Presenter's Name: Chen, Lina

Additional Authors: Chen L, Han Y, Liu P, Liu E, Zhang J, Huang Y, Ling C, Zhang Q

Abstract Title: Developing an Artificial Intelligence-Based End-to-End Mitosis Detection Tool

Mitotic count is an important feature for tumor grading and malignancy prediction in some tumors such as neuroendocrine tumor, breast cancer and soft tissue tumors. Currently, most pathologists manually examine histopathology images under high-resolution microscopes for the detection and counting of mitotic cells. However, the manual task is time-consuming, subjective, and tiresome. Previously, deep neural network algorithms to detect mitosis have been developed, but the speed and accuracy is still not satisfactory. To address this, we are developing an end-to-end mitotic-cell-detection method based on YOLOv4, the most advanced objective detection deep-learning platform. The deep learning algorithm was first trained on manually annotated mitotic images using pathology slides prepared at the Department of Pathology at LHSC. After the algorithm was verified and tested for best precision rate, we deployed this model to a web-based application (www.AI4Path.ca). We further validated the model using whole slide images (WSI) uploaded to the web application. The mitotic figures detected by the model are labeled on the WSI. Total mitotic counts, mitotic figures per mm2 and mitotic figures per 10 high power fields are reported. Re-label and re-train functions are under development, to enable pathologists to make corrections and fine-tune the deep learning models for continuous improvement.

POSTER PRESENTATIONS 3
3A: APPLIED CLINICAL RESEARCH 2

Presenter's Name: Halari, Moheem

Additional Authors: Shkrum M

Abstract Title: A Postmortem Study of Injury Patterns in Pedestrian and Cyclist Fatalities as a Predictor of Motor Vehicle Collision Dynamics and Pedestrian/Cyclist Kinematics

Introduction: The United Nations reports that annually 1.35 million fatalities occur worldwide due to road traffic collisions, more than half of which involve pedestrians, cyclists and motorcyclists. For Canada, the International Road Traffic Data and Analysis Group reported an increase of 21.1% and 7.3% in pedestrian and cyclist fatalities, respectively in 2016 compared to 2015. In Ontario, coroners' investigation of pedestrian and cyclist fatalities from motor vehicle collisions (MVCs) include post-mortem examinations by pathologists who document trauma to determine a cause of death and mechanism of injury. The purpose of this study is to understand these mechanisms by correlation of MVC findings with trauma patterns sustained by fatally injured pedestrians and cyclists. A predictive model using patterns of injury can be used by pathologists to assist coroners and police investigators in MVC reconstruction.

Hypothesis: We hypothesize that patterns of injury will assist in predicting MVC dynamics and pedestrian/cyclist kinematics and distinguish hit upright vs run over cases.

Materials and Methods: To identify the injury patterns sustained by pedestrians and cyclists involved in an MVC, a systematic medical literature review using databases such as Medline, CINAHL, EMBASE and Cochrane was done and an injury data collection form (IDCF) created. The form was used to extract data from cases within the province of Ontario including those examined at the Provincial Forensic Pathology Unit (PFPU) from 2013 to 2018.

The proposed study will tabulate injuries sustained by pedestrians and cyclists involved in MVCs and investigated by the Office of the Chief Coroner for Ontario and correlate this to real-world MVC data from Transport Canada using machine learning.

Results: Based on the literature review, the following injury patterns emerged:

1. Children (0-14 yr) – Head/Face/Neck, Thorax, Abdomen/Retroperitoneum, Lower Extremity
2. Youth (15-24 yr) – Head/Face/Neck, Thorax, Lower Extremity
3. Adults (25-64 y) – Head/Face/Neck, Thorax, Abdomen/Retroperitoneum, Lower Extremity, Spine
4. Elderly (≥ 65 yr) – Head/Face/Neck, Thorax, Pelvis, Lower Extremity

The literature described injuries in both fatal and non-fatal cases but could not link injuries to the type, speed and impact zone of the vehicle involved. The adult and elderly age groups were more frequently described in the literature. Their data were derived mainly in medicolegal settings.

After isolating specific injuries identified in the literature, a data collection form was created to identify patterns of injury that could be predictive of vehicle and collision types. It was used to extract data from approximately 700 post-mortems done from 2013 to 2018 within the province of Ontario available at the PFPU located in the Forensic Services and Coroners Complex in Toronto. Injury patterns are currently being analyzed based on pedestrian and cyclist age groups, and types of vehicle, speed and impact zones.

Discussion and Summary: The post-mortem trauma data of pedestrian and cyclists will be used to identify and correlate patterns of injury with pedestrian kinematics and collision dynamics. The results of our study will assist; clinical management of trauma patients due to MVCs, police in collision reconstruction, pathologists in medicolegal death investigations and promotion of safety features in motor vehicle design to prevent or mitigate serious injuries.

POSTER PRESENTATIONS 3
3A: APPLIED CLINICAL RESEARCH 2

Presenter's Name: Holder, Natasha

Additional Authors: McCord C

Abstract Title: Epigenetic Analysis of Glandular Odontogenic Cyst

Glandular odontogenic cyst (GOC) is an uncommon benign cystic lesion that occurs in the jaws. GOC is unique to most cysts found in the jaws in that it can have variation in clinical presentation and may behave aggressively. Aggressive lesions can lead to an increased risk of recurrence and extensive bony destruction. Based on the criteria we have available to diagnose GOC, it can be difficult to predict how individual lesions will behave. This study aims to use a molecular technique called DNA methylation analysis to examine epigenetic features of glandular odontogenic cyst, both in indolent and aggressive cases, and to compare these features to other lesions which show microscopic overlap with GOC (dentigerous cyst and intraosseous mucoepidermoid carcinoma). It is expected that the molecular profiles of GOC will be distinct from dentigerous cysts and intraosseous mucoepidermoid carcinomas, but any overlap may suggest a common link in the development and behaviour of these lesions. The results of this study have the potential to guide future clinical decision making.

POSTER PRESENTATIONS 3
3A: APPLIED CLINICAL RESEARCH 2

Presenter's Name: Kakar, Sachin

Additional Authors: Asfaha S, Flynn L

Abstract Title: Novel bioscaffold for intestinal stem cell culture

Introduction: Short-bowel syndrome is a disorder in which an extensive portion of the intestine is resected, resulting in malnutrition due to inadequate remaining bowel. Currently, there are few treatments for this condition, and there is a pressing need for cell-based therapies to enhance intestinal absorption. The discovery that intestinal stem cells can form "mini-guts" in a dish when cultured in Matrigel® has been an advancement in the intestinal stem cell field. However, the need to culture in Matrigel®, an extracellular matrix (ECM)-derived product sourced from mouse sarcoma cells, has hindered our ability to better understand the stem cell niche, and limited its future clinical utility. The ECM is a complex mixture of proteins and polysaccharides that are required for stem cell maintenance. The goal of this study is to develop a novel method for decellularizing mouse small intestine and to apply intestinal ECM in tissue-specific hydrogels for the culture of intestinal stem cells.

Hypothesis: The incorporation of intestinal-derived ECM within hydrogels will provide cell-instructive cues that can recapitulate the native intestine for organoid culture.

Materials and Methods: A novel decellularization protocol that included treatment with the detergent Triton X-100 and enzymatic digestion using DNase and RNase, combined with mechanical processing was developed in order to derive an intestinal specific ECM from the small intestine of C57BL/6 mice. Histological and biochemical analysis was used to confirm cell extraction efficiency and to assess the tissue-specific ECM composition relative to native tissue controls. The dimethylmethylene blue assay, hydroxyproline assay, and Picogreen assays were used to quantify sulphated glycosaminoglycans (sGAG), collagen, and DNA, respectively. Intestinal-derived ECM material was processed using pepsin-mediated digestion and used to generate ECM-alone hydrogels, as well as composites with alginate. Bioactivity of the newly derived intestinal ECM was assessed using a NIH 3T3 cells bioassay in which we assessed the effects on cell morphology and viability using a LIVE/DEAD assay.

Results: Picogreen assay revealed a significant decrease in DNA content in the decellularized intestine compared to native control. The hydroxyproline assay indicated there was a relative enrichment in collagen within the decellularized tissue compared to native controls. The dimethylmethylene blue assay showed retention of sGAG content in decellularized intestine relative to the native control. Following pepsin digestion, the solubilized ECM was used to generate SI hydrogels or incorporated within alginate to provide bioactive cues to modulate the response of encapsulated cell populations. 3T3-cell spreading was observed using LIVE/DEAD staining in ECM-incorporated hydrogels.

Discussion and Conclusion: The protocol for decellularization of SI was effective in eliminating DNA while retaining native ECM components. The ECM indicated bioactivity through a spreading phenotype in 3T3 cells. Ongoing studies are examining the cell-instructive effects of the ECM-derived hydrogels as potential 3-D culture platforms for intestinal organoids.

Presenter's Name: Lee, Wen Shen

Additional Authors: Chin-Yee I, Chin-Yee B, Lazo-Langner A, Iansavitchene A, Istasy P

Abstract Title: Examining the Impact of Artificial Intelligence on Health Equity in Oncology: A Scoping Review

Artificial intelligence (AI) draws from fields such as computer science, linguistics, psychology, and philosophy to develop technology with human-like cognitive functions. The applications of AI are numerous and broad, with some of the most promising benefits occurring in the healthcare sector, especially with regards to cancer diagnosis and patient care. There are also risks to consider, such as the potential for AI to be hacked, the ethical liabilities of AI decision-making, and the widening of health disparities. The literature discusses such advantages and benefits of AI but is limited in elucidating the impact of AI technology on health equity in cancer patients.

We hope to address such knowledge gaps and provide insight into how current AI technologies could impact equitable care for cancer patients. We will perform a scoping review to map out the current literary landscape of AI in cancer care and explore its impact on health equity. Literary searches with the electronic databases Medline, EMBASE, and arXiv will be done using keywords relevant to AI, socioeconomic inequity, gender/racial bias, and oncology. Finalized articles will be analyzed and our results will be presented in a qualitative and iterative manner to address project aims.

We expect heterogeneous literature, providing evidence for bias in AI technology contrasted with other applications that address health disparities. Currently, we have only screened 2019 article abstracts/titles from Medline, with 93 moving forward to the full-length review. To better prepare for a seamless transition and adoption of AI technology, it is crucial for society to understand the impact of this promising technology.

Our study aims to provide a framework for mapping out and identifying trends in the literature on AI and its impact on health equity in cancer patients. In doing so, we hope to also identify knowledge gaps in the literature for future research.

POSTER PRESENTATIONS 3
3A: APPLIED CLINICAL RESEARCH 2

Presenter's Name: Matyashin, Maxim

Additional Authors: Chin-Yee I, Chin-Yee B, Hsia C

Abstract Title: The Impact of SGLT2 Inhibitors on Secondary Erythrocytosis: A Report of Three Cases

Introduction: SGLT2 Inhibitors are a new class of antihyperglycemic pharmacological agents that are used in second-line therapy for the treatment and management of Type 2 Diabetes Mellitus. Increasing use of SGLT2 Inhibitors has created a need to identify and manage potential adverse effects associated with this class of medication. Recent case studies have implicated SGLT2 inhibitors in the development of erythrocytosis that warrants additional investigation. The Division of Hematology at London Health Sciences Centre has further noticed increased referrals of patients on SGLT2 inhibitors presenting with erythrocytosis, and laboratory data from this cohort was collected for review.

Cases: Three patients were include in this series, a 78-year-old male, a 61-year-old female, and a 66-year-old male, all referred to the hematology clinic for erythrocytosis. Two individuals presented with an increase in hemoglobin values following the initiation of their SGLT2 inhibitor. All three cases showed a resolution of the erythrocytosis within 3 months after discontinuation of the SGLT2 inhibitor.

Discussion: The discontinuation of SGLT2 inhibitors is associated with the resolution of the patient's erythrocytosis in three cases. Patients further underwent Next Generation Sequencing testing for myeloproliferative neoplasms, all of which were negative for JAK2 mutations. SGLT2 inhibitor use should be considered part of the differential diagnostic criteria and investigations for erythrocytosis. Physicians need to be aware of this association with these commonly used class of drugs to avoid unnecessary investigation for polycythemia and may consider discontinuation of SGLT2 inhibitors in individuals with elevated hemoglobin.

Presenter's Name: Peters, Jocelynn

Additional Authors: McRae S, Weir M, Zeman-Pocrnich C

Abstract Title: Cytotechnologist-cytopathologist interobserver variability in thyroid FNAB reporting

Introduction: Thyroid FNAB interpretation has been reported to have interobserver variability, but few publications examine variability between cytotechnologists (CT) and cytopathologists (CP). The purpose of this study was to evaluate and elucidate reasons for interobserver variability between CT and CP for thyroid FNAB diagnoses at our institution.

Methods: CT and CP diagnoses for thyroid FNABs from 2 non-consecutive years were compared. Diagnostic agreement was defined as CT and CP assigning to same category, with Suspicious/Positive for Malignancy (SFM/PFM) considered one. For the 100 Follicular/Hurthle Cell Neoplasm (FN/HCN) CT diagnoses which were changed to Benign by CP, descriptors pertaining to cellularity, colloid, architecture, thyroiditis, and atypia were compared between CT and CP.

Data and Results: Overall CT/CP diagnostic agreement was 73% (2749 cases) with significant differences ($p<0.05$) in diagnostic rates for: Benign 42% CT vs 50% CP; Follicular Lesion of Undetermined Significance (FLUS) 14% vs 11%; FN/HCN 11% vs 6%. CP reclassification rates were highest for FLUS 47%, Atypia of Undetermined Significance (AUS) 45%, FN/HCN 55% and SFM/PFM 22%. For 293 FN/HCN CT diagnoses, CP most frequently reclassified as Benign (34%) or FLUS (15%), where commonest reasons for benign reclassification were recognition of chronic thyroiditis (31%) and change in architectural pattern (43%).

Conclusions: Compared to CT, CP assigned Benign more frequently and FLUS and FN/HCN less. CT/CP interobserver variability was highest for grey categories. CP recognition of chronic thyroiditis resulted in FN/HCN reclassified as Benign. The findings will be used to promote CT and CP discussion and education within our department.

POSTER PRESENTATIONS 3
3A: APPLIED CLINICAL RESEARCH 2

Presenter's Name: Soparlo, Jeff

Additional Authors: Jackson-Boeters L, Khan ZA, Darling MR

Abstract Title: Evaluating the utility of S100A7 in identifying oral dysplastic lesions that will progress to oral squamous cell carcinoma

Introduction: Histomorphological evaluation by light microscopy is the gold standard for identifying oral epithelial dysplasia (OED). Prediction of malignant transformation by this method is difficult. Recently, S100A7 has been shown to be a potentially useful marker for identifying OED at risk of transformation. We hypothesise that lesions with increased expression of S100A7, are at high risk for transformation. The objective of this study is to evaluate the level of S100A7 expression in OED which have transformed into oral squamous cell carcinoma by qualitative and quantitative analysis. Correlation with dysregulation of phosphorylated MAPK pathway proteins will be evaluated.

Methods: Formalin fixed paraffin embedded specimens of OED at initial biopsy from 48 patients with oral squamous cell carcinoma and 35 patients with OED which did not transform were included in the study. 25 hyperkeratosis control patients were also included. Specimens were stained for S100A7 protein using a standard immunohistochemistry protocol. Expression of S100A7 was assessed semi-quantitatively, using an intensity and proportion scale, as well as by image analysis with application of a risk-predictive algorithm. Phosphorylated proteins in the MAPK signaling pathway (ERK1/2, p38, and JNK) were also evaluated via immunohistochemistry to correlate with S100A7 expression.

Results: Preliminary assessment shows that the location and intensity of S100A7 staining within the epithelium indicates the lesions at an increased risk of malignant transformation.

Discussion: S100A7 protein staining may be a reliable marker to determine the risk of malignant transformation in potentially malignant oral lesions and may aid in improved patient outcomes.

Presenter's Name: Sidahmed, Abubaker

Additional Authors: Freeman M, Muhammed R, Leckie S, Michel M, Sidahmed A

Abstract Title: Difficulties of a HLA-DPB1 alleles combination typing by using Luminex-RSSO

The human leukocyte antigen (HLA) complex on chromosome 6 is the most polymorphic region of the human genome, and although doubly polymorphic heterodimeric molecules are comparatively rare in human biology, this is a significant feature of the HLA class II gene antigens. The HLA-DQ and HLA-DP loci are characterized by having more diversity in genes for the alpha chains than HLA-DR. This results in less imbalance between levels of alpha and beta diversity; the ratio of beta/alpha known alleles is approximately 4:1 for these genes.

Aim: Design of hybridization probes and quality control for DNA HLA typing of certain alleles may be challenging due to a lack of DNA sequence with some known alleles to serve as quality control. Unexpected hybridization patterns in RSSO typing may suggest either the presence of novel alleles or non-specific probe hybridization. Recently, we experienced an unexpected typing pattern with a DPB1 typing.

Method: Subjects were potential solid organ donors and a recipient. Low resolution typing of HLA class I and class II were performed by RSSO (LABType kits from One Lambda) and real time PCR methodologies. High resolution typing of HLA-DPB1 was performed by using SSP.

RSSO testing revealed that the deceased and potential living donors plus recipient HLA typed as DPB1*20:01, DPB1*03:01 (Group 1, frequent on both alleles). These subjects were HLA typed again using SSP and real time PCR. In two of these cases the subjects HLA typed as DPB1*06:01 instead of DPB1*20:01. RSSO was repeated and again produced the same DPB1*20:01, DPB1*03:01 result with good bead reactivity including controls. The only way to achieve DPB1*06:01 instead of DPB1*20:01 involved adjusting 3 beads which included changing a very negative bead (#18) into a positive. One of the subjects typed as DPB1*20:01, DPB1*03:01 using RSSO and was confirmed by SSP and real time PCR.

The DPB1* typing was assigned using alleles in Group1, with no questionable beads. Investigation into the probes of DPB1*20:01 indicated that the probes are coated on beads 6, 7, 11, 20, 22, 23, 29, 36, 43, 60, 92 and 96 are within exon 2. All probes of DPB1*03:01 are coated on beads 6, 7, 11, 20, 22, 23, 24, 36, 60, and 96. Probes of DPB1*06:01 are coated on beads 7,11, 20 23, 29, 36, 43, 60. 92, 96 plus beads 18, 85 and 89 which in our case needed to be adjusted to assign DPB1*06:01 allele typing. It is possible that the beads involved in these RSSO reactions are not behaving as expected or that the presence of a second allele in this case DPB1*03:01, interferes with the ability of FUSION to analyze patterns differentiating between DPB1*06:01 and DPB1*20:01. See figure below.

Conclusions: Our findings suggest that the probes on beads 18, 85, 89 non-specifically hybridized to the allele of DPB1*20:01 or DPB1*03:01 instead of DPB1*06:01. These probes may require further improvement. It is important to be aware of these RSSO typing issues so that the DPB1* typing assignment can be confirmed by another molecular method. This finding may be discussed with the manufacturer and the DNA may serve for the QC.

POSTER PRESENTATIONS 3
3A: APPLIED CLINICAL RESEARCH 2

Presenter's Name: Sidahmed, Abubaker

Additional Authors: Michel M, Muhammed R, Leckie S, Freeman M, Sidahmed A

Abstract Title: Positive Flow cytometer crossmatch without measureable DSA is it false or real?

A 67-year-old AB+ve Caucasian male diagnosed with ESRD due to autosomal dominant polycystic kidney disease (ADPKD). He received a living unrelated kidney transplant in 2006. His renal allograft failed due to unknown reasons in 2019, so he is waiting for 2nd transplant. From March 2005 to April 2020, multiple sera for this patient were tested negative for class I and class II anti-HLA antibodies using one lambda single antigen beads (SAB). Nevertheless, FCXM(Flow cytometry crossmatch) with the living donor candidate (wife), were negative on T cells while positive on B cells, regardless of pronase treatment or not. The autologous crossmatch was negative. Current donor shares A3, B7, Cw7, DRB1*15:01, and DQB1*06:02 haplotypes with the 1st transplant. Different serum treatments, such as serial dilution, DTT, absorption, EDTA(routine or augmented concer tation), heat inactivation(56oC), failed to detect any positive anti-HLA antibodies. The sera also tested negative for anti-HLA antibodies using one lambda PRA bead, Reflex bead, and Immuncor SAB. We did not find positive antibodies to Non-HLA antigens, such as MICA, angiotensin II type 1 receptor, and 37 autoantigens in One Lambda autoantibody panel (Groups 1, 2, and 3). Historically, the patient had not received therapeutical antibodies, such as rituximab. To determine the allo-reactivity in patient's sera, surrogate FCXMs with 24 donors were performed. All B-FCXM were positive with 8 donors who had DR15 (repeated MM from 1st transplant) (Table 1). None of the B-FCXM with 16 DR15-negative surrogate donors were positive. So likely, alloantibodies specific to the product of genes closely linked to DR15 might cause positive B-FCXM. Considering shared mismatched with failed 1st transplant, the risk for these alloantibodies can not be overlooked.

In conclusion, positive FCXM in absent of detectable DSA with the solid-phase assay, requires interpretation with caution, rather than simply designated as false reactivity.