

UNDER THE AUSPICES OF

European Society of Pathology

Croatian Academy of Medical Sciences

Ministry of Science, Education and Sports of the Republic of Croatia - MZOS

Institute for Clinical Medical Research of Clinical Hospital Center Sestre Milosrdnice, Zagreb

City of Zagreb - City Office for Health

Ljudevit Jurak



27th Ljudevit Jurak

International Symposium
on Comparative Pathology
with One Health Session

FINAL PROGRAM & BOOK OF ABSTRACTS

May 31st and June 1st, 2019, Zagreb, Croatia

University Clinical Hospital Center Sestre milosrdnice

Dear colleagues,

The Organizing Committee cordially invites you to Zagreb to take part in the

**27th Ljudevit Jurak International Symposium on Comparative Pathology
with One Health Session**

This symposium summons international collaboration and involves an array of professionals within the field of pathology. What separates and defines this symposium is its collaboration with veterinary pathologists, and furthermore, comparison of pathologic changes in human and veterinary medicine. With this symposium we honour Professor Ljudevit Jurak, who founded the first pathology department in Croatia, in Sestre milosrdnice University Hospital Center. Professor Ljudevit Jurak contributed greatly to Veterinary and Human Pathology as well as Forensic Medicine and represents an historical and eminent figure in the field of pathology both in Croatia and worldwide (<http://www.kbcsm.hr/jurak/>).

The main topic of this Symposium is **NEW APPROACHES IN IMMUNOLOGY OF TUMORS AND OTHER DISEASES.**

All pathologists dealing with Human and Veterinary Pathology as well as Forensic Pathologists are invited to participate and support this multidisciplinary meeting. We are thrilled to call upon all professionals within the field of Pathology and Forensic Medicine to attend and engage in a variety of discussions, social gathering and enjoyment.

This year, for the first time, One Health Session will be also included in the program of the Symposium. The One Health concept is a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment (<http://www.onehealthinitiative.com/index.php>). Therefore, we are expanding our invitation at this point to all medical disciplines and other professions interested in sharing their interest in the ONE HEALTH arena at the 2nd day of the Symposium. The variety of topics presented by the international invited speakers will provide a framework for contributions of other participants.

Organizing Committee

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FRIDAY MAY 31st, 2019

**27TH LJUDEVIT JURAK INTERNATIONAL SYMPOSIUM ON
COMPARATIVE PATHOLOGY**

08:00 Registration

08:30 Opening ceremony

INVITED LECTURES

Chairpersons: Božo Krušlin, Hrvoje Čupić

09:00 George J. Netto (USA)
Immunogenomics on RCC
Discussion

09:40 Anna Yemelyanova (USA)
Molecular Diagnostics in Gynecologic Oncology
Discussion

10:20 **Coffee break**

Chairpersons: George J. Netto, Suzana Tkalčić

10:40 Luka Brčić (AUT)
The role of pathology in the era of immunotherapy
Discussion

11:10 Sanja Pleština (CRO)
Immunotherapy of lung cancer
Discussion

11:40 Donna Shettko (USA)
Interspecies Respiratory Disease Transmission: from humans to gorillas
Discussion

12:10 **Coffee break**

Chairpersons: Donna Shettko, Marina Kos

12:30 Suzana Tkalčić (USA)
Applied biomedical sciences: novel experimental approaches to tumor biopsy
Discussion

13:00 Elizabeth F. Schilling (USA)
Periodontal disease in veterinary and human patients
Discussion

13:30 Lunch

14:30 General Assembly of Croatian Society of Pathology and Forensic Medicine

Chairpersons: Marina Kos, Božo Krušlin

14:50 George J. Netto (USA)
Immunogenomics on bladder cancer
Discussion

15:30 Anna Yemelyanova (USA)
HPV-related Lesions of Gynecologic Tract: Current Challenges, Biomarkers, New directions
Discussion

16:10 Short break

Chairpersons: Suzana Tkalčić, Elizabeth F. Schilling

16:20 Fabio Del Piero (USA)
Comparative Pathobiology as Diagnostic and Research Tool for Advancements in Animal and Human Health
Discussion

17:00 Ivan-Conrado Šoštarčić-Zuckermann (CRO)
Prognostic factors of canine lymphomas and mast cell tumors
Discussion

20:30 Symposium dinner

SATURDAY JUNE 1st, 2019

INVITED LECTURES

Chairpersons: Suzana Tkalčić, Boris Habrun

- 08:30** Introduction
- 09:00** Tracey S. McNamara (USA)
One Health agenda: disease X
Discussion
- 09:30** Ante Cvitković (CRO)
One Health Symposium Slavonski Brod 2014
Discussion
- 09:50** Aleksandar Džakula (CRO)
Is the "One health" concept relevant for Croatia?
Discussion
- 10:10** John Tegzes (USA)
Novel and affordable antivenom on our doorstep
Discussion

10:40 **Coffee break**

Chairpersons: Tracey S. McNamara, Ante Cvitković

- 11:00** Boris Habrun (CRO)
CVI and One Health
Discussion
- 11:20** Pedro P. Diniz (USA)
Tick-borne diseases and their tricks
Discussion
- 11:50** Zdenko Sonicki (CRO)
Public Health and disease surveillance
Discussion
- 12:10** Diane McClure (USA)
Rethinking biosecurity with a one health perspective: design and assessment
Discussion
- 12:40** Suzana Tkalčić One Health Award and Closing ceremony

13:00 **Sightseeing trip to Zagreb surroundings**



kape | maske | ogrtači | kombinezoni | rukavci | navlake za cipele |
trljačice | brisači | sterilne prekrivke | sterilne navlake | sterilni setovi

STVARAMO DA ŠTITI!



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Lectures

IMMUNOGENOMICS ON RCC AND BLADDER CANCER

George J. Netto, M.D.

Professor and Chair of Pathology
The University of Alabama at Birmingham
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Bladder:

Two TCGA studies of bladder cancer have been completed that support a novel molecular taxonomy for MIBC. Stratified based on genetic, epigenetic and proteomic signature five molecular subclasses were identified: luminal papillary (35%), luminal infiltrated (19%), luminal (6%) and basal/squamous (35%), and neuronal (5%). The luminal-infiltrated and the basal/squamous subtypes show high levels of immune-expression signature and therefore tend to benefit most from treatment with checkpoint inhibitors. The luminal-papillary subtype displays FGFR3 activation (mutations, amplifications or overexpression) in 44% of the cases making them a target for treatment with FGFR3 inhibitors. While the luminal-papillary and luminal-infiltrated subclass tend to be resistant to chemotherapy, patients with tumors of the basal/squamous, and neuronal subclass are more likely to benefit for neoadjuvant chemotherapy.

The FDA has recently approved checkpoint inhibitor therapy (atezolizumab, nivolumab, pembrolizumab, durvalumab, and avelumab) for patients with locally advanced or metastatic disease post-platinum treatment. In the first line setting checkpoint inhibitors are only approved for platinum-ineligible patients. Further, the FGFR inhibitor erdafitinib was recently approved as a targeted therapy approach for selected post-platinum patients with somatic FGFR2/3 alterations.

Kidney:

Renal cell carcinoma (RCC) is a heterogeneous disease with clear cell RCC (ccRCC) being the most common histological variant. Once metastatic, RCC is challenging to treat as it is mostly unresponsive to conventional chemo- and radiotherapy. In ccRCC the von Hippel Lindau (VHL) tumor suppressor gene is frequently inactivated leading to overexpression of HIF-2 α and its downstream targets like VEGF. Tyrosine kinase inhibitors (TKI) are antiangiogenic therapeutic agents that target the VEGF pathway. Furthermore, renal cell carcinoma is an immunogenic tumor and is associated with innate host-mediated immune response. Therefore, in clinically advanced setting TKIs (e.g. axitinib, pazopanib and sunitib) and checkpoint inhibitors (e.g. ipilimumab, nivolumab, and pembrolizumab) are recommended treatment options for ccRCC. In patient with intermediate and poor risk, a combination of CTLA-4 inhibitor and PD-1 inhibitor (ipilimumab + pembrolizumab) was recently approved by the FDA. In non-clear cell RCC the preferred regimen remains TKI agent sunitinib. On-going clinical trials investigating immune therapy in non-clear cell histologic variants are forthcoming.

MOLECULAR DIAGNOSTICS IN GYNECOLOGIC ONCOLOGY

Dr. Anna Yemelyanova

Professor of Pathology, Director of Gynecologic Pathology Section, and an Associate Director of the Genomic Diagnostics and Bioinformatics Division at the Department of Pathology of the University of Alabama at Birmingham (UAB).

Molecular testing is becoming increasingly important in gynecologic oncology. Advances in sequencing technology has allowed for rapid discovery in genomics. Morphological classifications of gynecologic tract tumors that have been in use for decades are being replaced by newer classifications based on genomic profiles of the tumors. The Tumor Genome Atlas (TCGA) initiative provides strong foundation of knowledge regarding the molecular makeup of tumors of various sites and served as a starting point for further studies.

The discovery of the relationship between BRCA pathway alterations and tubo-ovarian cancer lead to the development of the concept BRCA-ness describing homologous recombination (HR) deficient tumors and supported introduction of PARP-inhibitors therapy into clinical gynecologic oncology practice. The classification of high-grade serous carcinoma of the ovary into four classes based on the expression profiles reproduces by several research groups describe tumors with unique molecular characteristics and different prognosis within the most common and highly aggressive ovarian cancer type.

In endometrial cancer, recognition of mismatch repair (MMR) deficiency in germline and sporadic setting led to implementation of Lynch syndrome screening programs. Furthermore, MSI-high tumors were shown to be responsive to immunotherapy with checkpoint inhibitors. TCGA data provided basis for classification of endometrial carcinomas into four subtypes with distinct molecular and clinical characteristics. These findings have been reproduced in subsequent studies that described algorithms for testing implementation into clinical practice. Further discovery and development of novel molecular targets will allow for truly personalized therapeutic approaches in oncology.

THE ROLE OF PATHOLOGY IN THE ERA OF IMMUNOTHERAPY

Luka Brčić

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Immunotherapy is rapidly becoming more and more important option for different solid tumors. For many years, it has been successfully applied in malignant melanoma, and recently was also implemented in therapy plan for lung carcinoma, urothelial carcinoma, head and neck carcinoma, and many other will soon be included. Having in mind the complexity of immune system, it is not easy completely to understand its effectiveness. However, it has proven to be a real game-changer, in regards of treatment, follow up and prognosis.

Unfortunately, one reliable, stable and reproducible biomarker, predictive for immune-check point inhibitor therapy, has not yet been found. In praxis, immunohistochemical PD-L1 expression on tumor cells and/or on tumor infiltrating immune cells, is used. However, this is not so straightforward, and is coupled with different issues. The main disadvantage is that PD-L1 is changing its expression in time, and can have heterogeneous expression within the same tumor. Furthermore, 5 different PD-L1 clones have been used in different clinical studies, with different cut-off values and different outcomes in regard to the therapy response. Recently, "agnostic" biomarkers for the immunotherapy have been introduced: mismatch repair deficiency /microsatellite instability and tumor mutation burden. Although, PD-L1 and microsatellite instability are the only markers approved by the Food and Drug Administration, analysis of the latter is also not as easy and as clear as expected. Tumor mutation burden has proved as a predictive biomarker for some (but not all) check-point inhibitors. Unfortunately, there are still no cross validation studies, the question what is high and what is low mutation burden is still not answered, analysis is costly and has a rather slow turn-around time.

Finally, the role of pathology in the era of immunotherapy is increasingly demanding. It includes not only introduction of new antibodies, but also completely new and expensive methods, with longer time for analysis and at the end of the day not straightforward results for clinicians.

IMMUNOTHERAPY OF LUNG CANCER

Sanja Pleština

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Lung cancer remains the leading cause of cancer deaths in the Croatia, with non-small cell lung carcinoma (NSCLC) accounting for most cases in men and women. Patients with NSCLC often present at late stages. Chemotherapy and/or radiotherapy have historically served as first-line treatments for advanced disease but have offered limited benefit with low response rates and low overall survival. Immunotherapy have changed the clinical practice of medical oncology bringing revolution in treatment of many different types of malignancies. Immune checkpoint inhibitors like the anti-PD-1 antibodies pembrolizumab and nivolumab, and anti-PD-L1 antibodies atezolizumab and durvalumab, have made significant inroads into the management of patients with advanced lung cancer.

The selection of patients for immunotherapy remains challenging given the lack of highly specific and highly sensitive biomarkers. The prediction of response to immunotherapy is extremely complex. PD-L1 immunohistochemistry (IHC) is the current predictive biomarker used to select patients for immunotherapy treatment. Programmed cell death protein 1 (PD-1) expression assessment is now established in routine practice and the efficacy of immune checkpoint inhibitors relies on the evaluation and scoring of PD-L1 expression by IHC.

New data from clinical trials that led to the approval of nivolumab therapy which compared nivolumab with docetaxel, both used as monotherapy in second-line treatment, show that patients who respond to the drug have better survival in the longer term. More than half of such patients were still alive after 4 years, what is the longest follow-up from phase 3 randomized trials of previously treated advanced nsclc, whereas the historical survival of patients with advanced nsclc has been 5% at five years. First-line pembrolizumab monotherapy improves overall and progression-free survival in patients with untreated metastatic non-small-cell lung cancer with a programmed death ligand 1 (PD-L1) tumour proportion score (TPS) of 50% or greater and without EGFR or ALK mutations comparing with platinum-based doublet chemotherapy treatment. Atezolizumab is a programmed death-ligand 1 (PD-L1) blocking antibody indicated for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose disease progressed during or following platinum-containing chemotherapy and recently in the first-line setting to treat non-squamous non-small cell lung cancer in combination with bevacizumab and chemotherapy.

Approximately one third of patients with non-small-cell lung cancer (NSCLC) have stage III, locally advanced disease at diagnosis. The standard of care for patients with a good performance status and unresectable stage III NSCLC is platinum-based doublet chemotherapy concurrent with radiotherapy (chemoradiotherapy). But, most patients have disease progression despite

definitive chemoradiotherapy. Using anti-programmed death ligand 1 antibody durvalumab as consolidation therapy in patients with stage III NSCLC who did not have disease progression after two or more cycles of platinum-based chemoradiotherapy significantly prolong progression-free survival (PFS). Patients whose tumors were positive for PD-L1 benefit the most.

With widening use of immune checkpoint inhibitors it is critical for clinicians to stay abreast of the unique adverse events associated with these agents. Treatment-related adverse events in patients treated with immune checkpoint inhibitor therapy differ significantly from adverse events of all other previous used systemic treatments. In general, immune checkpoint inhibitor use is associated with a spectrum of immune-related adverse events (irAEs) with the variety of clinical manifestations in multiple organs of the body. They can affect and are most commonly seen in the skin, GI tract, lungs and endocrine system, as well as in the musculoskeletal, renal, nervous, hematologic, cardiovascular and ocular systems. Most patients who are treated with immunotherapy are going to develop some adverse event, but it is usually mild to moderate. Severe irAEs require immediate discontinuation of immune therapy and initiation of systemic corticosteroids.

INTERSPECIES RESPIRATORY DISEASE TRANSMISSION: FROM HUMANS TO GORILLAS

Donna Shettko, DVM, MSN, FNP-C/PA-C

College of Veterinary Medicine, Western University of Health Sciences

Respiratory diseases are the second most common cause of morbidity and mortality among mountain gorillas in the wildlife preserve, showing a steady increase in prevalence over the last 29 years. (Cranfield) This trend may be due to the gorillas increased contact with humans or it may be secondary to improved monitoring and reporting. (Cranfield) A method for early detection of the respiratory disease occurrence or increase in the surrounding human villages can be used to predict when the gorillas may be at increased risk of disease transmission.

Gorillas are susceptible to many of the same infectious diseases that affect people due to the genetic similarity between the two species. Unfortunately, the gorillas are immunologically naïve to the pathogens to which they are exposed. The proximity of the gorillas to the people, which also includes the park rangers and the tourists, increases the likelihood of disease transmission, especially when it comes to respiratory diseases. (Muehlenbein) These close interactions may lead to alterations in animal stress responses, mainly immunosuppression, thus increasing susceptibility to infectious diseases. (Muehlenbein)

Recently, a first virus has been isolated in gorillas with respiratory disease that was linked to human infections. A 2009 study reports two mountain gorillas in Virunga that died of a respiratory infection caused by a human metapneumovirus. (Cranfield) Additional diagnostic testing found that the HMPV and the secondary bacterial pneumonia was related to human strains circulating among people in South Africa, suggesting tourists as a source of infection for gorillas in the park. (Science News)

With the health and welfare of the wild mountain gorillas in jeopardy, interventions must be implemented in order to prevent disease transmission and protect the gorillas. By investigating the pattern of respiratory infections in people that are in close contact with the mountain gorillas and comparing this information to respiratory disease patterns in the gorillas, information about disease transmission patterns can be determined. With this information, proper biosecurity measures could be instituted.

Cranfield, M., Gilardi, K. Human Virus Linked to Death in Endangered Gorillas. *Infection Control Today*. 2011. <https://www.infectioncontrolday.com/infectious-diseases-conditions/human-virus-linked-deaths-endangered-gorillas>

Muehlenbein, M.P. Ancrenaz, M. Minimizing Pathogen Transmission at Primate Ecotourism Destinations: The Need for Input from Travel Medicine. *Journal of Travel Medicine*, Volume 16, Issue 4, 1 July 2009, Pages 229-232, <https://doi.org/10.1111/j.1708-8305.2009.00346.x>

Science News. Fatal respiratory infections in endangered gorillas are linked to human contact. *Biology & Nature*. <http://www.mailman.hs.columbia.edu/>. Published: Wednesday, March 30, 2011 - 14:02

APPLIED BIOMEDICAL SCIENCES: NOVEL EXPERIMENTAL APPROACHES TO TUMOR BIOPSY

Suzana Tkalčić, DVM, PhD

College of Veterinary Medicine, Western University of Health Sciences, California

Advancement of modern day biomedical sciences and an exponential surge of biomedical data have resulted in an increase in advancements in biomedical technology. Veterinary medicine is partnering with technological companies in collaborating with pre-clinical and clinical research to assure safety and applicability/effectiveness of various devices. One of such devices include a sensor-integrated biopsy device for a real-time tissue metabolism analysis. Tissue metabolism is an important indicator of malignant transformations since it is closely associated with biochemical changes in situ and can act as an important hallmark of malignancy, adding the on-the-spot prognostic values to conventional tissue biopsy. The addition of chemical sensitivity to the biopsy needle can enhance diagnosis, prognosis and effective treatment by providing feedback on the metabolic and physiological state of the neoplastic tissue in the local environment. It can also aid to accuracy of the biopsy procedure by directing the biopsy towards areas with high concentrations of viable/most active tumor cells. The sensor integrated biopsy (SIB) needle is such an experimental device equipped with fiber optic sensors to measure oxygen, pH, and lactate levels as accurate indicators of tissue metabolism, primarily glycolysis and energy production by rapidly dividing cells. The preliminary data showed the multisensory probes were able to report the location of the needle with 100% accuracy, with 0% false positive, and 0% false negative results. It is also capable of demonstrating a clear differentiation between areas within/outside tumor margins and these of high concentrations of cancer cells, capacity that can expand further to surgical excisions of tumors.

PERIODONTAL DISEASE IN VETERINARY AND HUMAN PATIENTS

Elizabeth F Schilling DVM, Assistant Professor

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Periodontal disease (PD) is one of the most common disease states found in many species. By the age of 2, up to 80% of dogs and cats can already have PD. Globally, 20-50% of people also suffer from PD. PD manifests differently in horses than in species with brachydont dentition, and the prevalence varies greatly in different areas, but may range up to 25-50%. In dogs, cats and humans, plaque bacteria are the usual inciting cause for PD. The oral microbiome comprises commensal organisms, which combine with salivary glycoproteins to form a tenacious biofilm. In horses, whose dentition and diet have co-evolved, PD is not related to plaque but to malocclusions leading to feed stasis. Regardless of the initial etiologic origin, the local microbiome shifts from health to a diseased state. PD manifests across species as inflammation and infection. Unchecked, it can progress from gingival soft tissue to periodontal ligament to bone, leading to alveolar bone loss, osteomyelitis, pathologic fracture and, ultimately, loss of teeth. In addition, PB microbes can affect not just the local oral environment, but may have significant consequences on the overall health of the individual. Cardiovascular, renal and hepatic pathology can be associated with PD in dogs. In dogs and humans with diabetes, glycemic control is hampered and mortality is higher in the presence of PD. Horses with PD may have issues properly chewing their food, potentially leading to gastrointestinal challenges. Cardiac valvular disease has not been well documented, but is a concern, as is distant organ disease. In all species, treatment of PD involves primarily addressing infection and inflammation, and may also involve modulation of the host response. Restoration of periodontal tissue is the ultimate goal, but often realistically treatment goals are focused on arresting the current level of disease.

HPV-RELATED LESIONS OF GYNECOLOGIC TRACT: CURRENT CHALLENGES, BIOMARKERS, NEW DIRECTIONS

Dr. Anna Yemelyanova

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HPV-related cancers of the lower genital tract and, particularly their precursors are prevalent. The high prevalence of HPV-related disease reflects extremely high rates of HPV infection in certain age groups. Development and implementation of preventive vaccines is expected to lower the rates of significant HPV-related lesions, but it also brings additional challenges to screening approaches.

Most of the cervical cancer screening programs are based on the detection and treatment of precursor lesions. The terminology describing these lesions has evolved, and currently LAST recommendations advocate for a two-tiered nomenclature of squamous intraepithelial lesions to be used. In addition, LAST recommendations address biomarker use in diagnostic work up. Immunohistochemical analysis of p16 expression is widely used to assist with morphologic diagnosis of squamous intraepithelial lesions. Additional biomarkers of progressive HPV-related disease have been proposed. Particularly, HPV-methylation has been proposed as a potentially promising test that could be incorporated into screening programs.

The presence of a transcriptionally active virus and viral proteins in cancers allowed for development of not only preventive, but also therapeutic vaccines. Immunotherapy with checkpoint inhibitors is being increasingly used in oncology. PD-L1 inhibitor (pembrolizumab) has been approved for treatment of PD-L1 positive metastatic or recurrent cervical cancer. The new therapeutic modalities hold promise in fighting this potentially deadly disease.

COMPARATIVE PATHOBIOLOGY AS DIAGNOSTIC AND RESEARCH TOOL FOR ADVANCEMENTS IN ANIMAL AND HUMAN HEALTH

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The Department of Pathobiological Sciences (PBS - <http://www.lsu.edu/vetmed/pbs/index.php>) at the Louisiana State University School of Veterinary Medicine is fully engaged in research, diagnostic activity and teaching. Research emphasis is on infectious diseases, with strong programs in viral and bacterial pathogenesis, immunity and resistance to infectious agents, vector-borne diseases, and the use of Geographic Information Systems to study disease distribution and risk factors.

Comparative pathology is the science that considers human disease processes in comparison with those of other animals. Board certified veterinary pathologists have the appropriate qualifications to be protagonists in diagnostic activities and research in this field by fostering excellence in pathology, to protect and improve animal, human and environmental health for the betterment of society. The PBS ACVP board certified pathologists participate and collaborate to the departmental and extra departmental studies and in particular in the following projects and areas of investigation:

- Pathogenicity, pathogenesis of rickettsial diseases including *Rickettsia conorii*, *R. rickettsii* and *R. felis*; Roles of conserved Sca proteins from *R. conorii* and *R. rickettsii* in the interaction with endothelial cells; identification of mammalian receptors for Spotted Fever Group rickettsiae; generation of protective humoral immune responses against SFG rickettsiae using established models of infection;
- Viral vectors for vaccine development against Human herpesvirus 1 and 2, and Equine herpesvirus 1, Feline herpesvirus 1 and Bovine herpesvirus 1
- Viral immunology focused on innate immunity, dendritic cells and respiratory viruses studying the immune response to respiratory syncytial virus and human metapneumovirus;
- Molecular and cellular mechanisms responsible for neutrophil recruitment, priming, and activation in bacterial infected lungs, smoke-exposed lungs, and smoke-exposed lungs and organs followed by infection in the lungs and other organs/tissues; in particular determining the role of pattern recognition receptors (TLRs and NLRs) and their adaptors with the development of the innate immune response in the lung in animal models; bacterial pathogens studied include bacterial pneumonic agents such as *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Legionella pneumophila*.

- Pathogenesis of zoonotic endemic and foreign arboviral diseases including Zika, West Nile virus, Equine encephalomyelitis viruses
- Infectious diseases and vaccine development of aquatic animals, particularly *Edwardsiella ictaluri*, *Francisella asiatica*, and *Photobacterium damsela* sub.Piscicida
- Pathogenesis of natural diseases occurring in animals, particularly vertebrates from the necropsy and tissue biopsy submissions through the LA Animal Diagnostic laboratory (LADDL). The process includes evaluation of history and clinical signs, necropsy, histology and when necessary, immunohistochemistry, in situ hybridation, PCR and other techniques. The whole process is supervised by a case coordinator board certified pathologist.
- Forensic Pathology cases, via LADDL, since we are committed to the investigation of possible animal cruelty cases
- Diseases of endangered species and exotic animal collections, in particular avian and reptiles, in collaboration with the veterinary teaching hospital groups
- Collaborations with the Tulane Primate Center on vaccines, treatments and diagnostic tools for infectious diseases such as AIDS, Lyme disease, malaria and tuberculosis and other zoonotic infectious agents
- Collaborations with the College of Agriculture on animal nutrition studies such as special diets, hypovitaminosis and hyperammonemia in aquatic vertebrates

Other activities are performed and will be added in the future. Our continuous diagnostic activity on animals and tissue samples brings to our attention new and different aspects of natural pathological entities and infectious, degenerative, and neoplastic diseases.

PROGNOSTIC FACTORS OF CANINE LYMPHOMAS AND MAST CELL TUMORS

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Both lymphomas and mast cell tumors are very common tumors in dogs and be a cause of death of the affected animal. These tumors can share some similarities in their initial presentation, but from the standpoint of a veterinary pathologist, they require a completely different approach when establishing a prognosis.

Canine lymphomas are an example of a heterogeneous group of animal tumors where the human classification of counterpart tumors was successfully adopted since these tumors share many clinicopathological similarities (Velli et al., 2011). Precise classification with such adapted (WHO/REAL) classification of lymphomas is the main determinant of prognosis in canine lymphomas. Comparative similarities are further substantiated with the fact that many specific prognostic factors for particular subtypes in human lymphomas proved at least partially useful also for canine lymphomas.

On the other hand, mast cell tumors or tumors arising from mast cells, while very frequent in canines, are uncommon or rare in humans, and clinicopathologically distinct. Such a situation produced a need for human medicine-independent development of prognostic parameters. Canine mast cell tumors present only in two forms, the more common dermal form and less common subcutaneous form, and there is, therefore, no further need for classification. However, different microscopic appearance correlated with biologic behavior granted development of two widely used grading systems, namely the more recent 2 tier-system (Kiupel et al., 2011), and an older, today somewhat abandoned, 3-tier system (Patnaik et al., 1984). Additionally, immunohistochemical staining for c-kit and Ki-67 are used to more closely prognosticate these tumors. Recently, cost-prognosis favorable cytological grading systems were also developed (Camus et al., 2016).

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Kiupel M, Webster JD, Bailey KL, Best S, DeLay J et al. (2011): Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Vet Pathol*. 48(1):147-155.

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ONE HEALTH AGENDA: DISEASE X

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The world currently faces many global health security threats that urgently demand a One Health approach. Emerging pandemic diseases and antimicrobial resistance are all related to animals but continued failure to take a species neutral approach to biosurveillance leaves us all at risk. The World Bank recently updated its definition of One Health in its “Operational Framework for Strengthening Human, Animal, and Environmental Public Health Systems at their Interface” position document. They amended the definition to “highlight the discrete disciplinary involvement of human health, animal health, and environmental health, and focus on those infectious disease-related issues (including antimicrobial resistance) that undermine overall health and well-being.” Furthermore, recognizing that the majority of emerging infectious diseases have originated in animals, they make the argument that it is time to start “targeting disease threats upstream prevention at the source, or via early detection and response to help reduce the frequency and impact of emergencies the system has to react to” and that it is in each government’s financial interest to do. However, in Harvard’s “Global Monitoring of Disease Outbreak Preparedness: Preventing the Next Pandemic” make the point that there is “underinvestment in preparedness, and over reliance on reactive responses” that is “enormously costly in terms of both lives and dollars, and aggravates global risk” and that our typical response is reactive and costly in term of economics and human lives. They also point out that “the first line of defense is each country’s veterinary and human public health systems’ capacity to detect and promptly control an infectious disease outbreak”. Unfortunately, most countries have not invested in building veterinary capacity and infrastructure as needed. The Food and Agriculture Organization (FAO) released information on 13 SET (Surveillance Evaluation Tool) evaluations that were conducted in sub Saharan Africa in the fall of 2018 and arrived at the sobering conclusion that there is little to no veterinary surveillance taking place. Why? Because people in the field, who lack vehicles and gasoline, simply cannot get their samples to centralized labs for testing. Detection of zoonotic pandemic threats as well as antimicrobial resistance are predicated upon the ability to perform surveillance in domestic animals and wildlife. The question is, how will this be accomplished?

ONE HEALTH SYMPOSIUM SLAVONSKI BROD 2014

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The Symposium One Health Symposium was held in Slavonski Brod from 5-7 June, 2014. There were 39 lecturers coming from various institutions such as: The University of Georgia, College of Veterinary; The University of Georgia, College of Public Health; Institute of Public Health of Brod-Posavina County; General Hospital "Dr. J. Benčević", Slavonski Brod; University of Zagreb, School of Medicine; The University of Memphis, School of Public Health; School of Public Health "Dr. Andrija Štampar "; University of Zagreb, School of Medicine, Faculty of Veterinary Medicine Zagreb; University of Osijek, School of Medicine; University Hospital Center Zagreb; Croatian Institute of Public Health; Technical High School, Bjelovar; Medical University of South Carolina, College of Medicine, Department of Public Health Sciences. A series of topics from medicine and veterinary medicine has been presented. It was concluded that the continuation of this symposium should be held, but unfortunately the conclusions have not been realized.

IS THE "ONE HEALTH" CONCEPT RELEVANT FOR CROATIA?

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The Constitution of the Republic of Croatia guarantees the “right to health care in accordance with the law” to all citizens and that right is exercised through the health care system, governed by the Ministry of Health. The health care system includes mostly medical care of interest to the Republic of Croatia, but also public health services including environmental health, food safety, etc. Although The act on health care declares the state and general public administration as key authorities responsible to provide the comprehensive setting for health (including environment, food and water safety, social determinants of health, etc.), often these services are performed as a public service based on professional medical doctrine and with the use of medical technology and approach. In this presentation we will analyze specific settings and relations between the health care system/authorities and other “one health stakeholders” in Croatia. Existing public roles, accountability and relations between stakeholders are much more barriers than platform for sustainable “one health” care platform development. The policy process and some strategic activities, including the education, health promotion and political lobbying, have to be done to bring “one health” care approach to the policy mainstream.

NOVEL AND AFFORDABLE ANTIVENOM ON OUR DOORSTEP

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Despite poor data collection and availability, the World Health Organization estimates that 4.5 to 5.4 million people are bitten by snakes annually. Of these about 1.8 to 2.7 million develop clinical signs associated with envenomation, and that about 81,000 to 138,000 die each year. It is also estimated that among those who survive a significant percentage will require a limb amputation post-envenomation. These problems exist not only because of relatively poor availability of antivenom among those who need it most, but also because the efficacy of conventional treatments is variable and does not always address the variety of lesions induced by venom.

Traditional antivenom is produced with technology developed in France in the nineteenth century. Simply, venom is collected from snakes and injected into a host mammal that develops antibodies to certain components of the venom. The plasma is later collected from the host mammal and used to make antivenom. The process is long, expensive, and not without dangers for those who produce it. And even worse, antivenom is not always efficacious against all the clinical effects caused by venom, and patients are sometimes hospitalized for long periods of time. Complications can include poor wound management with limb amputations occurring in severe cases. Additionally, antivenom can trigger immune-mediated disease in both human and animal patients.

Our research program was developed to address several of the problems associated with traditional antivenom. Our aims are to formulate an antivenom that is faster to make, at a lower cost, and efficacious against multiple species of venomous snakes. Ultimately, we want antivenom to be readily available to those who need it, regardless if they live in Europe, North America, South American, Australia, Africa, or Asia. It is estimated that snakebite frequency is highest in sub-Saharan Africa, South Asia, and Southeast Asia, where most of the world's population lives.

We began our investigation by examining animals that appear to be innately immune to venom. In nature there are examples of reptiles and mammals that hunt and eat venomous snakes. Yet there is evidence that the animals sometimes get bitten by the snakes they are about to consume. We wondered about the mechanisms of resistance and whether these could be harnessed into a commercial antivenom. To that end, we began evaluating various species of king snakes looking specifically for neutralizing antibodies expressed in plasma against rattlesnake venom. After developing an ELISA method to quantify IgY antibody expression we surveyed various species of king snakes, pythons, and boas. From these we chose the species that expressed consistent antibody titers and used their plasma to develop an antivenom. To test whether this new antivenom was efficacious we completed a clinical trial in dogs naturally envenomated by rattlesnakes in southern California. The two-year study proved efficacy and safety in a clinical environment.

Ljudevit Jurak

The next phase of our research will explore efficacy against other species of venomous snakes in various animal species. It is our intention to evaluate the data collected with the possibility of moving into clinical trials in humans. To accomplish these goals, we have established a research laboratory in South Africa where we can first investigate safety and efficacy in animals envenomated by African venomous snakes. Ultimately it is our goal to create an antivenom that can be manufactured throughout the world near the places where people are frequently bitten by snakes, at a cost that the average snakebite victim can afford.

TICK-BORNE DISEASES AND THEIR TRICKS

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In humans, vector-borne diseases (VBDs) have increased in incidence for the last 20 years. The incidence of some VBDs in dogs and cats has increased in the last years, as well as their geographic distribution. Diseases such as anaplasmosis, ehrlichiosis, babesiosis, Spotted Fever Rickettsioses, and bartonellosis have wide geographic distribution. These diseases affect all breeds, causing hospitalization, debilitating or even fatal diseases. It is expected that the incidence of VBDs will continue to rise due to suburban development, climate change, dissemination of ticks, migration of infected animals from endemic areas due to natural disasters, and other causes. The true burden of VBDs in companion animals is unknown, as well as the variety of pathogens involved in disease causation. Depending on the species of pathogen, treatment is based on tetracyclines (such as doxycycline) or macrolides (such as azithromycin), although we have documented antibiotic resistance *in vitro* and *in vivo* in dogs and cats. This presentation will focus on selected “stealth” vector-borne pathogens, capable of causing chronic disease in humans and animals. Some of the pathogens’ strategies to evade the immune system include antigenic variation of major surface proteins, avoiding detection by Toll-like and NOD receptors of macrophages and neutrophils, translocation of virulent factors into host cells using type IV secretion systems, among others. Diagnosis of such diseases is challenging, due to limited immune response and antibody detection in several cases. In addition, molecular diagnostics are limited by the low-level of cyclic parasitemia. Clinical cases will be reviewed during a discussion about diagnostic strategies as well as limitations in current therapy protocols.

Keywords: Anaplasma, Ehrlichia, Bartonella, Type 4 secretion system, antigenic variation, stealth pathogen

RETHINKING BIOSECURITY WITH A ONE HEALTH PERSPECTIVE: DESIGN AND ASSESSMENT

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The definition of biosecurity is evolving and when designing or assessing one health biosecurity systems we must shift the paradigm and reach beyond the original definition of biosecurity as a set of measures designed to prevent the spread of infectious disease between humans and/or animals. The Epidemiologic triangle was useful to understand the relationship between the host, environment and the agent involved. If the goal is health management we must think beyond disease prevention or biosecurity measures that strive break the connection between infectious agent for a specific environment. Rethinking biosecurity means shifting the paradigm to include biorisk assessments in terms of multiple threats in a complex environment. Bioexclusion, surveillance, isolation and quarantine are not enough. In rethinking biosecurity design, a comprehensive approach is a paradigm shift that creates a mechanism for biosecurity protocols that can be applied from the micro to the macro environment in any setting. This systematic approach involves these key steps: 1) Define and fortify the health of the target population; 2) Define the perimeter and its permeability for health maintenance and health threats; and 3) Evaluate the environment and environmental controls. Examples will be presented to demonstrate a biosecurity audit system and application to One Health initiatives.

*Poster
presentation*

LUNG GOBLET CELL HYPERPLASIA/METAPLASIA QUANTIFICATION IN A MOUSE MODEL OF RSV A2-LINE 19F INFECTION USING PATHOLOGY DIGITAL IMAGE SOFTWARE

Maja Antolić, Anja Ognjenović, Dinko Relković, Ozren Majstorović, Ana Bruić, Ines Glojnarić and Snježana Čužić

Background & objective. Respiratory syncytial virus (RSV) is highly contagious RNA virus from the Paramyxoviridae family which usually causes acute lower respiratory tract infections. In addition, it can cause very serious and life-threatening complications in children under the age of 5, in older people and immunocompromised patients. Histopathology findings in a murine model of RSV pulmonary infection usually display oedema in intraalveolar space, damage of alveolar and bronchial epithelium, peribronchial and perivascular inflammation that consists mainly of lymphomononuclear cells, and goblet cell hyperplasia/metaplasia. Goblet cell hyperplasia/metaplasia results in increased mucin secretion. In addition to semi-quantitative histology score, mucin positive goblet cell quantification in lungs stained for Alcian Blue-PAS (PAB) is used to characterize murine RSV infection model.

Methods. BALB/c mice were infected by intranasal instillation of hRSV/A2-19F viral suspensions and treated with reference compound Ribavirin given at two doses, 30 mg/kg and 100 mg/kg, once a day from one day prior to infection until day 4 post-infection, at 24 hours intervals. Lungs for histopathology assessment were collected on days 4, 6, and 8 post-infection. For goblet cell quantification, lungs were formalin fixed, paraffin embedded, and stained for acid mucin by Alcian Blue - PAS (PAB) method. Mucin in bronchial goblet cells was stained dark blue. Presence of mucin positive cells was quantified using digital pathology image analysis software Calopix (TRIBVN, France) at the whole lung surface (without hilus). Between 30 and 40 different-sized bronchi were randomly chosen. Epithelial surface was marked from basal membrane to apical surface of bronchial epithelium. Pulmonary mucin expression was calculated as percentage of Alcian blue - PAS positive area per bronchial surface. This was calculated as \log_2 from μm^2 . Results were given for each bronchi per group.

Results. There was a significant increase of mucin expression in mice infected with RSV A2-line 19F on day 6 post-infection, compared to day 4. At the same time-point, Ribavirin treatment at both doses significantly reduced percentage of mucus-positive bronchial epithelial area. Sustained effect of Ribavirin therapy was observed on day 8 post-infection at the higher dose level.

Conclusion. Intranasal infection of mice with RSV A2-Line 19F induced goblet cell hyperplasia and metaplasia. Since goblet cells are not normally present in the epithelium of the smaller airways, changes are considered as goblet cell metaplasia. On the other hand, goblet cell appearance in large bronchi, can be defined as goblet cell hyperplasia. Digital image analysis can be helpful tool in analysing effect of substances on goblet cell hyperplasia/metaplasia in the mouse model of RSV induced pulmonary inflammation.

STUDENT SUBMISSION*THE POTENTIAL VALUE OF CASPASE 3 IN DIFFERENTIATING RENAL ONCOCYTOMA FROM CHROMOPHOBE RENAL CELL CARCINOMA****Fran Čakar¹, Ivan Pezelj², Božo Krušlin^{4,3}, Tihana Regović Džombeta^{4,3}**¹ School of Medicine, University of Zagreb;² Department of Urology, Clinical Hospital Centre Sestre milosrdnice, Zagreb;³ Department of Pathology and Cytology „Ljudevit Jurak“, Clinical Hospital Centre Sestre milosrdnice, Zagreb

Renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC) are epithelial neoplasms of the kidney that share many histological similarities. Differential diagnosis between the two is often difficult and is one of the main challenges of renal tumor pathology. Oncocytoma is a benign neoplasm of the kidney that may be treated conservatively, while chromophobe renal cell carcinoma is malignant neoplasm that rarely metastasizes but may undergo, in 2-8% of the cases, sarcomatoid transformation. After the diagnosis of chromophobe renal cell carcinoma, a partial or total nephrectomy is required. There is still no diagnostic method sensitive or specific enough to differentiate these two neoplasms. The aim of our study was to assess expression of apoptotic marker, caspase 3, in RO and in ChRCC and to determine if it can be used for differentiating these two neoplasms.

This study included twenty-four RO (11 female patients, 13 male, aged 46-82 years, mean age 64,9 years) and twenty-four ChRCC (12 female patients, 12 male, aged 26-76 years, mean age 52,1 years) from the archives of Ljudevit Jurak University Department of Pathology and Cytology, Clinical Hospital Centre Sestre milosrdnice and Department of Pathology, School of Medicine, Zagreb. To evaluate the level of caspase 3 expression, the percentage of positively stained cells and their staining intensity were graded on a scale of 0-3 and then multiplied to give the immunohistochemical staining index (ISI): 0=none; 1-3=low; 4-6=moderate and 9=high. All samples showed positive immunohistochemical reaction for caspase 3, with the majority of RO showing moderate ISI and the majority of ChRCC showing low ISI. Caspase 3 expression was significantly stronger in RO than in ChRCC (Mann-Whitney U test; $p < 0,01$).

This pilot-study shows a potential use of caspase 3 antibody in differentiating these histologically similar but biologically diverse tumors. Additional study on larger number of samples should be performed.

PROSTATIC STROMAL TUMOR OF UNCERTAIN MALIGNANT POTENTIAL: CASE REPORT

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ABSTRACT: Mesenchymal tumors of prostatic stroma are rare, making up to 1% of tumors arising in this location, but may represent a diagnostic challenge. Among them, stromal tumor of uncertain malignant potential (STUMP) is a rather distinctive lesion, designated under this term in 1998. It has been reported in about 100 cases worldwide. STUMP is usually an incidental finding, with peak incidence in 6th and 7th decade, has clinically indolent behavior and very rarely progresses to sarcoma, but its clinical significance and management are still uncertain due to its rarity and lack of long-term follow-up.

We present a case of 64 years old man who was diagnosed with STUMP in our hospital.

SMALL INTESTINAL GANGLIONEUROMATOSIS IN A MATURE DOG

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Ganglioneuromatosis is a rare disorder characterized by hyperplasia of intestinal ganglia including myenteric plexus and enteric nerve fibers. This disorder is generally described in children, but sporadic cases have also been described in adults. Most human cases arise in the colon and rectum. The disorder has also been described in dogs, mostly juveniles, but rarely in mature dogs. We report the first case in a mature dog from Croatia. A 13-year old female, mixed-breed dog had a history of diarrhea and weight loss. Ultrasound revealed focally-extensive markedly thickened small intestine. The changed part of the intestine, measuring 7 mm x 20 mm, was removed on laparotomy and delivered for histopathologic examination. Grossly, the intestine showed circumferential expansion of the mucosa. Microscopic findings included diffuse hyperplasia of myenteric and submucous plexus. So far, reports of ganglioneuromatosis in dogs included young dogs, and the oldest dog reported with this change was 9 years old. Our report shows that ganglioneuromatosis can be encountered in older dogs and it should be listed as one differential diagnosis in dogs where infectious and neoplastic etiology has been ruled out as cause of diarrhea.

PROTEIN PROFILE OF NONSEMINOMATOUS TESTICULAR GERM CELL TUMORS, PRSS21 ANALYSIS

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The world-wide incidence of testicular germ cell tumors (TGCT) has been continuously rising. Croatia is no exception to this, with the highest rise in incidence in the world, accompanied by a high rate of mortality compared to other countries.

TGCT's make up 95% of testicular tumors. TGCT are divided into pure seminomatous and non-seminomatous tumors, both considered to originate from germ cell neoplasia in situ (GCNIS) as a precursor lesion. GCNIS is considered to be driven by an interplay of genetic, epigenetic and micro-environmental factors leading up to an arrest of gonocyte differentiation.

Currently used biomarkers for testicular cancer lack specificity and sensitivity, and are used as accessories in diagnostics. A deeper look into molecular signature of nonseminomatous tumors is needed, due to their often times aggressive progression and cisplatin resistance. The aim of this study is to analyze protein expression in nonseminomatous tissue as a biomarker TGCT diagnostics and progression.

For immunohistochemical detection of PRSS21, 69 formalin-fixed paraffin-embedded non-seminomatous TGCT's from KBC SM and 9 non-cancerous testes were used. Slides were analyzed semi-quantitatively by pathologists. The data was analyzed in GraphPad Prism using the Mann-Whitney test.

The results have shown difference in PRSS21 expression between non-seminoma tissue and non-cancerous tissue. Expression of PRSS21 in non-seminoma is not uniform, with choriocarcinoma components showing a marked increase in expression.

The difference in PRSS21 protein expression between various non-seminoma and healthy testis tissue shows potential in TGCT diagnosis, and opens questions on the role of PRSS21 in non-seminoma development and differentiation.

IMMUNOHISTOCHEMICAL EXPRESSION OF $\beta 1$ INTEGRIN IN CANINE CUTANEOUS MELANOCYTIC TUMORS

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Canine melanocytic tumors in dogs present 4-20% of all skin tumors. They occur in older dogs, usually with darker hair and skin. Most of these tumors are benign. Malignant tumors can often be difficult to distinguish due to mimicking of other tumor types, high pigmentation or benign appearance of tumor cells but concurrently malignant biological behavior. Integrins are transmembrane receptors which mediate in cellular signalization between cells and its environment. Generally, $\beta 1$ integrin appears to be involved in tumor progression due to dynamic changes in its expression which depends on tumor type.

Histopathology and immunohistochemistry were performed on 48 cutaneous melanocytic tumors. According to the biological behavior tumors were subdivided in two groups (malignant melanoma, n=22; melanocytoma, n=26). Melan A was used to confirm a diagnosis only in amelanotic and scant pigmented malignant tumors. $\beta 1$ integrin antibody was used in all tumors. Chi-squared test was preformed for statistical analysis.

Malignant tumors showed expression of Melan A in 78%. Positivity of $\beta 1$ integrin was higher in malignant (77.3%) when compared to benign (30.8%) melanocytic tumors ($p < 0,05$) with mostly moderate to strong expression in malignant melanoma (64.7%). The results of Melan A were similar to previous studies. However, the results of $\beta 1$ integrin expression confirmed differences between melanocytic tumors according to their biological behavior. Its significantly higher expression in cutaneous malignant tumors suggests a possible role in tumor progression and consequently its metastatic potential when compared with benign counterparts.

LARYNGEAL TUBERCULOSIS MIMICKING NEOPLASM AND REVEALING UNRECOGNIZED PULMONARY TUBERCULOSIS

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Tuberculosis is the most common chronic granulomatous infection still posing major global health issue, especially in developing countries. However, laryngeal tuberculosis is uncommon condition which is considered to be seen in around 1% of patients suffering of this infection. It most often appears in male patients 40-50 years of age and is usually related to pulmonary form of the disease. Most common symptoms of laryngeal tuberculosis are hoarseness, odynophagia, sensation of foreign body in the throat, otalgia. It may macroscopically appear as polypous, ulcerative lesion, as leukoplakia, candidiasis, scleroma or may mimic neoplasm.

We present a case of a 29- year- old male suffering of hoarseness, cough and dyspnea who was initially suspected of having laryngeal neoplasm, but was later diagnosed with laryngeal and pulmonary form of tuberculosis. He was referred to ENT specialist due to his complaints of hoarseness and breathing difficulties for the past few months. His medical history was inconspicuous except for long- term smoking. Considering auscultatory respiratory findings, asthma was suspected and treated with corticosteroids and bronchodilators, relieving the symptoms. However, laryngoscopy demonstrated diffuse, bumpy thickening of the right vocal cord, therefore placing neoplasm as possible differential diagnosis. Subsequently, biopsy of the vocal cord was performed revealing chronic granulomatous inflammation with positive Ziehl- Nielsen staining of acid- fast bacilli and confirming the diagnosis of laryngeal tuberculosis. Due to the diagnosis, chest radiograph was performed demonstrating fibrocavous cavernous pulmonary lesions indicative of active specific inflammation. The patient was treated with antitubercotics and demonstrated symptom improvement during 4 months of therapy.

Although laryngeal tuberculosis is rare, physicians should be aware of its existence and connection to pulmonary form of the infection, especially in cases of unusual presentation of this condition.

BRAF MUTATIONS IN COLORECTAL CANCER

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INTRODUCTION: The survival of colorectal cancer (CRC) patients depends on early diagnosis and adequate choice of therapy. Besides surgery, chemotherapy and radiation therapy, a targeted therapy for epidermal growth factor receptor (EGFR) is nowadays a treatment option. EGFR initiates CRC progression via RAS-RAF-MAPK signaling pathway. Anti-EGFR targeted therapy is effective in the CRC with wild type RAS genes, thus mutation testing of RAS genes are obligatory prior such therapy. Unlike RAS testing, BRAF testing is not routinely performed on CRC. KRAS, NRAS and BRAF mutations are usually mutually exclusive and it is unlikely that patients with BRAF mutations will benefit from anti-EGFR therapy. The most common BRAF V600 variant is a result of a substitution of glutamic acid for valine in codon 600 causing a constitutive activation of the BRAF kinase.

MATERIAL AND METHODS: During the three-year period, we analyzed 327 cases of CRC for KRAS mutations using real-time PCR method. KRAS gene was mutated in 49.2% of cases and all non-mutated cases were further analyzed for NRAS and BRAF mutations. Eleven cases (3.4%) had NRAS mutation and twenty-one cases (6.4%) had BRAFV600 mutation. Patients with BRAF mutated CRC were men in 57.2% and women in 42.8%. Average age at diagnosis was 65 years, but women were younger than men (63.8y vs. 66.2y). In 81% of cases, cancer localization was on cecum or ascending colon (right-sided colon cancer). More than half of patients (52.4%) had liver metastases. In six cases using immunohistochemical staining, we analyzed mismatch-repair protein status, and in all six cases, we detected one or more protein lacking, which may indicate the microsatellite instability of CRC.

CONCLUSION: The incidence of BRAF mutations is more frequent than NRAS mutation in the CRC. According to literature BRAF^{V600} positive CRC has unfavorable prognosis and routine analysis of BRAF mutations in all KRAS non-mutated CRC may give more information about the biology of cancer and opens possibilities for combined targeted therapies for BRAF mutant disease.

COMPARISON OF THE EXPRESSION OF C-KIT AND OCT 3/4 IN THE TESTICULAR SEMINOMA.

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Testicular germ cell tumours (TGCT) represent 95% of all testicular neoplasia. Although testicular neoplasia represents only 1% of all neoplasia, TGCT affect young male population thereby representing well recognized challenge in the frame of male reproductive health. TGCT are divided into to groups - seminoma and nonseminoma. OCT 3/4 and c-KIT are used as a biomarkers for seminoma and embryonal carcinoma in routine diagnosis of TGCT. In this study, we wanted to compare the expression of c-KIT gene with the expression of OCT 3/4 in seminoma tissue.

52 seminoma tissue were collected in this study, embedded in paraffin and cut to 5 µm slides which were immunohistochemically stained. For visualization of OCT 3/4 and c-KIT we used specific antibodies. Pathologist performed morphometric analysis for OCT 3/4 and c-KIT expression.

In all seminoma tissue c-KIT and OCT 3/4 were expressed. Moreover, out of 52 tissue samples, in more than 50% of seminoma cells c-KIT was present in 34 patients while OCT 3/4 in just 18 samples analysed.

This study is a part of a bigger study in which we want to investigate different diagnostic, prognostic and possibly therapeutic biomarkers for testicular seminoma. According to our results, after the comparison of this two biomarkers which are already being used in the routine diagnosis for testicular seminoma, c-KIT has a higher specificity.

COMPONENTS COMPARISON BETWEEN HUMAN AND EXPERIMENTAL MOUSE TERATOCARCINOMA

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Testicular germ cell tumors are the most frequent malignancies among young male population. Teratocarcinoma (TCa) is a type of TGCT composed of teratoma, which consists of tissues derived from all the three germ layers, and embryonal carcinoma. This type of tumor can be obtained experimentally by transplanting 7.5 days old mouse egg cylinder beneath the kidney capsule. Although this experimental tumor histologically resembles human teratocarcinoma, detailed comparison of human and mouse experimental TCa have not been performed yet and therefore it is the aim of our study.

Twenty experimental mouse and eighteen human TCa formalin-fixed paraffin-embedded tissues were stained with hemalaun and eosin and their components were determined. Statistical analysis was performed by the Fisher's exact test.

Mouse experimental TCa is, in average, composed of more different components (6.95) than human TCa (4.33). Statistically significant difference was found in presence of cells of all four tissue types. Stratified squamous and columnar epithelium, skeletal muscle, adipose tissue, glia and neural tube were statistically significant more frequent in mouse.

Our results suggest that experimentally produced TCa diverge from human TCa in tissue types they are constituted of. Further analysis of proliferative and apoptotic activity, pluripotency and various cell type differentiation markers with immunohistochemical method and capillary western blot are in progress.

STUDENT SUBMISSION*INTRADUCTAL PAPILOMA OF MINOR SALIVARY GLAND
PRESENTING IN THE ORAL VESTIBULE****Jan Stranjik¹, Marko Velimir Grgić^{1,2}, Hrvoje Čupić^{1,3},
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Intraductal papilloma of minor salivary glands is a very rare benign tumor that usually presents in the lips and buccal mucosa. Here we present an even rarer localization of this tumor, described only once in the English literature.

A 58-year-old male was referred to our clinic with a painless submucosal mass, up to 13 mm in diameter, present for 2 weeks in the right upper oral vestibule. The lesion was excised under local anesthesia and sent to histopathological analysis. Microscopic examination revealed a unicystic cavity filled with papillary formations having fibrovascular stroma lined with uniform cylindrical epithelial cells. Mitotic figures, necrosis, significant atypia, as well as invasive growth were not found. Immunohistochemically the tumor cells were positive for CK-PAN, but negative for p63. Proliferative activity assessed immunohistochemically with Ki67 was up to 10% in the “hot-spot”. Based on these findings the tumor was signed out as intraductal papilloma of minor salivary gland, a variant of ductal papillomas. Due to pathohistological findings, a wider re-excision was made, revealing a microscopic foci of residual tumor. Three months after operation, clinical findings were stationary.

This example is in accordance with previously described cases but because of deficient data in the literature and rare localization of this particular tumor, it offers a unique learning point that can be useful for future research.

LOW-COST SOLUTION FOR AUTOMATING WHOLE SLIDE IMAGING

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Whole slide imaging (WSI) has significant potential to transform pathology practice. Its main function is the digitalization of glass slides, but through that process, it will also open a door for a wide array of other digital tools and algorithms, notably for machine learning (ML) and artificial intelligence (AI). In the last few years, these methods have demonstrated impressive results in image segmentation, object classification and clinical outcomes prediction, in some cases even exceeding human-level performance. Coupled with these tools, WSI will undoubtedly enhance pathology workflows. Unfortunately, due to the cost-prohibitive nature of commercial systems, relatively few pathology departments have implemented this technology, especially in under-privileged parts of the world. So, to make this method more accessible, we created a low-cost solution by automating an existing microscope. Using Arduino board with inexpensive stepper motors and a camera we managed to automate microscope stage and capture image data with sufficient precision to successfully stitch them into a large 2D digital slide. With 20X objective, the whole process of image acquisition and stitching takes, on average, 3.5h per slide. We believe that, when coupled with open-source image processing tools and algorithms, this system can provide a viable low-cost solution for incorporating WSI into a wide range of research programs.

THE ROLE OF REGULATORY T-LYMPHOCYTES IN COWS INFECTED WITH BOVINE LEUKEMIA VIRUS

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Enzootic bovine leukosis is a chronic contagious disease of cattle caused by bovine leukemia virus (BLV), an oncogenic RNA Deltaretrovirus of Retroviridae family. It has an important economic impact because it causes reduction in milk production, increased culling rate, shorter longevity and increased susceptibility to secondary diseases. According to the recent literature data, regulatory T-lymphocytes play an important role in progression of viral diseases by suppressing responses of other immune cells. Their role in enzootic bovine leukosis is still not sufficiently investigated.

The aim of this study was to compare number of Treg-lymphocytes in lymphatic organs of BLV-positive cows and BLV-negative cows in order to determine their potential role in pathogenesis of the disease.

Tracheobronchial and mesenteric lymph nodes, spleen and blood samples were collected in slaughterhouse from 26 serologically BLV-positive and 10 BLV-negative cows as negative control. Listed organs were grossly examined before sampling for histopathological analysis and immunohistochemical staining for FOXP3⁺ Treg-lymphocytes. Hematological analysis of blood samples was performed.

Since hematological analysis showed no increase in lymphocyte count and no lymphoma was observed on gross examination it was concluded that BLV-positive animals were in early stage of infection. In 13 BLV-positive cows, most common gross finding was enlarged tracheobronchial lymph node followed by enlarged mesenteric lymph node. Most common histopathological finding was reactive hyperplasia, mild in lymph nodes and moderate in spleen. Immunohistochemical staining showed higher average value of Treg-lymphocytes in tracheobronchial lymph nodes in BLV-positive cows (9,05%) compared to control group (7,06%).

Higher average value of Treg-lymphocytes in tracheobronchial lymph nodes of BLV-positive cows indicates their potential immunosuppressive function during early stage of infection.

INFLAMMATORY FIBROID POLIP - TWO CASE REPORT

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Inflammatory fibroid polyps are rare, benign, mesenchymal tumors that occur throughout gastrointestinal tract. Usually they appear as a small (few mm to 1.5 cm), solitary, sessile growths, though they can measure up to 9-10 cm. We report of 2 polyps, one arising in stomach and other in small intestine.

CASE 1: 70-year-old woman underwent surgery for removal of pedunculated gastric polyp protruding in duodenum, that was too large for endoscopic polypectomy.

We received polypoid tumor that measured 8,8x5,2x4 cm. Histologically, polyp was composed of uniform spindle cells in loose edematous fibrovascular stroma, with predominately eosinophilic and some mononuclear infiltrate. Mitotic activity was low; 1 mitosis/50 HPF. Immunohistochemistry revealed: CD34 and vimentin were positive; CD117, S-100, SMA, MSA and desmin were negative. Ki-67 index was 2-3% .

CASE 2: 52-year-old man with unspecific abdominal pain over couple of weeks had abdominal CT scan that revealed hypodense formation in ileum that rose suspicion of tumor process (GIST, adenocarcinoma, polyp), thus the patient was treated surgically.

Material sent for PHD was 30 cm long segment of small intestine containing gray tumor, 4.5 cm in diameter. Histologic examination showed tumor composed partially of spindle cells and partially of stellate cells in myxoid stroma, numerous blood vessels and inflammatory infiltrate with eosinophilic and plasma cell preponderance. 5 mitoses were found on 50 HPF, and immunohistochemistry showed: vimentin was positive; SMA was focally positive; CD 117, CD 34, MSA, S-100, desmin, synaptophysin and CK AE1/AE3 were negative. Ki-67 index was 15%.

In both reported cases inflammatory polyp was doubted and material was sent for consultation. Following morphologic characteristics and IHC profile diagnosis of inflammatory fibroid polyp was made.

We reported of 2 larger sized polypoid tumors that, although morphologically indicative of benign lesion, should be cautiously approached and immunohistochemically analyzed in order not to be replaced with other neoplasms such as GIST, vascular neoplasms, schwannoma, leiomyoma etc.

PD-L1 TESTING IN UNIVERSITY HOSPITAL CENTRE OSIJEK

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Lung cancer remains leading cause of cancer deaths worldwide, with poor prognosis. Hitherto, treatment therapies for non-small cell lung cancer (NSCLC) included surgery, chemotherapy, radiation and targeted therapy. Over recent years, following development of personalized medicine and progression in cancer immunology research, programmed death-ligand 1 (PD-L1) has emerged as a significant cancer biomarker and immunotherapy target.

Department for Pathology and Forensic Medicine of University Hospital Centre Osijek (KBCO) introduced PD-L1 expression testing in June, 2018, in addition to evaluation of EGFR and ALK. Test is performed using SP263 antibody clone on Ventana BenchMark Ultra platform, both on tissue and cell-block samples. Positivity is scored based on partial or complete membrane staining (at any intensity).

Thus far, PD-L1 expression has been analyzed in 62 patients; 45 male and 17 female (including patients from KBCO, as well as those that were sent for consultation). Majority of tests were done on tissue samples (74%). Expression of PD-L1 <1% was estimated in 37% of patients, and score of >50% was estimated in 24%. Furthermore, when applicable, EGFR and ALK tests were also performed. Test for EGFR mutation was done on 58 patients, with positivity in 5% of cases. For ALK immunohistochemistry test was applicable for 53 patients and scored positive in 2%.

To date, immunotherapy showed encouraging results in treatment of patients with NSCLC, therefore analyzing PD-L1 status has become integrated in pathologist work and clinical approach.

TISSUE MICRORNAS IN PROSTATE CANCER - (PRE)ANALYTICAL CHALLENGES

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With prostate cancer (PCa) being the most commonly diagnosed neoplasia among men all age, according to Globocan, there is a need for more sophisticated biomarkers for diagnosis, prognosis and management. MicroRNAs (miRNAs) have emerged as potential biomarkers for PCa that could allow its distinguishing from benign prostate hyperplasia, as well as aggressive from non-aggressive PCa. Even though it seems that the biggest miRNAs potential lies in their use as noninvasive biomarkers in liquid biopsies, research is mostly based on miRNAs in PCa tissue obtained by biopsy, both formalin-fixed paraffin-embedded (FFPE) and fresh frozen. However, questions are being raised regarding diverse (pre)analytical factors that influence miRNA analysis, such as FFPE block age. By examining published papers, we would like to present current knowledge and challenges in this area.

Preanalytical variables in research of miRNAs in FFPE tissue include time ex vivo, fixative, storage, microdissection and immunochemistry. Among those, FFPE storage represents a factor that highly differs between studies since blocks stored for different time periods were used in PCa research. Moreover, conflicting data was published regarding miRNAs stability in FFPE from different tissues. Some studies did not find significant change in miRNA levels depending on FFPE sample age, while others declared notable decrease in detected miRNA depending on sample storage time. Study of the effect of long-term storage on PCa FFPE tissue demonstrated significant loss of miRNA stability and suggested using sample block age rather than RNA quality when adjusting data. It also seems that FFPE tissue storage affects differently specific miRNAs, with

some being more stable than others over the years. However, majority of studies on PCa FFPE tissue did not report how long were their samples stored.

With raising research on miRNAs in PCa, there is a need for standardization of analytical procedures in order to be able to compare data from different studies. One of many aspects of this problem is FFPE PCa tissue storage which requires wildly excepted guidelines on storage conditions. For now, it seems advisable to consider adjusting data when analyzing miRNAs in FFPE samples stored for more than a decade.

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