on a wide range of malignancies and could be a valid option for hematological cancer.

Methods: We used one BL (RAJI) and five ALL (697, CEM, JURKAT, MOLT-4 and KOPN8) cell lines. Cells were incubated in absence or presence of different concentrations of PRT in single dose and daily administration. Metabolic activity was assessed by Resazurin Assay. Cell death was analyzed by Optical Microscopy and Flow Cytometry (FC) using Annexin V/7-AAD double staining and JC-1 probe. Apoptotic proteins levels (FAS, FAS-L, BCL-2, BAX and activated caspase 3), cell cycle and oxidative stress parameters (superoxide anion, peroxides and reduced glutathione through the DHE, DCFH2DA and mercury orange probes, respectively) will be evaluated by FC.

Results: Preliminary results showed that PRT reduces the metabolic activity in time, dose and cell line dependent manner, being the KOPN8 and RAJI cells the most sensitive and JURKAT cells the lowest. In fact, the half maximal inhibitory concentration (IC50) at 72 h was 2 μM for KOPN8 and RAJI, 3 μM for CEM, 4 μM for 697, 6 μM for MOLT-4 and 12 μM for JURKAT. These results may be related with the cell type and genetic background. Cell death analysis suggested that PRT induced apoptosis in these cell lines. Studies on the cell cycle and oxidative stress are still underway.

Conclusion: Our results suggest that PRT is a potential new targeted therapy in lymphoid malignancies, mainly ALL and BL.

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PS169

WNT/β-catenin and Hedgehog signaling pathways as therapeutic targets in B-cell neoplasms

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Aim: The goal of this study was to evaluate the therapeutic potential of WNT/β-catenin and Hedgehog inhibitors, IWR-1 and GDC-0449 respectively, alone and in combination, in B-cell neoplasms.

Introduction: B-cell neoplasms include, among others, the B-cell lymphomas and plasma cell disorders, such as multiple myeloma (MM), a malignant neoplasm originated by proliferation of monoclonal plasma cells; and diffuse large B-cell lymphoma (DLBCL), the most common form of non-Hodgkin lymphoma. Inappropriate activation of conserved embryonic signaling pathways, such as WNT/β-catenin and Hedgehog has been implicated in B-cell neoplasms. Hence, these pathways may constitute new potential candidate targets for MM and DLBCL therapy.

Methods: For this propose, H929 (MM) and FARAGE (DLBCL) cell lines, were cultured in absence and presence of different concentrations of IWR and GDC. Metabolic activity was evaluated using resazurin assay and cell death by optical microscopy (May-Grunwald staining) and flow cytometry (FC) (Annexin V/7-AAD staining). Cell cycle analysis was evaluated by FC, using a PI/RNAse solution. Proteins related to apoptosis and some molecules related to WNT and HH signaling pathways were tested by FC. The expression levels of AXIN and SMO genes were analyzed by RT-PCR.

Results: Preliminary results showed that IWR-1 and GDC-0449 reduced metabolic activity in a time,- dose- and cell line dependent manner, when administrated alone or in combination. The IC50 of IWR-1 and GDC-0449 in H929 cells was 40 μM and 70 μM, respectively, and 75 μM and 57 μM for FARAGE cell line, after 24 h of treatment. These compounds induce cell death mainly by apoptosis and showed an arrest in cell cycle at G0/G1. Complementary studies are still ongoing.

Conclusion: In conclusion, results suggest that IWR-1 and GDC-0449 are potential new targeted therapies that could be efficient in MM and DLBCL treatment.

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Physiology & Immunology Paralell Oral Session
Friday, September 15th, 14h00

PS099

Differences in aerobic capacity and spirometric parameters between athletes and nonathletes

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Aim: To investigate if there are differences in aerobic capacity and spirometric parameters between athletes and nonathletes, and also differences in these parameters between anaerobic and aerobic athletes.

Introduction: Physical fitness is defined as ability of organism to increase level of metabolic processes due to increased level of metabolic needs. Aerobic capacity is measured by maximum level of oxygen consumption (VO2max), and it can be expressed by absolute (l/min) or relative (ml/kg/min) value. Pulmonary capacity has great evaluation importance for sport and health of general population.

Methods: Number of participants was 45 males, aged 18–35 years, divided into 2 groups: athletes and nonathletes. Athletes were divided by sport type in aerobic and anaerobic group of athletes. Testing was consisted of anthropometric measuring, spirometry and measuring of aerobic capacity on ergobycicle with mask, by principle of ramp test.

Results: Value of VO2max in group of athletes (55.46 ml/kg/min, $p < 0.05$) was significantly greater than in group of nonathletes (37.78 ml/kg/min, $p < 0.05$). Compared between all groups, VO2max showed significant difference in both aerobic (58.88 ml/kg/min, $p < 0.05$) and anaerobic (52.04 ml/kg/min, $p < 0.05$) athletes in relation to nonathletes (38.78 ml/kg/min, $p < 0.05$). Spirometric parameters (FVC, FEV1) were significantly greater in group of nonathletes (5.481 L, 4.951 L, $p < 0.05$) than in group of athletes (4.874 L, 4.635 L, $p < 0.05$). Compared between all groups, we found significant difference in FVC between group of nonathletes (5.481 L, $p < 0.05$) and anaerobic athletes (4.807 L, $p < 0.05$), and in Tiffeneau index between group of anaerobic athletes (97.29%, $p < 0.05$) and nonathletes (90.82%, $p < 0.05$).

Conclusion: Values of anthropometric parameters are greater in group of nonathletes. Differences in body weight and body mass caused greater values of FVC and FEV1 in group of nonathletes. Values of aerobic capacity are increasing with training. The greatest values of aerobic capacity are shown by aerobic athletes.

Acknowledgements: To Department of Physiology, Faculty of Medicine, Novi Sad for using their resources; To Athletic Club "Vojvodina", Novi Sad and Triathlon Club "Tryogy" for participating; To Assist. Proff. Aleksandar Klašnja for mentorship.[1–15]

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PS070

The assessment of body composition, energy demands and muscle strength in people on different dietary regimes

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Aim: The aim of this study was to determine whether there are any differences in body composition, energy demands and muscle strength between people on different dietary patterns.

Introduction: There are numerous types of diets: vegan, vegetarian, and non-vegetarian. Considering the dietary pattern, the assessment of the body composition and determining the resting metabolic rate are a major challenge for many researchers. Regarding the muscle strength of physically inactive participants related to dietary patterns, there is no current data in literature.

Methods: The study was conducted at the Department of Physiology, Faculty of Medicine University of Novi Sad from November 2016 to February 2017. The study included 45 healthy, physically inactive randomly selected respondents (15 vegans, 15 vegetarians, 15 on a mixed diet) aged 20–30 years. All respondents practiced their dietary regime for at least 6 months before research. Firstly, the anthropometric measurements were done, and later the body composition was assessed using bioelectrical impedance and by measuring skin folds. The resting metabolic rate was estimated using the indirect calorimetric method. The muscle strength was determined using the isoaccelerating dynamometer.

Results: The values of body mass index (BMI) between the group on a mixed diet (23.9 ± 2.95 kg/m^2) and vegans (20.8 ± 2.58 kg/m^2) showed a statistically significant difference ($p < 0.05$). The BMI (21.3 ± 2.63 kg/m^2) for vegetarians did no differ from the other groups. Statistically significant differences between groups in other parameters of body composition, resting metabolic rate and muscle strength were not found. A negative correlation was observed between total body fat, resting metabolic rate and muscle strength in all groups.

Conclusion: Diet differences between tested groups affected only the value of BMI between vegans and non-vegetarians. The impacts of different diets on other parameters of body composition,

resting metabolic rate and muscle strength were not confirmed by this study.

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PS146

Titin phosphorylation by protein kinase G as a novel mechanism of diastolic adaptation to acute load

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Aim: To evaluate acute adaptions of myocardial stiffness to acute stretch and characterize the underlying mechanisms.

Introduction: Systolic adaption to myocardial stretch/volume overload is known, but whether the heart is also able to modulate its stiffness following such challenges remains unknown.

Methods: Left ventricle (LV) of intact rat Langendorff hearts, rabbit papillary muscles and myocardial strips from cardiac surgery patients were acutely stretched. Skinned cardiomyocytes from Stretched and Non-stretched myocardium were studied. Stretch by increased venous return or volume loading was assessed by echocardiography in healthy volunteers; pressure-volume hemodynamics in cardiac surgery patients and in a rat model of LV hypertrophy. Myocardial cGMP, phosphorylated vasodilator-stimulated phosphoprotein (VASP) and titin phosphorylation were quantified. Pharmacological studies assessed the role of NO and natriuretic peptides (NP).

Results: After stretch, end-diastolic pressure (EDP) or passive tension (PT) decreased over 15 min in all preparations. Skinned cardiomyocytes from Stretched hearts showed decreased PT – abrogated by protein phosphatase incubation – those from Non-stretched hearts showed decreased PT after protein kinase (PKG) incubation. Stretched samples showed increased cGMP levels and phosphorylation of VASP. Titin phosphorylation was increased in Stretched samples – attenuated by PKG inhibition (PKGi). PT decay after stretch was blunted by PKGi or by joint NP antagonism, NO synthase inhibition and NO scavenging. Moreover, it was remarkably attenuated in hypertrophic rat hearts which showed reduced titin phosphorylation and no increase with stretch. Healthy volunteers and cardiac surgery patients showed E/E' and EDP decrease after sustained stretch maneuvers, respectively.

Conclusion: We describe a novel physiological mechanism whereby myocardial compliance is increased in response to stretch/volume overload, by titin phosphorylation through cGMP-PKG signaling. The mechanism was translated to human physiology and may be abolished in the hypertrophic heart (potential role in the pathophysiology of heart failure with preserved ejection fraction).

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PS172

Cannabis sativa tetrahydrocannabinol (THC) impact on placental endocrine function

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Aim: The main goal of this work is to understand the impact of THC on placenta endocrine function.

Introduction: Cannabis sativa-based medicines have been used to help ease pain, nausea and loss of appetite in cancer and HIV patients. Endocannabinoid system plays an important role in the regulation of female fertility and pregnancy. This system is implicated in proliferation, differentiation and apoptosis of placental stem cells, the trophoblasts (1). These mediate critical steps such as hormone production, fetal immune protection and increase in maternal vascular blood flow. Previous studies have shown that cannabis consumption during pregnancy is associated with intrauterine growth restriction, preterm labor and low birth weight. Moreover, tetrahydrocannabinol (THC), the main psychoactive compound of cannabis, is able to cross the placental barrier. However its effect on trophoblasts turnover and hormone production are unknown.

Methods: Term placental explants were treated with THC [1–40 μM] for 24 h to 72 h. The relative mRNA levels of 3β-HSD, aromatase, leptin and PP13 were determined by qRT-PCR. The protein expression levels of 3β-HSD, aromatase and leptin were assessed by Western Blot. Progesterone, estradiol and β-human chorionic gonadotropin (β-hCG) levels were measured by ELFA.

Results: After 24 h, PP13 mRNA levels were significantly increased at 40 μM of THC, while for leptin this effect was observed at 10 μM. Moreover, after 72 h aromatase mRNA levels were increased, while there was no effect on 3β-HSD. No differences were observed regarding progesterone whilst, an increase in estradiol and β-hCG with 40 μM at 72 h was detected.

Conclusion: These findings suggest that THC may impair trophoblast turnover and endocrine function which may affect pregnancy outcome. Moreover these results may contribute to disclose the cellular effects of cannabis-derived drugs.

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PS219

Reactivity of the rat distal colon to autoantibodies targeting angiotensin type I receptors

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Aim: To describe the reactivity of the rat distal colon to AT1R-Abs and to compare it to that of Ang II.

Introduction: Agonistic IgG (IgG1 and IgG3 subclasses) autoantibodies against the angiotensin II type 1 receptor (AT1R-Abs) have been associated with hypertension, preeclampsia, placental ischemia, renal-allograft rejection and systemic sclerosis. It is though that AT1R-Abs mimic the action of angiotensin II (Ang II) and contribute to the physiopathology of several diseases and the associated complications.

Methods: Male Wistar rats (9–12 weeks of age) were killed by decapitation and strips of the distal colon were mounted in organ baths along their longitudinal axis. Tissues were stretch to 1 g of resting force and isometric responses to AT1R-Abs (25, 50 and 100 mg/dl) obtained from sera of systemic sclerosis and renal-allograft rejection patients and to Ang II (10pM-1 μM) were recorded on a polygraph. The response of Ang II were expressed as % of the response to 125 mM potassium chloride (KCl).

Results: AT1R-Abs caused a long-lasting response. Very often, AT1R-Abs induced an increase in the frequency and amplitude of distal colon spontaneous contractions. Occasionally, AT1R-Abs caused a slight decrease in the resting tone and, more rarely, they caused colonic contraction. The effects of the AT1R-Abs seem to be attenuated by candesartan. The pattern of the response to Ang II was different; Ang II caused a fast developing contraction of the colon with an Emax of 64.37 ± 12.68 (%KCl) and EC50 of 1.22 ± 0.20 nM.

Conclusion: AT1R-Abs change the normal rhythm of spontaneous contractions of the rat distal colon but more studies are necessary to evaluate whether this reactivity is mediated by AT1 receptors. Moreover, Ang II cause a marked AT1 receptor-mediated contraction of the rat distal colon.

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Public Health & Medical Informatics Paralell Oral Session
Friday, September 15th, 14h00

PS224

Intestinal colonization by antibiotic-resistant Gram negatives in children

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Aim: This study aims to further the knowledge of antibiotic-resistance in the commensal intestinal flora of children by studying the intestinal colonization by antibiotic-resistant Gram negative bacteria in portuguese children.

Introduction: Although it is known resistance to antibiotics is a growing problem worldwide, this scenario which constitutes a risk factor for infectious disease is an under-characterized reality in Portugal.

Methods: Faecal samples of 29 healthy children (4 months to 12 years-old) were collected from randomly selected localities of Portugal: Viana do Castelo ($n = 8$), Porto ($n = 6$), Braga ($n = 14$), Leiria ($n = 1$), from September 2016 to March 2017. Risk factors were assessed by questionnaire, namely antibiotic usage history and direct contact with dependent elders. Isolates were selected by spreading saline suspension (100 μL) on MacConkey agar and MacConkey agar with ampicillin (100 μg/mL), cefotaxime (2 μg/mL), and meropenem (1 μg/mL). Susceptibility profiles to β-lactam and non-β-lactam antibiotics were assessed by disk-diffusion methods according to the EUCAST. Presumptive identification of the isolates was performed with CHROMagar-Orientation culture media.

Results: In a total of 29 isolates (lactose fermenters ($n = 22$) and lactose non-fermenters ($n = 8$)), 28 showed resistance to amoxicillin and 13 to amoxicillin with clavulanic acid. Of the 29 children analysed, 17 showed resistance to at least one of the antibiotics studied. Four children were colonized with bacteria resistant to cephalosporins ($n = 8$), two of which have daily contact with elders.

Conclusion: The results indicate that young children might be an important reservoir of commensals with clinically relevant resistance mechanisms. The clarification of this reality in Portugal could prove essential in the fight against silent dissemination of these threats and persistent infections.

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PS187

Is the oral mycobiome of young adults influenced by the delivery mode?

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Aim: To investigate whether the mode of delivery influences the oral yeast colonization in young adults.

Introduction: The human microbiome is a complex ecosystem that varies considerably across the body and between individuals.[1] Postnatally the child is exposed to microorganisms from maternal and environmental sources and influenced by infant feeding, developing its own microbiome that will continue evolving throughout life.[2] Several studies have been carried out to determine the influence of the mode of delivery on the oral microbiome, and some influence on bacterial colonization has been verified.[3,4] However, the influence on oral fungal colonization is still unknown.

Methods: In 200 healthy students from the Faculty of Dentistry of University of Porto, colonization by yeast in the oral cavity was evaluated by collecting unstimulated saliva. Yeast isolation was performed by pour-plaque technique using Sabouraud Agar medium supplemented with chloramphenicol and ChromAgar Candida medium for species identification. Statistical analysis was performed using the chi-square test and t-test for independent samples.

Results: Participants' mean age was 21.61 ± 1.86 years old, with a total yeast prevalence of 37.5%. Candida albicans was the most isolated species present in 76.5% of the colonized participants. In comparison to caesarean section, the participants born by normal delivery presented higher oral yeast prevalence (41.6% vs. 25.8%, $p = 0.035$) and higher oral yeast load (13.68 ± 38.02 vs. 1.69 ± 0.62 Log CFU/mL, $p = 0.030$).

Conclusion: Our results suggest that delivery mode influences the oral mycobiome throughout life, specifically, normal delivery appears to promote the oral yeast colonization.

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PS034

Why, how and when are patients with Chromosomal anomalies hospitalized?

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Aim: We aim to describe Chromosomal anomalies (CA) related hospitalizations characteristics and specific trends in order to understand why, how and when are these patients hospitalized.

Introduction: CA affect approximately 2% of the world population.[1] Due to this low prevalence not many studies regarding hospitalizations are available in this set of conditions. Hospitalizations represent an overall health and prognosis indicator that may allow the implementation of specific health care policies regarding prevention measures to avoid CA-related hospitalizations.

Methods: A retrospective observational study was performed using a national hospitalization database that gathers all public hospital admissions between 2000 and 2014. CA were selected based on codes 758.0× to 758.7× codified by the International Classification of Diseases – 9th Revision – Clinical Modification. Birth date, sex, charges, admission/discharge date, discharge status, primary/secondary diagnoses were analyzed for each specific CA.

Results: CA related hospitalizations accounted for 0.08% of all the hospitalizations. Down syndrome represented 75.9% of all CA-related hospitalizations and 80.2% (approximately 30M€) of all the charges attributed to CA related hospitalizations. The median age of CA-related patients was 9.0 years old. The leading causes of hospitalization in different CA varied between pneumonia (3.6–18.6%) and live birth related diagnoses (7.9–52.5%). Mean number of hospitalizations ranged from 1.0 to 2.1 per patient and mean charges per hospitalization varied from 2 339 to 4 520 €.

Conclusion: CA hospitalizations have high mean charges per hospitalization, high length of stay and high in-hospital mortality. Down syndrome accounts for the majority of CA hospitalizations, representing the CA with higher economic burden in the health system. Klinefelter syndrome hospitalizations occur at a younger age than the described mean age of diagnoses in all Klinefelter syndrome patients, a novel finding not previously described.

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PS195

Efficiency of web application and spaced repetition algorithms as an aid in preparing to practical examination of histology

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Aim: The aim of this study is to evaluate impact of using web application on the results of histology practical exam as well as to check if the SuperMemo-based algorithm is a useful tool in medical education.

Introduction: Students in medical disciplines are looking for new learning strategies. Computer applications are becoming more popular as they use a variety of methods to improve efficiency of studying. One of them is spaced repetition algorithm like SuperMemo.

Methods: We prepared web application which shows the photography of histological slide. Students had to decide if they have recognized the slide and the program was measuring time of each answer. Then the algorithm allocated new slide to display.

Users were randomly divided into two groups: study – where difficult slides were shown more frequently (SuperMemo2-based algorithm) and control – where the slides were displayed randomly.

Quality of the student's answers was evaluated according to the 6-point scale, where 0 means incorrect answer, and from 1 to 5 – correct answer depending on time.

We also took into consideration results of histology practical exam (0–15 points).

The level of statistical significance was set at $p < 0.05$.

Results: The study involved 204 first year medical students. The study group ($n = 98$) and control ($n = 106$) were similar in terms of the average number of responses in application (901 vs. 858; $p = 0.73$).

We have shown a statistically significant difference which indicate obtaining higher examination score by students who used our application – 11.8 vs. 10.98 ($p = 0.016$).

There was no superiority of spaced repetition algorithm over the random allocation of slides, based on the examination results (11.7 vs. 11.9; $p = 0.73$).

Conclusion: The usage of computer programs can be a valuable complement to traditional teaching methods. As we showed in this study it may have a measurable effect on examinations results of the students.

Acknowledgements: The approval of the Jagiellonian University Bioethics Committee was obtained.

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PS008

The frequency of Human Parvovirus B19 infections in Vojvodina

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Aim: Determininating the seroprevalence of IgG antibodies among residents of Vojvodina, as well as the incidence of acute infections in different age groups and with different diagnoses, especially in women of generative age and pregnant women.

Introduction: Human Parvovirus B19 is a cause of infections in patients of all age groups. Clinical manifestations vary from asymptomatic to manifest infections such as erythema infectiosum, arthropathy, heart problems, and infections in immunodeficient patients. Acute infections during pregnancy present a distinct problem, which can result in intrauterine fetal death or hydrops fetalis.

Methods: The data presented in this study are the result of serological testing for the presence of HP-B19 infections performed at the Institute of Public Health of Vojvodina, Centre for Virology, in the period from November 2015 to November 2016. Detection of specific IgG and IgM antibodies was completed by analysing 472 serum samples. Samples were tested using the ELISA test manufactured by VIRION, Germany, in the VIRION Analyzer I-2P device.

Results: Of the total number of tested subjects, an acute infection was detected in 10.8% of the cases (11.7% of pregnant women, and 7.14% of children). An acute infection was confirmed in 13.9% of the patients in a febrile state, and 7.1% of the patients diagnosed with arthritis, immune deficiency, and heart failure. Seroprevalence of IgG antibodies was confirmed in 42.8% of the tested subjects, 36.8% of pregnant women, 60.78% of non-pregnant women of generative age, and 11.03% of children. In the total sample, 46.4% of the results were negative.

Conclusion: It can be concluded that Human Parvovirus B19 exist and circulates in the population of Vojvodina. The use of rapid serological tests enables a specific etiological diagnosis, timely implementation of appropriate infection control measures, and an appropriate treatment of patients, especially those belonging to high risk groups like pregnant women are.

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PS211

E-cigarette: An effective tool to quit smoking or an additional source of nicotine?

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Aim: We sought to evaluate the effectiveness of e-cigarette use as a tool to quit or reduce smoking.

Introduction: The electronic cigarettes (known as an "e-cigarettes") gaining on popularity, especially among young people. Available evidence regarding the relationship between e-cigarette usage as a tool in smoking cessation are inconsistent.

Methods: A population based survey was performed, in a group of 3800 students from three Universities in Katowice, Poland. Self-prepared, previously validated questionnaire, included questions on e-cigarette smoking habits.

Results: Completed questionnaires were obtained from 3000 students (response rate 78.9%; mean age = 21.5 ± 2.1 yrs) of which 70% were female (F) and 30% were male (M). E-smoking was declared by 3.5% of respondents (F: 3%, M: 4.9%; $p = 0.01$), wherein 1.5% of respondents smoked only e-cigarettes (F: 1.3%; M: 1.8%; $p = 0.3$) and 2.4% of subjects were dual smokers (F: 1.6%; M: 3%; $p = 0.01$). Almost one-third (33.7%) of e-smokers used e-cigarettes as an aid to quit smoking. Only 13.8% of e-smokers tired to give up e-smoking. Almost half of e-smokers (48.8%) tends to give up e-smoking in the nearest future. Reduction in cigarette consumption (mean 6.5 ± 5.0 cigarettes/daily) was observed by 50.8% of dual smokers. Only 4.4% of e-smokers used e-cigarettes without nicotine. Since they started e-smoking, constant concentration of nicotine in e-liquid was indicated by 61.4% of e-smokers, 12.5% increased (mean 8.7 ± 5.1 mg/ml) and 26.1% reduced (mean 8.2 ± 3.5 mg/ml) nicotine content in usually used e-liquid. Among e-smokers, 48.8% reported an addiction to e-cigarettes.

Conclusion: Smoking cessation was not the main reason for e-cigarette use among most of e-smokers. Low percent of e-smokers who use a non-nicotine e-liquid and almost half of e-smokers who declared addiction to e-cigarette, suggests that e-cigarette is rather an additional source of nicotine than effective tool in smoking cessation.

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Surgery Paralell Oral Session
Friday, September 15th, 14h00

PS130

The effect of medication intake on perforation rate in patients with colonic diverticulosis – A retrospective assessment

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Aim: The aim was to study the effect of drug intake on the frequency of perforation among patients with colonic diverticulosis.

Introduction: Diverticulosis is a common condition which incidence increases with age. One of the most severe complications with a high risk of late sequel and mortality is perforation of colonic diverticula. According to the current studies the use of some medications may affect the risk of perforation due to its influence on the intracolonic pressure and mucosal barrier function.

Methods: A retrospective review of 294 patients (mean age 68.6) with verified colonic diverticulosis was done. Included patients were admitted to 2nd Department of General Surgery JU MC during 2006–2016. Study enrolled 206 (70.1%) women and 88 (29.9%) men. Among investigated group 36 (12.2%) patients developed perforation.

The research regarded medications including NSAIDs, corticosteroids, calcium-channel blockers, statins, opioids, aspirin, anticoagulants and antiplatelet drugs. In addition, the data concerning comorbidity and the severity of the diverticulitis was collected.

Results: In the analysis the group of patients with perforation and the group with non-perforated diverticulosis were compared. Higher rates of the use of NSAIDs (13.89% vs. 3.88%; OR = 4; $p = 0.01$; 95% CI = 1.28–12.46), opioids (11.11% vs. 1.55%; OR = 7.94; $p < 0.001$; 95% CI = 1.89–33.3) and corticosteroids (22.22% vs. 8.14%; OR = 3.22; $p = 0.01$; 95% CI = 1.31–7.96) were observed among the patients with perforation. The results revealed an inverse relation concerning the use of statins (5.56% vs. 22.09%; OR = 0.21; $p = 0.02$; 95% CI = 0.05–0.89). Similar results were found in the review of available literature.

Conclusion: Medications used by patients with colonic diverticulosis affect the incidence of perforation. The administration of NSAIDs, corticosteroids and opioids is related to an increased rate of diverticular perforation. Conversely, statins may contribute to the decrease in the frequency of perforation. We can conclude that it is important to carefully administer drugs to patients with colonic diverticulosis.

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PS025

De novo atrial fibrillation following aortic valve replacement surgery is associated with decreased creatinine clearance and increased C-reactive protein levels

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Aim: We aim to assess the prevalence of de novo postoperative atrial fibrillation (POAF) in patients submitted to aortic valve replacement surgery (AVRS) and evaluate clinical and echocardiographic variables as predictors of POAF occurrence in this population.

Introduction: POAF is the most common complication following cardiac surgery, with peak incidence in the second day after the surgical procedure. Studies have demonstrated an increase in the incidence of stroke, hospital stay, health- associated costs and mortality in the group of patients experiencing POAF.

Methods: We conducted a cross-sectional study, that included all the patients submitted to AVRS during 2014 in a tertiary hospital, diagnosed with severe aortic valve stenosis without endocarditis, known history of atrial fibrillation, more than one major procedure, or other significant valve disease. Data were collected retrospectively and the statistical tests were performed according to the variable classification.

Results: The incidence of POAF in the 173 included patients was 45%, with the median time of occurrence being 2.4 ± 1.5 days. A univariate analysis showed that the group of patients who developed POAF was older ($p = 0.028$), had longer median in-hospital stay ($p = 0.008$), had a significantly higher C-reactive protein (CRP) peak blood level ($p = 0.025$) and a significantly lower minimum creatinine clearance ($p = 0.026$) in the post-operative period when compared with those who did not develop POAF. A multivariate analysis confirmed age to be an independent predictor of POAF. (OR: 1.04, CI 95%: 1.00–1.09).

Conclusion: Our study suggests age, peak post-operative blood level of CRP and creatinine clearance as predictors of POAF occurrence and supports the hypothesis that POAF may be the result of inflammation, being one of the few studies that focuses on a population with isolated aortic stenosis. Our findings on increased hospital stay reinforce the idea of risk stratification and the use preventive measures in the higher risk groups.

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PS050

Predictors of early reoperation after meningioma removal

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Aim: The aim of our study was to establish predictors of unplanned early reoperations after meningioma removal.

Introduction: Complications after neurosurgical procedures which lead to reoperation are associated with poor treatment outcome and costs. The knowledge of risk factors for complications might allow to implement specific preventive measures. However those factor are still poorly defined, especially in terms of benign brain tumours.

Methods: We retrospectively analysed 177 patients, with histologically confirmed meningiomas, hospitalized between 2014 and 2016 who underwent craniotomy. From medical records

we obtained detailed medical history (previous diseases, medications, tumour characteristics, blood test results, surgery's details). Completeness of tumour resection was assessed using Simpson Grade. Early reoperation was defined as reoperation during the same hospital stay. We used χ^2 test for proportional values; *t*-student test, Mann–Whitney *U* test for continuous variables. To determine the potential predictors of early reoperation we used univariate and multivariate logistic regression analyses.

Results: A total of 13 (7.34%) patients underwent unplanned early reoperation. Those patients significantly more often had retromastoid craniotomy (25.00% vs. 6.40%; $p = 0.047$). And significantly more often suffered from ischemic heart disease (66.67% vs. 6.64%; $p < 0.01$) and atrial fibrillation (60% vs. 6.25%; $p < 0.01$). Reoperated patients also more often took heparin (50% vs. 6.74%; $p < 0.01$) and anticoagulants (66.67% vs. 6.21%; $p < 0.01$). In multivariate logistic regression analysis anticoagulants intake (OR: 31.463; 95% CI: 1.139–868.604; $p = 0.04$) and retromastoid craniotomy (OR: 6.642; 95% CI: 1.139–38.73; $p = 0.034$) remained independently associated with higher risk of early reoperation.

Conclusion: Patients who underwent retromastoid craniotomy, those with history of ischemic heart disease or atrial fibrillation and those who take heparin and anticoagulants are more likely to require early reoperation. Retromastoid craniotomy and anticoagulatns intake are independent risk factors for early reoperation.

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PS095

Prevalence of foramen arcuale and its clinical significance: A meta-analysis of 55,985 subjects

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Aim: The aim of this study was to deliver the most complex study on the prevalence of the FA and its clinical significance.

Introduction: Foramen arcuale (FA) is an osseous prominence formed in place of a sulcus for the vertebral artery on the posterior arch of the atlas. The presence of an FA can make a threat during neurosurgery by giving a false notion of a wider posterior arch when viewed dorsally during C1 lateral mass screw insertion.

Methods: An comprehensive search of the major electronic databases was performed in order to find and identify all studies which reported relevant data on the FA. No date or language restrictions were applied. Data on the prevalence, type (complete and incomplete), side, gender, laterality, and morphometrics of the FA were extracted and pooled into a meta-analysis.

Results: A total of 127 studies ($n = 55{,}985$ subjects) were included into the quantitative analysis. The overall pooled prevalence of a complete FA was 9.1% (95%CI: 8.2–10.1), while the overall pooled prevalence of an incomplete FA was 13.6% (95%CI: 11.2–16.2). The complete FA was found to be most prevalent in North American (11.3%) and European (11.2%) populations, and least prevalent among Asian (7.5%) populations, especially Chinese (4.4%) and South Koreans (5.8%). In the presence of a complete FA, a contralateral FA (complete or incomplete) was found in 53.1% of cases.

Conclusion: The FA is a commonly present anatomical structure. Awareness of a complete variant of the FA during procedures performed on the atlas vertebra is essential in reducing the risk of iatrogenic injury. Therefore, risk for the presence of an FA should be considered by surgeons prior to procedures on the atlas in each patient according to gender and ethnic group. As such, we highly advise preoperative screening with CT as the gold standard for finding the presence of an FA.

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PS136

Quality of Life and aortobifemoral bypass – Importance of the hypogastric arteries

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Aim: Evaluate SD after AFB and assess the importance of patent hypogastric arteries before the procedure.

Introduction: The aortobifemoral bypass (AFB) is one of the best options to revascularize patients with Aortoiliac Occlusive Disease (AIOD). The impact of this procedure in sexual function (SF) is unpredictable, with 20–80% of the patients reporting sexual dysfunction (SD) after surgery. There's still insufficient data to safely predict the development of SD after AFB and what the role of hypogastric arteries.

Methods: The study includes only male population submitted to AFB due to AIOD. Patients with major amputations after the surgery were excluded. The development of SD was evaluated by phone call. The quality of life before and after the procedure was evaluated by a standardized index score questionnaire (15D). Pre-operative patency of hypogastric arteries was appraised by assessing the patients imaging file. The arteries with direct anterograde flow were considered patent.

Results: Of a total of 53 patients, 40 were included in the study – exclusion causes were intrahospital death, natural cause death and major amputation. In the included group, 37% reported worsening, 26% improved and 37% did not notice any change in SF after surgery. If at least one of the hypogastric arteries was patent before surgery, 58% described worsening in SF compared to only 13% in the group with no sustained anterograde flow to the hypogastric arteries. 92% of the patients was not warned of the possibility of SD after surgery, being that 26% of these would have refused the procedure if they knew.

Conclusion: SD is a prevalent and often overlooked complication after open aortoiliac revascularization and it remains a major taboo in the surgeon/patient relation. The existence of at least one hypogastric artery with preserved anterograde flow before

surgery can strongly predict a higher risk of sexual dysfunction after surgery.

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PS197

Compensatory renal overgrowth after unilateral nephrectomy in children

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Aim: The aim of the study is to investigate the intensity of renal overgrowth after unilateral nephrectomy in children's population, as well as to check dependency between kidney's dimensions and patient's age.

Introduction: Solitary kidney after unilateral nephrectomy tends to overgrow. In adult population the dynamic of overgrowth and maximal dimensions are identified. In childhood there are no described patterns of the process of solitary kidney overgrowth.

Methods: Patients who had undergone unilateral nephrectomy in the University Children's Hospital of Cracow were enrolled. The length of the solitary kidney was compared with control group which was based on ultrasound examination of the kidney (left $n = 1601$, right $n = 1635$) performed in the same clinic in children without kidney disease. All examinations were carried out with Philips Epiq 5G ultrasound unit with convex probe C5-1 MHz by a single physician (PS).

The comparison was analysed with t-student test for one or two means. 18 children (7 males) from the birth to the age of 17 who underwent in total 48 ultrasound examination after the nephrectomy were enrolled.

Results: There was significant difference between the mean of the kidney's length in patients after unilateral nephrectomy and control group. The difference was the most explicit in the groups at the age from 8 to 13 for the right kidney (difference range from 13 to 22 mm, $p < 0.05$) and in the groups at the age from 8 to 12 for the left kidney (difference range from 11 to 19 mm, $p < 0.05$). Solitary kidney in children after nephrectomy is significantly larger than in the control group.

Conclusion: The dynamic of solitary kidney overgrowth in children should be taken into consideration while performing the sonographic examination. Chronic kidney disease may be suspected when overgrowth of the solitary kidney is not present. Further research dealing with the dynamic of compensatory kidney overgrowth in children is indicated.

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Internal Medicine Plenary Session
Saturday, September 16th, 14h00

PS067

Cardiac effects of Ledipasvir plus sofosbuvir for Hepatitis C treatment in thalassemia

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Aim: This study was designed to evaluate the effects of Ledipasvir plus Sofosbuvir on cardiac function of thalassemia patients.

Introduction: Hepatitis C (HCV) infection is much more prevalent in thalassemia patients because of blood transfusion. Thalassemia patients may also have cardiac abnormalities due to congenital problems, anemia, and increased burden of iron in their myocardium. HCV treatment has been revolutionized after introducing new direct acting antiviral (DAA) drugs, and data is limited about effects of these new drugs on patients' cardiac function.

Methods: In this study HCV-infected thalassemia patients which were selected for treatment with DAAs in HepCC-2 trial (NCT03061032) were evaluated prospectively. Fixed dose daily tablets of 90 mg-Ledipasvir plus 400 mg-Sofosbuvir (12/24week, ±ribavirin) was prescribed for patients. All patients were evaluated by a unique echocardiography fellowship for collecting the echocardiography findings of before and after the treatment. Then effects of mentioned drugs on patients' cardiac function were evaluated.

Results: Thirty-two patients with mean age of 24.2 ± 6.4 years were evaluated. The treatment response, which was evaluated by rapid virological response and sustained virological response rates, was 100%. The patients' left ventricular end-systolic diameter (LVESD) and volume (LVESV), global longitudinal strain (GLS) of LV and average, and right ventricle (RV) size were significantly increased after finishing the treatment ($P < 0.05$). Changes in abovementioned parameters were not correlated with patients' myocardium iron load ($P > 0.05$). There were no significant differences in before-after comparison of other echocardiographic parameters ($P > 0.05$).

Conclusion: Ledipasvir-Sofosbuvir combination therapy was safe for our HCV-infected thalassemia patients and cause no serious cardiac events. But minimal changes in strain, size, and volume of left ventricle, and size of right ventricle may refer to needing more precise cardiac evaluations in these patients. Also, our patients' ejection fraction remained unchanged. Hence, we suggest more specific and long-term echocardiographic evaluations before and after treatment, if needed.[1–3]

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Neurosciences Plenary Session
Saturday, September 16th, 14h00

PS141

Paclitaxel-induced neuropathic pain: Unravelling the underlying mechanisms at the central nervous system

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Aim: Here we studied the effects of the cytostatic paclitaxel on: (i) the development of nociceptive and aversive behaviors; (ii) noxious-evoked-activation of spinal dorsal horn neurons and (iii) on descending noradrenergic modulation, which is the main spinal nociceptive inhibitory system.

Introduction: Chemotherapeutic drugs are widely used for cancer treatment but they also cause numerous deleterious side effects. Chemotherapy-induced neuropathy (CIN) is one of the most common side effects. The mechanisms underlying CIN are starting to be uncovered namely the alterations induced by cytostatics at the peripheral nervous system but the effects of these drugs at the central nervous system are still poorly studied.

Methods: Male Wistar rats were injected with paclitaxel (Taxol, 2.0 mg/kg) or the vehicle solution dimethyl sulfoxide on four alternate days. Nociceptive and aversive behaviors were assessed by the von Frey and conditioned place aversion (CPA) tests, respectively. Noxious-evoked-activation of spinal dorsal neurons was achieved at one month after CIN by evaluating the expression of c-fos expression upon cold stimulation. To study the descending noradrenergic pain modulation we assessed the effects of the α2-adrenoreceptor agonist clonidine at 1 and 10 μg administered intrathecally, on the von Frey test. We further assessed the expression of the α2-adrenoreceptor and dopamine-β-hydroxylase (DBH), a noradrenaline biosynthetic enzyme expressed in noradrenergic fibers, at the spinal dorsal horn.

Results: Paclitaxel induced mechanical allodynia and aversive behaviors. c-fos and DBH expression were increased in paclitaxel-treated animals while α2-adrenoreceptor expression remained unaltered. Clonidine induced antinociception at both doses with more pronounced effects in paclitaxel-treated animals.

Conclusion: Paclitaxel-treated animals showed neuropathic like-behaviors and increased spinal neuronal activation. It remains to ascertain if DHB upregulation results in increased spinal noradrenaline levels, but the increase of α2-AR antinociceptive potency in paclitaxel-treated animals indicates the recruitment of descending inhibition probably as a buffer to increased spinal sensitization.

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Oncology & Molecular Biology Plenary Session
Saturday, September 16th, 14h00

PS203

Is there horizontal transfer of the oncogene BCR-ABL mediated by extracellular vesicles released by chronic myeloid leukemia cells?

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Aim: The aims are to verify if: (i) EVs released by CML cells carry BCR-ABL in their cargo and if that BCR-ABL is captured by recipient cells; (ii) EVs released by a CML drug resistant cell line, with mutant BCR-ABL, may transfer mutant BCR-ABL and a resistant phenotype to sensitive cells.

Introduction: BCR-ABL, the fusion gene originated by the t(9;22) translocation, is responsible for Chronic Myeloid Leukemia (CML). BCR-ABL codes for a constitutively active tyrosine kinase (TK), deregulating downstream pathways and promoting cell survival. Imatinib mesylate (Gleevec), a TK inhibitor, is the gold standard treatment for CML; nevertheless, resistance to this drug often arises, mostly caused by additional point mutations on BCR-ABL and representing a major clinical drawback. It was recently suggested that drug resistance might be horizontally transferred by EVs, from resistant to sensitive cells.

Methods: A pair of drug-sensitive BCR-ABL+ cell line (KBM5), and its drug-resistant counterpart (KBM5-STI, harboring mutated BCR-ABL) were used in this study. EVs were isolated by ultracentrifugation and characterized by Dynamic Light Scattering, Nanoparticle Tracking Analysis, Transmission Electron Microscopy and Western Blot. The resazurin assay was used to assess drug response of drug resistant cells, drug sensitive cells and of drug sensitive cells following co-culture with EVs released by drug resistant cells. BCR-ABL levels were analysed by Western Blot.

Results: A dose-response curve to imatinib was performed in both cell lines, to confirm their different responses to the drug. Regarding EVs characterization, they had between 10 and 1000 nm and presented several markers of EVs with no evidence of cellular contaminants. Interestingly, BCR-ABL protein was detected in the EVs.[1–9]

Conclusion: These results suggest that there is selective packaging of BCR-ABL into EVs, promoting oncogenic protein shedding. Ongoing work will clarify if the EVs released by the resistant cells have mutant BCR-ABL and if they confer drug resistance to recipient sensitive cells.

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Physiology & Immunology Plenary Session
Saturday, September 16th, 14h00

PS030

Assessment of left ventricular systolic and diastolic function in diabetic rat model using Electrocardiography-gated 18F-FDG PET imaging

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Aim: In this study, we explore the potential of ECG-gated 18F-FDG PET to assess LV systolic and diastolic function in a well-stablished rat model of type 2 diabetes.

Introduction: Left ventricular (LV) diastolic dysfunction, defined as a disruption of the normal filling pattern of the ventricle but normal systolic function, is one of the early signs of cardiac involvement in diabetic patients.

Methods: List-mode gated 18F-FDG PET imaging was performed on a rat model of type 2 diabetes (ZDF fa/fa) ($n = 6$) and ZL control rats ($n = 6$) at age of 13 weeks 15–30 min after tracer-administration (37 MBq) via tail vein under hyperinsulinemic-euglycemic clamp using a dedicated small animal PET system (Siemens Inveon) with ECG signal recording for 20 min. List-mode data were sorted and reconstructed into tomographic images of 16 frames per cardiac cycle. PET images were resized to match human-scale pixels. Left ventricular functional parameters were calculated using standard clinical software program (Heart Function View).

Results: Hyperinsulinemic-euglycemic clamp and post mortem tissue analysis demonstrated the development of diabetes in the ZDF rats and of significant myocardial hypertrophy in ZDF rats at age of 13 weeks (994 ± 78 mg vs. 871 ± 44 mg in ZDF rats vs. ZL controls, $p < 0.01$, respectively). The PET images analysis showed a mild but significant decrease of LV PFR in the ZDF rats (10.4 ± 0.5 vs. 11.8 ± 0.4 EDV/s in ZDF rats vs. ZL controls, $p < 0.001$, respectively), whereas no significantly differences concerning LVEF and cardiac output (CO) could be detected between model and control rats (LVEF: 60.0 ± 4.5 vs. $63.7 \pm 4.1\%$, $p = 0.25$ and CO: 90917 ± 14015 vs. 85208 ± 17511 μl/min, $p = 0.90$, respectively).

Conclusion: In a rat model of type 2 diabetes, we demonstrated the ability of ECG-gated-18F-FDG PET together with a clinical ventricular edge detection software to assess reliable LV systolic and diastolic parameters and to detect the presence of a diastolic dysfunction in the diabetic rats.

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Public Health & Medical Informatics Plenary Session
Saturday, September 16th, 14h00

PS045

Prevalence of dietary supplements and over-the-counter drug use in patients with arterial hypertension

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Aim: Analysis of frequency of use of DS/OTC among patients with arterial hypertension as well as factors determining its use and patients' knowledge about possible interactions with conventional medication.

Introduction: Dietary supplements (DS) and over-the-counter drugs (OTC) are frequently advertised as a natural treatment of many disorders. DS/OTC can interfere with biotherapeutic action of prescribed medication and this is of particular concern in patients with cardiovascular disease, many of whom are on long term treatment.

Methods: The study was conducted in the Outpatient Hypertensive Clinic in the Tertiary Cardiac Center. Self-prepared questionnaire was administered among 151 hypertensive patients (58% females, age range 18–80 years). Regular DS/OTC use was defined as taking them at least 3 times per week.

Results: In the examined population regular use of DS/OTC was declared by 67% subjects. The most commonly, regularly used substances were minerals and microelements (60.4%), vitamins (48.5%), analgesics (18.8%), drugs increasing the immunity (18.8%), relieving the gastrointestinal symptoms (18.8%) and omega acids (18.8%). There were no differences in the frequency of DS/OTC use in relation to number of antihypertensive drugs, educational level, age and income. Women are more frequent regular users of DS/OTC than men ($n = 65$ vs. $n = 36$, $p = 0.03$). Only 38% of responders always consulted the use of DS/OTC with a doctor. The majority of responders (52%) is not aware of possible influence of DS/OTC on antihypertensive medication or blood pressure control. Cost of DS/OTC in 23% of responders is equal or higher than cost of prescribed drugs.

Conclusion: Two thirds of hypertensive patients are regularly using DS/OTC. Half of them are not aware of possible interactions with antihypertensive therapy and influence of blood pressure control. The perception that nonprescription therapies are unnecessary to report during medication history taking should be changed. DS/OTC are the important position in the responders budget.

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Surgery Plenary Session
Saturday, September 16th, 14h00

PS125

Do patients after bariatric surgery change their physical activity habits? A prospective one-year follow-up study

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Aim: The purpose of this study was to assess whether patients have changed their physical activity habits one year after the bariatric surgery.

Introduction: Vast majority of obese patients have developed their condition by overeating and insufficient physical activity. Severe obesity leads to problems with locomotor system and constraint movability, resulting in closure of the vicious circle of gaining weight. Bariatric surgery is an effective weight loss method, but it is still unclear whether this procedure influences modification of physical activity routine.

Methods: 54 patients (55.56% females, $n = 30$) who underwent bariatric surgery at 2nd Department of General Surgery JU MC in Cracow, Poland from November 2015 to June 2016 were enrolled to this prospective study. Mean age, BMI and absolute waist circumference of participants were respectively: 43.6 ± 12.2 y.o., 45.94 ± 6.35 kg/m^2 and 128.39 ± 13.45 cm (female), 146.9 ± 17.21 cm (male). One day before the procedure and one year later participants were asked to complete two standardized questionnaires: Paffenbarger Physical Activity Questionnaire and WHO Global Physical Activity Questionnaire, on the basis of which average physical activity in metabolic energy equivalents (MET-minutes) per week has been estimated

Results: One year after surgery MET-minutes has increased over 14 times (Me:299.75, Q1–Q3:225.78–358.38 vs. Me:4339.85, Q1–Q3:1590.6–7827.1, $p < 0.00001$). Average time sitting or reclining has reduced from 480 to 300 min per day ($p = 0.00118$). Mean pace of walking has changed from <3.2 km/h to average 3.2–4.8 km/h ($p = 0.00406$). Participants were also asked to rate on visual analogue scale their level of exertion during normal activities. This parameter has decreased from mean 5 (equivalent of strong effort) to mean 2.5 (equivalent of weak effort) ($p = 0.00004$).

Conclusion: Before the procedure none of participants has achieved recommended by WHO weekly level of 600 MET-minutes and after surgery 81% of them have exceed it. This data have shown significant positive changes in physical activity in patients who underwent bariatric surgery.

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Internal Medicine Poster Session
Friday, September 15th, 10h00

PS085

Comparison of liver biopsy and non-invasive APRI test in assessing the stage of liver fibrosis in patients with chronic HCV infection

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Aim: The aim of this study was to evaluate the performance of non-invasive APRI score in predicting significant fibrosis and cirrhosis in patients with chronic HCV infection who underwent liver biopsy.

Introduction: Determining the stage of liver fibrosis is essential in managing patients with chronic hepatitis C virus. In chronic HCV infection, liver biopsy is the gold standard method for assessing stage of liver fibrosis, but it is invasive with potential complications. Non-invasive markers have been proposed and APRI score (aspartate aminotransferase (AST)-to-platelet ratio index) has been shown as a simple and inexpensive marker of liver fibrosis.

Methods: This retrospective study included 142 patients with chronic hepatitis C who had undergone liver biopsy from January 2013 to December 2015. Liver fibrosis was staged according to METAVIR (F0-F4) scoring system. The diagnostic performances of APRI score in predicting significant fibrosis (F2-F4) and cirrhosis (F4) were evaluated and compared by ROC curves.

Results: Fifty-three (37.3%) patients had significant fibrosis and 18 (12.7%) had cirrhosis. The areas under the ROC curve of APRI for predicting significant fibrosis and cirrhosis were 0.76 and 0.81. Using recommended cut-off values for APRI test, significant fibrosis could be identified in 26% and cirrhosis in 22% patients, but specificity for significant fibrosis was 88% and for cirrhosis 91%. Results have also shown that lower platelets count in our patients is associated with higher stage of fibrosis ($p < 0.0001$).

Conclusion: APRI test shows low sensitivity and high specificity in the distinction between mild and significant fibrosis, and it shows good sensitivity in the evaluation of patients without cirrhosis and excellent specificity in patients with cirrhosis. Non-invasive biochemical tests and scores should be used only as additional criteria in differentiating the stage of liver fibrosis in chronic HCV infection, along with other non-invasive methods.

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PS144

Characteristics of patients with diagnosed chronic fungal rhinosinusitis surgically treated at the Clinic for otorhinolaryngology, Clinical centre of Vojvodina, in the past five years

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Aim: To determine the characteristics of patients with chronic fungal rhinosinusitis surgically treated at the Clinic for Otorhinolaryngology, Clinical Centar of Vojvodina, from 2011 to 2016.

Introduction: The incidence of fungal rhinosinusitis in European counties is steadily growing. The reason behind this is the increased usage of immunosuppressive therapy, antibiotics and changes in everyday behaviors (increased stays in rooms with air-condition).

Methods: The study included 21 patients diagnosed with fungal rhinosinusitis. The patient's data was collected from their medical history.

Results: The mean age of the patients was 45 ± 16.51, with females being more often affected (11/21). The most commonly affected sinus was the maxillary sinus (54.67%), after that the sphenoid (20.83%), posterior ethmoid (18.5%), anterior ethmoid (8.33), and frontal (4.17%). Aspergillus was the most common cause (57.14%), mucormycosis was found once (4.78%). Staphylococcus aureus was isolated in 7 (33.33%) patients. Clinical symptoms were dominated by intensive facial pain and nasal secretion (found in all patients). Nasal congestion was present in 85.71% patients, less common was loss of sense of smell, in 47.62%. Endoscopic results showed significant differences between the characteristics of mucosa and mucus of the healthy and affected side of the patient's face. Significant differences are present in CT scans in all sinuses when the sinuses of the healthy and affected side of the patient's face were compared.

Conclusion: Clinical symptoms of patients with fungal rhinosinusitis were dominated by facial pain, nasal secretion and nasal congestion. Endoscopy shows pathological changes in the mucosa of the affected side of the patient's face, with viscous mucous secretions. Intraoperative findings show unilateral affection of the sinuses in all patients, most commonly in the maxillary sinus. The sphenoid snius was less commonly affected, the ethmoid and frontal were rarely affected. Aspergillus is proven to be the most common cause.

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PS175

Predictors for recurrent spontaneous intracerebral hemorrhage: A retrospective study

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Aim: The aim of this work was to determine predictors for recurrence of intracerebral hemorrhage (ICH), which may allow the identification and more appropriate management of patients at higher risk of recurrent ICH.

Introduction: The prognosis of recurrent bleeding seems to be worse than that of the first ICH. However, only a limited number of studies report the frequency of recurrence in ICH and attempt to characterize which factors may be associated with tendency for repeated hemorrhagic events.

Methods: We analyzed data from 549 patients admitted for treatment of ICH at the Unit of Faro of the Algarve Hospital Center, followed over a period of 5 years. 189 patients with a Rankin at discharge equal to 6 were excluded from the analysis.

Results: We identified 24 patients (6.7%) with recurrent ICH. Recurrence was significantly more frequent in woman (10.9%) then in men (4.4%) ($p < 0.05$). By comparing patients with recurrent ICH with patients with isolated ICH, we found that recurrence was associated with more advanced ages at the time of the first ICH, but only for men (76 years for recurrent ICH and 68 years for isolated ICH) ($p < 0.05$). There was a tendency towards recurrence when the bleeding was lobar (33% of recurrence, and 24% for non-lobar bleedings), although no statistical significance could be found. Other factors, such as previous hypertension were not associated with increased risk of recurrent ICH.

Conclusion: In this series of patients, we identified sex and age as predictors for ICH recurrence.

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PS133

Randomized study to compare two methods of e-learning of ECG interpretation among medical students

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Aim: To compare the effectiveness of two methods of ECG e-learning among medical students: collaborative e-learning (C-el) and individual e-learning (I-eL).

Introduction: Electrocardiogram (ECG) interpretation is an essential skill in medicine. The best method of ECG education has not been determined.

Methods: Sixty 5th-year students from the Jagiellonian University Medical College were randomly assigned in a 1:1 ratio to the C-eL and I-eL groups. C-eL group students were further randomly divided into 6 subgroups of 5 students. Students from the I-eL group received by e-mail an ECG recording with comprehensive description every second day; at that time students from the C-eL group received the ECG recording without any description. C-eL students were encouraged to cooperate in analyzing the ECG in subgroups using internet platform and were expected to submit interpretation of the ECG recording to coordinator after 48 h. Afterwards they received comprehensive description of the ECG. Before starting the study all students participated in a pretest assessing their basic theoretic knowledge. The effects of e-learning were assessed at a final e-test. The main endpoint of the study was the number of students who passed the final e-test.

Results: Basic knowledge was similar in both study groups. Students from the I-eL group answered correctly to 9.0 ± 1.0 ($90 \pm 10\%$) and from the C-eL group to 9.5 ± 0.6 ($95 \pm 6\%$) questions, $p = 0.07$.

The main endpoint was achieved more frequently in the C-eL than in the I-eL group: 17 (63%) vs 10 (35.7%) students respectively, $p = 0.045$. C-eL group students, as compared to I-eL group students, achieved more points in the final e-test (12.3 vs. 11.0 points respectively, $p = 0.036$) and also better results in ECG interpretation (4.1 vs. 3.4; $p = 0.03$).

Conclusion: Collaborative e-learning of electrocardiography in 5th year medical students is superior to individual e-learning.

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PS096

The influence of smoking cessation-related weight gain on cardiovascular risk in patients treated with drug-eluting stent after acute coronary syndrome

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Aim: Aim of this study was to analyse association of smoking cessation influence on long-term clinical outcomes in patients with an acute coronary syndrome (ACS).

Introduction: Smoking and obesity are important cardiovascular risk factors. Patients often put on weight after quitting smoking.

Methods: 137 consecutive ACS patients of the Department of Coronary Heart Disease John Paul II Hospital in Cracow admitted between 2011 and 2013 were enrolled in the study. They had no previous history of Coronary Heart Disease and underwent Percutaneous Coronary Intervention with implantation of at least one Drug Eluting Stent. Telephone follow-up was carried out after a minimum time of 3 years. Patients were divided into 2 groups: patients who stopped smoking (91) and non-smokers (46) which were compared according to weight gain, increased of the BMI, morbidity of diabetes mellitus (DM), reasons of admission to hospital, occurrence of another ACS, stroke and neoplasm.

Results: The population consisted of 66% males, 34% females mean age 67 SD 11.29. Patients who used to smoke were significantly younger than non-smokers (64.,99 vs. 71.37; $P = 0.048$). Both groups did not statistically differ in terms of gender and frequency of DM. The ex-smokers were admitted more frequently due to STEMI while in the non-smokers NSTEMI and Unstable Angina predominated. The weight and BMI in both groups did not differ on the date of ACS. However after 3 years there was a statistically significant difference: ex-smokers put on weight on average 1.3 kg while non-smokers lost 2.17 kg ($p = 0.01$). There was no association between the patients' history of smoking and occurrence of stroke, malignancy or another ACS.

Conclusion: Smoking cessation does not appear to influence long-term clinical outcomes after ACS. However it associates with weight gain which obviously increases cardiovascular risk. Our findings need further investigation and follow-up in a larger cohort of ACS patients.

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PS059

Neonatal abstinence syndrome – Retrospective review

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Aim: To evaluate the characteristics of newborns diagnosed with neonatal abstinence syndrome (NAS) and the characteristics of their mothers in Vojvodina from 2012 to 2016., as well as the interrelationship of certain features.

Introduction: NAS is a collection of symptoms and signs that occur as a result of the sudden interruption of fetal exposure to certain substances (methadone, heroin, buprenorphine, etc.) that were used or abused by the mother during pregnancy.[1,2] It is manifested in a multitude of symptoms including central nervous system irritability, over-activity of the vegetative nervous system and dysfunction of the gastrointestinal tract.[3,4] The occurrence of NAS is closely related to the maintenance therapy of pregnant opioid addicts.[1]

Methods: This study analyzed medical records of women who gave birth at the Clinic of Ginaecology and Obstetrics in Novi Sad, whose children were diagnosed with NAS after birth, as well as the medical records of newborns treated at the Neonatology Department of the Institute for Child and Youth Health Care of Vojvodina diagnosed with NAS. Medical records included data from the medical history of the newborn and personal and gynaecological medical history of their mothers.

Results: A total of 41 cases of NAS were registered. An increase in incidence was noticed during the five-year period of about 15%. Mothers were mostly unemployed (80.49%). Slightly more than half of respondents (57.5%) during pregnancy were on one of substitution treatment modalities. The majority of newborns with NAS (75.61%) were male. The clinical picture was significantly more expressed in children whose mothers consumed methadone, compared to mothers who consumed heroin during pregnancy ($p = 0.0002$).

Conclusion: The incidence of diagnosed NAS cases is growing. Representation of male newborns with NAS is three times higher than female newborns. Methadone cause more NAS symptoms than heroin.

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PS176

Associations of epicardial adipose tissue thickness and cardiometabolic risk factors in STEMI patients treated with percutaneous coronary intervention

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Aim: To evaluate EAT thickness in STEMI patients treated with percutaneous coronary intervention (PCI) and its associations with body mass index (BMI), blood lipids and acute left ventricular dysfunction.

Introduction: Epicardial adipose tissue (EAT), located between the myocardium and visceral layer of pericardium is an emerging risk factor for cardiometabolic diseases.

Methods: The retrospective study consisted of patients hospitalised for STEMI treated with PCI from 2014 to 2016. EAT thickness was measured from the parasternal long-axis view at end-diastole. Cholesterol levels were determined in a blood sample. According to median patients were divided in two groups: thin EAT group (<2.27 mm, $n = 270$) and thick EAT group (≥2.27 mm, $n = 223$). Statistical analysis was performed with SPSS using Mann–Whitney test, *T*-test, logistic regression analysis. Values of cholesterol levels were evaluated by ROC curves. $p < 0.05$ was significant.

Results: Total 492 patients (332 males, 66.62 ± 12.24 year-old) were enrolled. Groups did not differ by age, gender, morbidity of diabetes mellitus and triglyceride levels. Patients had higher BMI (29.41 ± 4.97 vs. 28.13 ± 4.67 kg/m^2, $p = 0.009$), total cholesterol (>4.82 mmol/l: 35.2 vs. 26.4%, $p = 0.024$), low density lipoprotein cholesterol (>2.5 mmol/l: 45.8 vs. 33.3%, $p = 0.004$) and reduced high density lipoprotein cholesterol (HDL-C) levels (≤1 mmol/l: 24.4 vs. 10.4%, $p = 0.009$) in thick EAT group. Logistic regression analysis revealed that higher BMI (OR = 1.532, 95% CI 1.008–2.328, $p = 0.002$) and HDL-C ≤1 mmol/l (OR = 1.777, 95% CI 1.159–2.724, $p = 0.008$) were associated with thicker EAT. Killip class ≥III was more frequent (17.6 vs. 10.3%, $p = 0.02$) in thick than thin EAT group.

Conclusion: Increased EAT thickness was associated with obesity, cardiometabolic risk factors and influenced severity of left ventricular dysfunction.

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PS123

Evaluation of spleen volume: Practical diagnostic role of linear measurements, 2D and 3D coefficients in computed tomography

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Aim: The aim of the study was to find which linear measurements, field and volume coefficients correlate best with the real volume of the spleen and can be further used for determination of splenomegaly.

Introduction: Spleen is involved in a wide spectrum of abnormalities, which might lead to an increase in organ size. Splenic enlargement on CT is diagnosed basing on rather subjective criteria. The product of the length, estimated height and thickness of the spleen ("splenic index", cut-off ≥480) has also been proposed as an indicator for evaluating splenic size on CT.

Methods: Abdominal CT examinations of 153 patients' (77 females, 76 males) were retrospectively analysed in terms of maximal length, thickness, hilum thickness (axial plane), height (longest measurement in coronal plane), 90° height (maximum vertical height at coronal section), estimated height (number of axial scans where spleen was visible multiplied by the thickness of CT scans) (Impax Software) and real spleen volume (Vitrea software). Two-dimensional and three-dimensional coefficients were acquired through proper mathematical formulas. Splenomegaly cut-off: 314.5 ml. Pearson's correlation coefficient was calculated for the relationship between single, field, volume measurements and real volume (Statistica software).

Results: There was a statistically significant correlation between all single, field and volume measurements and real volume ($p < 0.05$). For single measurements, the correlation is the strongest for height ($r = 0.813$, sensitivity 65%, specificity 91.7%, PPV 71.4%, NPV 95.6%). For two-dimensional, it is the coefficient calculated from length and 90° height ($r = 0.918$, 85%, 94.7%, 70.8%, 97.7%). For three-dimensional, it is the coefficient calculated from length, 90° height and hilum thickness ($r = 0.919$, 75%, 96.2%, 75%, 96.2%). Cut-off for splenic index from our calculations was ≥1148.

Conclusion: Coefficient from length, 90° height and hilum thickness correlate best with the real volume of the spleen. Splenic index in our study is far from the perfection for clinical practice.

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PS128

Influence of blood inflammatory parameters to erythropoietin resistance in haemodialysis patients

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Aim: To evaluate the correlation between the inflammatory blood parameters and the resistance to EPO among the hemodialysis patients.

Introduction: Erythropoietin therapy is considered to be the standard treatment of anemia in chronic kidney disease patients, yet some patients do not respond well to this therapy. This is called EPO resistance and could be generally associated with the chronic inflammation.

Methods: A retrospective one single centre study, which analysed medical records of 30 HD patients who had advanced CKD and received EPO treatment in Vilnius University Hospital Santaros Clinics from 2016–2009 to 2016–2011. Data analysed – concentrations of C-reactive protein, neutrophils, lymphocytes, platelets, as well as EPO dose per kilo and hemoglobin concentration (measured at the beginning of the EPO therapy and one month after the treatment).

Results: Patients were grouped into 2 categories: 1 group ($n = 14$) – concentration of hemoglobin increased, 2 group ($n = 16$) – concentration decreased after treatment. In 1 group average concentration of platelets were statistically significantly ($p = 0.039$) higher (230.2 ± 73.70), compared to 2 group (174.1 ± 66.96).

Furthermore, platelets concentration among patients with hemoglobin level of >100 g/l ($n = 17$) after one month of treatment were statistically significantly ($p = 0.012$) higher (231.06 ± 56.41) compared to those patients with hemoglobin level of <100 g/l ($n = 13$) (160.08 ± 78.17) after treatment.

Additionally, patients with hemoglobin levels after one month of treatment 100–125 g/l ($n = 15$) were separated into two groups based on C-reactive protein level: >5 (1 group) and <5 (2 group). Average concentration of erythropoietin was statistically significantly higher in 1 group ($n = 9$) (223.82 ± 69.15 VV/kg) than in 2 group ($n = 6$) (116.68 ± 59.68 VV/kg).

Correlation analysis revealed that among patients with hemoglobin levels of <110 g/l after treatment there is a statistically significant positive correlation (+0.428) between change of hemoglobin levels before and after treatment and erythropoietin dose and statistically significant ($p = 0.023$) negative correlation (−0.481) with lymphocytes concentration in blood.

Conclusion: HD patients with a higher concentration of platelets respond to EPO therapy better than those with a lower concentration. Increased EPO dose results in higher Hgb

concentration, but the inflammatory environment could also lead to EPO resistance. Higher lymphocyte concentration in the blood results in lower Hgb concentration change during treatment. In order to achieve required Hgb change, the increase of CRP above the normal range may result in double the dose of EPO needed.

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PS061

The relationship between Calcium-Scor and the risk of coronary artery disease in patients with heart failure

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Aim: The purpose of this study was evaluating relationship between coronary calcium score in detecting the risk of coronary artery disease in patients with heart failure.

Introduction: Heart failure (HF) is an abnormality of cardiac structure or function leading to failure of the heart to oxygen delivery. Angiography is discussed as a gold standard for diagnosis of coronary artery disease but Cardiac CT-Scan recently is typical imaging technique which is low-cost and non-aggressive technique to determine coronary artery calcification.

Methods: This is case-control study that was conducted in Services Hospital. All Patients referring to Heart failure department were EF (Ejection fraction) ≤35% and all of them previously examined by Coronary Angiograghy or Coronary CT-Angiography to know the coronary artery status. The case group was patients with CAD related heart failure and control group was patients with normal coronary or Non-CAD Related-HF. All patients in both groups were evaluated with Conventional CT-Scan for calculated the Calcium score.

Results: Ninety patients with HF divided into case group ($n = 40$) and control group ($n = 50$). The average of EF in case group was 29.25 ± 5.05 and in control group was 27.7 ± 7.09. The amounts of calcium score in each Categories (Mild, Moderate, Severe and Extensive) in case group was 33%, 18%, 13% and 5%, but control group in Categories (Mild, Moderate, Severe) was 20%, 6% and 4% respectively.

There was a statistically significant correlation ($r = 0.835$; $p < 0.0001$) between calcium score and results of angiography. There was linear relationship between calcium score and age of patients with heart failure ($r^2 = 0.807$). No significant difference was found between genders in terms of calcium score ($p = 0.353$).

Conclusion: There was high correlation between calcium score and results of angiography. Calcium scoring is reliable tool for screening patients with CAD.

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PS083

Levels of 6-thioguanine nucleotides and clinical remission in inflammatory bowel disease – A systematic review and meta-analysis

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Aim: This systematic review and meta-analysis aimed i) to assess the clinical value of 6-thioguanine nucleotides (6-TGN) thresholds (200, 225, 230, 235, 250 and 260 pmol/8 × 108 RBC); and ii) to compare mean 6-TGN concentrations between patients with active disease and those achieving remission.

Introduction: Thiopurines are widely used as immunosuppressive drugs in the management of inflammatory bowel disease even though their minimum effective dose and dose–response relationship remain controversial. In addition, the monitoring of thiopurines' pharmacological active metabolites is currently reserved for particular cases namely in refractory patients or when non-compliance or toxicity is suspected.

Methods: Literature search was carried out following PRISMA and Cochrane Collaboration Guidelines and four databases were used (PubMed, Web of Science, ScienceDirect and the Cochrane Central Register of Controlled Trials). Statistical heterogeneity was assessed using the I2 statistic followed by subgroup and sensitivity analyses. Odds ratios (ORs) were computed under the random effects model.

Results: The systematic search identified 1384 records of which 25 matched the inclusion criteria and were retained for further analysis. From these, 22 were used in the cut-off comparisons while 12 were used in the 6-TGN mean differences analysis. The global OR for remission in patients with 6-TGN concentrations above the predefined thresholds was 3.95 (95%CI, 2.63–5.94; $p < 0.001$). When considering each of the six thresholds individually, the OR was significant for levels above 235 pmol/8 × 108 RBC (OR = 2.25) and 250 pmol/8 × 108 (OR = 4.71). Mean 6-TGN levels were significantly superior among patients achieving clinical remission, with a pooled difference of 63.37 pmol/8 × 108 RBC (95%CI, 31.81-94.93; $p < 0.001$).

Conclusion: These results reinforce that 6-TGN levels are related to clinical remission and give an insight into the thresholds that may be used to guide clinical decisions.

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PS020

Influence of energy drinks on hemodynamic parameters in young healthy adults – Randomized double-blind placebo controlled cross-over study

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Aim: Assessment of the influence of single dose of energy drink on blood pressure, heart rate, ECG, cardiac output and vascular compliance in healthy volunteers.

Introduction: An energy drink (ED) is a type of beverage containing stimulant drugs, caffeine, taurine, which is marketed as providing mental and physical stimulation. The popularity of product is increasing especially among teenagers and young adults. Some research suggest that its consumption may have negative effect on cardiovascular system.

Methods: A randomized double-blind placebo controlled cross-over study was conducted on 18 healthy volunteers (7 female, 11 male, mean age 23.67 ± 1.19). Subjects received: 500 ml of energy drink containing 160 mg of caffeine, 2 g of taurine and 50 mg of guarana or 500 ml of placebo. Participants drank beverages in random order during two different meetings. Drinks did not differ in taste, smell and color. In all participants before and after consumption of a drink following procedures were performed: peripheral and central systolic and diastolic blood pressure (SBP and DBP) measurement, ECG recording, echocardiography, and pulse wave velocity analysis – in the same sequence and time intervals for every participant.

Results: ED consumption was related to significant increase of SBP in 75 min of observation compared to placebo (ΔSBP for ED 5.7 ± 10.2 mmHg vs −0.3 ± 7.2 mmHg for P, $p = 0{,}03$). ED caused increase in central SBP (107.8 ± 13.2 vs 115.6 ± 12.1 mmHg, $p = 0{,}0005$), and central DBP (73.9 ± 11.9 vs 78.1 ± 10.2 mmHg, $p = 0{,}02$). However comparison between placebo and ED revealed no significant differences in these parameters. The ECG parameters (HR, PQ, QRS and QTc intervals, axis of P wave, QRS complex, T wave) did not reveal significant differences between groups. There were no differences in echocardiographically determined cardiac output and LVEF.

Conclusion: Single dose ED consumption increases peripheral and central SBP. This effect is probably mediated by vascular wall properties and not by cardiac performance.

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PS055

Analysis of genetic polymorphism 4a/b of the eNOS gene in infertile men

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Aim: The aim of our study was the analysis of genetic polymorphism 4a/b of the eNOS gene in infertile men with idiopathic infertility, correlation of genotype and phenotype in infertile men and comparing the results of testing of genetic polymorphism 4a/b with the results from the control group.

Introduction: Male infertility of unknown etiology represents a common medical and social problem, in whose basis lies a combination of genetic and environmental factors. Several recent studies have pointed to the possible connection of polymorphisms in eNOS gene and idiopathic male infertility.

Methods: The study included 50 infertile men with idiopathic infertility and 50 fertile controls. 4a4b polymorphism was detected by polymerase chain reaction (PCR).

Results: 4b4b genotype was detected in 27 (54%) patients and 36 (72%) controls, 4a4b genotype in 21 (42%) patients and 13 (26%) controls and 4a4a genotype detected in 2 (4%) patients and 1 (2%) control group participant. 4b allele frequency was 75% in the patient population and 85% in the control population, and frequency of allele 4a was 25% with patients and 15% in the control group. There was no statistically significant difference in the distribution of genotypes ($p = 0.062$) nor alleles ($p = 0.111$) between these two populations. Comparing 4a/b genotypes and serum concentration of FSH within patient group, we've detected a highly significant correlation ($p < 0.001$), where all carriers of 4b4b genotype had physiological concentration of serum FSH, while most of 4a4a and 4a4b carriers had higher serum FSH values.

Conclusion: Per our results VNTR (4a/b) is not connected to idiopathic male infertility in Serbian men, but they did show a highly significant correlation between serum FSH concentration and 4a/b genotype of infertile men.

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PS115

Intravenous iron treatment effect to patients on hemodialysis

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Aim: To evaluate the coherence between intravenous iron therapy and the inflammatory indicators to patients on hemodialysis.

Introduction: when the kidney function is failing, the number, of patients who has a final stage kidney disease with anemia, is increasing. One of the most important reasons of anemia is iron deficiency. The iron treatment may be intravenous or oral. Though the oral treatment is cheaper, it may cause gastrointestinal disorders. Intravenous iron therapy has a better tolerance, but earlier studies had showed that it increases the risk of infections to patients on hemodialysis.

Methods: The retrospective study included 33 hemodialysis patients who undergone the intravenous therapy during the 2016-10 and 2016-12 in Vilnius university hospital. The absolute numbers of neutrophils and lymphocytes, C-reactive protein and procalcitonin were assessed before the treatment with intravenous iron and a month after it.

Results: we analyzed 13 men and 20 women, the mean age 59 years, the mean creatinine 760 μmol/l, the mean hemoglobin 105 g/l. By the test of Wilcoxon signed rank the means of neutrophils and C-reactive protein increased after the start of the treatment with iron (the mean of C-reactive protein increased

from 12.8 ± 12.96 to 27.4 ± 41.17; $p = 0.07$; the mean of neutrophils increased from 4.45 ± 1.52 to 6.86 ± 12.11; $p = 0.59$). The *T*-test showed that the means of procalcitonin increased from 0.21 ± 0.07 to 0.23 ± 0.08, $p = 0.04$, the mean of lymphocytes increased from 1.35 ± 0.54 to 1.54 ± 0.62, $p = 0.1$. Pearson correlation coefficient showed statistically insignificant positive correlation between the dose of medication and variation of procalcitonin.

Conclusion: The study has showed that inflammatory indicators increased after the intravenous iron therapy to patients on hemodialysis.

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PS038

Distribution and quantification of elements of the enteric nervous system in the distal rectum of neonates and infants

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Aim: Analysis of variations in the ENS of distal rectum in neonates and infants under the age of 6 months, with no previous history of intestinal dismotility.

Introduction: The enteric nervous system (ENS) consists of numerous ganglia along the gastrointestinal tract. The most common disorder of ENS is Hirschsprung's disease (HD). Diagnostic problems may occur due to insufficient knowledge of the normal distribution of ganglion cells (GC) in the distal rectum.

Methods: The study analyzed ENS of distal rectum in autopsy samples of infants. The sections were stained with hematoxylin and eosin (H&E) and immunohistochemistry using the MAP-2 antibodies. All sections were analyzed at three levels: the level of anorectal junction (ARJ0), at 1 cm (ARJ1) and 2 cm (ARJ2) proximal to the ARJ0. We analyzed number of ganglia and GC, their distribution and thickness of the bundles of nerve fibers (BNF).

Results: GC were found at ARJ0 mainly within BNF of the intramuscular zone. Number of GC within BNF of intramuscular zone were lower at ARJ2 than ARJ1 (H&E: $p = 0.021$; MAP-2: $p = 0.017$). Number of GC in submucosal ganglia were significantly higher in ARJ1 and ARJ2 compared to ARJ0. In myenteric ganglia the number of GC were higher at ARJ1 compared to ARJ0 (H&E: $p = 0.002$; MAP-2: $p = 0.014$). Number of GC were significantly higher at ARJ2 compared to ARJ1 only in MAP-2 staining ($p = 0.009$). In submucosal plexus we observed higher number of ganglia at ARJ1 and ARJ2 ($p = 0.014$, both) compared to ARJ0 at MAP-2. In myenteric plexus there were higher number of ganglia at ARP1 compared to ARP0 (H&E: $p = 0.006$; MAP-2: $p = 0.014$). Individual thicker BNF were found in submucosa.

Conclusion: In distal rectum of neonates and infants there are significant variations in number of ganglia in the submucosal plexus up to ARJ2 and in myenteric plexus up to ARJ1.

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PS220

Inflammatory bowel diseases: Nutritional status and its significance for the course of the disease

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Aim: The aim of the study was to evaluate the association between the Body Mass Index(BMI) and the disease course of IBD patients.

Introduction: Inflammatory Bowel Disease(IBD) may lead to the underweight and malnourishment. However, the number of overweight and obese patients increases. Excess body weight connected with a pro-inflammatory state can modify the disease course.

Methods: Medical records from the University Hospital in Cracow Electronic System were screened from August 01, 2015 to December 31, 2016 in search of patients diagnosed with IBD. Data regarding the disease extension, occurrence of intestinal and extra-intestinal complications, number of days spent in the hospital annually and type of treatment was collected. The results were analyzed in the groups based on BMI (1 < 18.5; 2:18.5–25; 3 > 25 kg/m^2).

Results: 150 patients with Crohn's disease(CD) and 151 with ulcerative colitis(UC) were included. The median number of days spent in the hospital annually was significantly higher in the underweight group (13(IQR:11) vs 7(IQR:17) vs 7(IQR:12); $p < 0{,}01$). Overweight patients were less likely to receive anti-TNF or immunosuppressive treatment [anti-TNF (1:35% vs 2:38.36% vs 3:18.29%; 1 vs 3; $p = 0.02$; 2 vs 3; $p < 0.01$); immunosuppressive (1:40,00% vs 3:23, 17%; $p = 0.03$)]. Patients with BMI > 25 kg/m^2 developed fistulas and bowel strictures less often [fistulas (1:33.33% vs 2:27.04% vs 3:12.20%; 1 vs 3: $p < 0.01$; 2 vs 3; $p < 0.01$); strictures(1:25% vs 2:22, 64% vs 9.76%; 1 vs 3: $p = 0.01$; 2 vs 3: $p < 0{,}01$)]. Underweight UC patients had more extensive disease [left sided (1:25% vs 2:52.63% vs 3:49.02%; 1 vs 2: $p = 0.02$; 1 vs 3: $p = 0{,}04$); pancolitis (1:58.33% vs 2:26.32% vs 3:31.37%; 1 vs 2: $p < 0.01$; 1 vs 3: $p = 0{,}02$)].

Conclusion: Overweight seems to be associated with a milder clinical course of the disease in IBD patients. It is related to lower incidence of intestinal complications among CD and to less extensive intestine involvement in UC patients.

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PS023

Influence of glicoregulation and chronic degenerative complications of diabetes on bone mineral density

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Aim: The aim of this study is to determine the correlation between duration of diabetes, glicoregulation and chronic degenerative complications of diabetes, on one side, and bone mineral density, on the other side.

Introduction: Diabetes mellitus is a state of chronic hyperglycaemia. In late stages of the disease, especially if it is not regulated well, chronic complications may occur, dominating the clinical picture. Osteoporosis is characterized by bone loss per volume unit leading to microarchitectonics disorder of the bone. Connection between diabetes and osteoporosis is very complex.

Methods: Medical documentation collected at daily hospital of Clinic of endocrinology, diabetes and metabolic disorders is used in this study. Sample includes 60 patients which have been diagnosed with diabetes mellitus, with or without complications, who underwent densitometry measurement (DEXA). Glycosylated hemoglobin (HbA1c), fasting glucose and postprandial glucose are used as parameters of glicoregulation.

Results: Average duration of diabetes is 15.61 ± 9.63 years. Average value of HbA1c is $8.5 \pm 1.79\%$, average value of fasting glucose is 9.23 ± 2.94 mmol/l and average value of postprandial glucose is 11.35 ± 4.27 mmol/l. 67% of patients have one or more complications. Bone mineral density (g/cm^2) of femoral neck and total have significant negative correlation with HbA1c ($p < 0.01$). Bone mineral density of lumbosacral spine and femoral neck (g/cm^2, *T*-score) have light negative correlation with postprandial glucose.

Conclusion: Bone mineral density and parameters of glicoregulation have negative correlation. Statistically significant correlations between bone mineral density and chronic degenerative complications of diabetes were not found.

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PS022

Effect of autologous stem cell transplantation in patients with hematological malignancies

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Aim: The aim of this study is to analyse avaliable medical data of patients diagnosed with multiple myeloma (MM), lymphoma Hodgkin (MB) and non-Hodgkin (NHL) and acute leukemia (AL), who underwent ASCT, and to compare the results with the results from other scientific works.

Introduction: Autologous stem cell transplantation (ASCT) with high dose chemotherapy is effectible and safe approach in the treatment of different hematological malignancies. Nowdays, it is the standard therapy for multiple myeloma, lymphomas and acute leukemias.

Methods: Retrospective study included 84 patient diagnosed with MM, MH, LNH and AL who underwent ASCT in the period from 2004 to 2016. Data are presented in table and charts.

Results: In relation to the underlying disease, the distribution of respondents was as follows: 35 patients with MM, 24 with NHL, 20 with MH and 6 with AL. Large volume apheresis procedure had to 75 patients (89.3%), and 9 patients (10.7%) had conventional two-day procedure. The mean value of processed blood volume amounted to 13050 ml. The average number of MNC in the apheresis product was 7.8×108/kg bw, a CD34+ cells was 12.11×106 kg bw. After the application of conditioning regimens, depending on the underlying disease, neutrophils engraftment occurs at 11 day and platelets engraftment at 14 day.

Conclusion: Analyzing data of the patients with hematological malignancies and ASCT conducted, we conclude that the mentioned procedure is successful method of treatment, with low transplant mortality and complications caused by the mentioned procedure.

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PS237

Polymorphism of Kibra gene in patients with terminal renal insufficiency

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Aim: The aim of this study was to determine whether there is a difference in frequencies of genotypes and alleles of KIBRA gene polymorphism, rs17070145 between patients with terminal renal insufficiency and normal population.

Introduction: KIBRA gene has a role in signal transmission that regulates apoptosis, proliferation, and movements of the cytoskeleton of cells. Due to its most common expression in kidney and brain, the name of this protein is Kibra (KIdney, BRAin). Polymorphism rs17070145 (substitution of thymine with cytosine in the ninth intron of the gene) is associated with Alzheimer's disease and memory, while its connection with kidney's diseases has not been tested yet. It is thought that allele C is the factor of predisposition in TRI.

Methods: Polymorphism rs17070145 was analyzed with Real Time PCR method using TaqMan probes and 50 people with TRI were involved. Results of gene analysis for the control group were taken from previous research. Frequencies of genotypes and alleles between patients with TRI and healthy examinees was compared with χ^2 (chi-square) test.

Results: The frequency of CC genotype among patients with TRI is 76%, CT genotype 22% and TT genotype 2%. Based on frequencies of genotypes, we found that frequency of C allele is 87%, while the frequency of T allele is 13%.

Conclusion: Results of χ^2 test show extremely statistically significant difference in frequencies of genotypes and alleles in patients with TRI in comparison with healthy people ($P < 0.0001$). These results indicate that C alleles on locus rs17070145 in KIBRA gene are probably the significant factor of predisposition in the pathogenesis of TRI.[1–3]

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Neurosciences Poster Session
Thursday, September 14th, 16h00

PS088

D-Galactose high-dose administration and oral epigallocatechin-3-gallatte effects on the dendritic trees of developing neurons of young male rats

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Aim: In the present study, we aimed to explore the effect of D-galactose administration and epigallocatechin-3-gallatte (EGCG) on the dendritic trees of developing granule cells of the hippocampal formation (HF) of young male rats.

Introduction: The model of accelerated senescence with the administration of D-galactose is used in anti-aging studies. However, reports have questioned its effectiveness. To clarify this issue we used high-dose D-galactose on young rats and studied the immature granule cells stained with the neurogenesis marker doublecortin (DCX). We also used EGCG, a green tea catechin, to verify if there are neuroprotective effects in the D-galactose-treated animals.

Methods: At 4 weeks of age, male Wistar rats were allocated to a control group ($n = 7$), a D-galactose group (300 mg/kg body weight, intraperitoneally) ($n = 5$; GAL) and to a D-galactose + EGCG (oral solution, 2 grams/L) group ($n = 5$; gal + EGCG) during 4 weeks. After this period DCX immunocytochemistry was performed. The dendritic trees of immature granule cells were drawn with the aid of a camera lucida and a metric analysis of the dendritic segments of the dendritic trees was performed.

Results: No differences in all parameters quantified were found when controls and gal rats were compared. However, the results show that the total dendritic length of the dendritic trees of gal + EGCG rats was significantly reduced when compared with controls ($p < 0.03$). There were no differences in the others dendritic parameters quantified.

Conclusion: D-Galactose did not induce disturbance of the neurogenesis as shown by the absence of alterations in the dendritic trees confirming our previous studies. Surprisingly, the addition of EGCG led to a reduced total dendritic length. This unexpected effect can be explained if we consider that the addition of the catechin acted as a second aggression leading to a disturbed dendritic tree of the immature neurons.

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PS079

Analysis of variations in the F5, F2 and ACE genes among Latvian patients with ischemic stroke

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Aim: Evaluate thrombophilia causing genetic variants and ACE gene I/D variant impact on patients with ischemic stroke.

Introduction: Every year, 15 million people worldwide suffer a stroke that is the second leading cause of disability. Genetic variants in Leiden factor coding gene (F5) and in prothrombin gene (F2) cause inherited thrombophilia which is associated with increased risk of intravascular thrombosis, thromboembolism and cerebral stroke. Angiotensin-converting enzyme (ACE) coding gene I/D variant is discussed among numerous conditions including stroke.

Methods: In the study there were included 115 patients with mean age 70.3 ± 11.0 years, with diagnosed ischemic stroke. Control group for F5 and F2 gene variations consisted of 124 individuals with mean age 55.6 ± 14.6 years. And for ACE gene variation 248 individuals with mean age 56.8 ± 11.4 years. DNA was extracted from peripheral blood using standard phenol-chloroform method. Genotyping of F5 gene variant G1691A and F2 gene variant G20210A was performed using PCR-RFLP. ACE gene I/D variant genotyping were performed using PCR. Statistical analysis was performed using Fisher's exact test and SPSS v22.0 software.

Results: F2 gene variant were more frequent in patient group. Frequency in patients were 0.017 and in control group 0 ($p = 0.038$). F5 gene variant frequency in both patients and control group were 0.012 ($p > 0.05$). Seven patients (5.6%) had one variant in one of coagulation factors encoding genes comparing to three in control group (2.4%) ($p > 0.05$). Mean age for patients with identified variations in F2 or F5 was not significantly different comparing to other patients ($p > 0.05$). ACE gene I/D genotypes and allele frequencies in stroke patients were not significantly different from controls – I allele frequencies were 0.452 in patients versus 0.470 in controls ($p > 0.05$).

Conclusion: Prothrombin encoding gene variant G20210A could be risk factor for ischemic stroke. F5 and ACE gene I/D genotypes are not associated with ischemic stroke.

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PS205

The bioactive compounds from elderberry to modulate mitochondrial dysfunctions underlying Alzheimer's disease

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Aim: The specific objective of this work is to establish a correlation between the physical-chemical properties of the aqueous extract of elderberry (*Sambucus nigra* L.) and its ability to tune the cell redox state and to overcome mitochondrial dysfunctions, which are pathological events with high relevance in Alzheimer's disease (AD).

Introduction: Currently, there is no effective medicine to prevent or delay the progressive brain degeneration underlying cognitive decline and dementia that characterize AD. Previous works support the idea that the loss of mitochondrial functionality, connected with the decline of complex I activity, is able to promote AD phenotype through the activation of multiple pathophysiological pathways, including oxidative stress, neuroinflammation, and also tau and amyloid-beta pathologies. Thus, multi-targeted

approaches supported by mixtures of natural bioactive compounds should reveal more effectiveness than classical therapeutics for AD.

Methods: The polyphenolic profile of elderberry extract and of anthocyanin-enriched fraction was evaluated by HPLC-DAD, the optical properties by UV–vis and fluorescence spectroscopy and the redox behavior by cyclic voltammetry. Antioxidant properties were assessed in cell-free assays while the ability the elderberry extract to modulate the mitochondrial redox chain was evaluated in rat brain mitochondria.

Results: HPLC analyses showed that elderberry extract is a mixture of chemical compounds, particularly rich in anthocyanins. It exhibits intrinsic fluorescence properties with potential for bioimaging, reversible redox behavior and ability to scavenge DPPH, nitric oxide and superoxide radicals. The antioxidant, optical and redox properties of elderberry extract are strongly correlated to their content in anthocyanins. Bioenergetic studies show that elderberry extract has ability to promote the oxidation of NADH in aqueous phase and deliver electrons to ubiquinone or complex III in the inner-mitochondrial membrane, overcoming the complex I inhibition promoted by rotenone.

Conclusion: Elderberry anthocyanins have potential to be used in mitochondria-targeted formulations to modulate the pathophysiological changes underlying AD from their early stages.

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PS016

Effect of resveratrol on the cartilage and nociceptive system of Osteoarthritic animals

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Aim: This study aims to evaluate the effect of RV on the nociceptive behavior, histopathological alterations at the knee and DRG neurons of OA rats.

Introduction: Osteoarthritis (OA) is a common degenerative joint disease and arthritic pain is a prominent symptom associated with reduced quality of life. Peripheral pain mechanisms seem to be involved, with cartilage lesions showing a repercussion in Dorsal Root Ganglia (DRG) neurons. Resveratrol, a polyphenol with proven anti-inflammatory, anti-oxidant and neuroprotective properties, has been shown to prevent development of OA and act as an antinociceptive agent. However, its systemic effects once the disease has fully developed remain unclear.

Methods: To evaluate this, OA was induced in 18 male Wistar rats through intra-articular injection of mono-iodoacetate (MIA) (day 0). Animals were allowed to develop the disease for two weeks, after which followed a 4-week-long treatment with resveratrol or vehicle, administered intraperitoneally twice daily (10 mg/kg). Nociceptive behavior was quantified weekly using the CatWalk and Knee-Bend tests. Animals were sacrificed one week after the last treatment administration, their knees were dissected for histopathological analysis, and the DRG were dissected and processed for immunohistochemical evaluation of activating transcription factor 3 (ATF-3) neuronal expression.

Results: Resveratrol was unable to prevent cartilage degeneration but it significantly decreased ATF-3 expression. The nociceptive behavior of OA animals treated with resveratrol decreased during the first three weeks of treatment, in comparison to day 14 (before treatment was initiated), as shown by Knee-Bend scores. However, this tendency reverted as the disease progressed.

Conclusion: These results indicate that resveratrol may have antinociceptive effects in the early stages of the disease development, but it might not play such a relevant role once the disease has progressed. Thus, further studies are needed to fully understand the possible role of resveratrol in the different stages of OA.

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PS173

Bupivacaine treatment enhances the regeneration of the lesioned external urethral sphincter of the rat

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Aim: In this study we intent to verify if bupivacaine treatment can be used to enhance the repair of the lesioned urethral sphincter in rat.

Introduction: Stress urinary incontinence (SUI) is a major and frequent urinary dysfunction. It has been associated with external urethral sphincter (EUS) weakness due to several causes. Among them, ischemia and nerve lesion frequently associated with childbirth. The current treatments are mainly surgical but are far from being satisfactory. The local anesthetic bupivacaine is known to exert myotoxic action, followed by muscle regeneration with increased strength. This effect was already used in ocular muscles to treat strabismus. In the present study we evaluated the effect of bupivacaine application in the recovery of the damaged EUS.

Methods: A lesion of the external urethral sphincter (urethrolysis) was performed in adult female Wistar rats using established protocols. Two weeks after the lesion, the animals were injected in the EUS with 0.4 ml of 0.5% bupivavaine. Ten days later, the whole urethra was removed, fixed and sectioned in paraffin wax. Sections

were stained with hematoxylin and eosin, Massons's trichrome and immunoreacted for markers of striated and smooth muscle (sarcomeric actin and smooth muscle actin, respectively).

Results: Two weeks after urethrolysis, a marked reduction of muscle fibers in the EUS was detected. A recovery was evident in lesioned, bupivacaine injected animals when compared with lesioned and saline-injected controls.

Conclusion: Our data show that bupivacaine application in the lesioned external urethral sphincter accelerates its recovery. This finding opens a therapeutic opportunity to treat stress urinary incontinence.

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PS074

In vivo and in silico study of allicin as a stroke prevention

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Aim: To prove allicin effect to prevent stroke in insilico and invivo method.

Introduction: Stroke is a disease that can cause permanent disability, even death. Atherosclerosis is one of the cause of stroke. One way to prevent stroke is to treat atherosclerosis using allicin. Allicin works by inhibiting Integrin Alpha Beta-3 and ApoE proteins. Therefore, allicin can be considered as an alternative in stroke prevention.

Methods: This is true experimental study with two methods. Allicin was taken from pubchem, while ApoE with code (1EA8), Integrin Alpha Beta-3 with code (1JX5) taken from protein data bank. Afterwards, the ligands and macromolecule were docked with Pyrx. Analysis was done using Discovery Studio. Pharmacokinetic study, allicin compounds were analyzed with ACD/I-Lab. During invivo study, rats were induced with high fat diet for 8 weeks and were given allicin with dose 5, 10, 20 mg/kg BW during 6 weeks. Rat blood, carotid artery, and brain were analyzed for lipid profile, foam cells in blood vessels, and immunohistochemically to see BDNF.

Results: Pharmacokinetic results showed that allicin has oral bioavailability above 70%, distributed through lipoproteins and a few albumins. Allicin can penetrate through membrane and cytoplasm, affecting its target. Pharmacodynamically, allicin can bind to active site of ApoE on 149 leucine, and to active side of ApoE on 173 serine. Allicin bound with active site of ApoE will increase ApoE expression, thus lowering lipid profile except HDL. Meanwhile, allicin bound with active site of Integrin Alpha Beta-3 blocked platelet aggregation. Decreasing Integrin Alpha Beta-3 was proven by invivo results where foam cells were decreasing. These events caused a decrease foam cell in common artery, causing no brain hypoxia and increased BDNF. Invivo test showed a decrease in foam cells on 10 mg/kg BW. On the contrary, the brain showed an increase in BDNF amount on 20 mg/kg BW.

Conclusion: Based on insilico and invivo studies, allicin can be considered as a preventive treatment to stroke by inhibiting atherosclerosis development by increasing ApoE, lowering Integrin Alpha Beta-3 protein, and increasing BDNF.[1–5]

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PS148

Anxiety-like behavior in elevated plus maze upon sleep fragmentation of one light phase of rats' circadian cycle

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Aim: To assess anxiety-like behavior in rats after twelve hours of sleep fragmentation during the light phase of sleep-wake cycle, using the elevated plus maze test.

Introduction: Hallmark of sleep fragmentation is set of frequent, brief arousals, which modulates sleep architecture without significant diminishment of total sleep time. Anxiety is recognized as comorbidity in numerous disorders, including some of those related to sleep quality. Sleep fragmentation may be appropriate model of sleep alteration pattern in some disorders, yet its effects on behavioral alterations have not been broadly investigated.

Methods: Sleep fragmentation was achieved by treadmill method lasting for 12 h, during the light phase of the day (starting at 8 AM). Wistar albino male rats were randomly divided into: sleep fragmentation group (SF, $n = 8$, treadmill programmed to alternately work 30 s ON and 90 s OFF every 2 min); activity group (AC, $n = 8$, treadmill programmed to alternately work 10 min ON and 30 min OFF); and treadmill control group (TC, $n = 8$, rats stayed in the treadmill set to OFF mode and conditions equivalent to cages). Immediately after the sleep fragmentation regimen, elevated plus maze test was performed. To assess anxiety-like behavior, we measured time spent in the open arms, as well as number of transitions between open and closed arms.

Results: SF group spent significantly less time in the open arms compared to both, AC and TC group ($p < 0.001$). SF rats also had significantly less number of transitions between closed and open arms of the maze, compared to AC and TC group ($p < 0.05$). Moreover, no significant difference was observed in any of the measured parameters between TC and AC group.

Conclusion: The results of our study indicate that acute 12-h sleep fragmentation induced anxiety-like behavior in rats in elevated plus maze. Further research should help us better understand impact of this phenomenon on psychiatric disorders.

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PS082

Pain and bladder dysfunction in an animal model of multiple sclerosis

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Aim: Here, we investigated if MS-induced pain and bladder dysfunction can be attenuated by TRPV1 desensitization with RTX.

Introduction: Multiple sclerosis (MS) is the most prevalent neurological disorder in young people, causing irreversible disability and producing substantial economic and social impact. Among the most incapacitating symptoms, neuropathic pain and bladder dysfunction are reported by the majority of patients. The transient receptor potential vanilloid 1 (TRPV1) is a receptor described to have an important role in neuropathic pain, bladder dysfunction and inflammation. TRPV1 desensitization with agonists, such as resiniferatoxin (RTX), has been shown to improve bladder function and reduce behavioural signs of pain in various animal models of disease. In the context of MS, a recent study showed that TRPV1 knockout mice were protected from disease progressions, presenting delayed disease onset, myelin preservation and reduced clinical scores.

Methods: Experimental Auto-immune Encephalitis (EAE) was induced by a single injection in the flank of a solution of myelin basic protein (MBP) in Complete Freund's adjuvant (CFA). Behavioural tests were performed to evaluate symptoms. One month after MS-induction, animals were anesthetized and cystometries performed. Two other groups of MS animals received intrathecal RTX or vehicle and also submitted to behavioural tests and cystometries. At end of experiments, tissue was collected and processed.

Results: EAE rats developed neuropathic pain, as shown by the presence of mechanical allodynia and hypersensitivity to thermal stimuli. Cystometries performed at this time point showed signs of neurogenic detrusor overactivity. These clinical signs were accompanied by decreased spinal expression of MBP and increased activity of astrocytes and microglia. Preliminary observations suggest that intrathecal RTX improved cutaneous hypersensitivity and bladder function. These results suggest that TRPV1 might be involved in pain bladder dysfunction accompanying MS and that its modulation could have therapeutic relevance.

Conclusion: These results suggest that TRPV1 might be involved in pain bladder dysfunction accompanying MS and that its modulation could have therapeutic relevance.

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PS227

Neurogenesis in a rat model of sporadic Alzheimer's disease

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Aim: Characterize adult hippocampal neurogenesis in a rat model of the initial stages of sporadic Alzheimer's disease (AD).

Introduction: Sporadic late-onset AD is the most common cause of dementia, that can be characterized by a progressive cognitive decline, with a noteworthy episodic long-term memory impairment at early stages, accompanied by an excess accumulation of amyloid beta (Aβ) peptide in the brain. Present treatment options are very limited, so understanding AD pathophysiology is essential for exploring efficient therapies. Adult hippocampal neurogenesis is thought to play a crucial role in hippocampus-dependent cognitive abilities, namely learning/memory, although how this process is modulated in AD remains unclear.

Methods: An Aβ1–42 peptide solution was intracerebroventricularly injected into the rats' lateral ventricle (the same volume of vehicle was injected to controls). Moreover, rats were injected with 5-bromo-2′-deoxyuridine (BrdU) intraperitoneally to study cell proliferation and differentiation. Two weeks after Aβ1-42 injection, the open field (OF) test and the novel object recognition (NOR) test were performed. Further behaviour tests are currently being performed, including the elevated plus maze (EPM), the Y-maze forced alternation test, and the Morris water maze (MWM) test. Focusing on the dentate gyrus, immunohistochemical analysis is presently being performed to investigate cell proliferation, neuronal differentiation and neuroblast/neuron morphology. Additionally, the presence of Aβ1–42 monomers and oligomers will be assessed by western-blot and the eventual occurrence of Aβ1–42 aggregates by histology.

Results: Our results show that the Aβ1–42 injection did not affect locomotor activity, as assessed by the OF test. Furthermore, this injection did not affect exploratory drive or episodic long-term memory performance, as indicated by the NOR test.

Conclusion: Since the NOR test is dependent from several brain regions besides the hippocampus that might not be affected in our model, additional behaviour tests as well as cellular and molecular analysis are needed to further characterize this model.

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PS086

Hydroalcoholic extract of Dorema aucheri leaves prevents weakening of the brain antioxidant defense system and inhibits oxidative damage in rat model of ischemic stroke

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Aim: The aim of this article is Hydroalcoholic extract of Dorema aucheri leaves prevents weakening of the brain antioxidant defense system and inhibits oxidative damage in rat model of ischemic stroke

Introduction: The production of free radicals is the principal mechanism of brain injury in ischemic stroke. The present study tried to identify whether pretreatment with hydroalcoholic extract of Dorema aucheri (DA) leaves potentiates the brain antioxidant system and decreases brain infarction and oxidative damage during cerebral ischemia–reperfusion.

Methods: Three groups of rats were randomly selected (each group; $n = 12$); sham, control ischemic and ischemic pretreatment groups. Treated rats received freshly hydroalcoholic extract of DA (200 mg/kg/day) for 14 days. Then, cerebral ischemia–reperfusion was achieved by 90 minutes middle cerebral artery (MCA) occlusion followed by 24 h reperfusion. Infarct volume and contents of malondialdehyde (MDA), glutathione and nitrate (NOx) as well as superoxide dismutase (SOD) and catalase activities were assessed after 24 h reperfusion

Results: The contents of MDA and nitrate significantly increased in the ischemic hemispheres by 34% and 14%, respectively. Brain ischemia decreased the glutathione content (20%) and activities of catalase (38%) and SOD (14%) in ischemic hemispheres compared to sham rats. Treatment with DA before MCA occlusion significantly decreased the infarction in cortex and striatum by 63% and 75%, respectively, compared to control. DA considerably reduced the contents of MDA and nitrate in ischemic hemispheres by 28% and 11%, respectively, compared to control rats. Treatment with DA also increased the glutathione content (7%) and activities of catalase (46%) and SOD (16%) of ischemic hemispheres.

Conclusion: The present study revealed that pretreatment with hydroalcoholic extract of DA leaves prevents weakening of the brain antioxidant defense system and decreases the brain damage during cerebral ischemia–reperfusion.

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PS150

The Levator Auris Longus (LAL) muscle as an accessible system to study the effects of Botulinum Toxins in vivo

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Aim: In the present work, we aimed to find a reproducible model to study the effects of Botulinum neurotoxins (BoNTs), that allowed a widespread visualization of the intoxicated nerve terminals.

Introduction: Despite the well-established successful use of BoNTs to treat a variety of human conditions, their mechanism of action is still not fully understood. Thus, there is an emergent need of new and accurate models to study the effects of BoNTs. However, considering their potential lethality, it is challenging to find reproducible models to study the local application of BoNTs in living animals that allow a widespread visualization of the intoxicated nerve terminals.

In these work, we studied the innervation pattern and the effect of BoNTs in a group of small subcutaneous cranial muscles that are responsible for moving the pinna in rodents. Although all are easily accessible and manipulated, we focused on the levator auris longus (LAL).

Methods: Animals were injected subcutaneously with the indicated doses of BoNT/A, in the cranial muscles area. Muscles were then dissected and prepared for wholemount staining for Synapsin-I, cleaved SNAP-25 (synaptosome-associated protein of 25 kDa) and β3-tubulin.

Results: Detection of cleaved SNAP-25, the end-product of the catalytic action of BoNT/A, was possible even with injections as low as 0.1 ng. Mapping of the injected muscle showed the effect of BoNT/A in the majority of the endplate population. Also, seven days after BoNT/A injection, a sprouting process was evident, a landmark of regeneration.

Conclusion: BoNTs delivery to the LAL is a sensitive, simple and reproducible model to study the mechanisms of action of these toxins as it allowed the evaluation of BoNT/A effects throughout the entire muscle, without sampling bias. Thus, we forward that the LAL manipulation may constitute an excellent model to clarify the mechanisms of action of BoNTs in the neuromuscular system.

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PS188

NLRP3 inflammasome as a potential target to reduce epileptic-like activity

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Aim: Decipher how inflammation drives epilepsy and how NLRP3 targeting impacts epileptic-like activity.

Introduction: Epilepsy is one of the most common neurological diseases in worldwide. Inflammation was linked to the presence of inflammasomes, cytosolic multiprotein complexes, which promote the release of proinflammatory cytokines, namely Il-1β. Although a feedback loop has been described between inflammation and epilepsy, the role of inflammasomes in epilepsy is still unknown. NLRP3 is the most studied inflammasome,[1] activated by a two-signal process: 1) a priming signal (as lipopolysaccharides – LPS), which enhances the expression of NLRP3 and pro-IL-1β; and 2)

an activating signal (as ATP), which promotes the formation of the complex.

Methods: Organotypic slices were used to assess the interplay between inflammation and epilepsy. Slices were exposed to different concentrations of LPS (5, 10 and 20 ng/mL), either alone or in the presence of ATP (1 mM). LPS-induced inflammation was characterized using molecular-based assays, such as ELISA to quantify IL-1β, CBA to measure TNF-α, and western blot to assess the expression of Iba-1, GFAP, NLRP3/ASC, and αII-Spectrin. Field potential recordings were used to evaluate the epileptic-like activity of the slices and the effect of MCC950, a NLRP3 selective inhibitor,[2] was assessed.

Results: Results obtained by ELISA showed a significant increase in IL-1β concentration in slices exposed to 10 ng/ml LPS/1 mM ATP. TNF-α, assessed by CBA, was also significantly increased in this condition, corroborating the inflammatory phenotype. No changes in NLRP3 expression were observed by immunoblot analysis, but ASC, one component of the inflammasome, showed a decreased expression in LPS/ATP exposed slices, suggestive of its binding to NLRP3 and thus to complex formation.

Furthermore, epileptic-like activity, measured by field potential recordings, was blocked by MCC950 (10 μM).

Conclusion: We demonstrate that LPS induces an inflammatory phenotype in organotypic slices. NLRP3 blockade eliminated the epileptic-like activity of the slices.

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PS110

Study of the modulatory CNS regions in the visual circuit Retina-Superior Colliculus-Lateral Posterior nucleus triggering freezing behavior

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Aim: The goal is to understand the neuronal networks organization from the sensory input to the freezing behavior through the identification of modulatory brain regions that project to the Superior Colliculus.

Introduction: The behavior of an animal can be triggered by signals in its visual environment. Threating visual stimulus evoked innate defense behaviors as freezing behavior. This project is focused in one visual-guided behavioral circuit that links the retina visual information with the Lateral Posterior thalamic nucleus(LP) via Superior Colliculus(SC).

Methods: The experimental approach is based on retrograde viral tracing techniques. Using the stereotaxic surgery, the first injection with a Herpes Simplex Virus expressing TVA receptor and glycoprotein G was done at LP. After 21 days, the second injection was done at the SC with a Rabies Virus coated by EnvA and lacking of glycoprotein G. The combination of these viruses allowed the restriction of the viral tracing to the circuit of interest. Subsequently, the experimental procedure continued perfusing the mouse, slicing the brain and staining it. Finally, the slices were scanned using the fluorescent confocal microscope.

Results: The resulting images presented labeled cells in all brain areas that sent inputs to collicular neurons that are projecting to LP. The main nuclei identified were the Periaqueductal gray, the primary visual cortex and the Substantia nigra, suggesting their modulatory role in freezing responses.

Conclusion: The main areas labeled are sending excitatory projections to SC to reinforce the freezing behavior. Also, Ntsrl-GN209-Cre mice used in combination with flox-HSV for the first injection restricted more the viral tracing, specifically to the Ntsrl+-Wild-field neurons of SC which were already known that project to LP. The results were not completely consistent with the non-flox-HSV injections but the main nuclei named above were also labeled. These results suggest that the flox-HSV is necessary to exclude nonspecific labeling of projections from SC-LGN.

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PS048

The influence of antipsychotics therapy and sociodemographic characteristics on cognitive performances in acute phase of schizophrenia

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Aim: The main purpose of this research was to examine the influence of sociodemographic characteristics (gender, age, level of education, heredity, alcohol and psychoactive substances), and the effect of different therapies on cognitive capabilities of patients diagnosed with schizophrenia.

Introduction: Schizophrenia, as one of the most common psychiatric diseases, is characterized by generalized cognitive damage with various degrees and in all domains of cognitive functioning. Cognitive dysfunction is one of the main causes of poor social and professional functioning for patients with schizophrenia.

Methods: The research involved 50 patients with acute phases of schizophrenia from the Psychiatric Clinic in Novi Sad. The primary instrument for the research was the standardized test for examination of cognitive impairments, Mini-Mental Scale Examination (MMSE).

Results: Acquired data correlated with MMSE score, noting the degree of cognitive impairments in patients, particularly significant with relation to age and duration of illness. Gender, level of education and type of used antipsychotics were not significantly correlated with MMSE score.

Conclusion: During this research it is found that aging and longer illness duration bear significant correlation to higher levels of cognitive impairment.

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PS190

Voluntary inhibition of saccadic eye movements: EEG study

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Aim: The aim of our study was to find out EEG markers of inhibitory control in human.

Introduction: The voluntary inhibition is an important component of cognitive control. It is strong in healthy adults and weak in people with schizophrenia. The cortical mechanisms of inhibition are associated with event-related potentials (ERPs). In the case of a saccadic response some new EEG correlates of inhibition could be found.

Methods: Sixteen healthy right-handed subjects (18–22 years) participated in the study. We used a modified "Go/No go delay" paradigm with long interstimulus interval (2800–3000 ms). The task involved two types of target stimuli ("Go", "No go") with 50% probability. EEG and saccades were recorded simultaneously. ERPs were determined by means of coherent averaging relative to target stimulus onset. The EEG brain mapping was used to depict spatial dynamics of P1.

Results: P1 peak latency was 90–140 ms and tended to increase in cases of inhibition (by 6 ± 0.5 ms, $p < 0.05$). In the "No go" situation P1 amplitude was significantly lower than that in case of "Go" stimulus presentation (by 3.3 ± 0.7 mkV, $p < 0{,}05$). Regardless of the place where "No go" stimulus appeared, P1 amplitude was significantly higher on the right hemisphere, that is known to be the dominant one for inhibitory control. The EEG mapping data demonstrate the "bottom-up" spreading of P1 foci in "No go" conditions. It also indicates inhibitory processes.

Conclusion: The spatiotemporal parameters of P1 component in "Go/No go delay" paradigm reflect inhibitory processes. Therefore, P1 can be used as EEG marker of inhibitory violations in the clinical research. Our current research involves as subjects the patients with schizophrenia and ultra-high risk patients, as they demonstrate weakened the inhibitory processes. The data would contribute to the reliable diagnostics of schizophrenia at its early stages and to the plausible correction of cognitive impairments.

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PS135

Factors influencing the outcome of endovascular embolization of anterior communicating artery aneurysms

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Aim: The aim of the study was to assess the influence of morphologic parameters of anterior communicating artery aneurysms and the method of embolization on the success rate of procedure.

Introduction: Endovascular embolization of anterior communicating artery aneurysms is currently considered as primary management tool and the improvement of procedural success rate is crucial.

Methods: Treatment process of 109 patients undergoing endovascular embolization of anterior communicating artery aneurysm was retrospectively analysed. All procedures were performed between August 2006 and December 2016 in Department of Interventional Radiology of University Hospital in Cracow (Poland). The mean age of patients was 56.7 ± 15.2 years (range 28–91), 50.5% of patients were female. Used methods of embolization: coiling alone, balloon-assisted coiling, stent-assisted coiling, Y-stenting + coiling. Evaluated morphologic parameters: width of the neck, maximal height, maximal width, shape of aneurysm, dome orientation. The outcome of the procedure was assessed with Raymond–Roy occlusion classification. Data were analysed using chi-square test and Student's *t*-test. Statistical significance was set at $p < 0.05$.

Results: Coiling alone significantly improved outcome of embolization considered as better score in Raymond–Roy occlusion classification, compared to other methods (1.4 ± 0.5 vs. 1.6 ± 0.7; $p = 0.034$). In case of irregular aneurysms (85.7% vs. 34.6% (regular aneurysms); $p = 0.025$; OR = 2.615) and those with posterior orientation of the dome (76.9% vs. 36.5% (anterior orientation); $p = 0.005$; OR = 5.810) incomplete embolization (Raymond–Roy class II and III) was significantly more frequent. Within the group of discharged patients, only 33.3% undergone control radiologic examination – 40.7% conventional angiography, 59.3% MR angiography. In that group, 81.5% of aneurysms had better or the same class in Raymond–Roy classification and 18.5% had worse outcome. We did not discovered any statistically significant factor contributing to that phenomenon.

Conclusion: Coiling alone is the most efficient method in terms of the aneurysm occlusion rate. Irregular shape of the aneurysm and posterior orientation of the dome significantly hinder the embolization of aneurysm.

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PS097

Antidepressive potential of aqueous extract of common vervain (*V. officinalis* L. Verbenaceae) and molecular docking studies of its main components as potential antidepressive agents

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Aim: Assessment of antidepressive effect of aqueous extract (AE) of common vervain and its main constituent – verbascoside and elucidation of underlying mechanism of action.

Introduction: Common vervain is a plant used in traditional medicine. Its AE contains a vast number of compounds, hence its significant pharmacological potential.

The monoamine hypothesis is the central theory of depression, and a majority of conventional antidepressants act on the monoaminergic system.

Methods: Experiments were conducted on Swiss albino sexually mature male mice. There were 6-8 animals in each of 5 subgroups (imipramine; fluoxetine; two different doses of AE – AE I, II; and VS;). Forced Swimming Test (FST) and Tail Suspension Test (TST) were used to assess the antidepressive effect.

Molecular docking experiments were performed using the programme AutoDock 4.2, with 3D structures of crystallized proteins from the PDB database and 3D structures of ligands generated by the software Avogadro 2 0.8.0.

Results: Immobilisation time (IT) in FST after the administration of imipramine was shorter than the control, same as for subgroups treated with AE I, II and VS. In the subgroup treated with fluoxetine, IT in TST was shorter than the control time, and the same was observed in subgroups treated with AE I, II and VS.

Significant binding energies were found for Serotonin Reuptake Transporter (SERT) and verbenalin (−7.20 kcal/mol) and verbascoside (−6.61 kcal/mol), and for the Leucine Transprter (LeuT), the homologue of the noradrenaline reuptake transporter, and verbenalin (−6.27 kcal/mol) and caffeic acid (−5.85 kcal/mol).

Conclusion: In both pharmacodynamic tests the antidepressive effect of AE and VS has been confirmed. Verbenalin and verbascoside binding energies and poses in interaction with SERT were similar to those of paroxetine. For LeuT, verbenalin showed both a similar binding energy and pose to that of imipramine, whereas caffeic acid showed only a similar binding energy.[1–4]

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PS213

Thermal denaturation profiles of proteome and blood serum of rats with drug-induced dementia. A DSC study

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Aim: The aim of this study is to evaluate the effect of scopolamine on different brain segments using DSC.

Introduction: In this work, probes from different brain segments of rats with drug-induced dementia were characterized by differential scanning calorimetry (DSC) and their thermodynamic properties were determined.

Methods: Male Wistar rats were injected with scopolamine for 14 consecutive days in order to induce drug model of dementia. After being decapitated, their brains were divided into the following segments: telencephalon, mesencephalon and cerebellum. Afterwards, the brain supernatants of the latter 3 segments were examined by DSC and compared with the controls.

Results: The DSC measurements revealed large differences between the denaturation profiles of rat brain supernatants and blood serum. The thermograms of brain tissues displayed clearly expressed low-temperature exothermic transitions with peaks in the range 35–45 °C which are missing in blood serum samples. There were differences between the termograms of the separate brain segments as well. The thermodynamic parameters of the denaturation profiles were also determined.

Conclusion: These measurements show that DSC is an appropriate method with great potential for detection and characterization of the changes taking place at molecular level in different tissues, especially in brain tissues affected by neurodegenerative disorders.

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PS231

Effects of Vitamin D on the expression of markers of principal neurons, interneurons and astrocytes in cerebral cortex and hippocampus in gerbils exposed to transient global cerebral ischemia

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Aim: Examination of the effects of vitamin D pretreatment on the expression of markers of principal neurons (NeuN), inhibitory interneurons (PV) and astrocytes (GFAP) in cerebral cortex and hippocampus in gerbils who were exposed to transient global cerebral ischemia.

Introduction: Brain ischemia may cause serious damage to the cells in the central nervous system. Vitamin D has an important role in brain injury treatment due to its neuroprotective effects.

Methods: Gerbils were divided in 5 groups: control group; two groups that underwent ischemia and then reperfusion for three (I/R3d) and seven days (I/R7d) and two groups that were treated with vitamin D before I/R (vitD + I/R3d and vitD + I/R7d). Complete blood supply to the brain was cut off for 10 minutes and reperfusion lasted 3 and 7 days. They were daily treated with vitamin D for 7 days prior ischemia. Expression of proteins was detected using Western blot.

Results: No changes were detected in expression of NeuN markers in cortex of experimental groups, while there was increase in expression in hippocampus in groups I/R7d and vitD + I/R7d in comparison to the control group and group vitD + I/R3d. Expression of PV in cortex was significantly reduced in group I/R7d in comparison to group I/R3d, whereas in hippocampus the expression was significantly higher in group vitD + I/R3d than in group I/R3d. Expression of GFAP has significantly risen in all groups in comparison to the control group whereas in hippocampus there was a rise in groups vitD + I/R3d, I/R7d and vitD + I/R7d in comparison to the control group. There was also a rise of GFAP expression in groups treated with vitamin D (vitD + I/R3d and vitD + I/R7d) in comparison to those that have not been treated (I/R3d, I/R7d).

Conclusion: Vitamin D has positive effect on astrocytes in both structures of gerbils that underwent global cerebral ischemia, especially in hippocampal region.

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PS238

Identification of genetic modifiers of somatic CAG instability in Huntington's Disease by in vivo CRISPR – Cas9 genome editing

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Aim: To develop an experimental platform for *in vivo* investigation of candidate genetic modifiers of somatic CAG instability in Huntington's disease.

Introduction: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion within the huntingtin gene (*HTT*).[1] Despite being a monogenic disorder, for which the mutation has been known for some time now, no cure or disease-modifying therapy is available, indicating that novel approaches are critical.

Somatic CAG repeat instability, characteristic of mutant *HTT* alleles, is inversely correlated with patient age of onset and may contribute to HD pathogenesis.[2,3] This phenotype, common to other trinucleotide repeat disorders,[4] was previously shown to be DNA mismatch repair (MMR) dependent.[5] The DNA repair machinery was further implicated as a modifier of HD age of motor onset in a recent genome wide association study, underlining its promise as a relevant disease mechanism that could potentially be therapeutically targeted.[6]

In this study, we are developing a CRISPR/Cas9-based approach that will enable the investigation of candidate genetic modifiers of HD age of onset as potential modifiers of somatic CAG repeat instability in a HD mouse model.

Methods: We have developed CRISPR reagents against known and candidate genetic modifiers of somatic CAG instability in Huntington's disease. In preliminary experiments, we treated HD mice with CRISPR reagents against *Mlh1* and investigated the level of gene editing achieved as well as the impact on liver CAG instability.

Results: We were able to significantly suppress the CAG expansion process in the liver of HD mice by knocking out the *Mlh1* gene in our *in vivo* CRISPR platform. The efficiency achieved in modifying the instability phenotype makes us very confident that we will be able to test and validate additional candidate modifiers. To that end, we have already validated reagents for efficient knockout of a subset of known and candidate modifier genes and we have developed assays that will allow detailed characterization of gene editing at these sites.

Conclusion: We have successfully developed an *in vivo* CRISPR-Cas9-based platform that allows for knocking out genes of interest in the liver of adult mice, and consequently perturb the somatic CAG expansion process. We will next use this tool to test the role that candidate genes might play in that disease-relevant process. While the scope of this project was liver oriented, future work will also be aimed at targeting the striatum which is the main site of HD-related pathology.

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Oncology & Molecular Biology Poster Session
Sunday, September 17th, 10h00

PS021

Regulation of transcription factor MEF2C by RNA binding protein HuR

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Aim: We hypothesized that HuR RNA binding protein regulates MEF2C expression through association with MEF2C mRNA.

Introduction: MEF2C is earliest expressed member of the MADS-box super family during heart development. In the postnatal heart, decreased expression of MEF2C has been associated with myotonic dystrophy type 1 (DM1) heart disease. Hu proteins are known to regulate a wide range of gene expression by modulating mRNA's half-lives.

Methods: We use Human Fetal Cardiomyocyte cell line RL14. Cells are transfected with Superfect Transfection Reagent(Qiagen). And RNA Isolation performed by using RNeasy Plus Mini Kit. Real Time quantitative PCR (q-PCR) analysis performed using Fast SYBR Green Master Mix.

Results: Over expression of HuR in cardiomyocytes derived from primary human fetal ventricle increased MEF2C mRNA 47.3% ($p = 0.01$). Knocking down of HuR by siRNA decreased MEF2C mRNA by 62% ($p = 0.01$). RNA Immunoprecipitation showed HuR associated with MEF2C mRNA.

Conclusion: Our results suggest that RNA binding protein HuR associates with MEF2C mRNA in cardiomyocytes. And also HuR positively regulates MEF2C mRNA expression.

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PS024

The effect of prenatal Vitamin C deficiency on endochondral ossification in guinea pigs

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Aim: The aim of the research is to investigate the effect of prenatal vitamin C deficiency on endochondral ossification in guinea pigs.

Introduction: Vitamin C is an essential nutrient which inter alia enables the synthesis of collagen and therefore endochondral ossification. Throughout years a lot of research has been published investigating the exact role of vitamin C and the impairment developed due to its deficiency. However there is insufficient data about the effect of prenatal deficit of vitamin C on the developing bone structures.

Methods: The study encompassed 14 fertilized female albino guinea pigs. Their diet was comprised of vitamin C-free food and ad libitum water enriched with vitamin C. The 10th day of fertilization, experimental group was depleted of vitamin C. Deprivation lasted until the 50th day, after which the females were sacrificed and their fetuses were taken out. Forelegs of fetuses were fixed and dehydrated, after which they were embedded in paraffin and

longitudinal sections were made. The stain used for histology was Alcian&Alizarin.

Results: The development of long bones in vitamin C deficient guinea pigs are considerably stagnant. Hyaline cartilage models are significantly shortened. Ossification in the diaphyses of carpal and metacarpal bones are absent, and the organization of the epiphyseal plates is very irregular with the reduction of number of chondrocytes. Moreover, there are numerous haemorrhagic regions and subperichondrial bleeding with separation of perichondrium.

Conclusion: Deprivation of vitamin C during inrauterine period disables normal development of long bones. Disorder of hyaline cartilage models was seen, as well as the disorder of ossification.

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PS060

Peculiarities of expression of apoptosis markers in the tissues of primary fallopian tubes carcinoma

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Aim: immunohistochemical analysis of apoptosis markers in the tissue of PFTC.

Introduction: Primary fallopian tubes carcinoma is a rare case among oncological diseases of female genital organs, but the mortality rate is rather high. Nowadays, the prognostic factors of this neoplasia are not fully determined. The data on the p53 and bcl2 proteins expression and their use as prognostic factors in patients with malignant tumors of many locations are contradictory.

Methods: the study was conducted on 66 samples of fallopian tubes tumor tissue. To study the apoptosis peculiarities of tumor cells the mouse monoclonal antibodies for bcl-2 (clone 100/D5) and p53 (clone SP5) were used. Mathematic calculations were done using Microsoft Excel 2010 with AtteStat 12.0.5.

Results: The high expression of p53 was found in patients of all clinical stages. Mutations of p53 increased with spreading of the neoplastic process. Strong correlation of p53 presence in tumor samples and clinical stage of the disease was determined ($r = 0.77$). In contrast to the abovementioned protein the study of bcl-2 showed the moderate negative correlation between this protein and the stage of the disease ($r = -0.54$). Analysis of the dependence of p53 expression with the presence or absence of lymph nodes metastasis showed a direct correlation between the indicators ($r = 0.25$). Thus the level of p53 expression in patients with N1 was $80.6 \pm 2.7\%$ compared with the N0 group ($29.7 \pm 3.6\%$). The stage of neoplasia differentiation is in moderate direct correlation with p53 expression ($r = 0.58$) and in inverse with – bcl-2 ($r = -0.64$).

Conclusion: Expression of p53 depends on neoplasia spreading and stage of tumor differentiation. The expression of p53 is an independent prognostic marker for N-status and helps to classify the patients into "risk" groups.

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PS064

Analysis of combined impact of doxorubicin and menadione on human leukaemia Jurkat T cells

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Aim: The anti-proliferative effect and the mechanism of action of doxorubicin(DOX) in combination with menadione(MD) were studied in Jurkat T cells, a model for acute lymphoblastic leukaemia (ALL).

Introduction: Doxorubicin is a well-characterized and successful antineoplastic drug commonly used in various cancer treatments, including ALL. Menadione has proven a strong pro-apoptotic effect in Jurkat cells.[1–3]

Methods: Cell cycle, apoptosis/necrosis and the oxidative status were assessed by flow cytometry on propidium iodide, Annexin V-FITC/PI and CM-H2DCFDA/7-AAD labelled cells, respectively.

Results: Oxidative stress induced within 4 h by MD (IC50 = 11.5 μM) was reduced in the presence of 500 nM DOX (IC50 = 22.0 μM). After treatments of 18 h, DOX induced cell cycle arrest displaying a trimodal distribution; successive G2/M, S and G0/G1 blockage was produced with an IC50 of 49 nM, 464 nM and 1866 nM, respectively, whereas in the presence of 7.5 μM MD, increasing levels of DOX mainly induced S-phase arrest. Within 18 hours of exposure, DOX induced apoptosis in a biphasic dose-dependent manner (K_d = 335 nM and 3.29 μM, respectively). Addition of 7.5 μM MD enhanced apoptosis at <300 nM DOX, but reduced cell death at higher levels of DOX. However, 48 h after drug removal the apoptotic rate was considerably higher in cells exposed to DOX:MD, which also showed consistent fractions of early apoptosis (up to 44%). The efficacy of DOX was doubled by MD(K_d = 46.5 nM in the presence, and K_d = 99 nM and 143 nM in the absence of MD).

Conclusion: Data indicate that clinically relevant levels of MD and DOX in combined treatments can exert considerable cytotoxic impact on Jurkat cells, via cell cycle arrest and apoptosis induction. These findings could encourage new therapeutic strategies to improve the therapeutic index of doxorubicin in ALL treatments.

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PS068

Effect of symptom interval and demographic characteristics on initial stage of malignant tumors in children

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Aim: The aim of our retrospective study was to determine the influence of demographic and clinical characteristics of patients, initial stage of disease and tumor size on symptom period in children with malignant tumors.

Introduction: One of the main goals in pediatric oncology is timely diagnosis, cause it allows prompt and more effective treatment and significantly decreases the number of complications. The majority of children with malignant tumors have specific or non-specific symptoms certain time period before the diagnosis which can point towards malignant disease.

Methods: Our study included 296 children with malignant tumors, diagnosed and

treated between 2005 and 2016 in University Children's Hospital in Belgrade. Collected data included sociodemographic parameters, variety of symptoms and its duration, initial stage of disease and size of the tumor.

Results: The most frequent tumors were as follows: neuroblastoma, Hodgkin and non-Hodgkin lymphoma and kidney tumors. Non-Hodgkin lymphoma was diagnosed more frequently in boys, while Ewing sarcoma and primitive neuroectodermal tumors were seen mostly in girls. The majority was admitted at IV stage (30.1%) in opposite to 13.5% of patients in I stage. The average symptom interval was 87.7 days (median 46; SD = 164), from 5 to 2190 days. We have proven that following factors have significant effect on the extent of symptom interval: age ($p < 0.001$), type of tumor ($p < 0.05$), its localization ($p < 0.001$), specific symptoms ($p < 0.05$), and refferal from primary health care unit in comparison to secondary one ($p < 0.05$).

Conclusion: The results of our study give a new insight in symptom interval of children with malignant tumors in our country. More detailed comprehension of patients' characteristics, their diseases, healthcare system and their effect on symptom interval could significantly contribute to early diagnosis, as well as decreased number of complications at admission and during treatment.[1–6]

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PS069

Impact of prior malignancies on the outcome of colorectal cancer: Revisiting clinical trial eligibility criteria

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Aim: To study the impact of prior malignancies on the survival of subsequent CRC.

Introduction: Colorectal cancer (CRC) is the third most common cancer in the US.[1–3] Some studies have correlated a prior history of malignancy with an increased incidence of CRC. Patients with history of cancer are generally excluded in clinical trials. This practice, not only affects clinical trials accrual, but also limits the potential therapeutic options for this population. The rationale behind this exclusion is that a history of malignancy could potentially interfere with the study outcomes.[4] However, little is known about its real impact on survival of subsequent CRC.

Methods: We identified patients with CRC diagnosed between 1973 and 2008 using the National Cancer Institute's SEER database.[5,6] Outcomes of interest were overall survival and cause-specific survival of subsequent CRC in general, and specifically stage IV disease. Unadjusted Kaplan-Meier test and multivariable covariate-adjusted Cox models were used to assess the eligibility of enrollment of stage IV CRC patients in clinical trials.

Results: Overall, 550,325 patients with CRC were identified, of whom 31,663 patients had a prior malignancy. Both, history of prior non-leukemic malignancy and prior leukemia were associated with a worse overall survival (HR = 1.165 95% CI = 1.148–1.183, $P < 0.001$) and (HR = 1.825 95% CI = 1.691–1.970, $P < 0.001$), respectively. However, a history of any prior non-leukemic malignancy showed a favorable colorectal-specific survival (HR = .930 95% CI = .909–.952, $P < 0.001$). Analysis of stage IV CRC showed that a history of any prior non-leukemic malignancy was not associated with a significant difference in overall survival but having a history of leukemia showed a worse overall survival (HR = 1.535, 95% CI = 1.303–1.809, $P < 0.001$).

Conclusion: Clinical trials should take these results into consideration when including/excluding stage IV CRC patients with prior malignancies.

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PS071

Intervention of diabetes mellitus and metabolic risk factors in AMPK-PGC1α-SIRT3 pathway in the human corpus cavernosum

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Aim: This study aims to investigate the signaling pathway AMP-activated protein kinase (AMPK)-Peroxisome proliferator-activated receptor-gamma coactivator (PGC)1α-sirtuin (SIRT)3 in the human corpus cavernosum (HCC) between healthy individuals and those with cardiovascular disease risk factors (CVDRF).

Introduction: SIRT3 is a mitochondrial NAD+-dependent-protein-deacetylase involved in the regulation of cellular metabolism.[1,2] As a key factor in AMPK and PGC1-α activation in stress, the decrease in SIRT3 expression or activity is associated with diverse pathologies and aging. Actually, SIRT3 expression was found decreased in HCC of aged individuals with CVDRF.[3] CVDRF such as diabetes mellitus (DM), dyslipidemia, hypertension and obesity strongly associate to endothelial dysfunction, which early manifests as erectile dysfunction (ED).[4]

Methods: HCC's samples from individuals aged 40-60 years, submitted to programmed urological surgeries at Hospital São João-Porto, were divided in three groups (n = 4): (1)-controls without ED or CVDRF; (2)-DM patients; and (3)-patients with three or more CVDRF including DM. Dual immunolabelling of SIRT3 and superoxide dismutase (SOD)2 with alpha-actin was carried out. As well, levels of SIRT1, SIRT3, SOD2, PGC1α, NADPH oxidase (Nox)1, phospho-AMPK and AMPK were assessed by Western-blotting(WB).

Results: We observed SIRT3 and SOD2 expression in α-actin-labelled fusiform muscle cells in all groups. The semi-quantification by WB demonstrated a significant decrease in SOD2 expression in group 3 relatively to controls, as well as, an increased tendency of Nox1 and PGC1α and a decreasing trend in phospho-AMPK in groups 2 and 3. No differences in SIRT1 and SIRT3 were observed among groups.

Conclusion: This study suggests that CVRF including DM increase oxidative stress in HCC owning to a decrease in SOD2 expression and concomitant increment in Nox1. Further studies with an increased number of HCC samples will be necessary to elucidate the role of the AMPK-PGC1α-SIRT3 signaling pathway in the response to oxidative damage.[5]

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PS075

Examination of antiproliferative effects of the horseradish extracts

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Aim: The aim of the study was to investigate in vitro the antiproliferative effects of the horseradish juice and pulp using different solvents for the extraction.

Introduction: Horseradish (*Armoracia rusticana*, Brassicaceae) is a perennial herbal plant, which is widely used in human nutrition, as well as in a traditional medicine. Horseradish is a rich source of bioactive compounds such as isothiocyanates, that have proved to be significant antitumor agents.

Methods: Samples were prepared by the Kupchak extraction method, and the antiproliferative effects of the horseradish juice and pulp extracts were examined on the human tumor cell line MDA-MB-231 (ER−, human breast adenocarcinoma). Cell growth was determined by measuring the total protein by colorimetric sulforhodamine B assay. The obtained results (expressed as mean ± SD) were analyzed by Tukey HSD test and the differences were considered statistically significant at $p < 0.05$.

Results: According to the IC50 parameter (the concentration that inhibited the cell growth by 50%), as an important indicator of the antiproliferative effects, the most pronounced antitumor activity was observed for chloroform juice extract (IC50 = 5.52 ± 1.47 μg/ml). In addition, highly potent was chloroform pulp extract (IC50 = 19.44 ± 3.82 μg/ml), as well as the dichloromethane juice (IC50 = 26.50 ± 4.15 μg/ml) and pulp (IC50 = 26, 01 ± 2.45 μg/ml) extracts. On the other hand, significantly lower in vitro antitumor effect was noticed for the butanol pulp extract (IC50 = 114.52 ± 0.28 μg/ml). IC50 vales for butanol juice extract, as well as water juice and pulp extracts were higher than 500 μg/ml.

Conclusion: The obtained results suggest that *A. rusticana* is as a significant source of antitumor agents, especially liposoluble isothiocyanates and as such, it should be recommended for further use in a human nutrition and prevention of cancer.

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PS080

Contribution of the determination of numeric value of adc map in early detection of prostate cancer

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Aim: To define the range of ADC values for the absence of malignant disease, as well as to determine the threshold of ADC values for suspected prostate cancer.

Introduction: Prostate cancer is the second most diagnosed cancer, and the second most common cancer-cause of deaths in men worldwide. The apparent diffusion coefficient (ADC) derived from DWI has been shown to improve the detection of prostate cancer and is the primary imaging method for the differentiation between low to high grade cancers. ADC values show reduction with increasing Gleason's score.

Methods: Prospective study included 60 subjects. Male patients were divided into the groups with pathohistology verified benign and malignant lesions (aged, 46–81; average age, 67.7 years) with abnormal PSA values (>4 ng/ml), and into control group (aged, 44–81; average age 65.3) with normal PSA values (0–4 ng/ml). Prostates were first examined on MRI, determining the diffusion values on ADC map, by placing the region of interest (ROI), through the middle of lesions. Later, the TRUS-guided biopsies were perfomed. Three intersections of the prostate (apex, middle, and base) were observed, and at total of 12 places (4 places per section) the mentioned methods were indicated.

Results: Statistically significant difference ($p < 0.05$) between the groups of patients with malignant and benign lesions in relation to the ADC values of the apex, base and middle of prostate. ADC values of malignant lesions at apex were in range 952–1030, at base 859–977 $\times 10^{-6}$ mm^2/s, while in benign lesion values at apex where in range 1234–1336, and at base 1096–1183 $\times 10^{-6}$ mm^2/s.

Conclusion: Determination of the numerical value of ADC map represents a significant additional diagnostic parameter for prostate cancer. All values in the range of 1179–1229 for base, 1063–1139 for middle, and 1199–1379 $\times 10^{-6}$ mm^2/s for apex were considered normal. Values between the range of 857–1030 $\times 10^{-6}$ mm^2/s have been suspected for possible presence of the prostate cancer.

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PS101

Deoxycytidine kinase expression in AML blasts and its relationship to leukemia-free and overall survival

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Aim: Study the correlation of expression of genes involved in cytarabine metabolism to leukemia-free (LFS) and overall survival (OS) in AML.

Introduction: Cytarabine is the backbone of AML therapy. Understanding the roles and polymorphisms of genes involved in cytarabine metabolism and resistance in AML will facilitate development of novel therapeutics.

Methods: Adults less than 60 years with non M3 AML were included. Archived diagnostic marrow samples were studied for expression of 10 genes involved in cytarabine metabolism by RT-qPCR; gene expression normalized to GAPDH was compared using the unpaired t-test with Welch correction. SNP rs4694362 in deoxycytidine kinase (DCK) gene was tested using Taqman assay. Median time to relapse and survival was calculated by Kaplan Meier method.

Results: 21 Han Chinese patients (median age: 50) were identified; 15 were male; 16 had intermediate risk cytogenetics; 5 had a blast count of over 100 $\times 10^9$/L at diagnosis. 17 patients achieved CR; 12 after first induction. No difference in gene expression was seen between CR ($n = 12$) versus non CR ($n = 9$) with first induction. 17 CR patients were followed for a median duration of 67.5 months; median time to relapse was 15 months. 1 patient who underwent allogeneic transplant in CR1 was excluded. Higher mean DCK expression was seen in patients with LFS longer than ($n = 7$) versus less than 2 years ($n = 9$) (1.91 ± 0.67, p: 0.01) and in those with OS longer than ($n = 8$) versus less than 3 years ($n = 8$) (2.02 ± 0.69, p: 0.01). DCK rs4694362 TT genotype was less prevalent than CT in patients with >2 year LFS; but not statistically significant. (49% vs 60%, p: 0.6).[1,2]

Conclusion: DCK phosphorylates cytarabine to its active metabolite. Our work shows that higher DCK expression is correlated to LFS and OS in AML. The role of DCK SNP rs4694362 should be explored further in the Chinese.

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PS108

Analysis of MTHFR C677T polymorphism significance in patient preparation for the in vitro fertilization procedure

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Aim: The aim of this study is to determine if in relation to general population, there is a statistically significant difference in the frequency of alleles and genotypes of the C677T polymorphism of MTHFR gene, amongst women with unknown cause of infertility, who are undergoing in vitro fertilization preparation.

Introduction: Methylenetetrahydrofolate reductase (MTHFR) is an enzyme coded by MTHFR gene. Polymorphism of MTHFR gene C677T leads to decreased function of MTHFR enzyme, which is associated with high level of homocysteine and low concentration of folate, which can undermine female reproductive function and affect the outcome of in vitro fertilization.

Methods: The study included the experimental group consisted of 31 women and the control group consisted of 100 women. C677T polymorphism was detected via PCR/RFLPS method. The statistical difference in genotype and allele frequencies was conducted using the Chi-square test.

Results: The comparison of genotypes amongst the experimental and control group has not shown a statistically significant difference ($p > 0.05$). Frequency of the MTHFR677 TT genotype is 22.6% in the experimental group, and 12.0% in the control group, while the allele T frequency amongst the experimental group was

41.9%, and the frequency of the aforementioned allele amongst the control group was 34.5%.

Conclusion: The results of this study show that there is no statistically significant correlation between MTHFR C677T polymorphism in women with infertility of unknown cause, who are undergoing in vitro fertilization preparation, but also underline the need for further research.

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PS114

Assessing the oxidative modification of proteins in inflamed placenta combined with iron deficiency anemia in the pregnant through histochemical method with bromophenol blue based on Mikel Calvo

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Aim: To set features of OMB in the cytoplasm of decidua cells in basal plate of the placenta at chorioamnionitis with iron deficiency anemia in pregnant women by means of histochemical methods combined with computer microspectrophotometry.

Introduction: Decidua cells are important cells to the placenta, playing a significant role both in the physiology of pregnancy and during inflammation. The processes of oxidative modification of proteins (OMB) in inflammation are associated with increased levels of oxygen free radicals, which alter the properties of these macromolecules while oxidating amino groups of proteins. Anemic condition is accompanied by intensification of free radical processes in the blood and tissues, and iron deficiency additionally significantly modifies these processes.

Methods: 125 studied placentas, to compare the studied placental physiology of pregnancy and monitoring iron deficiency anemia without inflammation.

A histochemical reaction of bromophenol blue for "acidic" and "basic" proteins by Mikel Calvo was set in histological sections 5 μm thick.

Delta Optical Evolution 100 and Olympus SP-550UZ were used to obtain a digital copy of the image. Ratio R/B, which is the ratio between the amino and carboxyl groups in proteins, was determined by "ImageJ".

Unpaired Student's test calculated arithmetic mean and its error.

Results: When assessing visual histochemical preparations decidua cells are clearly stained, that is suitable for quantitative research, cell boundaries are defined through clear cell membrane coloring and contrasting color around decidua cells fibrinoid. Nuclei and nucleoli were visualized fairly well. "Basic" proteins prevailed in nucleoplasm, while "sour" in the nucleolus.

The decidua cells' cytoplasm specific color has been mostly granular in nature, and spectral characteristics and optical density of color varied greatly.

Factor R/B at physiological pregnancy ($n = 20$) was – 1.04 ± 0.008 and in iron deficiency anemia ($N = 21$) – 1.06 ± 0.009 $P > 0.05$. In acute chorioamnionitis ($n = 23$) – 1.08 ± 0.009, and combined with iron deficiency anemia ($N = 21$) – 1.09 ± 0.009 $P > 0.05$. Regarding chronic chorioamnionitis ($n = 20$) ratio – 1.24 ± 0.011, and combined with iron deficiency anemia ($N = 21$) – 1.64 ± 0.016 $P < 0.001$.

Conclusion: Conclusion. The intensity of OMB increases only in chronic form of chorioamnionitis in the decidua cells cytoplasm, and combined with iron deficiency anemia significant performance increase has been observed.

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PS121

Comparison of Ras/Raf/MAPK signaling pathway in primary tumour and lymph node metastases – A report on an experimental study of two colorectal cancer cell lines (SW480 and SW620) and tissue samples

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Aim: To compare the presence of mutations in essential genes of CRC pathogenesis pathway between tissues derived from the primary tumour site and lymph node metastases.

Introduction: Colorectal cancer (CRC) remains the third most commonly diagnosed malignancy worldwide and a leading cause of cancer - related death. One of the pivotal pathways leading to CRC development is Ras/Raf/MAPK which is regulated by the receptor for the EGF. Mutations in these genes predict lack of response to EGFR-targeting monoclonal antibodies. However it is a common practice to assess only the primary tumour site, while mutations in metastasis may also affect the response to treatment.

Methods: The study was conducted on 10 patient-derived tissue samples and two ATCC human CRC cell lines obtained from the same individual: SW480 (primary tumour) and SW620 (lymph node metastasis). Cell lines were cultured according to the protocol. Genomic DNA and RNA were isolated, and PCR and RT-PCR were conducted. Primers for PCR included the following fragments: KRAS (exons 2,3,4), NRAS (exons 2,3,4), BRAF (exon 15); and for RT-PCR: KRAS, NRAS, BRAF and EGFR. Restriction enzymes were used. Proteins were extracted, purified and Western-Blot (RAS, RAF, MAPK) was performed.

Results: For SW480 we detected a mutation in exon 3 of NRAS gene, whereas SW620 presented a wild type. The level of Ras protein remained the same. Raf protein expression was abundant in the primary tumour site as compared to the lymph node metastasis, whereas MAPK protein presented the opposite level of expression.

Conclusion: The analysis of Ras-Raf-MAPK pathway may suggest that along with the tumour progression, the dominating signal is located at deeper levels of signaling pathway. Due to existing differences in key molecular points between the primary tumour and its metastases, in the era of targeted therapy, pre-treatment assessment of both sites has a potential to become a standard of care.[1,2]

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PS122

PI3K-Akt and Ras-Raf-MAPK signaling in colorectal cancer – Comparison of activity in primary tumor tissues and primary tumour – Derived human colorectal cancer cell lines

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Aim: The study aimed to compare the differences in activity of PI3K-Akt and Ras-Raf-MAPK pathways, and changes in the Ras-Raf-MAPK activity after PI3K-Akt silencing, between different cell lines and tissue samples from primary tumour sites of human CRC.

Introduction: Alterations in EGFR-related Ras-Raf-MAPK and PI3K-Akt pathways are involved in the pathogenesis of up to 55% and 15% colorectal cancers (CRC) respectively. The Ras-Raf-MAPK pathway mutations are assessed before introducing a standard anti-EGFR treatment, as they indicate lack of response. However, the autonomic activity of alternative PI3K-Akt pathway may also have an impact on the effectiveness of targeted therapy.

Methods: The study was carried out on three ATCC human CRC cell lines derived from primary tumours (COLO320, SW480 and HT29) and ten patient tissue samples. Cell lines were cultured according to the protocol. Genomic DNA and RNA were isolated, PCR and RT-PCR were performed. Restriction enzymes were applied. Primers for the following fragments of genome were used: KRAS (exons 2, 3, 4), NRAS (exons 2, 3, 4), and BRAF exon 15 for PCR; KRAS, NRAS, BRAF, PIK3CA for RT-PCR. Proteins were extracted, purified and Western Blot was conducted. siRNA for Akt and specific PI3K inhibitors were used to silence PI3K-Akt activity.

Results: The analyzed material presented variable profiles of pathways activity. Interestingly, high expression of Ras protein was positively correlated with Akt protein level. In case of low level of Ras, Raf protein was dominating whereas Akt expression was significantly decreased.

Conclusion: Ras and Akt can simultaneously present a high level of expression. Thus, as PI3K- Akt is an alternative pathway to Ras-Raf- MAPK for EGFR signaling and its autonomic activity may affect the efficacy of anticancer treatment, it has a potential to be taken into consideration while planning a treatment and developing new anticancer agents.[1,2]

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PS124

The role of the hypoxic tumor microenvironment on the macrophage-tumor cell interplay

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Aim: The aim of this work is to unveil the role of the hypoxic microenvironment on macrophage-tumor cell interplay, using colorectal cancer (CRC) as a model.

Introduction: Microenvironment, in most cases hypoxic, is composed by cancer cells, extracellular matrix, stromal and immune cells, that cooperate and affect each other activities. Macrophages are one of the most abundant immune cells at the tumor microenvironment, acting as tumor suppressors or promotors. Previous research had shown that both hypoxia and immunosuppressive macrophages are associated with tumor progression. Nevertheless, these studies did not focus on the interplay between hypoxia and macrophage-cancer cell crosstalk.

Methods: To achieve our goal co-cultures of CRC cells and human macrophages, both in normoxia and hypoxia, were established. Macrophages were characterized functionally and phenotypically and their potential to induce cancer cell invasion was evaluated.

Results: Our results suggest that hypoxia, and the presence of cancer cells, decreases the cell surface expression of an anti-inflammatory marker (CD163), however the mRNA expression was not altered. Nevertheless, hypoxia induced an increase in the mRNA expression of the macrophage pro-inflammatory marker (CCR7).

Macrophages metabolic activity was not altered by hypoxia but decreased when co-cultured with cancer cells. In addition, lactate production decrease in co-culture while glucose consumption increased. Notably, macrophages in normoxia presented a more rounded morphology while in hypoxia are more elongated with evident cellular protrusions, suggesting dynamic alterations at the actin cytoskeleton organization. Interestingly, MMP-2 and MMP-9 activity profiles were not altered by the presence of cancer cells or hypoxia. Nevertheless, cancer cell invasion ability increased in the presence of macrophages, suggesting that other MMPs might be involved.

Conclusion: Findings in normoxia regarding macrophage potential to induce cancer cell invasion are consistent with those previously described by our group. Interestingly, we demonstrate now that hypoxia potentiates the invasive behavior of cancer cells and also macrophage pro-invasive ability.

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PS129

Ethnopharmacological use of *Cymbopogon citratus* (DC.) Stapf and *Cymbopogon schoenanthus* (L.) Spreng.: Anti-inflammatory potential of phenol-rich extracts

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Aim: The aim of this work consisted on expanding the knowledge on the chemical composition of different extracts from *Cymbopogon* spp., and on the evaluation of their anti-inflammatory potential in cell and cell-free systems.

Introduction: The ethnopharmacological use of Cymbopogon spp. dates back from ancient times. Traditionally used in tropical and semi-tropical countries for the repellent properties of their essential oil, the consumption of Cymbopogon spp. infusions is growing all over the world. This is not only due to the unique aroma, widely appreciated by the consumers, but also because of the antimicrobial, anti-inflammatory and sedative properties.[1]

Methods: The chemical characterization of infusions and ethanol:water (50:50, v/v) extracts from Cymbopogon citratus and Cymbopogon schoenanthus was achieved by HPLC-DAD. The anti-inflammatory potential of the extracts was assessed by cell and cell-free assays.

Results: HPLC-DAD analysis allowed the identification of several caffeic acid derivatives and flavonoids in the infusions and in the ethanol:water extracts of both species. The different extracts displayed scavenging activity against superoxide anion and nitric oxide (NO) radicals, and capacity to significantly reduce NO production by LPS-stimulated macrophages (RAW 264.7 cell line). In addition, the extracts were able to prevent hyaluronic acid degradation via inhibition of hyaluronidase, an enzyme recognized to participate in a number of physiological and pathological processes, including inflammation.[2] No toxicity was observed on human gastric adenocarcinoma and hepatocyte carcinoma cell lines, at a maximum concentration of 2.0 mg lyophilised extract/mL.

Conclusion: This study provided scientific evidence on the ethnopharmacological use of Cymbopogon species on inflammatory conditions, encouraging infusion consumption and future incorporation of Cymbopogon spp. extracts into nutraceuticals.

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PS140

Cytototoxic effects of novel synthesized polyoxometalates on human neuroblastoma SH-SY5Y cell line

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Aim: Investigation of cytotoxic effects of newly synthesized and untested polyoxometalates Pd1 and Pd2 on human neuroblastoma cells SH-SY5Y.

Introduction: Polyoxometalates (POMs) are transitional metal complexes, which are important in medicinal chemistry, as potent anticancer, antiviral and antibacterial agents. Inefficiently selective drugs and problems with dosing of usual chemotherapeutics directed the research towards investigation of new agents, such as POM.

Methods: Effects on viability rate of treated cells was tested using acid phosphatase assay. The mechanism of a cell death was examined using flow cytometry. JC-1, dihydroethidium, ApoStat, propidium iodide and acridin orange stainings were conducted in order to elucidate mitochondrial depolarisation, production of superoxide anion, caspase activation, DNA fragmentation and intracellular acidity.

Results: Pd1 and Pd2 have shown dose and time dependent decrease in cell viability rate. Complexes induced mitochondrial depolarisation after 2 h of treatment, which was shown as increase in FL1/FL2 ratio from 1 to 1.3 (Pd1, 6 μM) and from 1 to 1.7 (Pd2, 40 μM). Superoxide anion production was increased after 5 h of treatment using Pd1 and 2 h of treatment using Pd2. Pd1 complex exhibits increase in percentage of cells with fragmented DNA (subG0) and activated caspases after 24 h treatment. Pd2 complex induced increase in SubG0 and S phase without caspase activaction after 24 h treatment. POMs have shown intracellular acidification after 48 h (FL3/FL1 ratio: control 1, Pd1 2.3, Pd2 1.8).

Conclusion: POM complexes indicated cytotoxic effects on examined cell line. The mechanism by which these complexes exert those effects differ from one another. It was shown that both induce oxidative stress and mitochondrial depolarisation, accompanied by activation of caspases and DNA fragmentation in Pd1-treated cells, all indicative of apoptosis. In Pd2-treated group there was no increase in activation of caspases. Complexes have shown increase in intracellular acidification, which may suggest autophagy.

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PS153

HuR prevents c-fos mRNA degradation by proteasome-associated ribonuclease in vitro

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Aim: To estimate HuR protective activity against proteasome-associated ribonuclease for c-myc and c-fos mRNAs.

Introduction: Proteasome-associated proteins are attractive targets for multiple myeloma treatment. One of them is HuR protein known to selectively bind ARE-containing mRNAs and protect them from degradation. HuR is supposed to play a role in cancerogenesis since its expression is elevated in many cancer types and it stabilizes a lot of mRNAs encoding proteins involved in oncogenesis. Previously, it was shown that proteasome in addition to its main function – protein degradation – may act as a selective RNase. Moreover, HuR and proteasome have common targets – c-myc and c-fos protooncogene mRNAs.

Methods: HuR-GST fusion protein has been cloned, expressed and purified by affinity chromatography. Fragments of c-myc and c-fos were cloned and mRNAs have been transcribed in vitro. Proteasomes have been isolated from K562 cell line (human proerytroleykemia) and Im-9 cells (human multiple myeloma). mRNAs were treated by proteasomes in presence and absence of HuR. The estimation of mRNA cleavage was held by gel-electrophoresis.

Results: GST-HuR has specifically bound ARE-containing fragments of c-myc and c-fos mRNAs. Proteasomes extracted from Im-9 and K562 cells cleaved target mRNAs in absence of HuR. It was shown that HuR prevents degradation of c-fos mRNA by proteasomal endoribonuclease, whereas c-myc mRNA was cleaved in the

same conditions. GST protein didn't bind with target mRNAs and didn't affect proteasome cleavage activity.

Conclusion: HuR protects c-fos mRNA from proteasome ribonuclease cleavage in vitro, but can't prevent c-myc mRNA degradation. HuR and proteasome compete with each other for manifestation of their opposite activities. Thus, a new mechanism of regulation of proto-oncogenes expression was observed. However, the functional role of this process in vivo should be evaluated in further studies.[1–4]

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PS156

Metformin interferes with glucose cellular uptake by both estrogen and progesterone receptor-positive (MCF-7) and triple-negative (MDA-MB-231) breast cancer cell lines

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Aim: Transport experiments with 3H-DG, culture growth and proliferation rate assays were performed.This work aimed to investigate the possible interference of metformin with glucose uptake by MCF-7 and MDA-MB-231 human breast adenocarcinoma cell lines as a mechanism contributing to its anticarcinogenic effect.

Introduction: Breast cancer, the most common cancer among women, remains one of the leading causes of mortality among women worldwide.[1] Metformin has been widely used as a treatment for type 2 diabetes for over 40 years.[2] The first report of a reduced risk of developing cancer for diabetic patients treated with metformin was published in 2005.[3] Several mechanisms of action of metformin appear to be implicated in this effect.[2,4]

Methods: Transport experiments with 3H-DG, culture growth and proliferation rate assays were performed.

Results: Acute (26 min) exposure of MCF-7 cells to metformin significantly inhibited uptake of 3H-deoxy-D-glucose (3H-DG) (maximal inhibition found with metformin 0.5 mM: 27 ± 2% reduction). Chronically (24 h), metformin induced a concentration-dependent increase in 3H-DG uptake (maximal increase observed with metformin 1 mM: 81 ± 15% increase). Acute (26 min) exposure of MDA-MB-231 cells to metformin slightly inhibited uptake of 3H-DG (maximal inhibition found with metformin 1 mM: 10 ± 3% reduction). Chronic (24 h) exposure to metformin significantly increased 3H-DG uptake by MDA-MB-231 cells (maximal increase observed with metformin 1 mM: 30 ± 8% increase).

Chronic (24 h) exposure of both cell lines to metformin (1 mM) decreased culture growth/cell mass; in contrast, it increased cell proliferation rates. Combination of metformin (1 mM) with the facilitative glucose transporter (GLUT) inhibitor kaempferol (30 μM) did not result in a more marked effect on culture growth and cell proliferation rates.

Conclusion: Summarizing, chronic exposure of MCF-7 and MDA-MB-231 cells to metformin induces a marked increase in glucose uptake, associated with an anticarcinogenic effect of the drug. We suggest that the increase in glucose uptake is a compensatory mechanism to cellular energy depletion induced by metformin.

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PS162

Endocannabinoids induce placental trophoblast reticulum stress

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Aim: We aim to investigate in cytotrophoblasts whether these effects on cell viability loss are due to endoplasmic reticulum (ER) stress mediated apoptosis.

Introduction: Placental development relies on a balance between proliferation, differentiation and apoptosis of trophoblasts, a process tightly regulated by growth factors, cytokines and hormones. Endocannabinoids (eCB), such as 2-arachidonoylglycerol (2-AG) and anandamide (AEA) may play a role in these processes. We previously demonstrated that both eCB induced trophoblast cell death.[1,2] Here we investigated in cytotrophoblasts whether these effects on cell viability loss are due to endoplasmic reticulum (ER) stress mediated apoptosis.

ER stress is caused by the accumulation of unfolded proteins leading to an unfold protein response (UPR) triggered by transmembrane ER signaling proteins including: pancreatic ER kinase (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (Ire1) and Activating transcription factor 6 (ATF6). The dissociation of Grp78 (BiP) from these sensors triggers a series of mechanisms that can restore homeostasis or lead to apoptosis. Placental stress has been implicated in the pathophysiology of pregnancy complications, including growth restriction and pre-eclampsia.

Methods: BeWo cells (ATCC, USA), an accepted model of cytotrophoblast stem cells were treated with AEA or 2-AG (10 micromolar) for 24 h. Through quantitative real time polymerase chain reaction (qPCR), we evaluated mRNA levels of ER stress markers: CHOP, Grp78, ATF4 and spliced mXBP1. Protein expression of CHOP was evaluated by western-blot.

Results: After 24 h of treatment with both eCB, we found an increase in mRNA levels of ER stress markers: CHOP, Grp78, ATF4 and spliced mXBP1. Protein expression of CHOP also increased in both cases.

Conclusion: These results suggest that cell viability loss promoted by 2-AG and AEA was associated with ER-stress since both PERK and IRE1 arms of UPR are activated. Prolonged ER-stress, contributes to the expression of pro-apoptotic proteins, such as CHOP.

These findings shed light to the impact of endocannabinoids induced-ER stress which may negatively affect trophoblast cell turnover and pregnancy outcomes.

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PS163

Analysis of imaging characteristics, incidence, and prognosis of brain metastases from thyroid cancer

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Aim: The main objectives of this study were to evaluate the incidence, imaging characteristics, and prognosis of parenchymal brain metastases originating in thyroid cancer.

Introduction: While thyroid cancer is a relatively common type of cancer, it is usually highly curable.[1] Brain metastases from thyroid cancer are rare and their imaging appearance has not been well defined.[2]

Methods: Review of case records of thyroid cancer patients within the IPO Porto data base from 2005 to 2015 was conducted in order to identify the patients with thyroid cancer and evidence of brain metastases.

Results: We identified 3175 patients with thyroid cancer, with only five having evidence of brain metastases (two from papillary thyroid cancer, two from follicular thyroid cancer and one from poorly differentiated thyroid cancer). At the time of brain metastases detection, 100% of the patients had concurrent lymph node metastases, 80% lung metastases and 60% osseous metastases. Of those brain metastases, 60% were multifocal and 40% presented as partially cystic/necrotic. Of the two cases in which the patients died, the median overall survival after brain metastasis detection was less than one year.

Conclusion: Brain metastasis from thyroid cancer remains a rare phenomenon that most frequently occurs in the setting of widely disseminated lymph node disease. The imaging appearance is highly variable and the prognosis is poor.

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PS166

The association of GSTP1 genotype with the risk and survival in ccRCC patients with advanced tumor stage

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Aim: The aim of this study was to evaluate specific role of glutathione S-transferase P1 (GSTP1) gene variants as determinants of ccRCC risk in patients with advanced tumor stage (pT3 and pT4). Furthermore, we evaluated the effect of GSTP1 gene variants on postoperative prognosis in these patients.

Introduction: Renal cell carcinoma (RCC) accounts for up to 90% of malignant kidney tumors with clear renal cell carcinoma (ccRCC) being the most frequent and the most aggressive subtype of sporadic RCC in adults. Unfortunately, most RCCs are asymptomatic in early stages, whereas symptomatic RCC correlates with aggressive histology and advanced disease. Aside from known risk factors for RCC, evidence suggest that the development of RCC can be partially explained by genetic variations among the populations. Highly polymorphic cytosolic glutathione S-transferases are known to be involved in both the development and the progression of renal cell carcinoma.

Methods: GSTP1 genotype was determined in 99 ccRCC patients and 326 matched-controls by qPRC method, using TaqMan® SNP Genotyping Assay. The risk for disease was computed by odds ratios (OR) and 95% confidence intervals (CI) using logistic regression analysis Furthermore, overall survival was analyzed as well by Kaplan-Meier method and Cox proportional hazard regression model.

Results: GSTP1-variant genotype was associated with 5-fold increased risk for ccRCC in comparison with GSTP1-wild type genotype ($p < 0.001$). Moreover, survival analysis clearly indicated shorter overall survival in ccRCC patients with GSTP1-variant genotype, however without reaching statistical significance ($p = 0.166$). Additionally, ccRCC patients with GSTP1-variant genotype had a 7-fold higher hazard ratio ($p = 0.177$), compared to the carriers of GSTP1-wild type genotype.

Conclusion: GSTP1-variant genotype contributed independently towards the risk of ccRCC in our patients. Moreover, GSTP1-variant genotype is associated with poor postoperative prognosis in ccRCC.

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PS171

Neuroimaging analysis of rare brain metastases from prostate cancer

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Aim: Our main study focus was to evaluate the incidence, imaging characteristics, and prognosis of parenchymal brain metastases originating from prostatic tumors.

Introduction: Prostate cancer is considered the second most commonly diagnosed cancer.[1] In addition, it is considered the fifth leading cause of cancer death amongst males.[2] A small percentage (2%) of patients with prostate cancer are found to be castrate-resistant and to develop brain metastasis, a rare complication which is associated to an advanced systemic state of the disease when the tumor has already spread to other sites.[3] Although, there is not much evidence on optimal management of these patients.[4]

Methods: A review of case records of prostate cancer patients within the IPO Porto data base from 2013 to 2015 was conducted in order to identify the patients with prostate cancer and evidence of brain metastases. As criteria of exclusion, cases transferred to other hospitals without follow up and cases that were incorrectly categorized were excluded.

Results: We screened 2194 patients with prostate cancer, with only one having evidence of brain metastasis. Additionally, one case was identified with bilateral orbital metastatic lesions. The patient with evidence of brain metastasis aged 48 years old. Magnetic resonance imaging showed six metastatic lesions, three infratentorial and three supratentorial. The largest lesion was found in the parieto-occipital region. These brain metastasis were detected 42 months after the initial diagnosis of prostate adenocarcinoma. In addition, by the time of brain metastasis detection, the patient already had bone metastatic lesion.

Conclusion: Brain metastases from prostate cancer are rare, with only a few cases described in the literature. Variable magnetic resonance imaging characteristics are described. Brain metastases are also associated with a poor prognosis, with a mean survival of 1–7.6 months.

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PS177

Studies towards the synthesis of dicarboxylic acid metabolite of mitoxantrone

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Aim: The objective of this work was to synthetize the dicarboxylic acid metabolite of mitoxantrone (MTX) to further investigate its cardiotoxicity in H9c2 differentiated cells.

Introduction: Drug metabolism can result in active or toxic metabolites that can lead to side effects, namely cardiotoxicity.[1] MTX, an antineoplastic that belongs to the synthetic anthracenediones,[2] is used to treat breast cancer, acute leukaemias, and acute lymphomas in adults.[3] Nowadays, it is also used to treat aggressive multiple sclerosis. One of the most frequent and relevant MTX side effect is cardiotoxicity. Previously, we found the MTX-naphthoquinoxaline metabolite (NAPHT) to be less cardiotoxic than MTX.[4] One of the main human MTX metabolites was identified as the dicarboxylic acid resulting from the oxidation of the terminal hydroxyl groups of the side chains.[5] However, its putative cardiotoxicity was not yet assessed. Herein, the synthesis and structure elucidation of the dicarboxylic acid metabolite will be presented.

Methods: The total synthesis of the metabolite involved five steps, starting from chrysazin. The enzymatic reaction was accomplished through the horseradish peroxidase (HRP)-catalysed H_2O_2 and the studies of the oxidative reactions involved sodium tungstate, chromium trioxide, sodium nitrite, and potassium permanganate.

Results: In order to obtain the carboxylic derivative, several approaches were undertaken, namely, total synthesis from a commercial available anthraquinone, as well as enzymatic and oxidative reactions from MTX. Different derivatives were obtained. The structure elucidation of the intermediates was established by spectroscopic techniques and the characterization of the dicarboxylic acid metabolite is ongoing.

Conclusion: The synthesis of the dicarboxylic acid metabolite was only achieved by the multistep approach. Future work will involve cardiotoxicity studies of this metabolite in H9c2 differentiated cells.

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PS179

Human papillomavirus in the etiology of oropharyngeal carcinoma

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Aim: The aim of this retrospective survey was to investigate the association of HPV status in patients with oropharyngeal cancer with tumour staging and other clinical features.

Introduction: Infection by human papillomavirus (HPV) stands for the most frequent viral carcinogenesis in the world. Overexpression of cell oncoprotein p16 is routinely diagnosed by immunohistochemistry (IHC) as the surrogate marker of viral activity.

Methods: Records from the oropharyngeal cancer patients treated in the Department of Otorhinolaryngology-Head and Neck Surgery in Bratislava from January 2013 to December 2016 were retrospectively analysed. Patients were divided, according to IHC results on oncoprotein p16, into p16 positive, considered HPV-positive, and p16 negative as HPV-negative. The incidence of oropharyngeal carcinoma, location, T and N staging, age, gender of patients was compared based on HPV status.

Results: From 129 oropharyngeal cancer patients with p16 examination were 52 (40%) considered as HPV positive. HPV positive group consisted of 45 (86.5%) men and 7 (13.5%) women. The primary tumour in HPV-positive patients originated from the palatine tonsil and base of the tongue in 96% of cases. The peak of occurrence of HPV-associated carcinoma was found between 50 and 59 years of age. HPV positive tumours were diagnosed in early T stage (T1/2) in 52%. Both HPV positive and negative patients were predominantly diagnosed with advanced-stage cancer, 90.4% in HPV-positive and 87% in HPV-negative group.

Conclusion: Early T stage in HPV positive carcinomas was approved, as well as more advanced regional spreading and prevalence of men and non-smokers. Wide variations in numbers of diagnosed patients during years of study may be caused by relatively small size of the studied group. Survey is focusing at HPV status as the most important prognostic factor in oropharyngeal cancer and systematized introduction of HPV status examination as progressive approach to effective and targeted therapy.

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PS181

The effects of cannabinoids in exemestane-resistant breast cancer cells

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Aim: Considering that the development of resistance is the main reason for endocrine treatment failure, our group decided to explore the ability of three cannabinoids, Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and anandamide (AEA), to reverse resistance to exemestane. The THC and CBD are phytocannabinoids derived from the plant Cannabis sativa (marijuana) whereas AEA is an endocannabinoid. For that, it was used LTEDaro cells, a long-term estrogen deprived ER+ breast cancer cell line that mimics resistance to exemestane.[1] These cells were treated with exemestane in combination with two phytocannabinoids, CBD and THC, and the endocannabinoid AEA.

Introduction: Exemestane is one of the aromatase inhibitors (AI) used as first line treatment for estrogen-receptor positive breast cancer in post-menopausal women. Exemestane acts by inhibiting aromatase, the enzyme responsible for the conversion of androgens to estrogens[2] and also by promoting apoptosis of breast cancer cells.[3] Nevertheless, despite its therapeutic success, this AI, after prolonged treatment, can induce acquired resistance, which causes tumor relapse. Therefore, it is important to find new strategies to overcome resistance in order to improve breast cancer treatment.

Methods: The presence of CB1 and CB2 in LTEDaro cells was confirmed by Western blot analysis and the effects of the combination of cannabinoids with exemestane were evaluated by MTT and LDH assays. Cell morphology was analyzed by Giemsa and Hoechst staining.

Results: Our results demonstrate that all the cannabinoids induce a decrease in viability of exemestane-resistant cells, in a dose- and time-dependent manner, without LDH release. These results indicate that the studied cannabinoids, mainly THC and AEA, revert the resistance to exemestane, probably by inducing apoptosis, as observed in Giemsa/Hoechst stain by the presence of typical morphological features of apoptosis.

Conclusion: This study highlights the efficacy of using cannabinoids as a potential adjuvant treatment to revert resistance to AIs.

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PS184

Quantitative structure-property relationship (QSPR) of thiazolidin-4-one derivitives as RTIs of HIV virus

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Aim: The aim of this study is to build a quantitative structure-property relationship (QSPR) of 66 thiazolidin-4-one derivatives in order to predict their log *P*.

Introduction: Performing computational drug design is an important step for their synthesis and properties characterizations. In this work, quantitative structure-property relationship (QSPR) of 66 thiazolidin-4-one derivatives was examined in order to predict their logP which is the most commonly used measure of lipophilicity in chemical molecules. These group of compounds act as non-nucleoside reversed transcriptase inhibitors of HIV.

Methods: Two different quantum mechanics approaches including HF and DFT were applied for energy minimization of structures and different classes of molecular descriptors including quantum chemical descriptors were generated for prediction of their LogP. Numbers of descriptors which showed high correlation with each other were removed by MATLAB software. The model between structures and their LogP was built for both methods with performing Multiple Linear Regression (MLR) in Spss package

Results: Statistical results and application of developed model to the test set demonstrates that the DFT model is reliable with good predictive accuracy.(R2cal = 0.90, R2cv = 0.88) The lack of significant difference between the original and modeled values of logP reveals the validity of the built model which was built with 2D and 3D descriptors. The coefficients of model are statistically significant.

Conclusion: QSPR models can be used to predict molecular properties such as LogP. That will be beneficial in drug design processes. In this research, MLR model was built in order to correlate structure of 66 compounds with their LogP. Molecules that were optimized by DFT method showed better correlation than HF method that indicates the accuracy of the built model with 2D and 3D descriptors.

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PS185

Lung branching morphogenesis, in the chicken model, is accompanied by temporal metabolic changes

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Aim: In this work, we characterized, for the first time, the metabolic profile of chick lung branching in early stages of development: b1, b2 and b3 (1, 2 or 3 secondary bronchi, respectively).

Introduction: Pulmonary development is a complex process that depends on the activation of conserved signaling pathways that regulate cellular processes such as proliferation, differentiation and migration.[1–3] These cellular processes require high amounts of energy and nutrients to form new biomass.[4,5] However, the metabolic changes that occur during lung branching morphogenesis have not been described so far.

Methods: Ex vivo lung explant culture was performed and the medium collected to analyze the production/consumption of metabolic intermediates associated with glucose catabolism (lactate, acetate, alanine), by 1H-NMR. qPCR was performed to assess the expression levels of key enzymes and transporters from the correspondent metabolic pathways.

Results: The results showed that the major variations occur from stage b1 to stage b3. In b3 there is an increase in lactate and acetate production. Still, glucose consumption is maintained from b1 to b3 stage, with a concurrent decrease of glucose transporter 3 (glut3) transcript levels. Hexokinase 1 (hk1) levels also decrease in b3 stage (as compared to b2). This phenomenon suggests an increase in the glycolytic efficiency and a shift to lactic acid production (in detriment of mitochondrial respiration). In fact, we observed a decrease on pyruvate dehydrogenase B (pdhB) and an increase in lactate dehydrogenase A (ldhA) expression levels in b3 stage (as compared to b2), while lactate dehydrogenase B (ldhB) levels decrease.

Conclusion: This study describes, for the first time, the temporal metabolic changes associated with chick pulmonary branching. It seems that glycolytic efficiency is increased and Krebs cycle metabolism shifts to lactate production along development. Furthermore, acetate and lactate are potentially seen as metabolic biomarkers of lung development.

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PS186

Epigenetic modifications as targets to new therapies for Chronic Lymphocytic leukaemia – A preliminary study

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Aim: This study aimed to clarify the involvement of epigenetic modifications in chronic lymphocytic leukaemia development and analyse the therapeutic potential of epigenetic modulators.

Introduction: CLL is the most common type of leukaemia found in adults and is an extremely variable and heterogeneous disease. The CLL aetiology is unknown and it natural history is heterogeneous. However, epigenetic modifications may play an important role in CLL.

Methods: This study enrolled 18 CLL and 7 controls. To perform primary CLL cultures, peripheral blood mononuclear cells from CLL patients were isolated using Ficoll gradient and incubated with the hypometilants, Azacytidine and Decitabine, and deacetylase inhibitors, Panobinostat and Vorinostat, in monotherapy (single dose and daily administration) and in combination for 24 h/48 h. The cytotoxic/cytostatic effect of drugs was evaluated by fluorometric microculture cytotoxicity assay (FMCA). Cell death and cell cycle were determined by flow cytometry using Annexin V and PI/RNAse, respectively. CD5 and CD19 antibodies were used to identify normal (CD5−/CD19+) and neoplastic cells (CD5+/CD19+). Methylation pattern was determined by MS-MLPA. Data were analysed using univariate approaches.

Results: Preliminary results show that patients appear to be more sensitive to Azacytidine and Vorinostat than Decitabine and Panobinostat, on single dose administration. Combination of Panobinostat with Azacytidine and Decitabine induced higher cytotoxicity than single dose. For all drugs, daily administration schedule reduced more effectively cell viability/proliferation than the same doses in single administration. These drugs induced cell death mainly by apoptosis with specificity to neoplastic cells. Moreover, CLL patients had a significant higher methylation frequency of PAX5 (70%), KLLN (80%), WT1 (100%), THBS1 (90%) and GATA5 (90%) gene promoters when compared with controls (all genes demethylated, except MSH6). Furthermore, all CLL patients had at least one methylated gene.

Conclusion: The preliminary results suggest that methylation of tumour suppressor genes is a common event in CLL patients and that epigenetic modulators induce a cytotoxic effect, reducing cell viability/proliferation, in a time- and dose-dependent manner. Therefore, these results are promising and encourage further studies in CLL.

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PS191

Imaging features of brain metastases from testicular cancer

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Aim: Our study evaluated the incidence, imaging characteristics, and prognosis of brain metastases originating from primary testicular tumors.

Introduction: Approximately 95% of testicular tumors are testicular germ cell tumors (TGCT).[1] Sertoli cell tumors are rare non-germ cell origin tumors and account for less than 1% of testicular cancer.[2] Brain metastases from germ cell tumors are very uncommon, occurring in less than 2–3% of patients.[3] In testicular cell cancer, it is estimated that the incidence of brain metastases is 1–2% in all TGCT, whereas in advanced stages of TGCT the incidence rises to about 10–15% [4–9]

Methods: Case records of testicular tumors patients within the IPO Porto data base from 2006 to 2015 were reviewed to identify patients with testicular tumors and evidence of brain metastases.

Results: 368 patients with testicular tumors were identified, with only four having evidence of brain metastases.

Histopathological evaluation reveled that one of the patients had a non-germ cell tumor, a Sertoli cell tumor, while others had mixed germ cell tumors. Half of them had only a single right frontoparietal lesion (21 mm) or right occipital (42 mm), both were heterogeneous in T1WI and T2WI, and with intense and heterogeneous enhancement with gadolinium. The other two patients had multiple lesions. One of them had left frontoparietal (2.2 mm, hyperintense in T1) and right occipital (1.8 mm, hypointense in T1) lesions, both heterogeneous and predominantly hypointense in T2 and T1WI with no enhancement. The other had right temporal (5 mm) and left occipital (11 mm) lesions, both isointense in T1W1 and T2WI with intense and homogeneous enhancement. There was no diffusion restriction in all three cases and all four cases were hypointense in T2*.

Conclusion: Although the imaging features of brain metastases differ in some aspects, they all have a hemorrhagic component and a very low survival rate after diagnosis.

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PS196

Synthesis and tumor cell growth inhibitory effects of the marine product analogues of fiscalin B

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Aim: The aim of this work was to synthesize fiscalin B, to pursuit the development of a library of derivatives and to investigate the derivatives for their potential antitumor activity.

Introduction: Marine organisms provided numerous novel compounds with sensational multiple pharmacological properties. The necessity of novel therapeutics has gain more importance especially because of the resistance associated to the current therapeutics and the inexistent treatments for incurable diseases. Fiscalin B is a fungal metabolite with a pyrazino[2,1-b]quinazoline-3,6-dione system reported to have significant biological activities.

Methods: Two methods were studied for synthesis – double cyclization and microwave assisted procedures. First method started with coupling reactions to form tripeptide of tryptophan methyl ester linked to N-Fmoc-valine via anthranilic acid. Then, the dehydrative cyclization was performed using formamide to form the intermediate oxazine. The coupling reaction to form the fiscalin B were achieved after deprotection.[1] The second method is the coupling of anthranilic acid with N-Boc-valine to form Boc-protected benzoxazin-4-one by thermal heating conditions. Then, the addition of tryptophan methyl ester hydrochloride led to 4-quinazoline-3,6-dione scaffold by microwave irradiation.[2] The cell growth inhibitory effect was investigated by the Sulforhodamine B assay.

Results: The use of amino acids with different configurations and different side chains or even the derivatization of the existing functional groups were enable the application of this synthetic methodology for a library of fiscalin B analogues. The formation yields of fiscalin B analogues were low, ranging from 3 to 16%. Eight derivatives were tested on non-small cell lung cancer (H460), colon adenocarcinoma (HCT15) and breast cancer (MCF7) human cell lines and showed moderate cytotoxic effects, with GI50 concentrations ranging from 30 to 80 μM.

Conclusion: Significant differences were obtained between enantiomeric pairs.

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PS207

Heterocyclic chalcone derivatives: Synthesis and biological activity evaluation

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Aim: Synthesis of new heterocyclic chalcone derivatives with promising antitumor activity.

Introduction: Natural chalcones have been intensively studied for their wide range of biological activities, namely antitumor.[1] Possessing two electrophilic reactive centers at α,β-unsaturated ketone group, chalcone derivatives can participate in addition reactions leading to the synthesis of promising bioactive compounds with a more rigid structure, like isoxazoles and pyrazoles.[2]

Methods: Chalcones were synthesized by base catalysed Claisen Schmidt condensation via microwave assisted organic synthesis (MAOS). The antiproliferative activity was assessed using sulforhodamine B assay.[3]

Results: Seventeen chalcone derivatives were synthesized and identified as having in vitro growth inhibitory activity on three human tumor cell lines from breast, lung and melanoma (MCF-7, NCI-H460, and A375-C5).

Conclusion: Most of the synthetized chalcones revealed to be promising growth inhibitors of human tumor cell lines. The molecular mechanisms involved in their antiproliferative effect are being evaluated.

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PS209

A posttranslational modification in histones as prognostic/predictive marker in Estrogen-Positive Breast Cancer

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Aim: This work aims to evaluate H3K27me3 expression in luminal-like breast tumors, using immunohistochemistry assay, to assess the prognostic value of this epigenetic alterations in estrogen positive breast cancer (BrC).

Introduction: BrC is the second most incident cancer worldwide. In Portugal, in 2012, BrC was simultaneously the leading cancer in incidence and mortality in women.[1] Around 70% of all BrC are hormone-receptor positive, that is the major part of breast tumors is luminal-like.[2] H3K27m3 is a gene repression marker[3,4] and is associated with gene silencing, playing a crucial role in cell proliferation and differentiation.[3] H3K27me3 may have some clinical value in several types of cancer since it can be used as a biomarker. This histone modification has been associated with poor prognosis of some BrC subtypes.[5]

Methods: It was used a cohort of BrC patients of the Portuguese Oncology Institution of Porto (IPO-Porto), diagnosed between 1994 and 2002. A total of 102 luminal-like tumor cases were assessed by immunohistochemistry, to H3K27me3 expression. To verify the prognostic value of H3K27me3 levels, Cox regression with a log rank test was performed for both disease-specific and disease-free survival.

Results: Through the result analysis, it was established that only tumor grade ($p = 0.021$) was significant associated with disease-specific survival. Nevertheless, both luminal subtype ($p = 0.016$) and H3K27me3 expression ($p = 0.012$) were significantly associated with disease-free survival. Indeed, H3K27me3 high expression is associated with higher recurrence risk, especially in Luminal A.

Conclusion: We could confirm the prognostic value of H3K27me3 expression in luminal A subtype BrC patients. Therefore, higher H3K27me3 expression in luminal A tumors is associated with a greater probability of recurrence.

However, studies in larger cohorts are mandatory to validate its clinical utility.

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PS212

Is P-glycoprotein relevant for the release of microvesicles by tumor cells?

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Aim: In this study, we aimed to verify if MDR cells without expression of P-gp also produced more microvesicles and less exosomes than their DS counterpart cells.

Introduction: Cancer multidrug resistance (MDR) is a major cause of chemotherapy failure and is highly associated with overexpression of drug-efflux pumps such as P-glycoprotein (P-gp). The identification of mechanisms specific of P-gp overexpressing cells may contribute to the identification of biomarkers of MDR.

It was recently discovered that a drug-resistant phenotype may be horizontally transferred from drug-resistant (DR) to drug-sensitive (DS) cells, mediated by the cargo of extracellular vesicles (EVs) released by DR cells and captured by DS cells. These EVs include smaller exosomes and larger microvesicles. Our previous work showed that MDR cells with overexpression of P-gp released more microvesicles than exosomes, unlike their DS counterpart cells. However, it is not known if this phenomenon is restricted to MDR cells with overexpression of P-gp or if it is extensive to all DR cells (with other mechanism of drug resistance).

Methods: Drug-response curves of MDR and DS counterpart cells were obtained, using resazurin and trypan blue assays, to confirm the resistant or sensitive phenotype of the cell lines. Confirmation of their P-gp status was possible by Western-Blot. EVs released by both DS and MDR cells were isolated by ultracentrifugation and characterized by transmission electron microscopy, dynamic light scattering, nanoparticle tracking analysis and Western blot analysis.

Results: We confirmed that MDR cells without expression of P-gp release EVs with similar sizes to the ones released by their DS counterparts.

Conclusion: So, P-gp may be associated with the release of larger EVs by MDR cells. These results will be further confirmed by characterizing the EVs released by P-gp overexpressing MDR cell lines following downregulation of P-gp expression and the EVs released by DS cell lines following transfection of P-gp.[1–4]

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PS215

Uterine protein oxidative modifications may condition trophoblast function

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Aim: Evaluate whether protein carbonylation resulting from uterine altered redox imbalance interferes with extravillous trophoblast viability.

Introduction: Local redox homeostasis is believed to have a pivot role in uterine transformation necessary for blastocyst implantation and placenta development. By contrast, redox status imbalance plays a role in deficient placentation and the development of pregnancy-related complications (e.g. preeclampsia or gestational diabetes) with increased incidence in older women. Thus, it was hypothesized that at an older reproductive age, loss of redox homeostasis is a contributor to disruption of foetal/placental interactions and the development of such complications.

Methods: Uterine human samples were collected at delivery by elective caesarean section. The protocol was approved by the ethical committee of "Centro Materno-Infantil do Porto", volunteers gave written consent to be included in the study. Total protein carbonylation was detected by oxyblot and protein expression was quantified by western blotting. Specific protein carbonylation was verified by immunoprecipitation. Albumin was carbonylated using hydrogen peroxide (H2O2), followed by dialysis, and western blotting to confirm carbonylated albumin. HST-8SV neo extravillous trophoblasts were treated with carbonylated/non-carbonylated albumin, followed by cell viability assay. A P value less than 0.05 was assumed to denote significant difference.

Results: At the placental site, carbonylated albumin normalized to total albumin expression showed a positive and significant association with maternal age. ($r = 0.6909$, $P = 0.0021$) In vitro, carbonylated albumin displayed a cytotoxic effect, at concentrations ranging from 10 to 100 μg/ml. Lower concentrations did not affect trophoblast viability.

Conclusion: Uterine aging is accompanied by selective albumin oxidative modifications, which appears to interfere with trophoblast ability to invade and transform the maternal placental site.

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PS223

Advances on photodynamic therapy through new pyridine-fused diphenylchlorins as photosensitizers for melanoma treatment

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Aim: Assessment of cytotoxicity of four new photosensitizers intended for photodynamic therapy (PDT) against melanoma cells (A375 cells).

Introduction: Melanoma is the rarest form of skin cancer. PDT combines a photosensitizer with light culminating in the production of reactive oxygen species leading to cellular death. A new type of stable 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused tetraphenylchlorins1, proved to be very active as photodynamic agents. Thus, looking for a new generation photosensitizers with optimized properties for PDT we synthesized new diphenylchlorins.

Methods: The human melanoma cell line A375 was seeded in 48 well plates. The photosensitizers NAMP103A, NAMP103B (the tetraphenylchlorins monoester), NAMP263A and NAMP263B (the tetraphenylchlorins alcohol) were administered ranging 5 nM to 10 μM. Irradiation was performed after 24 h ($\lambda < 560$ nm). MTT and SRB assays as well as flow-cytometry were performed 24 h after the PDT.

Results: MTT assay results allowed to obtain dose-response curves and to calculate the concentration that inhibits the cultures by 50% (IC50). Phototoxicity (10J) was dependent on the chlorines concentration. Moreover, NAMP263B was significantly more cytotoxic than NAMP103A ($p = 0.037$) and NAMP103B ($p = 0.042$). From SRB assay we verified that with a 125 nM concentration the NAMP103A, NAMP103B, NAMP263A and NAMP263B produce a cellular viability of 36.9%; 33.2%; 18.3% and 18.8%, respectively. Flow cytometry studies confirmed the decrease of viability associated with cell death by apoptosis and necrosis. Loss of mitochondrial membrane potential, apoptosis hallmark, was also observed. An imbalance of ROS, namely superoxide anion and peroxides, was observed for all photosensitizers studied with an exhaustion of antioxidative intracellular defenses (GSH).

At low PS concentrations (5 nM), metabolic activity was variable with light energy (5 J, 10 J and 20 J) with lower values for higher fluence. Dark toxicity studies revealed photosensitizer dependence of irradiation.

Conclusion: We hereby conclude that the photosensitizers are indeed very promising, which rouses plans for following proceedings to verify in vivo outcome.

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PS226

Angiogenesis and inflammation at the crossroads between diabetes and cancer

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Aim: To study fibrosis, angiogenesis, oxidative stress and inflammation markers in diabetic and non-diabetic patients with gastric cancer (GC).

Introduction: Type 2 Diabetes mellitus (DM2) is a major health problem, with 415 million people diagnosed worldwide.[1] Evidence regarding its association with various types of cancer has been reported, including GC.[2] Some hypotheses have been suggested to explain how DM2 could enhance the risk of cancer development, such as hyperglycemia, hyperinsulinemia, oxidative stress, vascular disturbances and a chronic low inflammation state.[3–5]

Gastric cancer (GC) is the fifth most common cancer worldwide and ranks as the third leading cause of cancer-related death.[6] GC is frequently associated with infection by Helicobacter pylori and inflammation plays a central role in the carcinogenic process. Such chronic inflammatory state, linked with angiogenesis imbalance, oxidative stress and metabolic signaling, suggests that also DM2 might be a major risk factor in initiation and progression of GC, demanding further investigation.

Methods: A series of GC from DM2 ($n = 22$) and nonDM2 ($n = 21$) patients were studied. Immunohistochemistry (IHC) using antibodies against CD31 and 3-Nitrotyrosine was performed, to assess density of vessels and oxidative stress status. Histochemical staining with Sirius red was performed to determine the percentage of fibrosis in the tumor and non-neoplastic adjacent mucosa. Based on assessment of tumor inflammatory cell infiltrate and tumor stroma

percentage, a semi-quantitative evaluation of Glasgow Microenvironment Score[7] was performed. Also, Glasgow Prognostic Score, that is widely known as a systemic inflammatory-based marker, was determined for each patient.[8]

Results: Diabetic patients presented a significant higher glycaemia than the control patients (190.1 ± 13.6 mg/dL vs 98.2 ± 3.6 mg/dL, $p < 0.001$, respectively). Decreased survival rates were observed in diabetic patients (611.5 vs 916.0, p = ns). Tumours exhibited increased fibrosis relatively to the adjacent mucosa in both groups and diabetic patients (N: 9.362 ± 1.337; T: 12.29 ± 1.407) presented higher fibrosis levels than the non-diabetic patients (N: 7.165 ± 1.017; T: 10.97 ± 1.076).

Conclusion: Expected results: Identifying the distinct features that characterize GC of DM2 patients compared to nondiabetic patients (namely fibrosis, angiogenesis, inflammation, and oxidative stress biomarkers) will enable to study this subset of GC patients and unravel key mechanisms behind the relationship between DM2 and GC.

Acknowledgements: Funding: This work was supported by the project Diabetes & obesity at the crossroads between Oncological and Cardiovascular diseases – a system analysis NETwork towards precision medicine (DOCnet) – A multi-omics approach to decipher diabetes-related molecular targets in cancer: a step towards precision medicine. NORTE2020 - "Programa Operacional Regional do Norte" (NORTE-01-0145-FEDER-000003) (Jan 2016-Dez2018).

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PS229

Circulating EVs for AML minimal residual disease biomarkers detection

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Aim: We propose to evaluate the feasibility of a peripheral blood EV-based liquid biopsy method for AML disease monitoring in real time with molecular precision.

Introduction: Acute myeloid leukemia (AML) is a hematopoietic stem cell disorder with high mortality rate mainly due to the high frequency of post-treatment relapse. Minimal residual disease (MRD) determination in AML patients receiving treatment is useful to assess chemotherapy response and predict relapse. One approach to upgrade the current invasive MRD monitoring (traditionally based on bone marrow aspirates/biopsies) is to use methods that identify cancer-associated biomarkers in patients' blood. Recently, extracellular vesicles (EVs) have been increasingly recognized as a potential source of biomarkers, since the levels of EVs are markedly increased in cancer patients' blood and those EVs potentially carry molecular signatures associated with specific cancer phenotypes.

Methods: The profile of EVs isolated from AML patients' blood plasma collected from paired AML diagnostic and complete remission samples is being compared and correlated with clinical data. A size-exclusion chromatography (SEC) method was optimized to isolate the plasmatic EVs. The EVs profile is then characterized according to their size, plasmatic concentration, morphology and protein content.

Results: EVs with decreasing size were successfully isolated between SEC fractions 3 to 6, with a size ranging from 300 nm to 30 nm, respectively. Fraction 7 presented the smaller EVs, although mixed with some plasmatic protein contaminants. The expression of EVs markers such as CD63, HSP70 or Syntenin-1 was confirmed and allow to distinguish EV subpopulations between fractions 3 to 7. The expression of leukemia-specific markers is currently being studied in the EVs isolated from the paired AML blood samples.

Conclusion: The presented EV-based liquid biopsy proposed method for AML monitoring could unravel biomarkers for diagnostic and prognostic purposes in AML patients.

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PS232

The association of Generalized Epilepsy with Febrile Seizures plus (GEFS+) with FEB1 gene: A new insight to the etiology of GEFS+

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Aim: We aimed to carry out a linkage analysis in 6 Iranian families to find an association between the FEB1 and GEFS+.

Introduction: Generalized Epilepsy with Febrile Seizures plus (GEFS+), is a group of genetic epilepsy syndromes, likely to commence in the first year of life, in which, patient presents with febrile and tonic-clonic seizures. GEFS+ is associated with an autosomal dominant pattern and is caused by mutations in SCN1B which encodes the beta 1 subunit of sodium channels.

Methods: We conducted a case–control study in January 2017, with 6 families, with a total of 35 members entering the study with convenience sampling method, within which, 12 members were as the case group, diagnosed with autosomal dominant GEFS+, hospitalized in Ali Asghar Children's hospital, Iran University of Medical Sciences. 23 family members with no diagnosed GEFS+ were as the control group. Written consent was obtained from all family members according to the protocols of the ethics committee of the university. Afterwards, using D8S533 marker for FEB1 gene, with a Logarithms of Odds (LOD) of 3.16, two-point linkage analysis and haplotype reconstruction was carried out using MLINK program and Simwalk2 respectively.

Results: Haplotype reconstruction analysis in the case group revealed a haplotype associated with GEFS+. However, in the control group, not such an haplotype was seen and the difference between two groups was significant ($p < 0.05$).

Conclusion: In this study we reported a strong linkage between GEFS+ and FEB1 gene. This may clarify the etiology underlying GEFS+ and gives us chances in GEFS+ screening using FEB1.

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Physiology & Immunology Poster Session
Thursday, September 14th, 16h00

PS037

Genetical variability of VP1 gene of BK virus in HIV-infected patients

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Aim: The aims of this study were: to determine the prevalence of BK viruria in HIV-infected patients, to determine the distribution of BKV subtypes and the presence of nucleotide substitutions and mutations in the VP1 gene of BKV isolates.

Introduction: A broad range of diseases associated with BK virus (BKV) such as nephritis, haemorrhagic cystitis, encephalitis, retinitis and pneumonia have been reported in HIV-infected patients over the last few years. However, these diseases do not occur in all HIV-infected patients, suggesting that other factors, such as genetic variability of BKV, can contribute to their occurrence. Mutations in the BC loop of the VP1 gene may lead to selection of more aggressive variants of BKV.

Methods: The study included 50 HIV-infected patients. Semi-nested PCR was used for amplification of 290-nt fragment within the VP1 gene and all the positive PCR products were then directly sequenced. The sequence analysis was performed by using the appropriate bioinformatics tools.

Results: The frequency of BK viruria in HIV-infected patients was 56%. The predominant BKV subtype was I, followed by subtype IV. The majority of mutations were located within BC loop of VP1. The most frequent mutation was E82D.

Conclusion: The increased levels of BKV replication are associated with a higher incidence of mutations in the BC loop of VP1, and mutations in this domain may lead to changed tropism and the selection of more aggressive variants of BKV. Further studies are needed in order to select the patients with a higher risk of developing BKV associated-diseases.

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PS182

Cellular interaction in central and peripheral immune organs due to chronic light stress

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Aim: Study cellular interaction in central and peripheral immune organs at prolonged all-day illumination in an experiment on rabbits.

Introduction: Prolonged all-day illumination is considered nowadays as one of the stress-factors for the living organism and causes malfunctions of the neuroendocrine system and may initial immune dysfunction.

Methods: Experimental rabbits ($n = 10$) were in artificial lighting in the day and electric lighting at night during 12 months. Control animals ($n = 5$) were kept in natural day and night lighting conditions. Cell density in immune organs (thymus, bone marrow, spleen) were measured in surface area which was determined by a rectangle 100×100 μm. The results were processed with standard statistical methods and reported as mean ± standard deviation (SD).

Results: The cell density in the thymus and the bone marrow was decreased: in the cortex of the thymus was 359.6 ± 2.9, in the medullar part – $250{,}8 \pm 2{,}9$, in the bone marrow – 176.4 ± 2.9 (cells in 100×100 μm). An intensified formation of the connective tissue, an increasing of involutive processes and degenerative changes of lymphocytes were microscopically found in the spleen and the thymus. The cell density in the spleen was decreased too: in T zone – 235.8 ± 3.7, in B-zone – 159.5 ± 1.9 (cells in 100 μm × 100 μm). The causes of these changes, probably, may be decrease of the differentiation and migration of lymphocytes as result negative influence of the prolonged light on central immune organs.

Conclusion: These changes in organs of the immune system indicate both a premature aging of the spleen and the thymus and probably of all the immune system. Significant reduction in cell density in the immune organs associate with negative effects of the chronic light stress and leads to expressed immune dysfunction.

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PS217

Intermittent low-level lead exposure causes anxiety and cardiorespiratory impairment

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Aim: To characterize behavioural and cardiorespiratory changes in a new, intermittent low-level lead exposure animal model.

Introduction: Lead (Pb) is a cumulative toxic metal affecting all body systems that are particularly vulnerable during developmental phase. Permanent lead exposure has been defined as a cause of behavioural changes, cognitive impairment, sympathoexcitation, tachycardia, hypertension and autonomic dysfunction. However, no studies have been performed to describe a new, intermittent low-level lead exposure profile, that has been increased in the past years.

Methods: Foetuses were intermittently (PbI) exposed to water containing lead acetate (0.2%, w/v) throughout life until adulthood (28 weeks of age). A control group (without exposure, CTL), matching in age and sex was used. At 26 weeks, behavioural tests were performed for anxiety (Elevated Plus Maze Test) and locomotor activity (Open Field Test) assessment. Blood pressure (BP), electrocardiogram (ECG), heart rate (HR) and respiratory frequency (RF) rates were recorded at 28 weeks of age. Baroreflex gain (BRG) and chemoreflex sensitivity (ChS) were calculated. Student's *T*-test was used (significance $p < 0.05$) for statistical analysis.

Results: An intermittent lead exposure causes hypertension (increased diastolic and mean BP), increased RF, decreased baroreflex function and increased ChS, without significant changes in HR, when compared to CTL group. Regarding behavioral changes, the intermittent lead exposure model showed an anxiety-like behaviour without changes in locomotor activity.

Conclusion: Intermittent low-level lead exposure induces changes on the cardiorespiratory profile characterized by hypertension, carotid chemosensitivity and baroreflex impairment. According to behavioural tests results, this study also shows that the exposure to lead during developmental phases causes anxiety in adult animals without locomotor activity impairment.

In summary, this study brings new insights on the environmental factors that influence nervous and cardiovascular systems during development, which can help creating public policy strategies to prevent and control the adverse effects of Pb toxicity.

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PS120

Antihypertensive effects of two novel dihydropyridine derivatives

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Aim: Treatment of hypertension.

Introduction: Mebudipine and dibudipine are two novel derivations of dihydropyridine (DHP) Ca2+ channel blockers. Previous studies have shown that these two compounds have relaxant effects on vascular smooth muscles. In addition, DPHs are able to reduce contraction force of cardiac muscle in rat. In this study we decided to evaluate the antihypertensive effects of these two novel DHPs in hypertensive rat.

Methods: Male Sprague-Dawley rats were used in the study (8–10 weeks old). The rats were randomly divided to 4 groups of 10 rats (one control and 3 test groups). Blood pressure was measured by Tail cuff method. Left kidneys of the rats were removed by nephrectomy and sodium chloride 1% was added to the drinking water of animals and desoxycorticosterone acetate 20 mg/kg (SC) were injected twice a week. During and after 4 weeks, blood pressure of animals was evaluated to confirm the hypertension. Blood pressure of the animals was measured before i.p. injection of mebudipine and dibudipine (1–8 μmole/kg) and 2 min after the drug administration.

Results: Mebudipine and dibudipine significantly reduced the systolic blood pressure. Mebudipine was more effective than dibudipine and nifedipine in hypertensive animals and has significant results.

Conclusion: Previous studies showed that i.p. injection and oral usage of mebudipine and dibudipine decrease systolic hypertension in normotensive animals, on the other hand vasodilation effects of DHPs have been proved on aorta. Both novel drugs showed significant reduction in systolic blood pressure in hypertensive animals and mebudipine was more potent than dibudipine and nifedipine (as a standard drug uses). It is remarkable that, two new DHPs have similar efficacy and safety profile, but have higher efficacy compared to nifedipine in present study. The brilliant point is that DHPs as calcium channel blockers are more effective in hypertensive animals compared to normotensive animals.

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PS062

Biological processes of polyphenols in the cardiovascular system: A bioinformatics approach

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Aim: In this study, we aimed to evaluate the cardiovascular system-related biological processes (BP) modulated by polyphenols in rodents and humans, and to verify which of them are specie-

[a] Augusto Rachão and Ana Filipa Silva contributed equally to this work.

specific, in order to understand which outcomes for cardiovascular diseases (CVD) could be translated from animal to human studies.

Introduction: CVD stand as a great cause of morbi-mortality worldwide and polyphenol-rich diets have been associated with improved cardiovascular risk profiles. Although rodent models have been a resourceful means of understanding the CVD mechanisms and possible outcomes of the use of polyphenols in that context, most experimental models do not fully reproduce human CVD.

Methods: Database searching was carried out on PubMed and Google Scholar using specific keywords concerning CVD, retrieving close to 300 publications. After excluding irrelevant results, proteome data was organized in Excel® spreadsheets and the Cytoscape platform, ClueGo + CluePedia and Venny 2.1.0 were used to explore the biological processes influenced by flavonoids in the approached CVD.

Results: This study was mainly focused in the species Rattus norvegicus and Homo sapiens and in flavonoids, a polyphenol subgroup. Only about 5% of the BP influenced by flavonoids were common to both species and they were mostly related to the maintenance of blood pressure and the fatty acid metabolic process. Nevertheless, these effects were accomplished through different proteins/pathways and different subgroups of flavonoids.

Conclusion: Our research highlights the need for a careful translation of the flavonoids' effects observed in rat models to clinical trials, since different proteins and subgroups of flavonoids mediated the observed actions. Though this type of studies can provide insights to help choosing the most adequate polyphenols as preventive approaches or therapies for human CVD, further investigation should be performed to clarify the described effects. Besides, pharmacokinetic aspects of the flavonoids' action should also be considered when planning clinical trials.

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PS167

Affinity of *Listeria* sp. proteins to cAMP and role in virulence

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Aim: The aim of this study was thus to identify Lm proteins capable to bind cAMP.

Introduction: Infectious diseases are still a major cause of death worldwide. To infect a host and survive the environment, bacteria have to sense their surrounding and adjust their behaviour. In this adaptation process, cAMP (cyclic adenosine monophosphate) is known to be an important player in pathogens such as *Pseudomonas* spp., *Vibrio* spp. or *Mycobacterium* spp. The small molecule cAMP is a cyclic nucleotide that relays information from receptors to one or more effector proteins within a bacterial cell, functioning as a second messenger. To mediate a response, cAMP allosterically interacts with cAMP-binding proteins. Understanding how this happens is fundamental to predict how bacteria will adapt/act to/in a given context.

Methods: We recently showed that the human foodborne pathogen Listeria monocytogenes (Lm) produces cAMP. The aim of this study was thus to identify Lm proteins capable to bind cAMP. To do this, four candidate proteins selected by bioinformatics analyses were expressed, purified and studied biochemically. Three approaches were used: cAMP affinity chromatography; competitive cAMP affinity chromatography; and isothermal titration calometry (ITC).

Results: Among the four tested proteins, CbpA displayed cAMP-binding ability on the three approaches used.

Conclusion: Hence, our preliminary results showed that CbpA binds to cAMP. It is now mandatory to understand the relation between cAMP and CbpA, to determine the function of the protein itself and in complex with cAMP, and to understand the importance of this signalling system for virulence.

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Public Health & Medical Informatics Poster Session
Saturday, September 16th, 10h00

PS044

Assessment of ECG interpretation skills among Polish medical students, nursing, emergency medicine and English Division medical students

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Aim: The aim of the study was to evaluate ECG interpretation skills among study population, and analyze factors determining their score.

Introduction: The electrocardiogram examination is one of the most frequently performed diagnostic test. Correct interpretation of the ECG, particularly in life-threatening scenarios (LTS) may influence the decisions on appropriate actions and consequently have an impact on the lives and health of patients. It is important for medical, nursing and emergency medicine students to acquire this skill.

Methods: ECG interpretation skills were assessed by self-prepared questionnaire including questions about demographic data and 20 ECG problems with 17 cases. In 6 cases there were LTS. Three questions evaluated basic knowledge about rhythm, heart rate and axis. The survey was conducted via Internet. Study population consist of 551 medical, nursing and emergency medicine students.

Results: The overall score among Polish medical students is 46% which is higher than nursing and emergency medicine students (22% and 37% respectively; $p < 0.001$ in both). English division students scored almost similarly (49%; $p = 0.27$). Polish medical students scored better in LTS than the nursing students (37% vs 23%; $p < 0.001$). Among Polish medical students: Students in year "4–6" scored higher than those from year "1–3" (overall score: 51% vs. 31%; $p < 0.001$, LTS: 41% vs 25%; $p < 0{,}001$). In addition, members

of cardiology scientific groups scored higher than the rest (57% vs 43%; $p < 0.001$).

Conclusion: There is low level of ECG interpretation among medical students and quality of ECG training should be improved. Various factors influences ECG interpretation knowledge among students.

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PS126

Assessment of safe injection practice among nurses in Port Said General Hospital

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Aim: Improving safe injection practice in Port-Said General Hospital.

Introduction: A safe injection is one that, "does not harm the recipient, does not expose the provider to any avoidable risk and does not result in waste that is dangerous for the community". In developing countries, about 16 billion injections are administered each year.

Methods: Through-out March 2015, a cross-sectional, descriptive study was conducted to assess safe injection practice among 150 nurses in Port-Said General Hospital. Data collected by observational CDC Checklist and another checklist for unit evaluation.

Results: Regarding needle disposal 77% of nurses got rid of the needle in safety box, 1% threw it in the pin while 22% threw it in a barrel. Regarding hand washing 41% of nurses washed their hands before preparing medication, while 23% of nurses washed their hands before touching patients and 51% of them washed their hands after touching patients. 57% of nurses wore gloves while 43% didn't. We found 52% of nurses didn't have HBV vaccine. We found also 77% of nurses were trained on safe injection while 23% weren't trained.

Regarding observation, 73% of medication areas were cleaned while 27% weren't. 83% of nurses used single dose vials, ampoules or bottles of intravenous solution for only one patient while 17% didn't. Regarding to hospital unites, only 44% of unites had written policies or procedures for safe injection.

Conclusion: Our evaluation results are good regarding clean medication area,needles for one patient,new needles and syringes,using single dose Vail and using medical connectors for one patient, while are poor regarding disinfecting rubber septum of vial,dating multi dose vials' for 28 when opened and keeping multi dose vial in a centralized medication area and not to enter it in the immediate patient area, these poor results may be due to some untrained nurses.

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PS199

Frequency of analgesic drugs use and patients' awareness of their possible interactions with antiplatelet therapy in coronary heart disease

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Aim: The aim of the study was to assess the prevalence and frequency of analgesic drug use in patients with coronary heart disease, as well as to assess patients knowledge about possible interactions of these drugs with conventional cardiac therapy.

Introduction: Nonsteroidal antiinflammatory drugs (NSAIDs) are commonly used in the management of pain in a variety of conditions. Available data clearly indicate that the NSAIDs use is associated with a number of adverse effects especially in patients with cardiovascular disease.

Methods: The study group consists 93 patients hospitalized in the tertiary cardiology center (mean age 67 ± 11 years,30% females). Thirty nine subjects were hospitalized with diagnosis of acute coronary syndrome and 54 underwent elective coronary intervention. Self-prepared questionnaire was used to collect data.

Results: In the examined group 56 patients (60%) declared the use of analgesic drugs with regular use (defined as at least 3 times per week) reported by 25 subjects (27%). The most frequently used analgesics were NSAIDs ($n = 37$), paracetamol ($n = 36$), less commonly patients reported the use of tramadol ($n = 6$) or metamizol ($n = 9$). The majority of patients using analgesic are not aware about possible interactions with antiplatelet therapy (the answer "yes" for the question about knowledge of possible interactions of analgesic with cardiac treatment gave only 21% of responders, while majority answered "do not know" (72%)). Only 20% of patients admitted that they received the information about analgesics from their doctor. Majority of patients do not consult the use of analgesics with the physician (72%).

Conclusion: The regular use of NSAIDs/analgesic by 27% of hospitalized patients with coronary heart disease is a significant concern. Patients with coronary heart disease should be provided with detailed information and recommendation about safe analgesic therapy and alternatives for NSAIDs.

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PS094

A new route for Medical Education: Rethinking Anatomy's learning strategies

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Aim: The aim of this work is to analyze the advantages of the introduction and diversification of pedagogical strategies in Anatomy Education, as a comprehensive model of Medical Education.

Introduction: Medical Education has suffered a paradigmatic shift that led to curricular reforms. Due to scientific and technological development, Medical curriculum has been adopting a vertical integration model, in which basic and clinical sciences coexist during medical instruction. This context favours the introduction of new complementary technology-based pedagogical approaches. Thus, even traditional core fields of medical curriculum, like Anatomy, are refocusing their teaching/learning standards.

Methods: This work presents the main conclusions of a bibliographic review that reflected on Medical Education's current pedagogical trend, by analyzing the advantages of the introduction and diversification of pedagogical approaches in Anatomy Education.

Results: Anatomy Education's status quo is characterized by less available teaching time, increasing demands of 2D perspective of human anatomy from radiology and endoscopy imaging and other invasive and non-invasive medical techniques, increasing number of medical students and other logistical restrains. The traditional learning approach, mainly based in the cadaveric dissection, is drifting to complementary newer technologies as 3D models or 2D/3D digital imaging to examine the human anatomy. Also, knowledge transference is taking different channels, as learning management systems, social networks and computer-assisted learning and assessment are assuming relevant roles.

Conclusion: The future holds promising approaches for education models. Artificial Intelligence, Virtual Reality and Learning Analytics may provide analytic tools towards a real-time and personalized learning process.

A reflection on Anatomy Education, as a comprehensive model, allows us to understand Medical Education's complexity. Therefore, the present Medical Education context favours a blended learning approach, based on multi-modality pedagogical strategies.

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PS174

Human *Dirofilaria* (Nochtiella) *repens* infection in Serbia

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Aim: The aim of this study was to present and describe cases of *D. repens* infection in Serbia from 2013 to 2016. The cases were reported in National Reference Laboratory for Parasitic Zoonoses in Belgrade.

Introduction: Dirofilariasis is a vector-borne parasitic zoonosis caused by Dirofilaria genus which uses female mosquitoes as vectors. One of the important species is *Dirofilaria repens*. Dogs are main reservoir hosts for *D. repens*, whilst humans can be accidental hosts. The most common site of infection is ocular region. Human dirofilariasis is a rare infection, however, the number of reported cases is increasing in Europe and Serbia.

Methods: Retrospective study was performed including data from National Reference Laboratory for Parasitic Zoonoses in Belgrade. The results were processed and selected parameters were described: sex and age of the patients, locality, location of the lesion, clinical signs and characteristics of parasites. Diagnostic and therapeutic procedures, previous diagnosis were also described.

Results: Seven cases were reported during selected period. The mean age of patients was 44.9 years. Six patients were females (85.7%) and five patients were from Belgrade (71.4%). The most frequent site of infection was ocular region in four patients – 57.5%, while the other sites were skin of abdomen and limbs. All patients had nonspecific clinical signs. The most common cause of the infection was immature female worm in five cases (71.4%).

Conclusion: The number of cases of human *D. repens* infection is increasing in Serbia, and the most common site of the infection is ocular region. Considering the fact that cases are mainly misdiagnosed, it is important to point out the significance of this infection in differential diagnosis of different diseases.[1–4]

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PS104

The multidimentional approach to suicide done through self-mutilation with an overview of wounds

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Aim: The aim of the study was to evaluate the methods and wounds of suicide done by self-injury.

Introduction: Hanging and drug overdose are the most common ways of suicide. However, there are also more painfull methods of dying. This study considers: stabbing, cutting with a knife, ingestion of sharp foreign body, self-shooting, self-arson, crushing.

Methods: There were 65 recorded cases ($M = 56$, $F = 9$, mean age: 49.96 ± 15.78) of self-mutilation as a way of death in archives of the Department of Forensic Medicine of Jagiellonian University Medical College in Cracow in years 2011–2016. All of them were studied in terms of the method, trial and mortal wounds (number, area, type), condition of clothing, prior psychiatric treatment, prior suicide attempts. All calculations were done with the usage of Statistica software.

Results: The most common methods of suicide were self-shooting (38.46%), cutting (26.15%), stabbing (16.92%). There was 1 case of foreign body ingestion and 1 of head crushing in a blacksmith machine. There were 6 cases of self-arson. Trial wounds were observed in 29.23% cases, all of them were recorded in

cases of either stabbing or cutting. The places of mortal wounds: 43.4% head, 20.8% thorax, 18.9% upper limb, 9.4% neck, 3.8% lower limb, 1.9% abdomen, 1.9% digestive tract. The majority of patients (72.3%) had no previous mental treatment and prior suicide attempt (84.61%). In 57/65 cases the place of the wound was exposed.

Conclusion: People in their fifties commit suicide with self-injury. It happens most often with either a gun or a knife. The trial wounds were observed in cases of stabbing or cutting. 4 of 6 cases of self-arsony were accompanied by previous psychiatric treatment. Females commit suicides through self-mutilation more rarely than males.

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PS160

Intestinal colonization of residents of long-term care facilities and nursing homes in Braga area with Multidrug-resistant Gram-negatives

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Aim: The aim of our work was the detection of Enterobacteriaceae isolates producing extended-spectrum beta lactamases (ESBL) and isolates with reduced susceptibility to carbapenems, in the intestinal flora of institutionalized-residents in extra-hospital-health-care facilities in Braga region.

Introduction: Care of aging population has been a growing challenge to public-health and health-care providers. Due to the disabilities of older people, there is a growing need for long-term care facilities (LTCF) and nursing homes (NH). This brings a new paradigm for the spread of bacteria showing multidrug-resistance (MDR) to antibiotics.

Methods: Fecal samples of 27 residents of these institutions were collected (September-to-December, 2016). One gram of each sample was suspended in 10 mL of saline and 100L of the suspension was spread on MacConkey agar with ampicillin(100 mg/L)/cefotaxime(2 mg/L)/meropenem(1 mg/L). Susceptibility to antibiotics was determined by disk-diffusion methods, according to CLSI. ESBL-producers were detected by the double-disk-synergy-test and/or clavulanic-acid addition and PCR was performed for detection of blaTEM, blaOXA, blaSHV, blaCTX-M-group-1, blaCTX-M-group-2, blaCTX-M-group-8, blaCTX-M-group-9, blaCTX-M-group-25, tetA, tetB, aac(3)-II, sul1, aac(6)-Ib and qnrB genes.

Results: The study revealed 6 ESBL-producing Enterobacteriaceae colonizing 2 residents in LTCF (2-Escherichia coli/1-Klebsiella, Enterobacter, Serratia and Citrobacter (KESCgroup)) and 3 residents in NH (2-Escherichia coli/1-KESCgroup). Isolates showed positive for blaCTX-M-group-1, blaCTX-M-group-9, blaTEM, blaSHV, blaOXA, tetA, tetB, aac(3)-II, sul1 and aac(6)-Ib. These isolates showed resistance to non-beta-lactam antibiotics, namely to tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin and amikacin. We detected 6 MDR-bacteria isolates and 1 isolate with reduced susceptibility to carbapenems.

Conclusion: Our results show the dissemination of ESBL-producing-Enterobacteriaceae in intestinal colonization of LTCF/NH patients, who may act as vehicles of MDR-bacteria within the health-care-facilities and community.

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PS202

Social desirability in medical school admission: Differences between students from regular contingent and graduate admission

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Aim: Our work targeted the degree of social desirable answers among two different contingents with the objective of providing useful statistically insight about how this variable may be responsible for the differences among both groups. With the new evidence found in this study we hope to provide useful insight to help improving the selection process of the applicants.

Introduction: The relation between social desirability and medical school applicants may be of interest when analyzing the results of questionnaires in medical school admission. Our study analyzes the difference in social desirability between graduates admitted to a medical school, and students enrolling in the school by the regular admission process.

Methods: We used a resumed version of the Marlow-Crowne Desirability Scale to compare the social desirability between students from the regular and graduate admission. After collecting 181 questionnaires between 2005 and 2006, all the data was analyzed using R software. The sample was described by performing *t*-test between regular and graduate admission in the following variables: gender, marital status, childbearing, working status, previous residence and age.

Results: Statistically significant differences were found in marital and working status as well as within. The main outcome – social desirability – was also statistically different among both groups, indicating that students with graduate admission had given more desirable answers. A analysis of subgroups according to martial and working status was made, being the results also significant.

Conclusion: Our study suggests that graduate admission students have more social desirability than regular students. However, more research is needed in order to find in what way other factors, as age or working status, for example, might have influenced these results.

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PS105

Headache among medical students in Bukovina Region of Ukraine

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Aim: To study the prevalence of headache among medical students in Bukovina region of Ukraine.

Introduction: Headaches are the most prevalent neurological disorder and among the most frequent symptoms amoung medical students. Headache disorder is a major public health issue and is a great burden for a person, health care system, and a society. Identifying of headache risk factors is necessary for treatment and effective prevention.

Methods: A cross sectional study has been conducted in duration from 30th of January to the end of March 2017. 146 students of Bukovinian State Medical University aged 19–26 years were interviewed by using specially designed questionnaires. All students with a headache who completed questionnaires were examined by neurologist. The type of headache was determined according to the diagnostic criteria of the classification of the International Headache Society, 2003.

Results: Our study has found that headaches bother 121 (82.8%) students. Among respondents periodic headache was observed in 71.9% (105 students). 16 (10.1%) students experienced chronic headaches that bothered them more than 15 days a month. Among girls the prevalence of headache was significantly higher than among men – 62.1% and 38.9% respectively. Among the students the most frequent headache (58.7%) was tension headache, 9.1% of respondents had migraine headache, 32.2% had other types of headaches. According to the students' responses the most prevalent causes of headache were stress – 36%, sleep disturbance – 20%, and weather changes – 14%. The other causes included skipping meals – 11%, tea or coffee overdrink – 8%, alcohol consumption – 6%, and menstrual cycle disturbances in 2% students.

Conclusion: By means of the research there was found a high prevalence of headaches among medical students. There was determined the prevalence of primary cephalgias, namely, migraine without aura and episodic tension-type headaches. There was shown a lack of awareness of students about the causes of headache reflected in inadequate symptomatic treatment in most cases.

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PS194

New health problems: Assessment of nutritional and metabolic profile from indigenous citizens in the reserve park of Xingu

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Aim: To identify the prevalence of metabolic syndrome, dyslipidemia, systemic arterial hypertension and type 2 diabetes mellitus in the adult population.

Introduction: In the last 20 years, there have been many changes in the way of life of the Indians of the Xingu Indigenous Park (PIX), resulting from their contact with our society. Factors such as the increasing consumption of processed foods in substitution of the traditional diet based on natural products, the reduction of physical activity by the incorporation of technology and the monetarization of the economy have produced changes in the epidemiological profile of these populations. Previously nonexistent diseases like type 2 diabetes mellitus, systemic arterial hypertension, obesity and dyslipidemias have been increasingly observed. In this work, preliminary results of data collected between February and March 2017 are presented.

Methods: Individuals over 18 years old of both sexes undergoing physical examination (clinical and anthropometric), bioimpedance test to evaluate the percentage of body fat and blood collection by digital puncture for lipid profile and fasting glucose of 8–12 h.

Results: The metabolic profile of 188 indigenous people of the Pavuru polo showed: High index of overweight and dyslipidemia in both populations; high central obesity in females; Increased number of hypertensive and fasting blood glucosealtered among men; 25% have intermediate or high cardiovascular risk in the next 10 years among men, almost twice as many women.

Conclusion: Based on the data obtained, we can conclude that the process of socio-cultural transition to which the residents of the 4 villages studied are inserted has a direct impact on the their health, making it imperative to indetificate and delineate the magnitude of the problem, seeking to develop strategies for the primary and secondary prevention of diseases related to nutritional and metabolic alterations and their consequences among the people living in the PIX.[1–5]

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PS149

Late diagnostics of Alzheimer's disease and other dementias, retrospective research in Vilnius (Lithuania)

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Aim: To gain a deeper understanding around the prolonged diagnosis time for Alzheimer's desease.

Introduction: The world population is getting older. Correspondingly the number of old age sicknesses (like Alzheimer's disease (AD), dementias) is rising in the populations. These patients require custody, medicine and that takes a lot of money and resources from the country.

Methods: There were two multiple choice/short answer surveys done. One for doctors (psychiatrists, neurologists, general practitioners) and the other for the the patients care givers. The surveys were administered in the hospitals in Vilnius. The data were evaluated by SPSS program ($\alpha = 0.05$).

Results: 50 doctors completed the surveys. Doctors emphasize that just about 14% of patients' care givers are aware of the possibility for the genetic screening for AD and are interested in pursuing it. Moreover, according to the surveys, patients with cognitive disorders seek help when they are about 65–75 years old and 70% of

the doctors believe that this is too late. 68% of the doctors agree that the society does not have enough knowledge about dementias.

50 patients' caregivers completed the surveys: 26% – AD, 28% – vascular dementia, 46% – non-defined. The first contact with their doctors varied greatly among different dementia patients: AD – waited for 2.8 y, vascular dementia – 1.46 y, non-defined – 0.87 y. Even though patients' caregivers indicated that they received enough information from their doctor, they admitted that they had no or not enough knowledge concerning the disease before. They also highlighted that it was hard to find information in Lithuanian language.

Conclusion: The research showed that people do not have enough knowledge about dementias and have limited access to information. This is one of the main reasons why the diagnostics is late. Considering the mental health is a stigmatic topic in the world, the fact that people do not know about the dementias might be the problem in the whole world.[1]

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PS010

Obsessive-compulsive behaviour tendencies among medical students in Poland

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Aim: Determination of a need for educational program, focused at students' knowledge and awareness of OCD.

Introduction: As medical students, we are familiar with terms such as professional burnout and workaholism on an every day basis. However other psychiatric disorders seem to be still stigmatized and not well perceived for this profession. It does not mean that they do not exist among future physicians.

Gabbard and Mayers believed that perfectionism is one of the most common personality traits for medics. Moreover, they assumed that perfectionistic physician might perform more often "obsessive triad". Namely: self-doubt, guilt feelings, exaggerated sense of responsibility. Those personal features can lead to obsessive-complusive behaviour. Authors also suggest that those traits are already widely pre-existing for medical students. However no studies supporting this thesis were conducted.

Methods: Anonymous self-completion questionnaire, completed by students from different faculties who study at the Polish universities. Questions based on structuralized clinical questioner designed by the Polish psychiatrists (dr Bryńska and Wolańczyk, 2005).

Results: The questionnaire was completed by 855 students out of whom: 393 medical students (46%). 53% (454) students declared performing specific acts, although not purposedly or despite their will (e.g. checking if the door are locked, cleaning hands, counting. Only 49.6% (194) of medical students answered positively, in respect to 56% (259) non-medical students. Persistent thoughts and fantasies, which are not wanted but occur very often and persistently return – admitted 53.5% (457) questioned. Among the future doctors only 43.5% (170) confirmed to have those thoughts. In contrary to 63% (287) non-medical students.

Conclusion: At first glance it seems that medical students less frequently admit to perform compulsive behaviour or to have obsessive thoughts. But what is the cause? Are they less prone to those behaviours? Or, as Gabbard and Mayers suggest, they have to be seen as "perfect" in front of themselves or others? To answer those questions further research is needed.

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PS132

Assessing the prevalence of HBV and HCV infections in children under going hemodialysis and the related risk factors in a children's Medical Center

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Aim: Assessing the prevalence of HBV and HCV infections in children under going hemodialysis.

Introduction: Chronic hemodialysis is a life saving process in patient with end stage renal disease. Hemodialysis patients are at high risk for viral hepatitis infections due to the high number of blood transfusion sessions, prolonged vascular access and the potential for exposure to infected patients and contaminated equipments. Approximately 8% and 20% of hemodialysis patients have B hepatitis and C hepatitis respectively and this data varies from country to country. Hepatitis B virus (HBV) and hepatitis C virus(HCV) infections are important causes of morbidity and mortality in hemodialysis patients.

Methods: Based on the information gathered from the 149 hemodialysis children files, some special questionnaires were filled in. the obtained data was assessed and analyzed in SPSS software.

Results: A total of 149 hemodialysis patients with mean age 8.8(range: 0.24–16.74) years were enrolled in the study. Out of the total 149 patients, 74 were male and 75 were female. The majority of the patients (51 people) were in the 7–10 years age range. After glomeropathies (34 cases – 22.8%),reflux nephrophaties(24 cases – 16.10%) were the main reasons in charge of renal impairment in our study population.

The results of our study in hemodialysis patients referring to the children's Medical Center of Iran from 1991 to 2009 suggests that prevalence of B and C hepatitis were both 2.04%and the prevalence of the concurrent infections (B and C hepatitis) were 2.72%.

Conclusion: This study confirms that the prevalence of B and C hepatitis among hemodialysis children reffered to children's medical center are much lower than the adult hemodialysis patients in Iran and worldwide. This might indicate the higher health standards and the absence of intravenous drug abuse and unsafe sex among our study population. Screening donated bloods, treating anemia with erythropoietin, avoidance of dialyzer reuse, assignation of dedicated dialysis rooms, machines, and staff for infected patients, new disinfection methods, screening the patients before entering dialysis program and vaccination of susceptible patients and staff all have been reffered to as means of limiting hepatitis transmission within our dialysis unit.

The results of this study can be used in health programming and budget allocating for this group.

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PS078

Evaluation of differences in attitudes of service users about private and public health system of Serbia

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Aim: To evaluate attitudes of service users towards public and private health system of Serbia and how they influence users.

Introduction: It has been noticed that there are differences in attitudes towards public and private health system in Serbia. This attitudes influence service users in the process of choosing their medical treatment.

Methods: 400 users of the public and private health system (50% male and 50% female users) in Serbia filed up online questioner that consisted of 9 questions.

Results: 49% off users had more trust in doctors in public sector and 51% of users had more trust in doctors in private sector. For users that had more trust in private health sector, a perfect doctor was a male (for 14% of users) under age of 35 (for 50.2% of users), if they had a health problem 54.8% of them would go to a doctor, 44% of this users thought that private health system in Serbia is too expensive, and 31.3 of them did not understand their diagnosis after the visit to the doctors. For respondents that had more trust in public health sector, a perfect doctor was a male (for 10% of users) under age of 35 (for 54.3% of users), if they had a health problem 41.3% of them would look it up on the internet, 32% of this users thought that private health system in Serbia is too expensive, and 31.2% of hem did not understand their diagnosis after the visit to the doctors.

Conclusion: The biggest differences among users of the private and public health system are seen in the manner of what would be the best source of information for their health problems, other similarities can be explained by the fact that same doctor worked both in public and private health sectors.

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PS106

Non-consumable alcohol poisoning in the material of the Forensic Medicine Department in Cracow in 2007–2016

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Aim: To analyse cases, find correlation and compare results with other Departments in the future.

Introduction: Non-consumable alcohols – ethylene glycol, methanol, isopropanol and acetone – are the products of everyday use, though cases of lethal poisoning are not very frequent. They may be found in mixture with comestible ethanol.

Methods: The subject of the study was 50 lethal cases (8 women), which had been caused by direct or indirect poisoning connected with consumption substances in the period 2007–2016 in the material of the Department of Forensic Medicine in Cracow.

Results: The mean of cases for study period is 5,1 cases/year, with the notable rise of incidents in 2012 (16). Methanol poisonings were reported in 23 cases (46%), with the prevalence of men (78.3%). The blood concentration in both sexes ranged from 0.45 mg/l to 5340 mg/l. In period 24.10.2012–07.01.2013 the highest frequency of methanol poisoning was observed. It is linked to 2012 Czech methanol poisoning scandal, and influence of this event on Polish alcohol market. Ethylene glycol poisonings are reported in 9 cases (1 woman). The concentrations of alcohol in blood of males varied from 8 mg/l to 5710 mg/l; in female was 96.7 mg/l. The average age of victims was 45.6 years. Isopropanol and acetone poisonings were observed in 17 cases. The concentration of isopropanol ranged from 160 to 5589 mg/l. It is worth noticing, that acetone may partially be a product of metabolic transformation of isopropanol. The average age of victims is 45.6 years.

Conclusion: The vast majority of lethal non-consumable alcohol poisonings of victims were adult men with chronic overconsumption of alcohol. Nevertheless, some mean blood alcohol concentrations in women are comparable to those in men. There is an obvious time correlation between lethal alcohol poisonings and information about non-consumable alcohol appearance on the market.

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PS026

Scientific support of the most widespread and socially significant diseases in 2016

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Aim: Analysis of scientific support of the most widespread and socially significant disease in 2016.

Introduction: Scientific and technical activity is carried out by academic programs, scientific directions, scientific problems and research tasks, which are defined by perspective, practice and necessities.

Methods: The regulatory and legal materials in healthcare of Ukraine are analyzed. The methods of systems analysis, statistical, structural and logical analysis are applied.

Results: In 2016 year 177 scientific-research works were being done in 36 establishments (institutions) ofMinistry of Health ofUkraine; including 85.4% applied researches and 14.6% fundamentalones. Among the total number of establishments 19 scientific and research institutions provided 83 research projects (46.9%), including 77 applied and 6 fundamental researches. In 17 medical educational establishments 94 research projects (53.1%)were being done, including 74 applied and 20 fundamental researches.

In 2016 year 69 scientific-research works were mainly focused on the development and improvement of existing methods of diagnostics, treatment and especially prevention of the most widespread and socially significant diseases, among them 27 works were devoted to researching of cardiovascular diseases, 24 – to cancer pathology, 3 – to tuberculosis, 4 – to HIV/AIDS, 4 – to diabetes, 7 were devoted to issues of scientific evidence of medical and rehabilitation provision of soldiers who took part in anti-terrorist operation, especially those, who were injured and became disabled.

Conclusion: Thus, the results of scientific researches of leading scientists will have real impact on the quality of medical care, improvement of indicators and health care, accelerate the reform of the industry through the scientifically based measures.

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PS228

Is more stressful to become a physician or a pharmacist? A study on medical and pharmacy students' psychological state

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Aim: The objective of this study was to evaluate stress, anxiety, depression and happiness in medical and pharmacy students and to explore similarities and differences between them.

Introduction: Higher levels of depression, anxiety and stress have been found in medical and pharmacy students when compared to general population,[1,2] varying across year in school and gender. Well-being during school years conversely may decrease depressive symptoms, boost happiness and life satisfaction, and contributes to resilience to stressful academic experiences. Students awareness of symptoms and consequences of distress may foster the search for psychoeducation and psychotherapy which offer effective strategies to improve mental health and academic performance.[3,4]

Methods: A cross-sectional study included 420 students of Faculty of Medicine of the University of Porto (FMUP) and 200 students of Faculty of Pharmacy of the University of Porto (FFUP). Assessment included sociodemographic characterization, screening for anxiety and depressive symptoms - Hospital Anxiety and Depression Scale (HADS), stress - Perceived Stress Scale (PSS) and subjective wellbeing - Subjective Happiness Scale (SHS). One-way analysis of variance (ANOVA) and the independent paired t-test were applied to compare demographic and psychological characteristics from within each group.

Results: Statistically significantly higher number of anxiety and depressive symptoms were found in medical students ($p < 0.001$), and pharmacy students presented significantly higher PSS scores ($p < 0.001$). Interestingly, medical students showed statistically significantly higher SHS scores than pharmacy students. Female students revealed significantly higher levels of anxiety, depression and stress in pharmacy school, but in medical school female students presented uniquely higher stress levels.

Conclusion: Attending a faculty degree is a challenging experience which involves life changing experiences and poses different personal and academic problems according each specific school. These findings demonstrate the need to better understand the balance between students' stressful experiences and happiness to identify students at risk in both schools.

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PS234

The relationship between socio-economic determinants and incidence of most common types of cancer in Poland

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Aim: To establish the link between incidence rates of cancer and selected socio-economic variables.

Introduction: Geographical analysis of cancer incidence rates shows significant regional diversity and can be viewed as an approximation of the actual risk of particular types of cancer.

Methods: The absolute numbers of new registered cases of lung, breast and colon cancer in Poland in 2014 by voivodeships (Polish provinces) were obtained from the Polish National Cancer Registry. The situation in individual voivodeships in terms of social isolation, social capital, religious activity and poverty was assessed based on the results of the Polish Social Cohesion Survey for 2015. The Spearman's rank correlation coefficient (rS) was used to test the association between incidence rates of types of cancer (number of cases/100 inhabitants) and social variables. The significance level was set at $p < 0.05$ (2-tailed tests).

Results: Spearman's correlation analysis showed a statistically significant strong positive correlation between lung cancer risk and: social isolation ($rS = 0.73$; $p < 0.0013$), living conditions poverty ($rS = 0.55$; $p = 0.028$), poverty resulting from the lack of budget balance ($rS = 0.72$; $p = 0.0015$), and low/no involvement in religious activity ($rS = 0.7$; $p = 0.003$). Strong negative correlation with $rS = -0.64$ and $p < 0.008$ exists between lung cancer risk and high level of association-based social capital. In colon cancer, only negative correlation between colon cancer risk and high level of friend- and neighbour-based social capital ($rS = -0.56$; $p = 0.020$) was statistically significant. Breast cancer risk was statistically significant for strong negative correlation with high level of friend- and neighbour-based social capital ($rS = -0.74$; $p = 0.0009$) and for a fairly strong positive correlation with low/no involvement in religious activity ($rS = 0.53$; $p = 0.04$).

Conclusion: Our findings provide important evidence for the link between social and economic environment and the risk of most common cancer sites in Poland, and highlight the need to address these determinants as part of national cancer preventive programs.

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Surgery Poster Session
Friday, September 15th, 10h00

PS093

Multimodal analgesia after total knee joint arthroplasty surgery: Intrathecal morphine vs. local infiltration with ropivacaine

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Aim: To assess and compare effectiveness and side effects of postoperative anesthesia methods, using intrathecal morphine and

local infiltration of ropivacaine, a day after knee joint arthroplasty operations with spinal anesthesia.

Introduction: Inadequately chosen postoperative anesthesia method after knee joint arthroplasty surgery might cause prolonged hospitalization period, readmissions due to pain and overall increased cost of care.

Methods: In 2016 a prospective research was conducted in Vilnius University Hospital Santaros Clinics. 25 patients undergoing knee joint arthroplasty surgery with spinal anesthesia were enrolled in the study. Group1 – local soft tissue ropivacaine infiltration anesthesia around the knee ($n = 13$; dose 300 mg); Group2 – inthratecal morphine sulfate analgesia ($n = 12$; dose 0.1–0.2 mg). Pain intensity (using VAS) at rest and in motion, patient's satisfaction and side effects - nausea, vomiting, itch, urinary retention – were assessed at time intervals – 1, 2, 4, 6, 12, 18, 24 h postoperatively.

Results: In the first 12 h mean values of VAS were $1.8 \pm 2.6/1.4 \pm 1.7$ in Group1 and Group2 accordingly. After 12 h period a downtrend occurred and values were $1.7 \pm 1.1/1.1 \pm 1.5$, respectively ($p > 0.05$). Examining pain in motion 12 h after the surgery pain intensity values were $2.5 \pm 2.7/3.3 \pm 2.7$ and after 24 h in both groups pain intensity was $3.2 \pm 1.5/3.6 \pm 2.1$, resp. ($p > 0.05$). Zero episodes of nausea/vomiting were registered in Group1, while 58.3% ($n = 7$) of Group2 patients experienced nausea and 5 of them also vomited. Even 66.7% ($n = 8$) patients in Group2 had itch while none patients of Group1 indicated this side effect. It was difficult to assess urinary retention as 30.8% ($n = 4$) Group1 and 66.7% ($n = 8$) Group2 patients were catheterized prior surgery. Finally, satisfaction level of both groups were evaluated very similarly: $8.2 \pm 1.7/8.2 \pm 1.3$ ($p > 0.05$).

Conclusion: VAS values at rest were very similar in both groups, but pain relief efficiency compared to the intensity of pain during movement was better with local ropivacaine infiltration, also patients with ropivacaine analgesia experienced no side effects.

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PS134

The role of cerebroplacental ratio in prediction of neonatal outcomes and route of delivery

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Aim: The aim of our study was to check the appropriability of cerebroplacental ratio (CPR) measured within 48 h before delivery in prediction of route of delivery and adverse neonatal outcomes.

Introduction: The cerebroplacental ratio is an important obstetric ultrasound tool used for assessment of foetal oxygenation. It is also a valuable predictor of adverse pregnancy outcomes. CPR is calculated by dividing the Doppler pulsatile indices of the middle cerebral artery (MCA) and the umbilical artery (UA).

Methods: The retrospective study included 1328 pregnant women who gave birth in Department of Obstetrics and Perinatology Jagiellonian University Medical College, Cracow, Poland. Main inclusion criteria were: singleton pregnancy and the interval between ultrasound examination and delivery within 48 h. Exclusion criteria consisted: active labour, multiple pregnancy, preeclampsia, foetal growth restriction and evidence of intrauterine infection. CPR value lower than 1.08 was classified as pathological. Participants were divided into 2 groups: control ($CPR \geq 1.08$, $n = 1228$) and study ($CPR < 1.08$, $n = 100$). The differences in socio-demographic factors between control and study group were not statistically significant. Data were analysed using chi-squared test, independent sample 2-tailed T-test and logistic regression. p value < 0.05 was statistically significant.

Results: In study group was observed statistically significant increased risk of delivery provided by cesarean section (OR = 1.8; $p = 0.015$), preterm delivery (OR = 2.91; $p = 0.0001$), birth weight < 2500 g (OR = 5.87; $p < 0.00001$) and APGAR score < 7 in 1st (OR = 6.56; $p < 0.0001$), 3rd (OR = 7.04; $p < 0.0001$) and 5th (OR = 5.4; $p = 0.017$) minute after delivery, compared to control group. Moreover, low CPR was associated with lower incidence of foetus birth weight within normal limits (OR = 0.37; $p < 0.0001$) and on-term delivery (OR = 0.61; $p < 0.0001$).

Conclusion: Detection of low value of CPR in every case should be alarming signal for obstetrician. Normal CPR appears to suggest better foetal tolerance to the stress of labour. CPR may be used to stratify the risk of pregnancy before labour.

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PS072

Chronic subdural hematoma in aging population – How the age influence the outcome after surgical treatment

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Aim: The aim of our work is to examine how the age influence the outcome after surgical treatment of chronic subdural hematoma.

Introduction: Chronic subdural hematoma (CSDH) is a common condition, characterized by the collection of hemolyzed blood between dura and arachnoid mater of the brain surrounded by two pathological hematoma membranes - internal and external. The number of CSDH incidence increases with age and it is why more attention should be directed for surgical treatment in elder patients group.

Methods: Data on management and outcomes for patients with CSDH were collected retrospectively from years 2014–2017 and investigated using statistic methods. The study group was divided into two subgroups according to the age: <75 years and ≥75 years old. Age, gender, comorbidities, neurological status on admission and at discharge, pre-/postoperative epilepsy, surgical technique were investigated.

Results: We analyzed 257 patients with a diagnosis CSDH. Analyzed subgroups have not differ significantly except the gender and concomitant diseases according to the Chi2 and exact Fisher tests. We found craniotomy in patients ≥75 years old increases the risk of postoperative epilepsy compering to the bur-hole (logistic regression analysis: 9.8 [95% CI: 1.9–49.8], $p = .006$), same as the internal hematoma membrane removal during surgery (logistic regression analysis: 10.3 [95% CI: 2.0–52.15], $p = .005$). These dependencies do not occur in the younger age group. Type of treatment have not influenced the mRS in patients younger than 75 years old. In elder patients reoperation and removal of the internal membrane of the hematoma worsened outcome measured in mRS (logistic regression analysis: 5.5 [95% CI: 1.4–20.90], $p = .013$ and 3.1 [95% CI: 1.4–7.2], $p = .007$).

Conclusion: Craniotomy and internal membrane removal increase the risk of epilepsy in elder CSDH patients. Reoperation

and hematoma internal membrane removal are the risk factors of unfavorable outcome in patients ≥75 years old.

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PS103

The frequency of MINS (Myocardial Injury after Noncardiac Surgery) and others postoperative complications in different age groups of elderly patients who underwent endovascular aortic repair because of abdominal aortic aneurysm

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Aim: The aim of our study was to estimate frequency of MINS and others complications after endovascular aorta repair because of AAA in different age groups.

Introduction: Nowadays, endovascular aneurysm repair (EVAR) is the most common technique for repair of abdominal aorta aneurysm (AAA). This procedure involves less complications than open surgery, nevertheless they still occurs.

Methods: The study group consisted of 143 patients (85.3% men), aged 76.8 ± 7.7 with AAA who had endovascular aneurysm repair between January 2015 and May 2017 in the Department of Vascular Surgery and Angiology. Patients were divided into two groups depending on age: group I ≤75 years (60 patients, aged 69.3 ± 4.5), group II >75 yrs (83 patients, aged 82.2 ± 4.2). We considered coexistent diseases, some laboratory tests and Revised Cardiac Risk Index for Pre-Operative Risk (Lee index). Statistical analysis was performed with U Mann-Whitney and Chi2 tests.

Results: The study groups were comparable regarding the coexistent diseases and preoperative risk. Older patients had higher mean creatine level on admission than younger patients (group I: 103.29 ± 84.10 vs. group II: 118.5 ± 61.9 umol/l, $p < 0.005$) and lower eGFR (80.6 ± 27.6 vs. 61.61 ± 21.9 vs. 1.73 ml/min/m^2, $p < 0.001$). The mean concentration of haemoglobin and amount of white blood cells were also lower in elderly patient (13.19 ± 1.93 vs. 12.9 ± 18.3 g/dl, $p = 0.01$; 8.23 ± 2.74 vs. 7.49 ± 2.7 × 103/μl, $p = 0.04$).

Frequency of some complications such as acute kidney injury, pneumonia, sepsis, stroke or intrahospital mortality were similar in both groups.

However, we observed a statistically significant difference in the frequency of MINS (26.67% vs. 45.78%; $p = 0.04$). Older patients also needed red blood cells concentrate transfusion after surgery more often than younger (6.67% vs. 19.28%; $p = 0.03$).

Conclusion: MINS is the most common complication after EVAR. Age seems to be a significant feature which increases the frequency of MINS in compared groups despite similar coexistent diseases and preoperative risk assessment determined by Lee index.[1–3]

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PS210

Evaluation of clinical characteristics as indicators for shunt procedure in patients with medulloblastoma

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Aim: Determing clinical characteristics and parameters reliable as predictors of the need for the shunt installation and their relation to the treatment outcome.

Introduction: Medulloblastoma represents the most common pediatric tumor, that most frequently involves posterior cranial fossa and often manifests as hydrocephalus. Current therapy involves tumor excision and posterior cranial fossa decompression, with or without temporary external drainage of cerebrospinal fluid, endoscopic ventriculocisternostomy and ventriculoperitoneal (VP) shunt placement.

Methods: This retrospective study included 36 patients treated in the period from January 1st 2007 to December 31st 2015 in the Clinic of Neurosurgery, Clinical Center of Serbia. Basic demographic data, symptoms and signs at admission, degree of tumor resection and disease outcome information were analyzed.

Results: 22 patients (61.1%) were male and 14 (38.9%) were female, most of them 4–14 years old (58.3). Sex and age showed no significant corelation with VP shunt installation, or timing of shunt installation. VP shunt was installed in 92% of patients, in 33.3% prior to and in remaining after surgery. The most frequently observed symptoms on admission were cerebellar symptomatology (91.2%), headache (75.7%) and vomiting (68.5%), which showed no significant correlation with the VP shunt installation and shunt installation timing. In 83% of patients total resection was achieved. The degree of tumor resectability and VP shunt installation were significantly related ($p < 0.001$). Correlation among shunt installation and treatment outcomes, as well as the shunt installation timing and outcome showed a statistical significance ($p < 0.001$).

Conclusion: No clinical characteristics reliable as prognostic parameter for VP shunt installation in medulloblastoma patients have been found. Shunt placement is recommended in all cases of incomplete tumor resection, unless already placed preoperatively. Patients with a shunt placed prior to surgery have had significantly better outcome.[1–35]

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PS009

Percutaneous cholecystostomy in the management of acute cholecystitis

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Aim: The aim of this study is to clarify the role of percutaneous cholecystostomy in calculous acute cholecystitis treatment and to elucidate about its association with the surgical treatment.

Introduction: Laparoscopic cholecystectomy is the gold-standard treatment in acute cholecystitis. However, percutaneous cholecystostomy stands as an alternative therapeutic approach among the elderly or patients with several comorbidities.

Methods: In December 2016, a systematic database search on PubMed, Scopus and Web of Science was conducted to identify articles on percutaneous cholecystostomy published from January 2013 to November 2016, using the query "(acute cholecystitis OR severe cholecystitis) AND (cholecystostomy OR percutaneous cholecystostomy OR cholecystostomy tube)". In total, 290 articles were found and submitted to inclusion and exclusion criteria.

Results: A total of 13 records involving 1130 patients from 10 different countries met all inclusion criteria and were therefore included in this systematic review. All studies found eligible concluded percutaneous cholecystostomy is a potentially safe and effective therapeutic approach among high-risk surgical patients in the setting of acute cholecystitis. Percentage of patients undergoing percutaneous cholecystostomy followed by cholecystectomy varied between 7.2% and a maximum of 66.7%, with a conversion rate fluctuating between 0.0% and 66.7%. Complication and mortality rates ranged from 2.2% to 41.7% and 0.0% and 43.2%, respectively.

Conclusion: Percutaneous cholecystostomy is generally considered safe and effective among high-risk surgical patients diagnosed with acute cholecystitis.[1,2]

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PS092

The influence of maternal age and parity on perinatal outcomes – A preliminary study

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Aim: The aim of the study was to compare maternal, perinatal and neonatal outcomes depending on maternal age and parity.

Introduction: Advanced maternal age at childbirth has been associated with adverse perinatal and neonatal outcomes. As mean maternal age in developed countries is increasing decade by decade, the issue of perinatal outcomes among older patients seems to be of utmost importance.

Methods: It is a preliminary study that enrolled 243 women who gave birth in the Department of Obstetrics and Perinatology of the University Hospital in Kraków, Poland, during a one-month period (in May 2017). The patients were divided into 2 groups: >30 and ≤30 years old. The two groups were subsequently subdivided into 4 subgroups. Maternal, perinatal and neonatal outcomes were compared between all the subgroups.

Results: Comparison of women at age >30 and ≤30 revealed that advanced maternal age may constitute a predisposing factor for stillbirth, preterm delivery and congenital disorders. At the same time, the patients in the first group were at lower risk of SGA (small for gestational age) and LGA (large for gestational age)

as well as lack of progress in labour. Nevertheless, none of these parameters reached a statistical significance. Primiparas both ≤30 and >30 year-old had a much greater risk of lack of progress in labour (OR = 11.3; $p = 0.0015$ and OR = 19.5; $p = 0.00027$) as well as emergency caesarean section (OR = 5.6; $p = 0.00056$ and OR = 2.5; $p = 0.027$) and a lower risk of elective C-section (OR = 0.4; $p = 0.0027$ among women ≤30 years old) when compared to multiparas. These results met statistical significance criteria.

Conclusion: Advanced maternal age can undoubtedly be associated with several adverse perinatal outcomes. However, the results of the study do not seem to be unequivocal. Further investigation of the influence of maternal age on the course of pregnancy and delivery should be conducted.

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PS118

Varicose veins surgical treatment: Endovenous laser ablation versus open surgery

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Aim: Compare long-term outcomes of varicose veins treatment between endovenous laser ablation and open surgery.

Introduction: 25–33% of the Western population adults is suffering from varicose veins and surgical treatment is characterized by high recurrence rate of 60% after 5 years follow-up observation. That leads to look for the most effective treatment option.

Methods: A retrospective study of 182 patients who had varicose veins endovascular or open surgery during 2015-06–2015-12 were made. Patients' demographic and clinical data were registered. Patients were contacted and asked to evaluate surgery outcomes subjectively. Patients were divided in two groups: I – ones who had endovenous laser ablation and II – those who had open surgery.

Results: 136 (74.7%) patients agreed to participate in the study: 26 (19.1%) men and 110 (80.9%) women; average age – 48.36 years (±1.087). 65 (47.8%) – group I, (41 (63.1%) were operated because of the symptoms, 24 (36.9%) were operated because of the esthetical reasons), 71 (52.2%) – group II, (58 (81.7%) were operated because of the symptoms, 13 (18.3%) were operated because of the esthetical reasons.) The symptoms renewed: group I – 9 (22%) patients, group II – 25 (43.1%). Esthetical relapse: group I – 3 (12.5%), group II – 5 (38.5%). Patients assessed esthetical view: group I – 48 (73.8%) were completely satisfied with the results, 15 (23.1%) were partly satisfied and 2 (3.1%) were unsatisfied, respectively in group II the results were 44 (62%), 26 (36.6%) and 1 (1.4%) ($p = 0.202$). Patients also evaluated their symptoms after treatment. Group I: 53 (81.5%) patients were completely satisfied, 9(13.8%) – partly satisfied, 3 (4.6%) – unsatisfied, respectively in group II – 52 (73.2%), 14 (19.7%) and 5 (7%) ($p = 0.513$). According to the EQ-5D-3L questionnaire patients treated in group I evaluated their health – 80.29 (±13.199), II – 74.56 (±16.615).

Conclusion: Chronic venous insufficiency symptoms more often renewed to patients who had open surgery. Patients' were more satisfied after endovenous laser ablation. Also, those patients who were treated with endovascular procedure evaluated their health better.

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PS147

Patients' quality of life after surgical treatment of oral cancer

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Aim: To evaluate the quality of life of patients after surgical treatment of oral cancer.

Introduction: Oral cancer surgery may have resulted in damage to the act of chewing, swallowing, speech and communication, lead to social incompetence and decline in the quality of life of patients.

Methods: The study included 30 surgically treated patients previously diagnosed with oral cancer at the Department of Maxillofacial Surgery, Clinical Center in Novi Sad. The survey and assessment of Karnofsky index ofpatients was conducted within the regular control visits to a specialist clinic for maxillofacial surgery. The questionnaire is in addition to general information included questions related to the primary tumor localization, harmful habits, stomatognathic system function and psychosocial condition after surgical treatment of oral cancer.

Results: Mean age of patients was 63 years, of which 73.3% were male and 26.7% female. The most common localization of oral cancer was under the mouth of 9 (30%) patients. Alcohol and cigarettes were consumed by 70% of patients. In 50–60% of patients,stomatognathic system function was reduced to varying degrees of severity. Chewing function wasstatisticallysignificantly worse in patients after marginal resection of the mandible ($p < 0.05$). Depression was observed in 18.2% of men and 12.5% women, and the problem of appearance in public places had 40% of patients. Karnofsky index of 25 (83.3%) patients was 100% while the remaining was lower as a result of comorbidity.

Conclusion: The quality of life in terms of stomatognathic system function and psychosocial status was reduced to varying degrees of expression in many patients after surgical treatment of oral cancer. Therefore, the detection of diseases in an early stage is crucial to the survival and quality of life for patient.

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PS221

Long-term outcomes in simultaneous pancreas-kidney transplant recipients: Retrospective single centre study

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Aim: The aim of this study is to present long-term outcomes of SPKT.

Introduction: Simultaneous pancreas-kidney transplantation (SPKT) is the treatment of choice for patients with end-stage renal failure due to type 1 diabetes mellitus (DM1).

Since the 1980s, pancreas transplant has become the most effective strategy to restore normoglycemia in patients with DM1.

Methods: We performed a retrospective analysis of 73 SPKT recipients who underwent transplantation between 1988 and 2015.

Results: 50.68% of patients were male. During the time of surgery the mean age was 37.8 ± 7.44 years. DM1 was diagnosed average 25 ± 6.08 years before SPKT. For 21.9% it was pre-emptive transplant. 60.9% and 17.19% were on haemodialysis and CADO respectively (the mean dialysis time was 29.05 months). Reoperation due to pancreatic and kidney complications amounted respectively 23.3% vs 8.3%. DGF was observed in 9.6% of kidney graft recipients. Mean HLA - A, – B, – DR mismatches were: 1.42, 1.58, 1.27. All patient received induction of immunosuppression (polyclonal immunoglobulins: ATG/Thymoglobulin – 64% or monoclonal: daclizumab/basiliximab – 36%). Kidney graft survival at 1, 5, 10, 15 years 100%, 97%, 85% and 67%; and pancreas survival is 95%, 92%, 87% and 67% respectively. There was noticed tendency to increase creatinine level (from 1.18 at 1 year to 1.78 at 15 years) and decrease of haemoglobin level (from 13.84 at 1 year to 12.65 at 15 years). Patients with longer time of dialysis were more commonly infected by HCV ($p = 0.004$), more often hospitalized due to cardiovascular complications ($p = 0.004$) and had shorter survival time ($p = 0.03$). HBV infection correlated with longer time of hospitalization during transplantation procedure ($p = 0.006$), more often delay grant function of pancreas ($p = 0.008$), higher serum level of CRP ($p = 0.04$) and more frequent hospitalizations in subsequent years ($p = 0.003$).

Conclusion: Shorter dialysis time improves patient prognosis after SPKTx. HBV and HCV infection is associated with more frequent complications and worse prognosis. Cardiovascular complications are more likely to affect dialysis patients.

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PS031

The impact of suturing hemostasis on ovarian reserve during conserving surgeries on the ovaries

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Aim: To determine the effects of using suturing hemostasis in cases of cystectomy of unilateral endometriomas and mature teratomas (MT).

Introduction: Nowadays there is a noticeably growing rate of benign ovarian tumors requiring urgent treatment. It is known that ovarian tumors lead to diminished ovarian reserve (OR). Conserving surgeries bring to a further reduction of OR.

Methods: The study involved 66 patients with endometriomas and 69 with MT. The mean age was 28.07 ± 5.3. All patients underwent laparoscopic cystectomy. The methods to stop bleeding were: ligature hemostasis with absorbable polyglycolic suture, USP 2-0 (I group) and bipolar coagulation (BPC) – Autcon II 350, current power 35 W – (II group). Before and 6–12 months after surgery serum levels of Antimüllerian hormone (AMH) were evaluated; by ultrasound (Toshiba Aplio 500, 3.6–8.8 MHz) we measured the volume of healthy ovarian tissue (Vcm^3), antral follicle count (AFC), their site and diameter.

Results: At the pre-surgical stage patients with endometriomas had reasonably lower ultrasound and biochemical markers than patients with MT. 6 months after suturing hemostasis patients with endometriomas had a 1.8 times higher AFC, its diameter and, as a result, the volume of ovarian tissue of the operated gonad compared to the group after BPC. Studying similar indices by the patients with MT showed the difference of 1.3 times respectively. Deformed follicles with small diameter (3–4 mm) ousted to the periphery were located on the echograms. Six months after laparoscopy the AMH level of all patients decreased, the biggest reduction (1.7 and 1.9 times correspondingly to groups) was noted by the patients with endometriomas.

Conclusion: To preserve women's reproductive potential after conserving surgeries on the ovaries, intracorporeal suturing is a preferred hemostatic method over bipolar energy. Enucleation of endometriomas and MT leads to diminished OR regardless of the energy type used as a hemostasis.

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PS102

Complications after open surgery for the abdominal aorta and its branches depending on patients' age

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Aim: Age is one of the risk factors for postoperative complications in open surgery of the aorta. The awareness of their frequency may lead to earlier diagnosing and referral for procedure in order to avoid negative results of surgery and further therapy.

Introduction: The aim of our study was to assess the dependent of age frequency of postoperative complications among patients undergoing primary open surgical procedure within abdominal aorta due to aneurysm (AAA) or/and peripheral arterial disease (PAD).

Methods: The study group consisted of 249 patients (84.7% men), aged 69.1 ± 8.2 with AAA or/and PAD who underwent open abdominal aorta surgery between August 2015 and January 2017. Patients were divided into three groups depending on age group I < 65 years (61 patients, aged 58.8 ± 5), group II 65–74 yrs (118 patients, aged 68.5 ± 2.8) and group III >74 yrs (70 patients, aged 79.1 ± 3.4). We considered coexistent diseases, smoking habit, Revised Cardiac Risk Index for Pre-Operative Risk (Lee index), some laboratory tests, type and mode of surgery (elective vs urgent/emergent). Statistical analysis was performed with Kruskall Wallis and Chi2 tests.

Results: Frequency of some complications such as myocardial infarction, pneumonia, sepsis, stroke or bleeding was similar in compared groups. Nevertheless, we observed a statistically significant difference in the frequency of acute kidney injury undemanding dialysis (respectively, for groups I–III: 3.28% vs. 17.80% vs. 20.00%; $p = 0.013$), multi-organ failure (1.64% vs. 10.17% vs. 14.29%; $p = 0.039$) and intrahospital mortality (1.64% vs. 11.86% vs. 18.57%; $p = 0.009$). The groups were comparable regarding the coexistent diseases – the only differentiative feature was hypertension that occurred less in group I (62.30%) than in other groups (82.20% and 81.43%, $p = 0.01$). There was no significant difference between groups in preoperative risk determined by Lee index and mode of surgery procedure. However, older patients had higher mean creatine level on admission than younger patients (72.2 ± 21.5 vs. 91.3 ± 34.2 vs. 94.4 ± 37.7 umol/l, $p < 0.005$), lower eGFR (109 ± 31 vs. 84.5 ± 29.1 vs. 73.8 ± 24.7 ml/min/1.73 m^2, $p < 0.001$) and more

often underwent procedure due to ruptured aneurysm (4.92% vs. 9.32% vs. 11.43%, $p < 0.001$).

Conclusion: Age is a significant feature which increases the frequency of acute kidney injury, multi-organ failure and intra-hospital mortality in compared group despite similar coexistent diseases, mode of surgery and preoperative risk determined by Lee index.[1,2]

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PS076

Modern treatment and diagnostics for submucosal tumors of the upper third of the esophagus. Analysis of preoperational and postoperational data

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Aim: This study was to evaluate diagnostic data and outcomes of endoscopic treatment for submucosal tumors (SMTs) of the upper third of the esophagus.

Introduction: Esophageal submucosal tumors (SMTs) are very rare, with prevalence of 0.5% in autopsy series. Among them leiomyomas are the most common, they originate from the muscularis propria (4th EUS layer) or muscularis mucosa (2th EUS layer) of the esophageal wall.[1] Submucosal lesions of the upper third is very rare and occur in 4% of cases.[2] Submucosal tunneling endoscopic resection (STER) and endoscopic submucosal dissection (ESD) are modern techniques for treating SMTs. The choice between them depends on layer of origin of the tumor.

Methods: In this study were included 2 patients with SMT of the upper third of the esophagus. For diagnostics we used esophageal symptoms questionnaire, endoscopic ultrasonography (EUS) and/or computed tomography (CT) to determine layer of origine, size and relation of lesions to the surrounding structures and organs. Esophageal manometry were used to identify problems with movement and pressure in the esophagus. Immunohistochemistry and histological analysis were performed postoperatively.

Results: Both patients were asymptomatic, tumors were found accidently during rutine esophagoscopy. Although in both cases manometry of the esophagus revealed increased distal latency (DL) comaring with mean value in patient without esophageal SMT. In the first case tumor arised from 4th EUS layer, hence we used STER, subcutaneous emphysema of the neck occured during operation. in the second case lesion originated from 2th EUS layer, therefore ESD was performed. En bloc resection was achieved in both cases, histological diagnoses were leiomyomas.

Conclusion: Upper third of the esophagus is the most difficult location for performing endoscopic techniques. Determination of the layer of origin is crucial, as on that depends the choice of treatment tactics. Increased DL in such patients requires further study.

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PS035

The role of the state of uterine-placental-foetal circulation on the clinical course of gestational process and its impact on perinatal outcome

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Aim: To identify the relationship between the condition of utero-placento-foetal circulation with the clinical course of gestational process and its impact on perinatal outcome.

Introduction: The period of foetal development before birth is so extensive however, only a small part of the duration of this period, which largely determines the quality of his later life. It is a proven fact that the events during the prenatal period effects the outcomes of pregnancy which are favourable in childbirth, later leading to diseases in adulthood.

Methods: We analysed the course of pregnancy, delivery, the condition of the foetus and newborn from 72 pregnant women (24–41 weeks of gestation) with placental dysfunction at the 3rd Maternity Hospital, Zaporozhye.

Results: According to CT, distress of the foetus were confirmed in 22.7% of pregnant women with impaired hemodynamics I-A degree, 24.8% with impaired hemodynamics, at 30.6% with circulatory disorders of the II degree. On analysis of the hemodynamics in the system of maternal-placento-foetal revealed violations of IPC(I-A) in 46% of cases, ACC(I-B) at 28.7%, IPC and SPC(II) at 12.7%, critical blood vessels PPK(III) and 3.4% of cases. The frequency of caesarean section in pregnant women with dysfunction of placenta was 28.2%, of which the foetal distress was 22.4%, vacuum extraction of the foetus were used in 3.2%. The analysis of the development of newborn from mothers with placental dysfunction, identified the violation of their status at birth and Apgar score 7–5 points received at birth 11.2% of newborn.

Conclusion: Analysis of indicators of physical development of newborns in the early neonatal period were distinguished by the presence of signs of functional immaturity. Clinical and statistical analysis conducted revealed a high frequency of complications of pregnancy and childbirth in women with dysfunction of the placenta.

Acknowledgements: I would like to express my special thanks of gratitude to my associate professor Nataliya Victorovna Gaidai as well as my co-authors Varahabhatla Vamsi and Katnam Sahithi who gave me this golden opportunity to do this wonderful project on the topic. The role of the state of uterine placental foetal circulation

on the clinical course of gestational processes and it's impact on perinatal outcome. Secondly, I would also like to thank my parents and friends who helped me a lot in finalizing this project within the limited time frame.

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PS014

The possibility of optimization of hemodynamics in the fetoplacental pool as a factor of influence on perinatal outcome

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Aim: To study the possibility of optimization of hemodynamics in the fetoplacental pool as a factor of influence on perinatal outcome.

Introduction: Endothelial dysfunction in uteroplacental pool is a universal response of placenta to adverse effects of hypoxia, which leads to a high percentage of obstetric complications. Recreation is a way of optimization of hemodynamics in fetoplacentar complex in the interests of antenatal protection of the foetus.

Methods: The study was conducted at the 3rd Maternity hospital, Zaporozhye including 40 pregnant women with VD with age group of 21–36 years(Primapara – 52.5%, multipara is 47.5%). 40 pregnant women with chronic venous insufficiency to restore homeostasis used the IR thermo-camera, designed and implemented by the Department of Clinical Pathophysiology, Institute of physiology. Pregnant women of the main group underwent 3 sessions of IR sonotherapy(1 time per week), lasting 30 min at temperature of 35 °C.

Results: Pregnant women with VD after using sonotherapy in the infrared heat chamber in the complex sanatorium treatment, on comparison with the control group, a more pronounced therapeutic effect of lowering body weight by $22.3 \pm 1.2\%$,and decrease of systolic 14.6 ± 0.2 mmhg and diastolic 15.1 ± 1.1 mmhg pressure. Ended pregnancy in a core group of women, the birth of full-term newborns with no signs of distress, with an Apgar score of 7–9 points, body mass 2980–4000 g. 1 in the case of birth by caesarean section for obstetric indications.

Conclusion: The research conducted in the sanatorium "Velikii Lug", confirms the effectiveness of the use of the IR sonotherapy in optimizing antenatal protection of the fetus against the background of endothelial dysfunction.

Acknowledgements: I wish to express my sincere gratitude to my scientific supervisor Mrs. Nataliya Viktorovna Gaidai for providing me an golden opportunity to be a part in this project.I also sincerely thank my co-authors Mr. Vamsi Varahabhatla and Katnam Sahithi for their guidance and encouragement in carrying out this project work. Finally, I would like to thank my family for supporting me financially and morally.

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PS090

Bile duct injuries after cholecystectomy: A retrospective tertiary centre study comparing outcomes of different types of surgical treatment

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Aim: Evaluation of long-term outcomes after different types of surgical management of postcholecystectomy bile duct injuries (BDI).

Introduction: Cholecystectomy is one of the most routinely performed procedures in abdominal surgery. Despite the growing experience of surgeons and benefits of minimal invasive approach, BDIs still occur. The treatment of this complication is challenging.

Methods: This was a single-center retrospective study. The outcomes of 64 consecutive adult patients, surgicaly treated after postcholecystectomy BDI 2002–2016, were reviewed. The newest EAES ATOM classification was used to describe injuries. The anatomic characteristics of the injury and long-term treatment outcomes were evaluated.

Results: 48 (75%) BDI followed laparoscopic cholecystectomy. 26% of injuries were detected intraoperatively, 58% detected <7 days, 16% >7 days after the procedure. The injury of non-main bile duct was diagnosed in 10 (16%) cases. The injuries of main bile duct: choledochal duct 22 (34%), hepatic duct 22 (34%), bifurcation with right-left communication preserved 5 (8%), bifurcation with right-left interrupted 1 (2%), right/left hepatic duct 4 (6%). 26 (41%) patients with a cystic stump leak or partial division of duct were managed endoscopically. This treatment was successful for 7 (88%) cystic stump leaks and 8 (58%) partial divisions. 13 (20%) partial divisions of duct were closed by suture. 8 (73%) patients had complications which later required endoscopic management or hepaticojejunostomy. End-to-end anastomosis (6 (10%)) or hepaticojejunostomy (16 (25%)) was initially performed after the complete division with or without loss of substance was detected. End-to-end strategy was successful in 4 (67%) cases, others finally required hepaticojejunostomy. The complication rate after initial hepaticojejunostomy - 25%.

Conclusion: Endoscopic treatment is optimal for cystic stump leaks and partial divisions of ducts. Complete divisions with or without loss of substance may be treated by hepaticojejunostomy and end-to-end anastomosis with similar long-term outcomes. While end-to-end anastomosis is more physiological, this strategy should be considered when possible.

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PS041

Perinatal loss in multiple pregnancies

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Aim: The aim of the study was to analyze causes of perinatal loss in multiple pregnancies.

Introduction: In population rate of multiple pregnancies varies from 0.7 to 1.5%. Multiple pregnancies are complicated by perinatal loss 4–9 times more frequently than singleton pregnancies.

Methods: Retrospective study of medical histories was carried out. Thirty patients with twin pregnancy and perinatal loss of one or both fetuses were included. Thirteen (43.3%) twins were monochorionic (MC), 17 (56.7%) – dichorionic (DC). At 11–14 week of gestation chorionicity was diagnosed by ultrasound; transvaginal measurement of cervix was performed at 19–21 week; biometry was done to identify degree of fetus' discordance.

Results: Complications of DC pregnancy: discordant fetal growth – 17 (100%), fetal growth restriction – 7 (41.2%), cervical insufficiency – 4 (23.5%). Discordant fetal growth was diagnosed in 17 DC twins: 8 (47.1%) – ≤20%, 9 (52.9%) – >20%. 8 (47.1%) patients with discordance >25% had highest degree of fetal growth restriction (estimated fetal weight <5‰).

Perinatal loss in patients with DC twins was 61.8% (21 of 34 children). Highest mortality [10 of 21 (47.7%)] was among newborns at 22–27 week of gestation with DC type of placentation: 7 – intrauterine death, 3 died postnatally. Seventeen cases of intrauterine death were diagnosed: 7 (41.2%) – 22–27 weeks, 3 (17.6%) – 28–31 weeks, 5 (29.4%) – 35–36 weeks, 2 (11.8%) – at term.

Complications of MC pregnancy: discordant fetal growth – 13 (100%), twin-to-twin transfusion syndrome (TTTS) – 11 (84.6%), fetal growth restriction – 9 (69.2%), cervical insufficiency – 4 (30.8%). Discordant fetal growth was diagnosed in 13 MC twins: 7 (53.8%) – ≤20%, 6 (46.2%) – >20%. Four (30.8%) patients with discordance >25% had selective fetal growth restriction.

Perinatal loss in patients with MC twins was 80.8% (21 of 26 children). Highest mortality [13 of 21 (61.9%)] was among newborns at 22–27 week of gestation: all of them died antenatally. Nineteen cases of intrauterine death were diagnosed: 13 (68.4%) – 22–27 weeks, 4 (21.0%) – 28–31 weeks, 1 (5.3%) – 35–36 weeks, 1 (5.3%) – at term.

Conclusion: There were 1.3 times more perinatal losses in MC twins than in DC twins (80.8% vs. 61.8%). Regardless of chorionicity, perinatal losses were observed more frequently at 22-27 weeks of gestation: DC (47.7%) and MC (61.9%) twins. Causes of perinatal loss in DC twins: prematurity – 52.9%, discordant fetal growth (>20%) – 52.9%, fetal growth restriction – 41.2%. Causes of perinatal loss in MC twins: TTTS – 84.6%, prematurity – 76.9%, fetal growth restriction – 69.2%, discordant fetal growth (>20%) – 46.2%.

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PS230

Hyaluronic acid solution as a treatment of adhesive intestinal obstruction in children – A positive effect

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Aim: To explore the possibility of using hyaluronic acid solution (HAS) for the treatment of intraperitoneal adhesions in children.

Introduction: Adhesive intestinal obstruction (AIO) has been found to be a challenging problem of abdominal surgery with increased occurrence in children worldwide. Intraperitoneal adhesions occur commonly after abdominal surgery and frequently cause intestinal obstruction. Current means of adhesion prevention includes good surgical technique and anti-adhesion barriers. This study is hence directed towards the effect of hyaluronic acid solution (HAS) in reducing the incidence and recurrence of adhesions.

Methods: 84 children were operated on for AIO. 21 children (25%) were operated on for early adhesive intestinal obstruction (EAIO), 63 (75%) – on late adhesive intestinal obstruction (LAIO) and 12 (14.29%) for recurrent AIO. Following surgery, these children were divided into two groups; group I (56 patients) and group II (28 patients). The Hyaluronic Acid Solution; Defensal was used. Follow-up on the children took place from 1 to 4 years.

Results: 13 children (23.21%) in group I were found to have adhesion syndrome in the first year after surgery. This increased to 20 (35.71%) patients over the 4 year period. Children in the II group who had undergone treatment for adhesion syndrome (cured conservatively using HAS) over a 2.5 year postoperative period were not found to have adhesive syndrome at the end of the follow-up period with the exception of 2 (7.14%) patients. When compared to group I patients who had no treatment by the HAS, group II patients showed a higher degree of recovery with minimal recurrence.

Conclusion: Although accompanied by a minimal recurrence rate, HAS shows effectiveness as a treatment for adhesive intestinal obstruction in children. This serves as a step further towards a complete prevention of postoperative adhesion common in children.[1–11]

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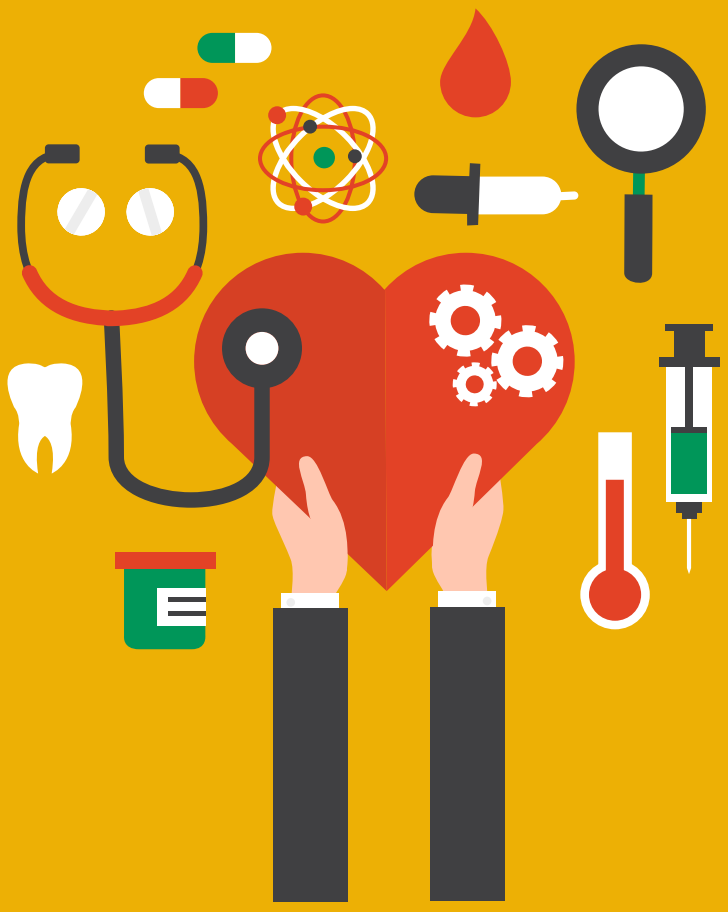

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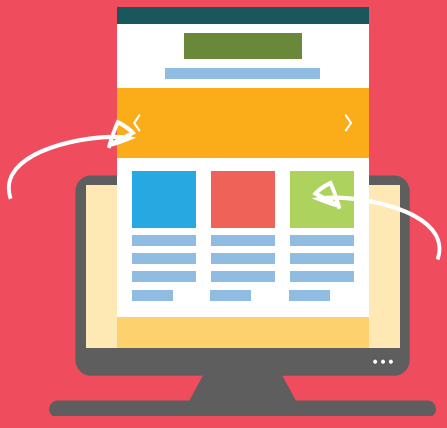

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