Dubrovnik Summer School on Molecular Biosciences in Medicine
with the
International Oxidative Stress Symposium

Dubrovnik, September 11-15, 2023

SVEUČILIŠTE U DUBROVNIKU
UNIVERSITY OF DUBROVNIK

THE BOOK OF ABSTRACTS

Doktorski studij
Molekularne bioznanosti

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Dubrovnik Summer School on Molecular Biosciences in Medicine with the International Oxidative Stress Symposium

University of Dubrovnik, September 11-15, 2023

The Final Program

Monday	Tuesday	Wednesday	Thursday	Friday
10:00 Welcome address Introduction to molecular aspects of oxidative stress in medicine (NZ*)	10:00 Lipid geometrical isomerism: from chemistry to biology and diagnostics (CC)	10:00 Uncovering proteotypic signatures in disease by proteomics (UR)	10:00 Evaluation of oxidative stress in a clinical setting (UR)	10:00 **International Symposium on Oxidative Stress** Conversion of proteins into DNA mimetics by lysine N-pyrrolation (KU)
Oxidative stress and the role of biomimetic radical chemistry in discovery of biomarkers (CC)	Oxidized LDL in atherosclerosis: An update (ANS)	Utilizing biological databases to identify potentially disease-driving PTMs on proteins (UR)	Oncogenetic mechanisms and energetic metabolism in malignant mesothelioma (SS)	Post-translational modifications of placental endothelial nitric oxide synthase (eNOS) in preeclampsia (ANS)
11:45 Coffee break - POSTERS	11:45 Coffee break- POSTERS	11:45 Coffee break- POSTERS	11:45 Coffee break- POSTERS	11:45 Coffee break
12:00 Introduction to Lipidomics (CF)	12:00 Crispr/Cas9 mediated targeted deletions to uncover etiologic disease-causing molecular switches (UR)	12:00 Purine lesions in DNA damage: chemical, analytical, biological and diagnostic significance (CC)	12:00 Molecular background of BPC 157 action (SS)	12:00 Essential trace element selenium and redox regulation: its metabolism, physiological function, and related diseases (YS)
13:00 Lunch	13:00 Lunch	13:00 Lunch	13:00 Lunch	13:00 Lunch
16:00 Lipidomics and Human Health: The impact of membrane lipidome in the inflammatory scenario; case studies (CF)	16:00 Lipid peroxidation in spontaneous regression of cancer (NZ)	16:00 Lipid peroxidation and skin photoaging (ANS)	16:00 Neoangiogenesis in atherosclerotic lesions: role of oxidized LDL and lipid peroxidation (ANS)	16:00 Structural library and visualization of endogenously oxidized lipids (KY)
17:00 POSTERS	Lipidomics and Human Health: membrane lipidomics in oncology (CF)	17:00 POSTERS	Lipidomics and Human Health: Quality of fats in overweight-obesity progression (CF)	Lipid peroxidation, vascular and systemic oxidative stress in aggressive COVID-19 (NZ)
Selected Oral Presentations	17:30 Poster session	Selected Oral Presentations	17:30 Poster session	Closing remarks
Welcome Dinner 20:00-22:30	SEMINAR: Valorization of fatty acid-based membrane lipidomics as a spin-off company: a successful story (CC)	Concert at the Sponza Palace 20:00-21:00	SEMINAR: How not to screw-up material for histological, immunohistochemical and molecular analysis (SS)	Farewell dinner 20:00-23:00

* NZ - Neven Zarkovic, Croatia
CC - Chryssostomos Chatgilialoglu, Italy & Poland
CF - Carla Ferreri, Italy
ANS - Anne Nègre-Salvayre, France
UR - Ulrike Resch, Austria
SS – Sven Seiwerth, Croatia
KU – Koji Uchida, Japan
YS – Yoshiro Saito, Japan
KY – Ken-ichi Yamada, Japan

REGISTRATION: Sunday 10th 17:30-19:00 and Monday 11th 8:30-10:00

LECTURES

Introduction to molecular aspects of oxidative stress in medicine

Neven Žarković, Rudjer Boskovic Institute, Laboratory for Oxidative Stress, Bijenička cesta 54, 10000 Zagreb, Croatia.

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Abstract:
Oxidative stress implies excessive production of reactive oxygen species (ROS), notably of oxygen free radicals, which overcome the antioxidant defense mechanisms. Similar to many other pathophysiological processes, oxidative stress can be either acute or chronic, harmful causing irreversible damage to the bioactive macromolecules or physiological in case of energetic metabolism, production of some signaling molecule, regulation of growth or metabolic pathways or even as essential component of the organism's defense through inflammation.

It is commonly believed that the border between desirable and dangerous oxidative stress is crossed during lipid peroxidation (LPO). However, in case of polyunsaturated fatty acids, LPO results in production of reactive aldehydes, which are not ROS, but are considered to act as "second messengers of ROS". Among these, the most convenient representative, due to its bioactivities and biomedical relevance, could be 4-hydroxynonenal (HNE), which has strong affinity to bind to proteins thus changing their structure and function. Consequently HNE can regulate bioactivities of proteins as well as cellular processes, in a concentration and the cell-type dependent manner.

Some options of monitoring oxidative stress, in particular HNE, and their relevance for pathophysiological processes and major human diseases will be briefly presented.

References:

1. Neven Žarković (2003) 4-Hydroxynonenal as a bioactive marker of pathopysiological processes. Mol Asp Med, 24:281-291

2. Anne Negre-Salvayre, Nathalie Auge, Victoria Ayala, Huveyda Basaga, Jordi Boada, Rainer Brenke, Sarah Chapple, Guy Cohen, Janos Feher, Tilman Grune, Gabriella Lengyel, Giovanni E. Mann, Reinald Pamplona, Giuseppe Poli, Manuel Portero-Otin, Yael Riahi, Robert Salvayre, Shlomo Sasson, Jose Serrano, Ofer Shamni, Werner Siems, Richard C. M. Siow, Ingrid Wiswedel, Kamelija Žarković, Neven Žarković (2010) Pathological aspects of lipid peroxidation. Free Radical Research, 44:1125-1171

3. Kamelija Zarkovic, Antonia Jakovcevic, Neven Zarkovic (2017) Contribution of the HNE-Immunohistochemistry to Modern Pathological Concepts of Major Human Diseases. Free Radic Biol Med, 111: 110-125

Title: Oxidative stress and the role of biomimetic radical chemistry in the discovery of biomarkers

Author: Chryssostomos Chatgilialoglu[1,2]

Institutions: [1]Istituto per la Sintesi Organica e la Fotoreattività, Consiglio Nazionale delle Ricerche, Bologna, Italy; [2]Center for Advanced Technologies, Adam Mickiewicz University, Poznan, Poland

Abstract: Free radicals are generated in the biological environment as a result of normal intracellular metabolism. Reactive oxygen and nitrogen species (ROS/RNS) function as physiological signaling molecules that participate in the modulation of apoptosis, stress responses and proliferation. ROS/RNS can also have a negative effect by causing damages to biomolecules. Therefore, the estimation of the type and extent of damages, as well as the efficiency of the protective and repair systems, are important subjects in life sciences. When studying free radical-based chemical mechanisms, it is very important to establish biomimetic models, which allow the experiments to be performed in a simplified environment, but suitably designed to be in strict connection with cellular conditions. The biomimetic modelling approach has been coupled with physical organic chemistry methodologies and knowledge of free radical reactivity, in order to gather substantial insights of biological processes relevant to health, such as biological damages and repair, signaling and biomarkers, biotechnological applications and novel synthetic approaches.
In particular, free radical processes such as lipid geometrical isomerization in cell membranes, cyclopurines formation in nuclear and mitochondria DNA, and protein desulfurization have been studied in our laboratory, finalizing this research toward biomarker discovery. In the first part of this lecture, I will present an overview of reactive oxygen and nitrogen species (ROS/RNS) with main emphasis to the presence of free radicals and their role in various biological conditions. In the second part, I will give the background of some important reactions of radicals with lipids, proteins and DNA by using biomimetic models based on liposomes, amino acids and oligonucleotides.

Title:

Introduction to Lipidomics

Author(s): **Name Surname**[1]
Carla Ferreri

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

Consiglio Nazionale delle Ricerche, ISOF – Bologna CNR Research Area, Italy

Abstract:

In this lecture an overview of the field of Lipidomics will be given starting from the the different classes of lipids and their distribution in the human body, together with their main structures and functions. Lipids as macronutrients, together with proteins and carbohydrates, are present in human nutrition and their "dynamics" from food to cells are the main focus of the lipidomic approach targeted to cell membranes. Membrane lipidomics and the development of a molecular medicine approach will be explained. The outline of the lecture is:

- Importance of Lipids for the cell structures and functions
- The role of fatty acid diversity: focus on cell membranes
- Membrane Lipidomics: the dynamics of adaptation and remodeling
- Membrane Lipidomic Profile: analytical and functional insights

Title:

Lipidomics and Human Health: The impact of membrane lipidome in the inflammatory scenario; case studies

Author(s): **Name Surname[1]**
Carla Ferreri

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

Consiglio Nazionale delle Ricerche, ISOF – Bologna CNR Research Area, Italy

Abstract:

In this lecture we enter in the scenario of the human metabolism considering inflammation as a common denominator for many cellular processes. The event of inflammation will be explained by the membrane lipidomic approach, based on the knowledge of molecular processes which link metabolism and nutrition with health status. The role of membrane asset for inflammatory responses will be deepened in skin responses, with case studies in human cohorts and in single patients. The protocol of a membrane lipid intervention and the follow-up of membrane response will be presented.

The outline of the lecture is:

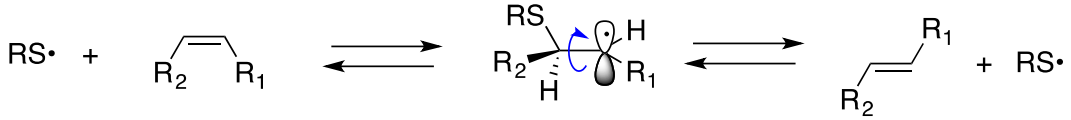
- Membrane Lipidomics to understand inflammation and the natural control of pro- & anti-inflammatory signals
- Nutritional-based unbalances affecting inflammation: skin status, parenteral nutrition, & others
- A protocol for membrane lipid therapy

Title: Lipid geometrical isomerism: from chemistry to biology and diagnostics

Author: Chryssostomos Chatgilialoglu[1,2]

Institutions: [1]Istituto per la Sintesi Organica e la Fotoreattività, Consiglio Nazionale delle Ricerche, Bologna, Italy; [2]Center for Advanced Technologies, Adam Mickiewicz University, Poznan, Poland

Abstract: Unsaturated fatty acid moieties of various classes of lipids present the stereochemical requirement of cis geometry at the carbon-carbon double bond. Cis lipid geometry is essential for life, ensuring membrane fluidity and properties. The importance of lipid geometrical isomerism and the formation of unnatural trans geometry have a great applicability to very different fields, including biological, biotechnological, and medical areas. This subject originated from the merging of lipid and free radical research and fostered the development of biomimetic radical chemistry, to provide models for conducting mechanistic and product studies, in conditions strictly related to the complexity of the biological systems. During the last two decades, we investigated in details the cis–trans isomerization of double bonds catalyzed by thiyl radicals by means of product distribution, tailored mechanistic studies, kinetics and theoretical approaches. Double-bond isomerization occurs via the addition–rotation–fragmentation cycle reported in the following Scheme.



Scheme. Mechanism of thiyl radical catalyzed cis-trans double bond isomerization of unsaturated fatty acid moieties in lipids depicted as a consecutive addition-elimination process.

We applied the free radical-catalyzed cis–trans isomerization of unsaturated lipids under biomimetic conditions, leading to the identification of such process in the cellular environment. We also studied models of tandem protein–lipid damage and discovered that sulfur-containing proteins can undergo a chemical mutation under radical stress producing diffusible thiyl radicals thus causing cis–trans isomerization in unsaturated lipid vesicles. We extended our studies to the reaction of thiyl radicals with a variety of polyunsaturated fatty acids (PUFAs). By a suitable geometrical trans fatty acid library new insights have been acquired, including (a) the mono-trans isomers of arachidonic acid as biomarkers for the endogenous origin of trans lipids in cells, animals, and humans, and (b) the mono-trans isomers of the PUFA omega-3 with important applications in lipid metabolism and nutraceutical industry.
The lecture will provide an overview of these topics embracing life sciences, from organic chemistry to biological and medical applications.

Title:

Oxidized LDL in atherosclerosis

Author(s): **Name Surname[1]**

Anne NEGRE-SALVAYRE

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

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Abstract:

Atherosclerosis is a chronic and inflammatory disease of medium and large arteries, and a main cause of cardiovascular morbidity and mortality worldwide. Its pathogenesis involves a number of risk factors including hypercholesterolemia, endothelial dysfunction, and vascular inflammation, with a main implication of reactive oxygen species (ROS) produced by vascular cells. The oxidative theory of atherosclerosis relies on the modification of low density lipoproteins (LDL) by ROS, an early event in atherogenesis. Endothelial dysfunction promotes an increased permeability to LDL which accumulate on the extracellular matrix in the intima of lesion-prone areas , and undergo oxidative modifications. Oxidized LDL are thought to play a key-role in the formation of foam cells and fatty streaks, the early atherosclerotic lesions. Oxidized LDL exhibit a large panel of biological properties which may contribute to the development of more advanced lesions, depending on the extent of lipid peroxidation, the nature of oxidized lipids (oxidized phospholipids, oxysterols, malondialdehyde, α,β-unsaturated hydroxyalkenals), their local concentration and uptake by vascular cells and macrophages via scavenger receptors (e.g. CD36, LOX-1, SRA) that signal through different transduction pathways. Low concentrations of oxidized LDLs are proinflammatory and mitogenic whereas higher concentrations are proapoptotic, which theoretically may contribute to plaque growth, instability, complication (intraplaque hemorrhage, proteolysis, calcification, apoptosis) and rupture. The role of oxidized LDL in atherogenesis is consensual, whereas their implication in advanced lesions and atherothrombotic events is more debated, in part because antioxidant supplementation failed to prevent coronary disease events and mortality in intervention randomized trials.

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

- Förstermann U et al. Circ Res. 2017;120(4):713-735.
- Nègre-Salvayre A et al. Free Radic Biol Med. 2017, 106:118-133.
- Negre-Salvayre A et al. Free Radic Biol Med. 2020;149:8-22.

Crispr/Cas9 mediated targeted deletions to uncover etiologic disease-causing molecular switches

Ulrike Resch[1], Huriye Ercan[1], Bernhard Moser[1], Judit Mihaly-Bison[1], Goran Mitulovic[1], Maria Zellner[1], Margarethe Geiger[1],

[1] Medical University of Vienna, Department of Vascular Biology and Thrombosis Research, Schwarzspanierstrasse 17/I, A-1090 Vienna, Austria

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Abstract:
The dynamic regulation of a balanced, functional proteome, termed proteostasis, is vital in protein biogenesis, folding, function and trafficking. Proteostasis is achieved via the dynamic and complex interplay between proteases and their inhibitors. In earlier studies we found a reduced protein abundance of Serpin A5 (also known as protein C inhibitor, PCI) in prostate cancer cells (DU145) as compared to non-malignant prostate cells (RWPE). In contrast, protein levels of the cysteine-protease Cathepsin L (CTSL) displayed the opposite abundance pattern. This suggested that CTSL and PCI counter regulate each other. CTSL is a family member of lysosomal located proteinases capable of processing and/or degrading a variety of proteins important in autophagy, immune response and in secretory pathways. Abnormal activities of CTSL have been observed in several pathologies including arthritis, cardiovascular diseases and tumorigenesis. Previous research showed that CTSL acts at several key molecular switches. For example, in the nucleus it regulates cell cycle progression of cancer cells, in the cytosol it regulates metabolic networks. In the extracellular space, its activities shape a cells´ matricellular secretome and consequently the extracellular matrix.

To gain more insight into the prostate-cell type specific function of CTSL, we employed Crispr/Cas9 methodology to delete CTSL protein expression, performed biochemical analysis and proteome abundance profiling by mass spectrometry. With this seminar I aim to share and discuss “expectations” and “reality” when planning and performing such a research project. We will also discuss different ways of data analysis and interpretation and consequently its multifaceted outcomes.

Lipid peroxidation in spontaneous regression of cancer

Neven Žarković, Rudjer Boskovic Institute, Laboratory for Oxidative Stress, Bijenička cesta 54, 10000 Zagreb, Croatia.

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Abstract:
Defined as disease of uncontrolled, destructive and persistent proliferation of transformed cells, cancer is generally considered also as disease that is hardly curable, except if diagnosed early enough to allow complete removal and destruction of the transformed cells. In spite of that, there are numerous well documented cases of spontaneous partial or complete regression of cancer recorded for decades and their number is increasing including almost any type of malignant neoplasm. Majority of such cancer regression cases include pathophysiological processes associated with oxidative stress, such as inflammation.

Carcinogenesis is a long lasting and complex process often associated with oxidative stress. Therefore, cancer is also assumed to be example of persistent oxidative stress. However, majority of anti-cancer therapies rely on oxidative stress, assuming that cancer cells are more sensitive than their counterpart normal cells to cytotoxic effects of the mediators of oxidative stress, especially reactive oxygen species (ROS).

The efforts trying to explain these paradoxes brough in focus of research peroxidation of polyunsaturated fatty acids generating reactive aldehydes, notably 4-hydroxynonenal (HNE), which can act as the second messenger of free radicals, signaling molecule and the growth regulatory factor.

Hypothesis that HNE and granulocyte-mediated inflammation may be crucial factors of spontaneous regression of cancer will be presented.

References:
1. Žarković, N., Jaganjac, M., Žarković, K., Gęgotek, A., Skrzydlewska, E. (2022) Spontaneous Regression of Cancer: Revealing Granulocytes and Oxidative Stress as the Crucial Double-edge Sword. Frontiers in Bioscience – Landmark. 27(4),119. https://doi.org/10.31083/j.fbl2704119

2. Ana Cipak-Gasparovic; Lidija Milkovic; Suzana Borovic-Sunjic; Neven Zarkovic (2017) Cancer Growth Regulation by 4-Hydroxynonenal Article Type. Free Radic Biol Med, 111: 226-234.

3. Morana Jaganjac, Marija Poljak-Blazi, Kamelija Zarkovic, Rudolf Joerg Schaur, Suzana Borovic, Tanja Matijevic, Ana Cipak, Marina Cindric, Koji Uchida, Georg Waeg, Neven Zarkovic (2012) Elevated neutrophil elastase and acrolein-protein adducts are associated with W256 regression. Clinical & Experimental Immunology, 170:178-185

Title:

Lipidomics and Human Health: membrane lipidomics in oncology

Author(s): **Name Surname[1]**
Carla Ferreri

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

Consiglio Nazionale delle Ricerche, ISOF – Bologna CNR Research Area, Italy

Abstract:

In this lecture we explain the role of membrane lipid asset and lipidomic profiles in the onset and invasiveness of tumoral events. Tumoral growth needs cell replication, hence formation of cell membranes, for sustaining proliferation; also, the signaling of bioactive lipids generated from polyunsaturated fatty acids present in membranes is involved in tumorigenesis and cancer invasiveness. In all these aspects, lipids are considered strategical elements and biomarkers have been individuated for the follow-up of cancer patients.

The outline of the lecture is:

- Membranes and cancer: the need of phospholipids for cell replication and invasion
- Membrane biomarkers of inflammation & cancer proliferation
- Membrane lipidomics in cancer patients

Uncovering proteotypic signatures in disease by proteomics

Ulrike Resch

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Abstract:

Proteome analysis of body fluids, tissues and cells in a clinical setting gained increasing popularity during the past decade. It has been recognized that the relative static DNA/RNA sequence information often does not translate into the complexity of an organism´s phenotype, instead, this is achieved by the complex interplay and networking capabilities on all proteins in an organism. Disease states manifest due to over-or under-compensated mechanisms aimed to re-establish hemostasis. Identification of disease-specific proteotypic signatures has therefore become the joint enterprise in clinical research. In this seminar I will present two clinical proteomic studies, i) A comparative proteomic profiling of human heart tissue of over 100 patients undergoing heart transplantation and ii) Characterization of plasma microparticles (PMPs) lipid-and protein composition isolated from liver cancer patients before and after partial hepatectomy.

We will discuss the general problem of defining “healthy” versus “diseased” in such studies, the critical importance of study design, patient stratification and analysis workflows and practical considerations during planning and execution processes of such projects.

Utilizing biological databases to identify potentially disease-driving PTMs on proteins

Ulrike Resch

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Correspondence: ulrike.resch@meduniwien.ac.at

Abstract:
Proteins are the essential effectors of genes, and play major roles in all life processes at all organizational levels (cells, tissues, and organs) and thus are also involved in aetiology of diseases. Causal events can be abnormally altered protein levels due to single nucleotide polymorphism (SNP), differential splicing, infection, environmentally altered somatic mutations/post-translational modifications (PTM), or a mixture of all these parameters. PTMs occur on "vulnerable" amino acids (i.e. Lysine, Arginine, Histidine, Serine, Threonine, Tyrosin, Cystein) and result in covalent modification such as acetylation, glycosylation, nitrosylation, phosphorylation, palmitoylation or ubiquitination. The analytical accessibility of such PTMs in frequently used shotgun-proteomics approaches is extremely limited and to date, only the relative "conservative" 2D-DIGE methodology can give information on such proteoforms. This seminar is aimed to show the different kind of information on PTMs deposited on biological databases and I will give practical examples how comparative analysis of proteomes by shotgun and 2D-DIGE/Top-down proteomics will aid the identification biologically relevant PTM and proteomes.

Title: Purine lesions in DNA damage: chemical, analytical, biological and diagnostic significance

Author: Chryssostomos Chatgilialoglu[1,2]

Institutions: [1]Istituto per la Sintesi Organica e la Fotoreattività, Consiglio Nazionale delle Ricerche, Bologna, Italy; [2]Center for Advanced Technologies, Adam Mickiewicz University, Poznan, Poland

Abstract: 5′,8-Cyclopurine-2′-deoxynucleosides (cPu) are solely generated by the attack of HO• radicals on purine moiety *via* C5′-radical chemistry resulting in the formation of an additional C5′−C8 covalent bond; the structures of the four cPu are shown in the Figure. cPu can be removed only by the nucleotide excision repair (NER) pathway and different repair efficiency of the *R* and *S* diastereoisomers has been detected. On the other hand, the well-known 8-oxo-purines (8-oxo-Pu) lesions (see Figure for the structures), derive from the oxidation at the C8 position of adenine and guanine by a variety of reactive oxygen species (ROS), and can be repaired by the base excision repair (BER).

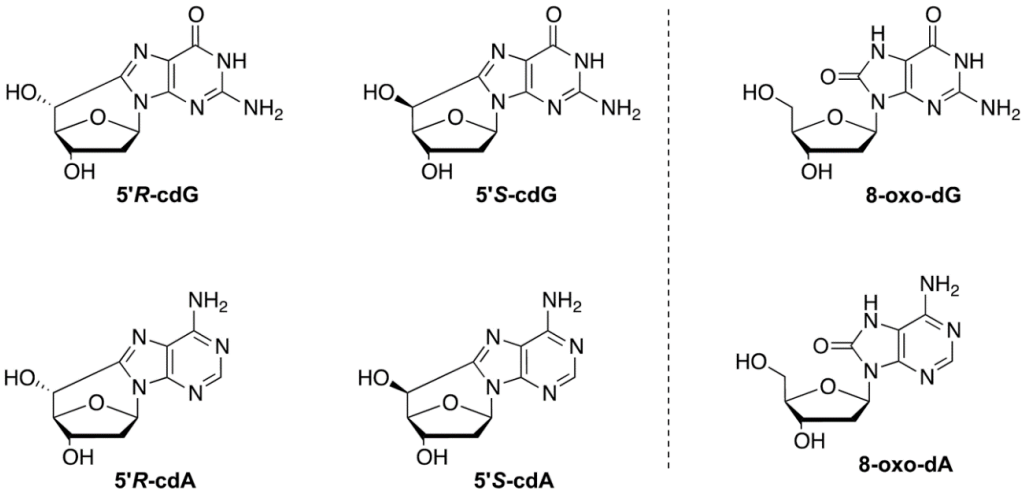

Figure. Structures of 5′,8-cyclo-2′-deoxyguanosine (cdG) and 5′,8-cyclo-2′-deoxyadenosine (cdA) in their 5′*R* and 5′*S* diastereomeric forms (left) and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) and 8-oxo-7,8-dihydro-2′- deoxyadenosine (8-oxo-dA) (right).

In this lecture we will discuss our most recent results on these purine lesions: (i) Radical-based synthetic strategies and Mechanistic insights; (ii) Analytical protocol based on LC-MS/MS method for quantification of these lesions in DNA; (iii) Comparison of analytical method efficiencies using irradiated samples of calf thymus DNA; (iv) Detection of purine lesions in mammalian cell cultures, human fluids and animal tissues; (v) Recognition of DNA damage by repair enzymes and mutagenic potential.

Title:

Lipid peroxidation and skin photoaging

Author(s): **Name Surname[1]**

Anne NEGRE-SALVAYRE

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

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Abstract:

Aging is a multifactorial process characterized by metabolic, functional, and esthetic changes resulting from the interaction of both genetic and environmental factors. Photoaging is an accelerated form of ageing affecting specifically the skin exposed to solar ultraviolet (UV) radiations. Clinically, photoaging is characterized by the progressive formation of deep wrinkles, a loss of skin tone, skin dryness, elastic tissue clumping, pigmentary alteration, and exaggerated bruising. Most clinical features result from UV-induced changes in the dermis, the predominant target of UV radiation. UV(A) radiations deeply penetrate the dermis and generate reactive oxygen species (ROS) which promote damage to DNA, lipids and proteins. The oxidative attack of polyunsaturated fatty acids generate lipid peroxidation products particularly reactive carbonyl species (RCS) such as α, β-unsaturated hydroxyalkenals (4-hydroxynonenal or HNE, acrolein or malondialdehyde or MDA). A main characteristic of these RCS is the formation and accumulation of adducts on free NH2 groups and thiol residues on amino acids in proteins, leading to carbonyl stress, a source of cellular and tissular dysfunction, inflammation, toxicity and senescence. RCS-adducts are detected in the dermis of skins exposed to UV-A radiations. Several RCS-targets have been identified in the extracellular matrix such as collagen and elastin, which could contribute to actinic elastosis lesions, a characteristic feature of photoaging. RCS-adducts accumulate in dermis fibroblasts,by forming adducts on histones, the sirtuin SIRT1, (leading to an accumulation of acetylated proteins), or the cytoskeleton protein vimentin. These modifications increase the expression of cellular senescence markers such as Histone γ-H2AX, a marker of DNA damages, and the activation of the senescence-associated beta galactosidase activity (SA-β-GAL), thereby promoting a senescence-associated phenotype of dermis fibroblasts. Photoaging lesions could be partly protected by the topical application of carbonyl scavengers such as carnosine, which neutralizes the formation and accumulation of RCS-adducts in the dermis. A better identification of carbonyl stress-targets in the skin may help to develop new therapeutic approaches for preventing photoaging.

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

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Swiader A, et al. Antioxidants (Basel). 2021;10(3):365.
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Evaluation of oxidative stress in a clinical setting

Ulrike Resch[1], Lore Schrutka[2], Konstantin Krychtiuk[2], Walter Speidl[2], Klaus Distelmaier, Maria Zellner[1], Willibald Wonisch[3], Franz Tatzber[3]

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[3] Department of Internal Medicine II, Division Cardiology, Medical University of Vienna, Währingerguertel 18-20, A1090 Vienna, Austria

[3] Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation, Chair of Immunology and Pathophysiology, Medical University of Graz, Graz, Austria.

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Abstract:

Oxidative stress (OS) results from an imbalance between formation and neutralization of reactive oxygen species (ROS). Excessive ROS cause damage to macromolecules such as proteins, lipids and DNA, perpetuating chronic inflammation with heterogenous pathophysiologic outcomes including cancer, autoimmune disorders and coronary vascular disease (CVD). Lp(a) is a circulating lipid-protein particle closely related to LDL, yet shown to constitute an independent risk factor for developing CVD. Among the independent factors carried by Lp(a) are not only its reportedly high levels of oxidized phospholipids, but also the fact that Lp(a) is endowed with an additional apolipoprotein (apo(a)) and a genetically determined variable number of kringle IV repeats resulting in numerous apo(a)-isoforms. This structural feature is highly similar to plasminogen, however, kringle IV in Lp(a) competes with plasminogen for binding to endothelial cells during fibrinolysis, thereby promoting atherogenic conditions such as increased lipid deposition in the vascular wall, increased ROS and consequently lipid-protein oxidation along with the formation of immunogenic OS neoepitopes (OSE). To date, therapeutic interventions reducing elevated Lp(a) levels are limited, as HMG-CoA reductase inhibitors (statins) inhibitors and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibtors, albeit efficiently reducing LDL-C, are much efficient in reducing circulating Lp(a) levels. **Research questions and methods:** Clinical studies investigating OS and OSE in subjects with atherogenic Lp(a) conditions are scarce due to a relatively low incidence/knowledge of elevated Lp(a) (levels >50mg/dl) and insufficient determination of apo(a)-isoforms as it were kringle IV repeats. In this study we evaluated the oxidative stress index (OSI) in a patient cohort displaying a broad range of elevated Lp(a) levels. OS and OSE were assessed by means of determination of total antioxidant capacity (TAC), resembling mainly small-molecule and antioxidant enzymes and total oxidant capacity (TOC), endogenous peroxide activities (EPA) as well as polyphenols (PPm) as a measure of plant and microbiota derived secondary, metabolites with radical scavenging properties. Concomitantly we evaluated immunogenic OSE through determination of IgG and IgM autoantibodies to oxidatively modified LDL (MDA and copper-oxidized), Lp(a) and oxidized Lp(a), native and oxidized Fibrinogen. **Results:** We found that PCSK9 inhibitors have a low effect on OS, but the distinctly elevated oxidized-to-native Lp(a) ratio in patients with high Lp(a) was modulated upon PCSK9 inhibitor intake.

Oncogenetic mechanisms and energetic metabolism in malignant mesothelioma

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Abstract:
Diffuse pleural mesothelioma (DPM) originates from pleural mesothelial cells. It is behavior. So far whole genome sequencing identified a subset of genes that were most frequently afected in mesothelioma: BAP1, NF2, CDKN2A. More advanced techniques also discovered minute deletions resulting in biallelic inactivations of SETD2, BAP1,PBRM1 and SMARCC1. There are also ongoing studies of epigenetic mechanism demonstrating that high expression of epigenetic modifiers (e.g. EZH2 and SUZ12) is significantly correlated with poor survival of individuals with DPM. Results of epigenetic studies suggest not only the importance of epigenetic regulation in mesothelioma biology, but also highlight the potential utility of epigenetic-based therapies such as EZH2 inhibitors. Epigenetic regulation seems also to be involved in tumor heterogeneity characterised by resistance to various types of therapy and poor survival. In spite of recent advances in understanding genetic identity of human mesothelioma, it remains unclear which molecular mechanisms eventually drive its oncogenesis and regulate its malignant behaviour. The shift from oxidative to glycolytic metabolism includes the activation of phosphoinositide 3-kinase and serine-threonine kinases (signalling pathway, phosphatidylinositol 3-kinase, serine-threonine kinase, PI3K/AKT), which is one of the most common changes in cancer cells in general, and DPM is no exception. In this we studied whether pharmacological modification of mitochondrial energy metabolism together with high glucose concentrations could potentially be used for a more successful treatment of malignant mesothelioma. The obtained results showed that all three tested mitochondrial energy metabolism modulators, UK- 5099, DNP and DCA, when used in higher concentrations, have a similar pattern of action; potentiating the cytotoxic effect of cisplatin and pemetrexed. This suggests a synergistic effect of antineoplastic agents and mitochondrial metabolism modulators on reducing mesothelioma cell proliferation. The expression of pluripotency factors is a key regulator of tumor differentiation status and cancer stem cells. We tested the expression of pluripotency factors OCT4/POU5F1, NANOG, SOX2, PI3K-AKT pathway and BCL2 genes in mesothelioma and normal mesothelium. Mitochondrial membrane potential, reactive oxygen species (ROS) generation and expression of pluripotency factors were also tested in human mesothelioma cell line. Human mesothelioma displays enhanced expression of NANOG, SOX2 and phosphorylated AKT proteins, while elevated NANOG expression correlates with poor differentiation of human mesothelioma. Mitochondria of mesothelioma cells have a large capacity to form ROS and thereby upregulate NANOG, leading to dedifferentiation of mesothelioma.

References:

1. Sedlić F. et al. Mitochondrial ROS Induce Partial Dedifferentiation of Human Mesothelioma via Upregulation of NANOG. Antioxidants. 2020, 9, 606.

Molecular background of BPC 157 action

Sven Seiwerth M.D. Ph.D. , Predrag Sikirić M.D., PhD[2], Sunčana Sikirić Ph.D., Marija Milavić Ph.D., University of Zagreb School of Medicine, [1] Institute of Pathology, and [2]Institute of Pharmcology, Zagreb, Croatia

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Abstract:
BPC 157, a synthetic pentdecapeptide equal to peptide naturally occurring and isolated from gastric juice has a wide array of beneficial effects in different animal models of human disease.
Vascular damage including widespread venous occlusion models as well as wound and tissue healing models were among the fields of BPC 157 action most thoroughly studied also on molecular level. The effect of BPC 157 on early stage expression of angiogenesis related genes was analysed in a rat skin-induced wound model. Analysed genes were: *Akt1, Braf, Egfr, Egr1, Grb2, Hdac7, Kras, Mapk1, Mapk3, Mapk14, Nos3, Pik3cd, Plcg1, Prkcg, Ptk2, Pxn, Src, Srf* and *Vegfa.* Our findings suggest that BPC 157 upregulates expression of several genes involved in signalling pathways important for angiogenesis: RAS-RAF-MEK-ERK/MAPK, PI3K-AKT-eNOS-VEGF, FAK-SRC-PXN and SRC-PLCγ-PKC-MEK-ERK. The interaction of BPC 157 with nitric oxide (NO)-system as a whole was studied in a series of very distinctive disturbances models, analyzing more than 80 targets, and based on the effects and mutual interaction of L-NAME-L and L-arginine. The results lead to the conclusion of an important role of BPC 157 in controlling of NO-system functions. This can be effectuated by BPC 157 inducing the release of the NO by its own, counteraction of the adverse effect of NOS-blockade (i.e. hypertension) or NOS-over- stimulation (i.e. hypotension), maintained thrombocytes function and many molecular pathways, controlling vasomotor tone and the activation of Src-Caveolin-1-eNOS pathway. Its function as a stabilizer of cellular junction was studied in the model of leaky gut syndrome. The significant mitigation of leakage was via increasing tight junction protein ZO-1 expression as well as transepithelial resistance increase and the inhibition of of inflammatory mediators (iNOS, IL-6, IFN, and TNF-alpha) mRNA, the expression of HSP 70 and 90 and antioxidant proteins.
In the model of tumor-induced cachexia BPC 157 induced the inhibition of catabolic pathways (IL-6, TNF-alpha)balanced with stimulation of anabolic pathways (FoxO3a, p-AKT, p-mTOR, and P-GSK-3β).
A model of radiation-induced liver damage demonstrated thet BPC 157 protective action was mediated trough KLF4.
In addition to molecular pathways studies a very rapid conformational change of protein secondary structure and lipid content was demonstrated by FTIR (Fourier transform infrared spectroscopy) in the vessel wall in different models of damage induced by NSAIDs and corticosteroids, an event consistent with the rapid-onset response envisaged in both – Robert's cytoprotection concept and Selye's stress concept.

References:

1. Daniel Gwyer et.al.; Gastric pentadecapeptide body protection compound BPC 157 and its role in accelerating musculoskeletal soft tissue healing. Cell Tissue Res 2019, 377, 153–159.
2. Bing-Shen Huang et al. Pentadecapeptide BPC 157 efficiently reduces radiation-induced liver injury and lipid accumulation through Kruppel-like factor 4 upregulation both in vivo and in vitro. Life Sciences, 2022, 310,121072.

Title:

Neoangiogenesis in atherosclerotic lesions: role of oxidized LDL and lipid peroxidation

Author(s): **Name Surname[1]**

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Abstract:

Atherothrombotic complications are a leading cause of morbidity and mortality in western countries. The neovascularization in atherosclerotic lesions plays an important role in the progression of atherosclerotic plaque and aggravates its vulnerability. Neoangiogenesis may generate intraplaque hemorrhages which are a main cause of plaque instability and rupture, in correlation with thin-cap atheroma, macrophage infiltration and large necrotic cores. The main angiogenic mechanism results from sprouting angiogenesis from vasa vasorum, in response to hypoxia and angiogenic or inflammatory factors secreted in the intima, including the vascular endothelial growth factor (VEGF), the Transforming Growth Factor Beta (TGF-beta), various growth factors, the sphingolipid sphingosine-1-phosphate (S1P), metalloproteases, oxidized LDL and lipids. The neovascularization of atherosclerotic lesions allows to supply nutrients and promote macrophage infiltration, vessel wall thickening, lipid deposition and inflammation leading to plaque progression. However, neoformed capillaries within the plaques are fragile, often leaky, thus may release intraplaque erythrocytes leading to hemorrhages. These events could be associated with the release of erythrocyte fragments, iron deposits, foam cells and cholesterol crystals, which aggravates oxidative stress, inflammation and endothelial cell apoptosis.

Oxidized LDL and lipids such as hydroxynonenal (HNE) exert a biphasic effect on angiogenesis depending on their local concentration, low concentrations being angiogenic whereas higher concentrations are cytotoxic. The angiogenic signaling evoked by these agents involves the production of reactive oxygen species and the production of S1P and VEGF. In vivo, low concentrations of oxLDL stimulate angiogenesis in animal models such as the Matrigel plug assay in mice or the chicken chorioallantoic membrane. High oxidized LDL concentrations are proapoptotic (a possible cause of neocapillaries fragility), while HNE-adducts could be detected in intraplaque hemorrhage area in human lesions. These properties suggest that oxidized LDL and lipids may contribute to intraplaque neoangiogenesis and complications toward intraplaque hemorrhage and instability.

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

Camaré C et al. Free Radic Biol Med. 2016 ; 93:204-16.
Camaré C. Angiogenesis in the atherosclerotic plaque. Redox Biol. 2017 ; 12 :18-34.

Title:

Lipidomics and Human Health: Quality of fats in overweight-obesity progression

Author(s): **Name Surname**[1]
Carla Ferreri

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

Consiglio Nazionale delle Ricerche, ISOF – Bologna CNR Research Area, Italy

Abstract:

In this lecture we focus on the importance of lipidomics and lipidomic profiles in the evaluation of the fat accumulation process. Lipid variations, not only as quantity but also as quality of fatty acids, will be explained with the differences observed in normal-overweight-obese conditions, connecting molecular and metabolic processes in a holistic approach. This approach allows to individuate the successful strategy to favor the reorganization of fat depots and recover the normal weight conditions.

The outline of the lecture is:

- Why quality of fats is important in the fat accumulation
- The revolutionary adipocyte transformation
- The timing of fat accumulation
- Membrane lipidomic profiles

How not to screw-up material for histological, immunohistochemical and molecular analysis?

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Abstract:
Pathology, as the science about diseases, is an analytical discipline devoted to knowledge collection and implementation. Historically Pathology evolved as the „translation“ rooting in basic sciences and serving the clinical needs around the patient.
Un this positon Pathology fulfills its teaching and scholarly role as well as the position of expert consultant.
In the syntagm "*mortui vivos docent*" is well synthetizing both positions in the learning process – the one of the teacher but likely the one of the learner broadening all the time individual and collective knowledge.
First it would be wise to define the term or realm of Pathology, at least for the purpose of this lecture. The term Pathology does not encompass equal elements in all organizational systems and schemes. The most comprehensive is the Anglosaxon system where basically all Laboratory medicine is gathered under the heading Pathology. It includes 12 fields beginning with microbiology and ending with blood banking. Most of European countries have adopted a system where Pathology is covering the fields of tissue and cell analysis, preferably using a visualization device (microscope) but recently also using the tools of molecular biology. In addition in Austria traditionally, Pathology also covers Microbiology.
In order to understand the process of optimizing collaboration and circumventing possible problems with Pathology it is advisable to first approach the different errors which can occur in this field.
There are several ways to approach the matter-none of the comprehensive and flawless.
One possibility is::

1) Errors of the pathologist
2) Errors of Pathology as discipline
3) Errors of the laboratory
4) Errors the pathologist discover during analysis
5) Communication errors

Another one is a complicated four – tier classification:

1. Depending on workflow: Pre-laboratory Laboratory, (Post-laboratory)
2. Impact on patient (or results): near misses, adverse events, sentinel
3. Type of error: misidentification of patient, specimen,
4. Depending on cause: human, environment, equipment; defective rules or procedures

In order to make it more practical a four category system has also been proposed: defective specimen, misidentification, misinterpretation, defective report.
From the above sad it is obvious that a plethora of possible errors is waiting to those walking the path of pathological analysis.
So, what are we going to cover in this lecture?
Histolological analysis, histochemical analysis, imunohistochemistry, different ISH techniques, electron microsopical analysis, molecular techniques analysis, cell/tissue culture, quantitative pathology, data presentation and archiving of results, material, slides and blocks are the structural elements of modern „Histopathology“ work-up. The we will focus on elements not

imanent to the laboratory or pathologist which can adversely influence the final results. Unexpectedly enough the potential „customer“ or „client“ seeking colaboration with a pathologist can negatively influence almost all of the aforementioned steps and processes.
At the beginning it is worthwhile to remember two golden rules spearing the optimistic researcher or eager clinical colleague a lot of problems:

1. Garbage in – garbage out (meaning that the quality of material and data entering the analytical process has paramount influence on the quality of final results)
2. Before entering new avenues, consult your favorite pathologist and afterwards stick to his suggestions.

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1. Monique Freire Santana and Luiz Carlos de Lima Ferreira ; Errors in Surgical Pathology Laboratory; In: Quality control in laboratory; Gaffar Sarwar Zaman ed. on-line version, 2018.

Conversion of proteins into DNA mimetics by lysine *N*-pyrrolation

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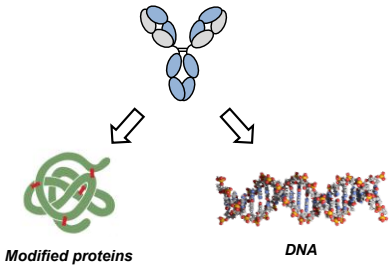
Endogenous reactive species, such as oxidized fatty acids and intermediates of glycolysis, mediate covalent modification of proteins under physiological conditions. The ε-amino group of lysine represents one of the targets of modification, which has a great impact on the chemical properties of proteins and has important functional and regulatory consequences. Lysine *N*-pyrrolation, converting lysine residues to N^{ε}-pyrrole-L-lysine (pyrK), is a recently discovered posttranslational modification. This naturally occurring reaction confers electrochemical properties onto proteins that potentially produce an electrical mimic to DNA and result in specificity toward DNA-binding molecules such as anti-DNA autoantibodies. The discovery of this unique covalent protein modification provides a rationale for establishing the molecular mechanism and broad functional significance of the formation and regulation of pyrK-containing proteins. This presentation summarizes the state of knowledge about the chemistry of this unique conversion reaction of proteins into DNA mimetics by reactive species.



Title:

Post-translational modifications of placental endothelial nitric oxide synthase (eNOS) in preeclampsia

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Abstract:

Preeclampsia (PE) is a multifactorial pregnancy disease, exclusively observed in humans and a leading cause of pregnancy complications worldwide. PE could be suspected after 20 weeks pregnancy, in patients presenting new-onset gestational hypertension with proteinuria or end-organ failure. PE pathophysiology could result from abnormal placentation due to defective trophoblastic invasion and impaired remodeling of uterine spiral arteries, leading to poor adaptation of utero-placental circulation. These events are associated with hypoxia/reoxygenation waves and oxygen gradient fluctuations, decreased antioxidant defence, increased oxidative stress and reduced of nitric oxide (NO) bioavailability. Among the causes of this decreased bioavailability, NO may react with the radical anion superoxide (O2•-), which produces peroxynitrite ONOO-, a powerful pro-oxidant and inflammatory agent. Another cause is that placental endothelial nitric oxide synthase (eNOS) could be inhibited by excessive reactive oxygen species (ROS) production, which leads to eNOS uncoupling, i.e. a switch of its activity from a NO-producing enzyme to a NADPH oxidase-like system generating O2•-. eNOS uncoupling potentiates ROS production and oxidative stress and aggravates the pathophysiology of diseases. Several events may lead to eNOS uncoupling, including a depletion of the eNOS substrate L-arginine due to increased arginase activity, an oxidation of the eNOS cofactor tetrahydrobiopterin (BH4), or eNOS post-translational modifications, for instance by S-glutathionylation. In PE placentas, eNOS is highly S-glutathionylated, suggesting its dysfunction and loss of NO bioavailability. In addition, eNOS could be post-translationally modified by lipid peroxidation-derived aldehydes such as HNE and 4-oxononenal (ONE) a highly bioreactive agent, able to inhibit eNOS activity and NO production. All these data point out the high sensitivity of placental eNOS to ROS, oxidative stress and lipid peroxidation products, and the potential consequences on NO production and PE pathogenesis.

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

Guerby P et al. Redox Biol. 2019;22:101126.
Guerby P et al. Redox Biol. 2021 ;40:101861.

Title:

Essential trace element selenium and redox regulation: its metabolism, physiological function, and related diseases

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Abstract:

The trace element "Selenium" plays a significant role in the antioxidative system *in vivo* (1). Selenium is very reactive and has potent toxicity; however, life utilizes its reactivity for redox reactions. A pathology associated with selenium deficiency was identified as Keshan disease with severe cardiomyopathy. The increased pathogenicity of coxsackievirus associated with selenium deficiency is deeply involved in the development of cardiomyopathy, suggesting the importance of selenium in the protection against viral infection. The biological function of selenium is mediated by selenoproteins, which contain selenocysteine, a cysteine analog that possesses selenium instead of sulfur. Sec is encoded by one of the stop codons "UGA" and is called the 21st amino acid that is translated. Twenty-five types of human selenoproteins have been identified, including glutathione peroxidase (GPx) for the reduction of hydroperoxides and thioredoxin reductase for redox regulation. Therefore, selenoproteins are a key factor in the antioxidant system. Our research group focused on a major selenium-containing protein "Selenoprotein P (SeP)" that is primarily synthesized in the liver and secreted to plasma. SeP is a multifunctional protein containing 10 selenocysteine residues: One N-terminal Sec residue forms an active site of GPx-like enzyme activity to reduce phospholipid hydroperoxide, while the nine C-terminal Sec residues function as a selenium transporter to deliver selenium from the liver to other tissues, such as those of the brain and testis. SeP functions to maintain antioxidative selenoenzymes in several tissues and plays a pivotal role in selenium metabolism and antioxidative defense. However, recent studies indicate that excess SeP exacerbates glucose metabolism and promotes type 2 diabetes. SeP levels are increased in type 2 diabetes patients, and excess SeP impairs insulin signaling, promoting insulin resistance. Increased levels of SeP disturb the function of pancreatic β cells and inhibit insulin secretion (2). Recently, the increase of SeP expression has been observed in cancer cells and its implication for cell proliferation is discovered.

This presentation focuses on the relationship between selenium metabolism and redox regulation, particularly on its physiological significance for the antioxidative system and defense against environmental stresses. The important role of selenoproteins to prevent lipid peroxidation and to protect from the toxicity of heavy metals is shown. Furthermore, the pathophysiological implications of excess SeP and diseases such as type 2 diabetes and cancer are presented. Particularly, the role of SeP expression in the progression of diseases and the application of its targeted molecules for these diseases are shown. Finally, the bifacial properties of selenium and SeP on the maintenance of homeostasis are discussed.

References:

1. Y Saito (2022) Essential trace element selenium and redox regulation its metabolism, physiological function, and related diseases. ***Redox Exp Med,*** 1, R149-R158

2. Y Mita and Y Saito, et al. (2017) Selenoprotein P-neutralizing antibodies improve insulin secretion and glucose sensitivity in type 2 diabetes mouse models. ***Nature Commun,*** 8, 1658

Title:

Structural library and visualization of endogenously oxidized lipids

Author(s): **Name Surname[1]**

Ken-ichi Yamada

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

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Abstract:

Recently, oxidized phospholipids have been reported to be involved in various diseases. For example, lipid peroxides induce a new cell death form, "ferroptosis," and epoxidized ω3 fatty acids, an oxidized metabolite, are engaged in worsening allergies. In addition, the complex between lipid peroxide-derived aldehyde and protein is involved in angiogenesis. Thus, although the importance of oxidized phospholipids is widely recognized in the induction of inflammation and cell death, the number of oxidized phospholipids available or detectable is limited. This lower number would be due to the lack of appropriate detection techniques.

Here, we have developed a fluorescent probe to detect "lipid-derived radicals," key molecules during the chain reaction of lipid peroxidation. Furthermore, since this probe can covalently bind to lipid-derived radicals, we have constructed an LC/FL/HRMS/MS system and have successfully analyzed the structures of 132 lipid-derived radical species1). In addition, the involvement of lipid-derived radicals in the vitamin K cycle has been clarified recently.

Next, a non-targeted analysis of phosphatidylcholine-derived oxidized lipids (oxPCs) was performed using a high-resolution mass spectrometer, and a library of 465 oxPCs was constructed 2). Furthermore, we detected 70 kinds of oxPCs in mice with acetaminophen-induced acute liver failure, and mass imaging of oxidized lipids was successfully performed.

In this symposium, I would like to introduce our recent research, including the detection and structural analysis of oxidized phospholipids and their application using animal models.

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

1. Yamada K., et al., Fluorescence probes to detect lipid-derived radicals. Nat. Chem. Biol. 12, 608-613 (2016)
2. Matsuoka Y., et al., Structural library and visualization of endogenously oxidized phosphatidylcholines using mass spectrometry-based techniques. Nat. Commun. 12, 6339 (2021)

Lipid peroxidation, vascular and systemic oxidative stress in aggressive COVID-19

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Abstract:

The SARS-CoV-2 virus caused the pandemics denoted as COVID-19, which was manifested by common respiratory symptoms, but also causing severed ARDS even leading to the death of patients who were otherwise in apparently good health.

While COVID-19 itself changed a lot during a period of two years, ceasing eventually after the appearance of Omicron, the relatively benign variant of SARS-CoV-2, it is still not really certain why was this virus so lethal in case of aggressive infections.

The research we did comparing the onset of disease in patients who eventually recovered and those who passed away within a week after admission to the hospital, but who did not have any comorbidities and did show any remarkable differences when they came to the ICU, we found that vascular oxidative stress might be the crucial pathogenic mechanism of the lethal outcome of COVID-19.

The results of several studies done on samples obtained from these patients will be presented briefly.

References:

1. Neven Žarković, Biserka Orehovec, Lidija Milković, Bruno Baršić, Franz Tatzber, Willibald Wonisch, Marko Tarle, Marta Kmet, Ana Mataić, Antonia Jakovčević, Tea Vuković, Danijela Talić, Georg Waeg, Ivica Lukšić, Elzbieta Skrzydlewska and Kamelija Žarković (2021) Preliminary Findings on the Association of the Lipid Peroxidation Product 4-Hydroxynonenal with the Lethal Outcome of Aggressive COVID-19. Antioxidants, 10(9), 1341; https://doi.org/10.3390/antiox10091341

2. Zarkovic, N., Jakovcevic, A., Mataic, A., Jaganjac, M., Waeg, G., Zarkovic, K. (2022) Post-mortem Findings of Inflammatory Cells and the Association of 4-Hydroxynonenal with Systemic Vascular and Oxidative Stress in Lethal COVID-19. Cells, 11(3), 444; https://doi.org/10.3390/cells11030444

3. Žarković, Neven, Anna Jastrząb, Iwona Jarocka-Karpowicz, Biserka Orehovec, Bruno Baršić, Marko Tarle, Marta Kmet, Ivica Lukšić, Wojciech Łuczaj, and Elżbieta Skrzydlewska. 2022. The Impact of Severe COVID-19 on Plasma Antioxidants Molecules 27, no. 16: 5323. https://doi.org/10.3390/molecules27165323

Oral Presentations

Monday September 11

Application of immunohistochemical methods (CKHMW, p63, AMACR and c-myc) in distinguishing benign pathohistological changes from prostate cancer

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[1] University Psychiatric Hospital Vrapče; [2] University Hospital "Sveti Duh"; [3] Croatian Institute of Transfusion Medicine

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Abstract:

The objectives of this study were to investigate the expression and the expression intensity of immunohistochemical markers CK HMW and p63 in prostate samples with the findings of hyperplasia of basal cells and PIN compared to the intensity of expression of prostate surrounding normal tissue, and to determine whether there is a difference in the intensity of expression of these markers between said entities. Then, compare the expression intensity of immunohistochemical markers AMACR with the PIN, ASAP and prostate adenocarcinoma, and examine the expression and the expression intensity of the immunohistochemical marker c-myc between prostate samples with the findings of ASAP, PIN and prostate adenocarcinoma.

The material is prostate tissue samples archived in the Clinical Institute for Pathology and Forensic Medicine of the Clinical Hospital Centre Osijek in a one year period.

Most of the adenocarcinoma diagnosed belongs to group grade 2. There was a statistically significant difference in the intensity of expression of markers CK HMW and p63between PIN and normal prostate tissue, and between HBS and normal prostate tissue. 17participants (29.3%) had a strong intensity of expression of the marker AMACR in adenocarcinoma, and 20 participants (43.5%) had a strong intensity of expression of the marker c-myc in adenocarcinoma.

Conclusion: Immunohistochemical methods were performed on all samples, what indicates its great help in differential diagnosis of various pathological prostatic changes.

Key words: adenocarcinoma; immunohistochemistry; intensity of expression; prostate; tumor Markers;

The Appearance of 4-Hydroxy-2-Nonenal (HNE) in Squamous Cell Carcinoma of the Oropharynx

Antonia Jakovčević [1], Irena Veliki Dalić [1], Kamelija Žarković[1], Neven Žarković[2]

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Abstract:
Tumor growth is associated with oxidative stress, which causes lipid peroxidation. The most intensively studied product of lipid peroxidation is 4-hydroxy-2-nonenal (HNE), which is considered as a "second messenger of free radicals" that binds to proteins and acts as a growth-regulating signaling factor (1). The incidence of squamous cell carcinoma of the oropharynx is associated with smoking, alcohol and infection of human papilloma virus (HPV), with increasing incidence world-wide (2). The aim of this retrospective study involving 102 patients was to determine the immunohistochemical appearance of HNE-protein adducts as a potential biomarker of lipid peroxidation in squamous cell carcinoma of the oropharynx. The HNE-protein adducts were detected in almost all tumor samples and in the surrounding non-tumorous tissue, while we found that HNE is differentially distributed in squamous cell carcinomas in dependence of clinical stage and histological grading of these tumors. Namely, the level of HNE-immunopositivity was increased in comparison to the normal oropharyngeal epithelium in well- and in moderately-differentiated squamous cell carcinoma, while it was decreasing in poorly differentiated carcinomas and in advanced stages of cancer (3). However, more malignant and advanced cancer was associated with the increase of HNE in surrounding, normal tissue. High concentrations of HNE are cytotoxic and mutagenic, while in physiological processes, present at low levels, HNE acts as a signaling molecule involved in growth regulation, interacting with cytokines and regulating the expression of cellular (proto) oncogenes. The cytotoxic effects of reactive aldehydes resemble the toxicity of ROS, but because of their higher chemical stability, these aldehydes can spread from the site of origin and react with major biomolecules. Because of high reactivity with biomolecules and its multiple biological effects, HNE is a popular LPO product in the field of molecular oncology. This study confirmed the onset of lipid peroxidation, generating HNE-protein adducts that can be used as a valuable bioactive marker of carcinogenesis in squamous cell carcinoma of the oropharynx, as well as indicating involvement of HNE in pathophysiological changes of the non-malignant tissue in the vicinity of cancer (3).

References:

1. Esterbauer, H. et al., Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 1991, 11, 81-128.

2. Cohan, D.M. et al.; Oropharyngeal cancer: current understanding and management. Curr. Opin. Otolaryngol Head Neck Surg. 2009, 17, 88-94.

3. Jakovčević, A. et al.; The appearance of 4-Hydroxy-2-Nonenal (HNE) in Squamous Cell carcinoma of th Oropharynx. Molecules 2020, 25,868.

Dubrovnik Summer School on Molecular Biosciences in Medicine with
The International Oxidative Stress Symposium 2023

The Abstract Form

Title:

Effects of Intergenerational Dietary Changes on Adipokine Levels in Female Sprague Dawley Rats

Author(s): **Name Surname[1]**

Marlena Brstilo-Čičković1, Nevija Brstilo1, Karla Rožac1, Robert Mujkić1, Nenad Čekić1, Željka Perić Kačarević1, Darija Šnajder Mujkić1, Mijač Sandra2, Anđela Grgić1

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author.**

1 Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
2 Children's Hospital Srebrnjak, Zagreb, Croatia

Abstract:

Background: Previous studies have shown that changes in feeding protocol between generations can significantly affect adipose tissue physiology (Šnajder et al., 2019).
Aim: To determine the effects of maternal and offspring diets on adipokine levels in all groups of rats.
Methods: Ten female rats were randomly divided into two groups, one fed a high saturated fat diet (HSFD), and the other fed a standard laboratory chow diet (CD). After the mating and lactation period, their offspring were randomly divided into two subgroups fed HSFD or CD, forming four study groups: a) CD-CD, b) CD-HSFD, c) HSFD-CD, and d) HSFD-HSFD. The dams and offspring were subjected to biochemical analysis of blood adiponectin, IL-6 and TNF-α levels at 37 and 18 weeks of age, respectively. Body weight was also determined, and body mass index (BMI) was calculated.
Results: There was no significant difference in body weight and BMI between groups. In the maternal HSFD group, a higher IL-6 concentration was measured ($p=0.009$) and a lower adiponectin concentration ($p=0.009$). Significantly higher IL-6 and TNF-α ($p=0.004$ and $p=0.004$, respectively) and lower adiponectin levels ($p= 0.004$) were observed in the HSFD-HSFD and HSFD-CD groups.
Conclusion: Consumption of a high saturated fat diet is not necessarily correlated with high body weight and BMI. Nevertheless, maternal HSFD consumption predisposes offspring to an increased risk of adipokine level disorders leading to metabolic abnormalities.

Keywords: adipokines, animals, body mass index, diet, oxidative stress, saturated fatty acids

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary.**

Šnajder D, Perić Kačarević Ž, Grgić A, Bijelić N, Fenrich M, Belovari T, Radić R. Effect of Different Combination of Maternal and Postnatal Diet on Adipose Tissue Morphology in Male Rat Offspring. J Matern Fetal Neonatal Med. 2019 Jun;32(11):1838-1846. DOI: 10.1080/14767058.2017.1419181.

Title:

Oxidative Stress and Inflammation in Female Lewis Rats in Relation to Diet and 13-cis-retinoic Acid Intake

Author(s): **Name Surname[1]**

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Abstract:

Background: Adipose tissue is characterised by infiltration of inflammatory cells such as monocytes, neutrophils, Th1 and Th17 lymphocytes, and M1 macrophages (anti-inflammatory), the proportion of which can be as high as 40% in obesity. Increased secretion of saturated fatty acids (SFAs) activates resting macrophages and adipocytes, leading to increased secretion of proinflammatory cytokines and chemokines. Macrophages are characterised by increased expression of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO). Nitric oxide is an important indicator of inflammation, oxidative stress, and polarisation of macrophages into the M1 phenotype. Arginase I reduces inflammatory activity by interacting with iNOS. The anti-inflammatory cytokines IL-4, IL-10, and IL-13 stimulate arginase I activity in macrophages, enhancing the anti-inflammatory effect (Adedeji et al., 2022).
Aim: To explore the relationship between diet and 13-cis-retinoic acid intake on oxidative stress and inflammation in female Levis rats.
Methods: The analysis included 36 Lewis rats, of which half were fed a high-fat diet (HFD, 45% saturated fat) for 30 days, and the other half were fed a standard laboratory diet (STD). The groups were divided into three groups (six rats each): two experimental groups received 13-cis-retinoic acid (13-cRA) orally daily for 30 days (7.5 mg/kg and 15 mg/kg, respectively), and the control group received distilled water. Animals were sacrificed after 60 days, and serum was analysed for inflammatory cytokines, arginase, and NO activity.
Results: The increase in serum concentration of IL-6, IL-1β, and IFN-γ in subjects fed a high-fat diet was observed in the control group and the groups to which 13 cRA was added in both concentrations. In the same groups, there was an increase in IL-10, which is part of the Th2 inflammatory response. In subjects receiving HFD with the addition of 13 cRA, there was a visible increase in serum, renal, and liver activity NO compared with the control group. Analysis of serum and renal arginase concentrations revealed decreased arginase levels in subjects receiving HFD.
Conclusion: According to the present results, 13 cRA and HFD affect metabolic parameters and inflammation and oxidative stress in the organism. Administration of cRA affects metabolic parameters differently depending on whether STD or HFD was used.

Keywords: 13-cis-retinoic acid, animals, anti-inflammatory agents, inflammation, oxidative stress, saturated fatty acids

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary.**

Adedeji TG, Jeje SO, Omayone TP, Agbonifo WO. Oxidative Stress and Inflammatory Response to High Dietary Fat and Carbonated Soda Intake in Male and Female Wistar Rats. Nutrition. 2022 Nov-Dec;103-104. DOI: 10.1016/j.nut.2022.111800.

Title:

Extracorporeal blood purification therapy role in COVID-19 ICU patients: a study of sequential EBP

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Abstract:

Critically ill patients with severe form of COVID-19 often suffered from dysregulated hyperactive immune response predisposing them to multiorgan disfunction. This "cytokine storm" is characterized by very high levels of interleukin 6 (IL-6), interleukin 10 (IL-10), ferritin, tumour necrosis factor alpha (TNF-α) and procoagulant factors, released either due to the illness itself, mechanical ventilation-associated lung injury (VILI) or due to extracorporeal membrane oxygenation (ECMO). These patients were also highly susceptible to bacterial superinfections, which further complicated their clinical course and added to the "cytokine storm". Extracorporeal blood purification (EBP) such as hemodiafiltration and hemadsorption are emerging as novel treatment support modalities for these patients. EPB eliminates endotoxins and pro- and anti-inflammatory cytokines through absorbent power of different filters. Absorbing mechanisms include hydrophobic interactions, ionic attraction, hydrogen bonds and Can der Waals interactions, specific for a type of filter. Traditionally, patients were treated with only one filter. However, sequential therapy with different types of filters is described, to harness different effects of each filter. EBP effect on the patient is described by "cytokinetic model", in which the reduction of circulating levels of cytokines returns the immune system to a less deranged state and allows it to redirect the immunocompetent cells to the source or site of inflammation, improving the immunological response of the host and supporting organ function.
In our centre, we conducted a prospective observational study on 68 patients with severe form of COVID-19 and confirmed bacterial superinfection, with evidence of systemic inflammation and organ dysfunction. They underwent either standard EBP with one filter, or sequential EBP with a combination of haemofilters. We then compared clinical and biochemical variables between groups. Sequential EBP was shown to be superior in reducing inflammatory markers Il-6, ferritin and c-reactive protein compared to standard EBP. Thus, a sequential approach may enhance the positive effect of EBP on organ dysfunction in this patient population.

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

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Wednesday September 13

Metabolomics of schizophrenia, depression and bipolar affective disorder

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Abstract:

Schizophrenia and depression are serious mental disorders that are often diagnosed in a long and complicated process due to phenotypic, biological and genetic heterogeneity, and complicated interactions of biological, external and genetic factors that cause the development of these disorders. Schizophrenia is a severe chronic mental disorder with a complex and complicated progression symptomatology. It is estimated that 1% of the population suffers from schizophrenia. Depression is a complicated and clinically heterogeneous mental disorder that affects about 10 -15 % of the population. Bipolar affective disorder (BAP) is classified as a mood disorder. It is characterized by alternating episodes of depression with episodes of mania or hypomania, unlike unipolar depression, in which hypomanic or manic episodes have never occurred.

Metabolomic analyzes have great potential for improving existing knowledge about pathogenesis and etiology of depression and represent a new approach to the discovery of biomarkers for diagnosis, prognosis and monitoring therapy.

Using a non - targeted metabolomics approach to investigate changes in biochemical pathways specifically related to the pathology of each individual disorder with the purpose of defining new and easily accessible indicators of the process that are in the background of these disorders and enable their reliable differentiation, up to date and accurate diagnoses and earlier and more successful therapeutic interventions, especially in the case of BAP.

Untargeted metabolomic analysis using gas chromatography paired with mass spectrometry (GC-MS) to identify metabolites whose levels differ in these disorders. Untargeted metabolomic analysis using liquid chromatography paired with mass spectrometry (LC-MS) to identify metabolites whose levels differ in individuals with the diagnosis of BAP in relation to respondents with depression and schizophrenia.

Key words: schizophrenia, depression, BAP, metabolome, mass spectrometry

Acrolein as a novel marker of bladder cancer aggressiveness: Pilot study on 49 patients

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Abstract:
Muscle-invasive bladder cancer (MIBC) has an aggressive clinical course and a high propensity for local and metastatic spread. Moreover, MIBC invariably develops resistance to all known therapies currently applied in the clinic. The high level of intratumor hypoxia and intracellular oxidative stress can hypothetically explain this phenomenon. Acrolein, a highly reactive aldehyde and potential marker of oxidative stress, was shown to be involved in bladder carcinogenesis. We investigated the presence of acrolein in archived bladder cancer tissue and correlated it with clinical variables. Within a single high-volume institution, an annotated cohort of patients with MIBC who underwent radical cystectomy with urinary diversion was prospectively followed for functional and oncological outcomes. Archive pathology blocks were retrieved for those patients, and their records were retrospectively analyzed. Additional immunohistochemical analysis was performed using monoclonal antibody 5F6 to detect acrolein presence on formalin-fixed, paraffin-embedded cystectomy specimens (1). A single expert pathologist provided distinct immunohistochemical analysis and scores for tumor stroma, nuclei, and cytoplasm, evaluating the distribution and intensity of acrolein immunostaining in the tumor and adjacent tissue samples semi-quantitatively (2). Descriptive statistic methods were used to associate the presence of acrolein with clinical factors (TNM stage, etc.) Forty-nine patients who underwent cystectomy in 2016 had available archive tissue and were involved in this pilot study. 40/49 (82%) were males; the median age was 74 years (range 54-84 years). 11/49 (22%) patients had professional exposure to harmful chemicals. 35/49 (71%) were smokers. TNM stages were S2 in 27/49 (55%) patients, S3 in 19/49 (39%) patients, and S4 in 3/49 (6%) patients. 35/49 (71%) had pure urothelial cancer. 40/49 (82%) had high-grade tumors, 3/49 (6%) were G2, and 6/49 (12%) were unassigned. Positive staining for acrolein was found in 48 patients (6/49 (12%) in stroma, 37/49 (76%) in cytoplasm, and 34/49 (69%) in nucleus). Diffuse staining was found in 31/49 (63%) patients. Cytoplasmic acrolein was more prevalent in high-grade than low-grade tumors (91% vs 33%, $p=0.005$). A significant difference between histology subtypes (small cell, squamous, variant, and pure urothelial) and acrolein presence was observed ($p<0.001$). Patients with more advanced tumors were more often acrolein positive ($p=0.02$). Patients with tumors advancing into the urethra were 100% acrolein positive compared to 68% in those without urethral involvement ($p=0.02$). In addition, a difference in acrolein presence was found for TNM stages 2, 3, and 4, respectively ($p=0.003$), with acrolein being more prevalent in the higher TNMS stage. After a median follow-up of 63 months, 15 patients developed distant metastases, nine developed local relapse, and 15 died from bladder cancer. The median PFS was 19 months, and the median OS was 24 months. No significant association was found for the presence of acrolein in local, distant, or combined relapse or overall survival, respectively (all $p>0.1$). In this small pilot study on bladder cancer patients, the presence of acrolein was associated with more histologically aggressive and advanced disease at the time of cystectomy. However, due to the relatively small group of patients included in the study, acrolein did not appear to be associated with survival or relapse.

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Profiling the metabolome of patients with dementia

Hrvačić Martina[1], Nedić Erjavec Gordana[2], Uzun Suzana[3] Nikolac Perković Matea[2]
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Abstract:
Dementia is a syndrome of global and progressive impairment of acquired cognitive abilities with preserved consciousness, caused by an organic disease of the central nervous system, in which memory, learning, abstract thinking, orientation and understanding of visual-spatial relationships are particularly impaired. The identification of new biomarkers that would enable the timely diagnosis of dementia and the differentiation of different types of dementia is essential for the development of more reliable diagnostic tests and new approaches to therapy. Metabolomics is one of the latest "omics" approaches that enables the monitoring of changes occurring downstream of genomic, transcriptomic and proteomic modifications. Detecting the level of endogenous metabolites, which represent the end point of all biochemical reactions, could be used as a fairly sensitive measure of an individual's overall health status. Metabolomic analyzes have great potential for improving existing knowledge about the pathogenesis and etiology of dementia and represent a new approach to the discovery of biomarkers for diagnosis, prognosis and therapy monitoring in patients with different types of dementia.

Changes in biochemical pathways will be investigated using a non - targeted metabolomic approach, with the aim of finding new and easily accessible indicators of the processes underlying these disorders. Through untargeted metabolomic analysis using gas chromatography coupled to mass spectrometry (GC-MS), we will identify metabolites whose levels have changed in people with dementia. Through untargeted metabolomic analysis using liquid chromatography coupled to mass spectrometry (LC-MS), we will identify metabolites whose levels have changed in people diagnosed with Alzheimer's disease compared to subjects with vascular dementia and corresponding controls. We will investigate whether the established changes in the metabolome are related to the decline of cognitive functions.

Key words: dementia, Alzheimer's disease, vascular dementia, mild cognitive impairment, metabolome

Effects of maternal cigarette smoking on antioxidative enzymes SOD and GPx and their association with trace elements in maternal-placental-fetal compartments

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Abstract:
Tobacco smoke is a mixture of numerous chemical substances with the potential to adversely affect human health, enhance oxidative stress and disturb the antioxidative balance. Maternal smoking during pregnancy is related to a wide range of pregnancy complications and adverse infant outcomes, such as preterm birth and low birth weight. Moreover, it may be associated with increased risks of overweight and obesity in childhood, characterized by metabolism impairment and antioxidative disbalance due to oxidative stress. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are key enzymes involved in reducing oxidative stress and a first-line defense against free radical species and their by-products in living systems. Both enzymes are metal-dependent enzymes and they need specific trace elements for their optimal functioning – Zn, Cu, and Mn for SOD and Se for GPx. Fe easily changes its oxidation state, which makes it a major catalyst for the production of reactive oxygen species. In excess, Fe may trigger oxidative stress and potentially limit the antioxidant capacity in the body. Besides their role in the antioxidative system, trace elements are crucial nutrients for growth and development.
The aim of this study was to investigate the associations between the activities of antioxidative enzymes SOD and GPx and trace elements Zn, Cu, Mn, Se, and Fe in relation to maternal smoking habits in the study cohort of healthy postpartum women and their infants (n=156) from Zagreb, Croatia. Maternal and cord blood and the placenta were collected immediately after spontaneous vaginal delivery at term in clinical hospitals and sampled following methods described earlier (1, 2). The activity of the antioxidant enzyme was analyzed spectrophotometrically and Zn, Cu, Mn, Se, and Fe were determined using the ICP-MS method. Based on self-reported data on cigarette smoking habits and confirmed by urine cotinine levels, three study groups were formed: never-smokers, former smokers, and active smokers.
Positive weak to moderate correlations (Spearman's ρ ranged from 0.2 to 0.4, p <0.05) were found between the activity of the SOD enzyme and the levels of Zn, Mn, and Se in the studied samples. GPx showed a positive association with Se levels (p <0.001) in all samples. However, an inverse association was found between placental Fe and antioxidative enzymes, which was strong (ρ=-0.8) for SOD and weak (ρ=-0.2) for GPx. In active smokers, a decrease in Fe levels in maternal plasma and the placenta, as well as decrease in Cu levels in cord plasma were found. No differences were observed in the activity of SOD and GPx between the groups of nonsmokers, former smokers, and active smokers.
More studies are required to explain the complex association between antioxidant capacity and maternal tobacco smoke exposure during pregnancy that may affect health of future progeny.
This study was funded as a part of the Croatian Science Foundation research project HRZZ-IP-2016-06-1998, METALORIGINS.

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Posters

Monday September 11

Immunohistochemistry of the epithelial tumors of the ovary

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Abstract:

Research objectives: The aim of this paper was to demonstrate immunohistochemical staining important for diagnosis and differential diagnosis of epithelial ovarian tumors, to investigate the number of diagnosed epithelial tumors and to classify them according to the type and malignant potential, and to evaluate the intensity of expression of immunohistochemical markers in relation to the intensity of expression of positive tests in relation to the type of epithelial tumor.

Materials and methods: The material consists of samples of ovarian tumor stored in a two-year period in the Archives of the Department of Pathology and Forensic Medicine of the University Hospital Osijek. They were processed by histochemical procedure and stained with hematoxilin and eosine (HE) and processed by an indirect ABC method with antibodies for CK7, CK20, WT1, Ca125 and CDX2. The expression of immunohistochemical markers in relation to the positive test has been described by light microscope.

Results: In a two-year period, a total of 102 epithelial ovarian tumors were diagnosed, out of which 59.80% were serous, 34.31% mucinous, 2.94% clarocellular, 0.98% Brenner tumors and 1.96% metastatic ovarian tumors. Out of 102 tumors, only 10 of them were treated by immunohistochemical methods, ie 9.80%. Tumor marker CA125 had on most preparationsweaker intensity of the positive tests, whereas in contrast, CK7 had on most preparations higher intensity, and the marker CK20 had on most preparations the color intensity equal to the intensity of the positive probe.

Conclusion: At the Department of Pathology and Forensic Medicine of the University Hospital Osijek the most commonly diagnosed were serous ovarian tumors of benign nature.

Immunohistochemical methods were performed on 10 tumors, meaning that in the diagnosis of ovarian tumors immunohistochemistry is not as widely used as histological methods. Different tumor markers showed different expressionin relation to the positive test.

Key words: immunohistochemistry; epithelial ovarian tumors; tumor markers; intensity of expression

Dubrovnik Summer School on Molecular Biosciences in Medicine with
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The Abstract Form

Title:

Mucoepidermoid carcinoma of the lung: When you are stuck with a diagnosis and the patient is young, FISH can help

Authors:

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Abstract

Introduction: Primary lung cancer is one of the most common in the world. Salivary gland–like carcinomas of the lung generally refers to a class of rare primary lung cancers to which mucoepidermoid carcinoma of the lung belongs. Mucoepidermoid tumor originates from submucosal glands of tracheobronchial tree and is usually very slow growing low grade malignant tumors. Surgery is the mainstay of treatment and rarely requires adjuvant therapy.

Case presentation: A 25-year-old man was hospitalized due to left-sided recurrent pleuropneumonia accompanied by mild respiratory insufficiency. During hospitalization, narrowing of the left main bronchus as well as the lobar branches by external compression was found. A lobectomy was performed and the lower left lobe with a solid whitish-yellowish tumor mass measuring 3.7x3x2.6 cm was removed. On histology the tumor was composed of 3 cell types: squamoid cells, mucin-secreting cells and cells of intermediate type. Mucin-secreting cells lined multiple partially dilated glandular structures, while squamoid cells admixed with intermediate cells formed some nests in a sheet-like pattern. Histology indicated a diagnosis of high grade primary salivary type lung cancer: mucoepidermoid carcinoma. On FISH MAML2 rearrangement was found which confirmed the diagnosis.

Conclusion: The MAML2 rearrangement is the one found in mucoepidermoid carcinoma and it tends to be more frequently found in low-grade PMEC than in high-grade PMEC. Although the morphological distinction of PMEC from its mimics can sometimes be challenging, the location, morphology, immunophenotype, and molecular genetics are important for the differential diagnosis between mucoepidermoid carcinoma and mimics, which is critical for therapeutic and prognostic considerations.

Title:

Comparative analysis of staining of MAIT cells in skin with two MR1 tetramers

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Abstract:

Mucosal-associated invariant T (MAIT) cells are a subset of unconventional T cells known to play a central role in immune surveillance at mucosal and epithelial surfaces. Canonical human MAIT lymphocytes use the semi-invariant TCRVα7.2 receptor to recognize microbial antigens in an MHC related class I (MR1)-restricted manner. The development of MR1 5-OP-RU tetramers enabled the identification of classical MAIT cells based on T-cell receptor specificity for ribityl antigens, but the integration of MR1-tetramers into routine tissue staining protocols remains limited. Therefore, the aim of this study was to optimize visualization of MAIT cells in skin samples with MR1-tetramers conjugated to either phycoerythrin (PE) or Alexa Fluor -488.

MATERIALS AND METHODS: Two psoriatic (PASI>10 and PASI<10) and one healthy skin samples were fixed in paraformaldehyde, cryoprotected in a sucrose gradient, and snap frozen, before sectioning on the cryostat. Each sample was stained with MR1 5-OP-RU PE and Alexa Fluor 488-conjugated tetramer, and nuclei were stained with DAPI. Slides were imaged under a fluorescence microscope and images were processes using ZEN lite software.

RESULTS: A total of 18 skin images were analyzed. MAIT cells were rare in both healthy and psoriatic skin, and were found along the basal membrane of the epidermis, with a more prominent distribution within the papillary dermis, especially in the papillary loops. The average number of MR1 5-OP-RU PE+ cells per image was 1.75 and 1.8 after MR1 5-OP-RU Alexa Fluor 488 staining. The maximum number of PE- or AlexaFluor-488-positive labeled cells per image was 4 and 3, respectively.

CONCLUSION: MR1-Alexa Fluor 488 skin labeling provides better stability and reliable visualization over a longer period of time, while PE-labeled tetramers show better performance due to lower background noise. Skin autofluorescence and photoconversion of DAPI hampered visualization of MAIT cells within the epidermis.

HFE Gene Mutation in Eastern Croatian Patients Suspected of Having Hereditary Hemochromatosis

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INTRODUCTION: Hereditary hemochromatosis (HH) is an autosomal recessive disorder with disrupted iron regulation. Toxic accumulation of iron in vital organs leads to development of cirrhosis, bone and joint disease, diabetes mellitus, heart disease, and malignancies, particularly hepatocellular carcinoma. There are four main types of HH that have been categorized based on the dysregulation of distinct proteins involved in iron homeostasis. The most frequent inherited form of iron overload, type 1, is divided into three subtypes depending on three point mutations in the *HFE* gene that can cause HH: type 1A, type 1B, and type 1C. Type 1A HH is recognized in patients who are homozygous for the point mutation C282Y (rs1800562, c.845G>A, p.Cys282Tyr) in the *HFE* gene. Type 1B HH patients are compound heterozygotes with two point mutations, C282Y and H63D (rs1799945, c.187C>G, p.His63Asp), with the C282Y/H63D genotype. Type 1C HH is characterized by a point mutation S65C (rs1800730, c.193A>T, p.Ser65Cys) (1).
OBJECTIVES OF THE RESEARCH: The aim of this study was to determine the frequencies of mutations C282Y, H63D and S65C in the *HFE* gene in a group of patients with HH and to compare them according to gender of the subjects.
SUBJECTS AND METHODS: The examined group consisted of 133 patients (91 males and 42 females) aged 7-79 who were suspected of having HH. Genomic DNA was isolated from whole blood samples with the anticoagulant K_2EDTA. Genotyping of the *HFE* gene mutations was performed using a real-time polymerase chain reaction. Fisher's exact test was used to compare allelic frequencies and the prevalence of HFE genotypes among groups by gender.
RESULTS: The most frequent genotypes for C282Y, H63D, and S65C were GG (74.0%), CC (68.0%) and AA (97.0%), respectively. Ten patients (7.52%) were found to be homozygous for the C282Y mutation, eight patients (6.02%) had compound heterozygous genotype C282Y/H63D, and six patients (4.51%) were homozygous for the H63D mutation. There were patients heterozygous for C282Y mutation (GA), for H63D (CG) and for S65C mutation (AT), 11.28%, 21.80% and 2.26%, respectively.
CONCLUSION: In the age category between 51 and 70 years of age, a significantly higher number of women were referred for molecular diagnostics of HH. There was no statistically
significant difference in the distribution of alleles and genotypes for mutations C282Y, H63D and S65C, nor in the distribution of *HFE* genotypes between male and female gender groups. The frequency of the C282Y mutation homozygous, genotype associated with type 1A HH, was represented by 6.6% in the male group and 9.5% in the female group. The frequency of the C282Y/H63D compound heterozygote, genotype associated with type 1B HH, was represented by 5.5% in the male group and 7.1% in the female group. There was no significant difference in the distribution of HFE genotypes between male and female groups.
KEYWORDS: Familial hemochromatosis; Gene Polymorphism; Molecular Diagnostics, *HFE*, C282Y, H63D, S65C

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Tuesday September 12

Dubrovnik Summer School on Molecular Biosciences in Medicine with
The International Oxidative Stress Symposium 2023

The Abstract Form

Title:

Clinical and laboratory biomarkers of COVID-19 outcome

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Abstract:

Several studies on coronavirus disease 2019 (COVID-19) aimed to detect key laboratory parameters as indicators of COVID-19 severity. Previous experience has shown that certain laboratory parameters such as interleukin-6 (IL-6), D-dimers and lymphocyte count can be used in the clinical assessment of the disease severity. However, the most adequate biomarkers for COVID-19 severity remain undefined.
In this retrospective study, we analysed the data of 1290 hospitalised COVID-19 patients. This mainly included analysing the association of patient outcomes with clinical and laboratory data collected on patient admission. Data were retrieved from medical records following standard ethical guidelines.
Out of 1290 analysed patients, 65.4% were male (median age 66 years, range 0.1-.98 years) and 25.9% of patients in this cohort died. The most common comorbidities were hypertension (58.9%), heart disease (25.4%), obesity (21.6%) and diabetes (21.6%). Logistic regression analysis revealed that heart disease (aOR = 2.05, $p < 0.001$), chronic kidney disease (aOR = 2.52, $p < 0.001$) and obesity (aOR = 1.41, $p = 0.031$) were associated with fatal outcome. Patients with fatal outcome exhibited higher modified early warning scores (MEWS) than surviving patients (medians 3 and 2, $p < 0.001$). Patients with fatal outcome showed differences in several laboratory parameters recorded on admission. This included higher levels of white blood cells (medians 8.4 and 7.2 /nL, $p < 0.001$), D-dimers (medians 1.5 and 0.9 ng/mL, $p < 0.001$), glucose (medians 7.8 and 6.9 mmol/L, $p < 0.001$), hs-troponin T (medians 0.02 and 0.01 µg/L, $p < 0.001$), N-terminal pro-brain natriuremic peptide (medians 961.5 and 407.0 ng/L, $p < 0.001$), C-reactive protein (medians 117.9 and 86.8 mg/L, $p < 0.001$), IL-6 (medians 76.6 and 45.0 ng/L, $p < 0.001$), lactate dehydrogenase (LD) (medians 423.0 and 313.0 U/L, $p < 0.001$) and urea (medians 9.0 and 6.0 µmol/L, $p < 0.001$). Patients with fatal outcome also exhibited lower oxygen saturation (medians 93% and 83%, $p < 0.001$), lymphocyte percentages (medians 7.2% and 11.0%, $p < 0.001$), platelet count (medians 180 and 200 /nL, $p = 0.002$) and albumin levels (medians 33.7 and 38.3 g/L, $p < 0.001$).
We constructed a random forest model to classify patients based on disease outcome using comorbidities, MEWS and laboratory parameters on admission. The best model utilized eight parameters in classifying patients with fatal outcome. These parameters were, by order of importance, oxygen saturation levels, platelet count, LD, creatinine, fibrinogen, D-dimers, albumin and MEWS. This model achieved an accuracy of 88.1% (sensitivity 72.3%, specificity 93.8%) and an AUC of 0.83 (95 % CI 0.77-0.89) on cross-validated data.
This study identified several key biomarkers recorded on patient admission which were effective in predicting COVID-19 outcome. Monitoring of these biomarkers in early disease stages could be key in evaluating COVID-19 severity and predicting the disease outcome. However, more research on the stated biomarkers in different patient cohorts is needed.

Dubrovnik Summer School on Molecular Biosciences in Medicine with
The International Oxidative Stress Symposium 2023
The Abstract Form

Title:

Vaccination and viral variants do not influence IgG glycome dynamics in COVID-19

Author(s): **Name Surname[1]**

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Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

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Abstract:

The pandemic of coronavirus disease 2019 (COVID-19) has become an ongoing public health problem. Consequently, numerous studies have been conducted on the pathogenetic mechanisms involved in COVID-19. However, the influence of glycosylation of immunoglobulin G (IgG) on the severity of COVID-19 remains largely unexplored.

In this study, we analysed the total IgG glycome in 806 longitudinally monitored COVID-19 patients, resulting in 2001 analysed sera. Glycosylation traits of IgG were evaluated using ultra-high-performance liquid chromatography. Longitudinal analysis of patient IgG glycome was done by implementing a linear mixed-effects model in which glycan measurement acted as a dependent variable and time was modeled as a fixed effect. Patient data were retrieved from medical records following standard ethical guidelines.

Out of 806 included COVID-19 patients, 64.1% were male (median age 60.1 years, range 0.1-94.7 years) and 8.9% of patients died. Furthermore, 50.1% of patients were admitted during the second pandemic wave (October 2020 – January 2021 – Wuhan variant) and 49.9% of patients were admitted during the third pandemic wave (March 2021 – July 2021 – Alpha variant). Additionally, 16.7% of patients were vaccinated. When considering disease severity, 37.9% of patients had mild COVID-19, 14.1% of patients presented with moderate disease severity, 34.5% of patients had severe COVID-19 and 10.8% of patients presented with critical COVID-19. We observed a significant difference in IgG galactosylation in patients with different disease severity ($p < 0.001$) and outcome ($p < 0.001$). Patients with severe and critical disease and patients with fatal outcome exhibited a sharp decline in IgG galactosylation levels, while patients with mild and moderate disease and surviving patients exhibited constant IgG galactosylation levels. We also observed constant IgG fucosylation, with an increase in average fucose levels after the 15th day of disease. Patients with fatal outcome exhibited a higher increase in IgG fucosylation levels, but this difference was not statistically significant ($p = 0.078$). Syalization levels of IgG were relatively stable during the disease course, with no significant differences between patients with different disease severity and outcomes ($p > 0.05$). Finally, we observed a decline in the presence of bisecting N-acetylglucosamine during the disease course that was larger in patients with fatal outcome. However, this difference was not statistically significant ($p = 0.078$). We also analysed IgG glycosylation in vaccinated and unvaccinated patients, as well as in patients hospitalized during the second and third pandemic wave. However, no significant differences were found ($p > 0.05$).

The recorded changes in galactose and bisecting N-acetylglucosamine levels in total IgG during COVID-19 suggest a shift to proinflammatory IgG function in patients with severe COVID-19. However, further research on specific anti-SARS-CoV-2 IgG glycome is required to better understand the implications of IgG glycosylation dynamics in COVID-19.

Molecular detection of carbapenemase in uropathogenic strains of *Klebsiella pneumoniae*

Vladimira Tičić[1], Elizabeta Jakovac[3], Lucija Vlahek[1], Maja Anušić[1], Jasmina Vraneš[1,2]

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Abastract:

Introduction: Multidrug-resistant and extensively drug-resistant uropathogens are increasingly common causes of urinary tract infections (UTI), especially in hospital and nursing home patients. In addition to β-lactamases, these strains also produce carbapenemases, which significantly complicates therapy. The aim of this retrospective study was to compare the carbapenemase production of *Klebsiella pneumoniae* isolated from the urine of patients with UTI in two periods over two years.

Methods: *K. pneumoniae* strains isolated from the urine of outpatients in Zagreb over an 11-month period were analyzed from July 15 to December 31, 2022, and from January 1 to June 15, 2023. K. pneumoniae was identified by the mass spectrometry method (MALDI-TOF). Strain sensitivity to antibiotics and carbapenemase production were tested according to current European guidelines (EUCAST). The bacterium *K. pneumoniae* is considered causative of urinary system infection when isolated at a count of ≥ 104 CFU/ml. The type of carbapenemases was determined by the loop mediated isothermal amplification method (LAMP).

Results: During the 2022/2023 observation periods, *K. pneumoniae* was detected in a total of 1636 patients (M:F=1:3.26). Carbapenemases OXA-48 production (9.41%) was detected in 152 strains, while carbapenemases producing strain OXA-181 was detected in only five patients. Of the total 1252 women in whom *K. pneumoniae* was identified as the cause of UTI, the OXA-48-producing strain was isolated in only 6/86 women aged 65 years or younger; all others were older women ($p < 0.01$). In contrast, no statistically significant difference in the production of OXA-48 was found in 384 men in whom UTI was caused by *K. pneumoniae,* and this carbapenemase was detected in 11 younger and 55 older men over 65 years of age ($p > 0.05$). There was no statistically significant difference between the detection of carbapenemases in the two observed periods, regardless of patient sex or age ($p > 0.05$).

CONCLUSION: *K. pneumoniae* strains producing the carbapenemase OXA-48 were significantly more likely to cause UTI in women over 65 years of age, whereas there were no significant age-related differences in men, as uncomplicated UTI are rare in younger men and complicated infections are usually recurrent and caused by resistant bacterial strains. A high prevalence of *K. pneumoniae* strains producing the carbapenemase OXA-48 was observed, especially in women older than 65 years.

KEYWORDS: *Klebsiella pneumoniae*; OXA-48; urinary tract infection

Title:

ApoE Gene Polymorphism in Patients Suspected of Dementia, Cardiovascular and Cerebrovascular Diseases at the University Hospital Centre Osijek

Author(s): **Name Surname[1]**

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Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

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Abstract:

Introduction: Apolipoprotein E (ApoE) is synthesised in various tissues, mainly in the liver and brain. ApoE is an essential component of plasma lipoproteins that plays a role in cholesterol and triglyceride homeostasis. Besides, it has an antioxidant and inflammation modulatory properties. The ApoE polypeptide is encoded by the ApoE gene located on chromosome 19 and consists of 4 exons spanning 3.7 kb in lenght. There are three allelic variants of the ApoE gene, designated E2, E3 (wild type) and E4, defined by SNPs rs429358 and rs7412. Several population-based studies suggest that ApoE allele distribution depends on ethnicity and varies with latitude. Allele E3 is the most common allele with the frequency of 75%-80% in healthy population, while E2 and E4 are relatively rare. The ApoE4 allelic isoform is associated with elevated cholesterol levels which contributes to an increased risk of cardiovascular and cerebrovascular diseases, Alzheimer's disease and other dementias.
Aim: The aim of this study was to determine the frequency of genotypes in patients referred for ApoE gene testing performed in the Laboratory of Molecular and HLA Diagnostics at the Clinical Institute of Transfusion Medicine, University Hospital Centre Osijek from 2017 to 2023.
Methods: The study included 241 patients (116 males, 125 females). Genomic DNA was isolated from peripheral whole blood using the „QIAamp® DNA Mini and Blood Mini Kit“ (Qiagen, Germany). The ApoE genotypes were determined by „LightMix Kit ApoE C112R R158C“ (TIB Molbiol, Berlin, Germany). Genotyping was performed using Light Cycler 480II (Roche Life Sciences, Mannheim, Germany) real-time polymerase chain reaction technology.
Results: In the studied group of 241 patients, the most prevalent ApoE genotype was E3/E3 (68.46%), followed by E3/E4 (18.67%), E2/E3 (9.13%), E4/E4 (1.66%), E2/E4 (1.24%) and E2/E2 (0.83%).
Conclusion: This study showed that dominant genotype was E3/E3. We did not observe a statistical difference between the genotype frequencies with regard to gender.

KEY WORDS: ApoE; Genotyping Techniques, Real-Time PCR

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

Wednesday September 13

Dubrovnik Summer School on Molecular Biosciences in Medicine with
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The Abstract Form

Title:

EPIDEMIOLOGY OF CERVIX CANCER IN ZADAR COUNTY

Author(s): **Name Surname[1]**

Petar Krešimir Lozo, MD

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author**

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Abstract:

Aim: The aim of the work was to evaluate the appearance and specific epidemiological features of cervix cancer of the Zadar County population from 2007 to 2016 and the possible differences with reference to other counties in Croatia.
Material and methods: The patient source of data were aggregated data from the Health-Statistics Chronicles of Zadar and Istria counties, Croatian Health Care Institute and Cancer Register. The Statistics State Institute was the source of data on the population. Standardized rates were calculated according to the 2013 European Standard for Population (ESP 2013).
Results: In the period from 2007 to 2016 Zadar County had a total of 537 cervix cancer patients. The median annual morbidity rate, which represented an approximate value of cervix cancer prevalence rate for Zadar County during the same period, was 62.17/100,000. New cases of cervix cancer in ZC from 2007 to 2014 amounted to a total of 99. The annual average is 12 new cases. The average annual age standardizes rate of new cases is 14.48/100,000. Starting from the age group 25-29 years in all the five-year-period up to the group of 85 years of age and more, new cases of cervix cancer have been registered. The youngest age of new cases was from 25 to 29 years, and the oldest was of 85 years and more. The highest number and the highest age-specific incidence rate were in the later fertile age from 40 to 49 years with 33 new cases which made up 1/3 of all new cases. A total of 31 patients died of cervix cancer in ZC in the 2007-2016 period. An average of 3 patients died each year. The rough average mortality rate of cervix cancer in ZC was 3.60/100,000. The highest number of deaths in one year was 5 deaths (years 2009 and 2015). There was no mortality result in year 2016. None of the cervix cancer deaths were under the age of 34. The youngest cervix cancer death was in the average fertile age, the age group of 35-39 years, with 2 deaths. The highest number of deaths according to age was 5 deaths in the age group of 40-44 years. In the fertile period of 15-49 years of age, there were 10 deaths, while in the post-fertile period of 50-64 years of age there were 11 deaths. In the age ? 65 there were 10 deaths. 2/3 (21) of the 31 deceased belong to the category of early death age prior to 65 years of age. The highest rough age-specific mortality rate was in the age group of 80-84 years (11.15/100,000). The 40-45 years of age group followed (9.25/100,000). The lowest rough age-specific mortality rate was in the age group of 35-39 years (3.65/100,000).
Conclusion: The results of this study have shown that in ZC, during the period from 2007 to 2016, the highest number and highest age-specific rate incidence of cervix cancer was in the late fertile age of 40-49 years. In that same period, a total of 31 ZC inhabitants died of cervix cancer, which, according to contemporary health protection standards in developed countries, should not have died.

Association of serum vitamin D level with disease activity, applied therapy and bone density in patients with axial and peripheral spondyloarthritis.

Mirna Kalombo Mbuyi[1], Simeon Grazio[2], Hana Skala Kavangh[2], Frane Grubišić[2], Ines Doko Vajdić[2]

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Abstract:

Spondyloarthritis (SpA) or spondyloarthropathies form a group of inflammatory rheumatic diseases that share some common genetic, clinical, serological, radiological and prognostic features. The international organization ASAS created classification criteria for SpA, dividing them according to dominant involvement into axial (axSpA) and peripheral (pSpA). The etiology of SpA is believed to be the result of the interplay of genetic and environmental factors as well as the microbiome and biomechanical stress, i.e. the body's abnormal immune response to infection through changes in the IL-12, IL-23 and Th17 axis. An association with the HLA-B27 locus was observed. Treatment modalities for SpA include physiotherapy, nonsteroidal anti-inflammatory drugs (NSAIR), conventional, targeted synthetic or disease-modifying biologic antirheumatic drugs (csDMARDs, tsDMARDs, and bDMARDs), including methotrexate and sulfasalazine for peripheral arthritis, and anti-TNF and IL-17A. Furthermore, osteoporosis is considered a frequent feature of inflammatory rheumatic diseases. Since the role of vitamin D in the regulation of the immune response and autoimmunity itself is known, the question arises whether vitamin D deficiency can lead to increased disease activity in spondyloarthritis. In this research, we observed the prevalence of vitamin D deficiency in patients with axSpA and pSpA, to determine whether there is a relationship between vitamin D deficiency and disease activity, treatment modality (NSAIDs, csDMARDs, tsDMARDs or bDMARDs) and reduced bone mineralization. We observed patients treated at the Clinic for Rheumatology, Physical Medicine and Rehabilitation, Sestre Milosrdnice University Hospital Center, in the period from 1st of January 2021 to 1st of June 2023, who were diagnosed with axial or peripheral spondyloarthritis according to the criteria of the ASAS classification. Patient history, physical examination and laboratory data from Archive of the Clinic were used. Socio-demographic, anthropometric data and those on habits were collected: age, gender and smoking of tobacco products. Also, the season of the examination, vitamin D supplementation, and SpA treatment modality were observed. As indicators of functional ability and disease activity in axSpA, standardized questionnaires were used: BASFI, BASDAI, ASDAS-CRP, VAS global assessment of disease activity, HAQ, MASES index, number of painful and swollen joints in pSpA cases, and laboratory indicators such as CRP and ESR levels. We also observed serum concentration of 25(OH)D as a metabolite of vitamin D, PTH, calcium and phosphates. Bone mineral density was measured by skeletal densitometry. The results may suggest the need of implementing vitamin D food fortification in Croatia, practice seen in countries such as Finland.

Hyperglycemia in patients with acute stroke

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Abstract:

Introduction: hyperglycemia often occurs in the early phase of acute stroke, in about 20 to 40 percent of patients (1), and is associated with greater brain damage, worse outcome, higher mortality rate and lenght of hospitalization. High blood glucose during ischemia can lead to membrane lipid peroxidation and cell lysis in damaged tissue through anaerobic metabolism, lactic acidosis and the formation of free radicals (2). Objective: to determine whether there is a link between hyperglycemia and acute stroke in out-of-hospital patients regardless of the presence or absence of diabetes mellitus in personal medical history and regardless of the time of the last meal. Methods: the study was based on data from the eHitna system and includes age, gender and blood glucose level in out-of-hospital patients who were diagnosed with an acute stroke and transported to the Emergency Department of General Hospital Dr.Tomislav Bardek in Koprivnica. The study included 114 patients over a one-year period in Koprivnica-Križevci County. Patients were divided into two groups; blood glucose level under 10 mmol/L- group 1, blood glucose level higher than 10 mmol/L- group 2. Results: the average blood glucose level was 8,2 mmol/L; in group 1 it was 6,9 mmol/L, in group 2 13,2 mmol/L. 20,2 percent of out-of-hospital patients had blood glucose level of more than 10mmol/L, which corresponds to previous studies of the connection between hyperglycemia and acute stroke. Conclusion: in addition to adequate glycemic control in the acute phase of the stroke (insulin therapy), preventive measures are extremely important, with which patients would change their lifestyle and thereby prevent significant tissue and organ damage (stroke, myocardial infarction etc).

Key words: hyperglycemia, acute stroke, blood glucose level, free radicals

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1. Kiers L et al. Stroke topography and outcome in relation to hyperglycemia and diabetes. J Neurol Neurosurg Psychiatry. 1992;55:263-270.

2. Lindsberg PJ. Hyperglycemia in Acute Stroke. Stoke. 2004;35(2):363-364.

Title: Inflammatory parameters and albumin concentration in major burns - initial values at the admission

Author(s):

Mirela Dobrić[1], Ana Miletić[1], Jure Pavešić[2], Ana Čizmić[3], Biserka Orehovec[4]

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Abstract:

Burn injuries are caused by thermal, electrical or chemical energy. A burn greater than 20% of total body surface area (TBSA) is considered major in an adult. One of the most important feature in the early stage of burns is a strong immune and stress response. Major burns trigger a pro-inflammatory immune response in the peripheral blood and affected tissues. The hyperinflammatory state is accompanied by increase in the number of immune cells and serum concentrations of inflammatory mediators, cytokines and acute-phase proteins. Hypermetabolic phase usually starts from 48h and may last up to one year. It is characterised by increase in concentrations of catecholamines, cortisol and glucagon, decreased insulin release, increased hepatic gluconeogenesis, lypolysis, glycogenolysis and proteolyses. Among the most important biomarkers are cytokines and growth factors, acute-phase proteins, metalloproteinases, reactive oxygen species, nitric oxide and parameters of the hemostasis.

Majority of patients with major burns in our Burn Unit had sepsis, prolonged hospital stay and high mortality rate. The laboratory data and clinical parameters were available from the hospital information system. The retrospective study includes 20 adult patients with major burns burned treated from January 2018 to January 2023. Patients with severe burns that occurred more than 24 hours before admission to the ward, as well as those with chronic kidney disease, malignant disease or inflammatory bowel disease, were excluded. The research aims to determine the initial laboratory values of inflammatory parameters at the admission of severely burned patients (leukocytes, C-reactive protein, procalcitonin, fibrinogen) and albumin. A secondary goal is to determine the association of laboratory values of inflammatory parameters and albumin at admission with the number of hospital days, the development of sepsis and mortality. The values of C-reactive protein, procalcitonin, WBC, fibrinogen and albumin at admission, D0, and on days 5 and 10, D5 and D10, were evaluated.

C-reactive protein is one of the markers of the acute phase of inflammatory processes, correlates with the severity of inflammation and indicates tissue damage. Laboratory values of C-reactive protein were normal or slightly elevated with an increase on the fifth and tenth days. In the majority of patients wound infection, urinary or respiratory tract infection and bacteriemia was subsequently proven by microbiological methods.

Procalcitonin is a known biomarker of the systemic inflammatory response and an important predictor of sepsis. Procalcitonin proved to be a good predictor of the development of sepsis in our study. At admission, most patients had normal procalcitonin values, and elevated values on the 5th and 10th days. Sepsis rarely occurred within the first 7 days of the injury, more often a week or weeks after the injury.

The WBC on admission was elevated in all patients, with a significant increase in the population of neutrophils in mature and immature forms.

The concentration of fibrinogen is elevated after trauma and surgical procedures, in sepsis and malignant diseases. In all patients, fibrinogen was elevated on admission, with an increase on days 5 and 10.

Significantly reduced serum albumin concentration on admission was found in patients with deep burns (3rd-degree burns) and patients with more than 20% of the body surface affected. Patients with very low serum albumin concentration, < 20 g/L, more often had multiorgan failure and death.

Thursday September 14

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The Abstract Form

Title:

Lipid Status and Obesity via Arg72Pro TP53 Genotypes Linked with Oxidative Stress in a Sample of People Aged 85 Years and Older in Croatia

Author(s): **Name Surname[1]**

Goran Slivšek1,2, Maja Šetinc3, Sandra Mijač1,2,4, Vesna Bišof1,2, Tatjana Škarić-Jurić3

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author.**

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2 Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
3 Institute for Anthropological Research, Zagreb, Croatia
4 Children's Hospital Srebrnjak, Zagreb, Croatia

Abstract:

Background: The tumour protein P53 gene (TP53) encodes the eponymous protein that responds to diverse cellular stresses to regulate the expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism, and it is often mutated in human cancers. Due to its tumour-suppressing functions, it plays an important role in the stability of the genome and is often referred to as the guardian of the genome. In response to oxidative stress, TP53 activates the transcription of many genes involved in the regulation of oxidative stress that can lead to oxidative damage to DNA, proteins and lipids. The Arg72Pro mutation—a missense variant in which arginine is replaced by proline at codon position 72 of exon 4—is a common human single nucleotide polymorphism (SNP) of the TP53 gene (rs1042522). Studies have investigated the association between this genetic variation and susceptibility to cancer and found a protective effect of the Pro-allele. Since TP53 regulates the entry of cells into senescence, it also plays a role in maintaining the proliferative capacity of cells throughout human life. Therefore, it is unsurprising that some recent studies point to an association between this locus and longevity (Šetinc et al., 2023).
Aim: To investigate the association of SNP rs1042522 with lipid, glucose status and anthropometric indicators of obesity linked to oxidative stress in the older adults Croatian sample.
Methods: The SNP rs1042522 of the TP53 gene was genotyped in a Croatian sample of the oldest old (85+ years, N = 314; CSF project IP-01-2018-2497). The association between their lipid status (LDL, HDL, LDL, VLDL, total cholesterol, triglycerides), fasting glucose and anthropometric indices of obesity and SNP rs1042522 genotypes was tested.
Results: Individuals with the ProPro genotype have significantly higher mean HDL values than those with ArgPro or ArgArg (1.6 vs 1.4 and 1.3 mmol/L), according to ANOVA results ($p = 0.009$). The ProPro genotype is significantly more common in individuals with lower mean values of body weight ($p = 0.010$), hip circumference ($p = 0.023$), upper arm circumference ($p = 0.031$) and body mass index ($p = 0.040$). The same trend was demonstrated when both genotypes containing the Pro allele were combined (ProPro and ArgPro), and a t-test was performed: Individuals with the ArgArg genotype had significantly lower mean values for HDL (1.3 vs 1.4 mmol/L; $p = 0.016$) and higher values for glucose (7.3 vs 6.6 mmol/L; $p = 0.030$), subscapular skinfold (18.67 vs 16.85 mm; $p = 0.038$), hip circumference (107.39 vs 104.71 cm; $p = 0.012$), waist circumference (95.15 vs 92.51 cm; $p = 0.045$), body mass index (28.19 vs 27.00 kg/m2; $p = 0.028$) and body weight (69.44 vs 65.56 kg; $p = 0.014$).
Conclusion: The results suggest an association of the most common ArgArg genotype with some cardiovascular disease risk factors, especially blood lipids and the obesity phenotype linked to oxidative stress.

Keywords: cardiovascular diseases, lipids, obesity, oldest old, reactive oxygen species, SNPs, TP53 gene

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary.**

Šetinc, Maja et al. Genetic Scores for Predicting Longevity in the Croatian Oldest-old Population. PLoS One. 2023 Feb; 18(2): 1-22. DOI: 10.1371/journal.pone.0279971.

Dubrovnik Summer School on Molecular Biosciences in Medicine with
The International Oxidative Stress Symposium 2023

The Abstract Form

Title:

Single Nucleotide Polymorphism rs2706372 and Cardiovascular Risk Factors Linked with Oxidative Stress in the Population 85 Years and Older

Author(s): **Name Surname[1]**

Goran Slivšek1, Matea Zajc Petranović2, Maja Šetinc2, Sandra Mijač1,3, Tatjana Škarić-Jurić2

Institution(s): **Following the order of authors by numbers as above, email of the corresponding author.**

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2 Institute for Anthropological Research, Zagreb, Croatia
3 Children's Hospital Srebrnjak, Zagreb, Croatia

Abstract:

Background: The DNA double-strand break repair protein gene (RAD50), which encodes the protein of the same name, is primarily involved in repairing DNA double-strand breaks and contributes to maintaining genome stability via the MRN complex. Studies suggest that it also plays a role in other cellular processes, such as response to oxidative stress, which occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the cell's ability to detoxify or repair the damage. This can lead to oxidative damage to DNA, proteins and lipids, resulting in cellular dysfunctions and the development of disease. Although RAD50 single nucleotide polymorphisms (SNPs) are usually associated with increased susceptibility to some diseases, the T-allele of SNP rs2706372, located in the extended RAD50/IL13 region on chromosome 5q31.1, has also been related to human longevity (Flachsbart et al., 2016).
Aim: To investigate the possible association between SNP rs2706372 and cardiovascular diseases (CVDs) and cardiovascular (CV) risk factors linked to oxidative stress in long-lived individuals.
Methods: The SNP rs2706372 of the RAD50 gene was genotyped in a Croatian older adults sample (85+ years, N = 314; CSF project IP-01-2018-2497). The association between their CVD status and SNP rs2706372 genotypes was tested using original and categorised variables (health-related answers, anthropometry, blood pressure measurements, blood glucose and lipid levels).
Results: Individuals with the SNP rs2706372 TT genotype have significantly higher mean values for VLDL ($p = 0.020$) and triglycerides ($p = 0.023$) than those with other genotypes. They also have higher mean values for obesity-related traits: Waist-to-height ratio ($p = 0.011$), subscapular skinfold ($p = 0.035$) and waist circumference ($p = 0.038$). Accordingly, the sex-specific values in the upper quartile ($p = 0.010$) and above the median ($p = 0.037$) of the waist-to-height ratio are also more common in those with genotype TT. When two less common genotypes are combined, individuals with the most common CC genotype are more prone to hypertension ($p = 0.037$) and familial hypertension ($p = 0.014$). They are also more likely to take acetylsalicylic acid regularly for thromboprophylaxis ($p = 0.043$), have a higher risk of stroke ($p = 0.057$) and exhibit above median blood glucose levels ($p = 0.087$). Individuals with the genotype CC have higher mean height values (157.4 vs 54.9 cm; $p = 0.019$), and the chi-square test showed the same association for sex-specific values of height above the median ($p = 0.004$) and fourth quartile ($p = 0.001$).
Conclusion: The most common SNP rs2706372 genotype CC in the older adults appears to be associated with several CVDs, but the longevity-related TT genotype is also more likely to be associated with some CV risk factors related to oxidative stress. The results suggest a complex and diverse impact of the SNP rs2706372 genotypes across the lifespan.

Keywords: cardiovascular diseases, DNA repair enzymes, lipids, oldest old, reactive oxygen species, SNPs

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary.**

Flachsbart, Friederike et al. Immunochip Analysis Identifies Association of the RAD50/IL13 Region with Human Longevity. Aging Cell. 2016 Jun; 15(3): 585-8. DOI: 10.1111/acel.12471.

The Abstract Form

Title:

Postoperative values of miR-151 in the patients undergoing elective cardiovascular surgery

Author(s): **Name Surname[1]**

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Abstract:

Background: miR-151 was found to correlate with neurological impairment (1). The aim of this work is to confirm whether there are a changes in the miR-151 levels after cardiac surgery under general anesthesia.
Material and methods. In the group of 37 patients undergoing on-pump coronary artery bypass grafting (CABG), blood samples were taken from peripheral blood before, 1 hour after surgery and 24 hours later in the ICU. All surgical procedures were done in balanced general anesthesia using inhalation anesthetics, sufentanil, and rocuronium.
RNA isolation. Total RNA was extracted from 300 µL of serum using Macherey-NagelTM NucleoSpin miRNA Plasma kit (Fisher Scientific, Göteborg, Sweden) according to the manufacturer's instructions. RNA was eluted by adding 30 µL of RNase-free water. The concentration and purity of RNA were determined using a SpectraMax QuickDrop spectrophotometer (Molecular Devices, San Jose, CA USA).
cDNA synthesis and real-time quantitative PCR analysis. Expression of the miR155 and miR16 was analysed using TaqMan microRNA assay (Applied Biosystems, Carlsbad, USA). 10 ng of RNA was reverse transcribed in 15 µL reactions using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Carlsbad, USA) according to the manufacturer's instructions. Complementary DNA was processed in quantitative PCR with the TaqMan Universal PCR Master Mix, no AmpErase UNG (Applied Biosystems). Levels of miR151 were calculated relative to reference miR16 using the 2 method. The qPCR amplification was performed in a LightCycler 480II real-time PCR System (Roche, Basel, Switzerland).
Results: Postoperative values of miR-151 were significantly lower compared to baseline (0.45 [0.28-0.86, $P<0.001$), while after 24 hours there was a statistically significant increase compared to initial values and values measured 1 hour after surgery (1.23 [0.67-1.68], $P<0.001$).
Conclusion: The changes in circulating miR-151 values observed in this study may be related to neurological damage, which was frequently observed in the patients after CABG. In order to confirm this possibility, it is necessary to correlate these values with the neurological outcome and cognitive tests in each individual patient.

References (not necessary): **Any style or format, first author only et al., corresponding in-text citation is necessary**

Reference: Liang J, et al. MicroRNA profiling of different exercise interventions for alleviating skeletal muscle atrophy in naturally aging rats. J Cachexia Sarcopenia Muscle. 2023;14(1):356-368. doi:10.1002/jcsm.13137

Dubrovnik Summer School on Molecular Biosciences in Medicine with
The International Oxidative Stress Symposium 2023
The Abstract Form

Title:

EFFECTS OF 5G RADIOFREQUENCY ELECTROMAGNETIC RADIATION ON ANTIOXIDANT STATUS OF IN VITRO EXPOSED SEMEN OF BREEDING BOARS

Author(s): Name Surname[1]

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Abstract:

The majority of research has focused on the negative impact of radiofrequency electromagnetic radiation (RF-EMR), emitted by various devices (mobile phones and laptops, and wireless internet technologies such as Wi-Fi and 5G routers), on the human reproductive system. RF-EMR, in addition to leading to excessive reactive oxygen species generation or oxidative stress, can also cause a change in the activity of antioxidant enzymes in semen. Data on the effect of RF-EMR on the reproductive system of male domestic animals are scarce. Also, so far there is no data on the effect of RF-EMR on antioxidant status indicators in vitro exposed semen of breeding boars. The aim of this study was to investigate the effect of RF-EMR of the 5G frequencies on antioxidant status indicators of in vitro short-term exposed semen of breeding boars.

The research was performed on semen samples of 8 breeding boars of the pietren breed and 4 breeding boars of the German Landrace breed, aged from 1.5 to 3.5 years. Sampling and exposure of semen were carried out in three periods in the winter. Freshly diluted semen of each boar and each period was divided into a control sample (n = 12) and a test sample (n = 12) that was exposed. The test samples were exposed to continuous RF-EMR at three different 5G frequencies (700, 2500 and 3500 MHz) at a field level of 10 V / m for 2 hours using gigahertz transverse electromagnetic (GTEM) chamber. In addition to the GTEM exposure chamber, an HP 8657A signal generator and an RFGA0101-05 linear amplifier were used to obtain electromagnetic field level. The test and control samples after exposure were centrifuged and the supernatants were removed afterwards. The remaining spermatozoa were washed three times with saline and preserved to - 80 °C until analyzed. After thawing the spermatozoa samples, spermatozoa lysates were prepared. The activities of glutathione reductase, glutathione peroxidase (GSH-Px), total superoxide dismutase and the concentration of total antioxidant status and malondialdehyde were determined in the spermatozoa supernatants lysates.

GSH-Px activity was not measurable in the supernatants of spermatozoa lysates of control and test semen samples. The values of malondialdehyde and all antioxidant status indicators investigated except GSH-Px did not differ significantly between control and test boar semen samples.

GSH-Px activity was not measurable in the boar spermatozoa lysates, probably due to a very poor enzymatic antioxidant protection system in the boars, which is probably even lower in the winter. RF-EMR at 5G frequencies has no significant effect on value of spermatozoa antioxidant status indicators after in vitro (2 h) exposed semen of breeding boars. The reason probably lie in the diluent added to the semen, which may contain additives with positive properties on the spermatozoa survival and motility and their oxidative status.

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