DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

DÉPARTEMENT DE PATHOLOGIE ET DE MÉDECINE DE LABORATOIRE

JOURNÉE ANNUELLE DE LA RECHERCHE ANNUAL RESEARCH DAY 2014

Université d'Ottawa
University of Ottawa

ANNUAL RESEARCH DAY PROGRAM
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE
UNIVERSITY OF OTTAWA
Thursday May 8th, 2014

ROGER GUINDON HALL
ROOM 1007
HEALTH SCIENCES BUILDING

8:30-8:40	**Coffee**
8:40-8:45	**Welcome**
8:45 – 9:00	**Radiological and pathological features associated with early disease progression and death in newly diagnosed anaplastic astrocytic tumours** ***Jason K. Wasserman,*** Santanu Chakraborty, Garth Nicholas, Rebecca Yaworski, Gerard Jansen, John Woulfe , and Thanh Nguyen
9:00 – 9:15	**An explanation for pathological utero-placental blood flow in placenta creta** ***Christopher G. Ball,*** David Grynspan, Raymond W. Redline, Bojana Djordjevic, Julien Yockell-Lelievre, Andree Gruslin, Joseph de Nanassy
9:15 – 9:30	**"Seeing is believing": direct autofluorescence visualization to guide breast specimen grossing – a proof-of-concept** ***Soufiane El Hallani,*** Catherine Poh, Shaheed Hakim, Pierre Lane, Denis Gravel, Susan Robertson, Shahidul Islam
9:30 – 9:45	**Pathological phenotyping of uterine leiomyomas from patients with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) syndrome** ***Sarah Strickland***, G. Graham, C. Gilpin, I. Teo, E. Belanger, B. Djordjevic
9:45 – 10:00	**Epithelial to mesenchymal transition in melanoma cells** ***Lauren St-Germain,*** Sarah Schock, Bill Staines, Shahidul Islam, John Bell, David Grynspan
10:00 – 10:30	**Break & poster viewing (Atrium, 2nd Floor)**

10:30 – 10:45 **Tumour-Infiltrating Lymphocytes as a Predictor of Response in Neoadjuvant Endocrine Therapy in Breast Cancer**
Nina Chang, Shaheed W. Hakim, Zuzana Kos, Angel Arnaout, Susan J. Robertson

10:45 – 11:00 **Tumor infiltrating lymphocytes are correlated with RCBI and Ki67 in post neoadjuvant breast cancer**
Shaheed W. Hakim, Nina Chang, Mark Clemons, Angel Arnaout, Denis H. Gravel, Susan J. Robertson

11:00 – 11:15 **Mechanism for Iron Delivery to Heme: Endosome-Mitochondria Interactions Augment Following Erythroid Differentiation**
Tariq Roshan, Daniel Garcia Santos, Anne Mason, Alex Sheftel, Prem Ponka

11:15 – 11:30 **Tumor regression in mammary high grade ductal carcinoma in situ is associated with hormone status but not invasion**
Jason K. Wasserman and Carlos Parra-Herran

11:30 – 11:45 **Lymphatic endothelial mimicry in papillary thyroid carcinomas: hidden evidence of lymphatic invasion and histopathogenesis of cystic metastasis in cervical lymph nodes**
Christopher G. Ball, Soufiane El Hallani, Chi K. Lai, Bibianna Purgina, Kien T. Mai

11:45 – 12:00 **Nuclear H&E staining pattern in flat epithelial atypia of the breast predicts presence of carcinoma on excision: a digital image based histopathological analysis**
Phillip A. Williams, Bojana Djordjevic, Yasmine Ayroud, Shahidul Islam, Denis Gravel, Susan Robertson, Carlos E. Parra-Herran

12:00 – 13:30 **LUNCH AND POSTER VIEWING**
(ATRIUM 2ND FLOOR, FACULTY OF MEDICINE)

13:30 – 14:30 **Keynote lecture**
Dr. Wedad Hanna
Professor
Sunnybrook Health Science Centre
University of Toronto

Title: Ductal carcinoma-in-situ: an update

14:30 – 14:45 **The prognostic significance of c-MET and EGFR overexpression in gastric carcinomas**
Aleksandra Paliga, Horia Marginean, Bibianna Purgina, Basile Tessier, Derek Jonker, Esmeralda C. Marginean

14:45 – 15:00 **Resolution of maternal D typing using serology and genotyping**
Philip Berardi, J. Hannon, G. Clarke, T. Alport, G. Growe, D. Lane, R. Fallis, J. Cote, G. Ochoa, M. Goldman

15:00 – 15:15 **Antimicrobial resistance and serotype distribution of Streptococcus pneumoniae in Eastern Ontario**
Chelsey Ellis, R. Robinson, M. Desjardins, B. Toye

15:15 – 15:30 **Prostatic ductal adenocarcinoma (PDCa): an aggressive variant that is underdiagnosed and undersampled on transrectal ultrasound (TRUS) guided biopsy**
Previn Gulavita, Nicola Scheida, Susan J. Robertson, Kien T. Mai, Eric Belanger, Trevor Flood

15:30-15:45 **Evaluation of efficiency of histological criteria in detecting Lynch syndrome (HNPCC) in colorectal cancer**
Phillip A. Williams, E Tomiak, LE Bourns, F. Halwani

15:45 – 16:10 **Break**

16:10 – 16:30 **ANNOUNCEMENT OF PRIZE WINNERS AND CONCLUSION**

- **Nadia Mikhael Award for Best Paper presented by a Junior Resident**
- **2nd Best paper by a Junior Resident**
- **Virbala Acharya Award for Best Presentation by a Senior Resident or Fellow**
- **2nd Best paper by a Senior Resident or Fellow**
- **Best Poster Presentation by a Graduate Student**
- **2nd Best Poster Presentation by a Graduate Student**
- **Best Poster Presentation by a Resident**
- **2nd Best Poster Presentation by a Resident**
- **Dr. M. Orizaga Award for Best Teacher**

POSTERS

1. **Molecular regulation of early myogenesis**
   Hamood Alsudais and Qiao Li

2. **Molecular mechanism of p300 activity during early myogenesis**
   Munerah Hamed and Qiao Li

3. **Characterization of impaired insulin signalling in Alzheimer's disease**
   Yuka Sai, Wandong Zhang and Qiao Li

4. **Histochemical and immunohistochemical analysis of metabolic enzymes and co-factors in calf muscle of rats exposed to brominated flame retardant (BFR) Hexabromocyclododecane (HBCD)**
   Syed Aziz, ***Clarine Chan***, Ivan Curan, Virginia Liston, Don Caldwell, Kamla Kapal, Rekha Mehta

5. **The Effects of 3-Methylfuran on the Calf Muscle of Fischer 344 Rats**
   Syed Aziz, ***Sana Aman***, Meghan Kavanagh, Michael Barker, Kamla Kapal, Wendy Cherry, Rekha Mehta and Santokh Gill

6. **Pseudo-outbreak of *Mycobacterium fortuitum* due to Contaminated Ice Machines**
   N. Sant, M. Desjardins, V. Chirip, R. Ettinger, I. Gorn, V. Roth

7. **Basal-like variant of non-invasive urothelial carcinoma: a variant of urothelial carcinoma with immunohistochemical features of basal-like urothelial cells associated with high rate of recurrence and invasion**
   Christopher G. Ball, Trevor A. Flood, Eric C. Belanger, Kien T. Mai

8. **Boundary conditions for three placental flow fields that may predict and cause intra-uterine growth retardation**
   Christopher G. Ball, Andree Gruslin, David Grynspan

9. **Novel technique of sampling the urinary bladder in radical cystectomy for urothelial carcinoma**
   Christopher G. Ball, Joanne Swift, Trevor A. Flood, Eric C. Belanger, Kien T. Mai

10. **Porous media flow models for maternal placental circulation**
    Christopher G. Ball, David Grynspan, Andree Gruslin

11. **Orbital IgG4-related disease with superimposed Graves' ophthalmopathy mimicking an orbital tumor**

Soufiane El Hallani, Susan J. Robertson, Paula Blanco, James Farmer, Manisha Lamba

12. **Tumor and endothelial cell hybrids contribute to glioblastoma vasculature: fact or artefact**
Soufiane El Hallani, Carole Colin, Ahmed Idbaih, Karima Mokhtari, Jean-Yves Delattre

13. **Role of fine-needle aspiration in the surgical management of pancreatic neuroendocrine tumors: utility and limitations in light of the new World Health Organization classification**
Shaheed W. Hakim, Wayne S. Kendal, Avijit Chatterjee, Derek J. Jonker, Jean A. Maroun, Laval Grimard, Wael Shabana, Richard Mimeault, Terence N. Moyana

14. **Superficial invasive urothelial carcinoma of large nested variant with regional or distant metastases: a variant of urothelial carcinoma with hidden evidence of stromal invasion**
Shaheed W. Hakim, Christopher G. Ball, Joelle Levac, Fawaz Halwani, Trevor A. Flood, Eric C. Belanger, Kien T. Mai

15. **Improving the autopsy service through a pathology resident-led educational initiative for clinical residents**
Sarah Strickland, Aleksandra Paliga, Marcio Gomes

16. **GATA3 expression profile in invasive breast carcinoma post neo-adjuvant systemic chemotherapy**
Phillip A. Williams and Shahidul Islam

17. **Mean Platelet Volume and Immature Granulocyte Count in ICU Sepsis Patients and Disposition at 30 days**
Vito Sanci, **Ruth Padmore**

18. **The Prognostic Effect of MLH1 Loss in Endometrial Endometrioid Adenocarcinoma**
Bojana Djordjevic, Amanda Bruegl, Bryan Fellman,Su-Su Xie, Diana Urbauer and Russell Broaddus

19. **Challenges in the diagnosis of the aleukemic prodrome of pediatric acute lymphoblastic leukemia**
Elaine Leung, Mylene Bassal, Luke Shier

20. **The ability to detect peripheral blasts in children with newly diagnosed or relapsed leukemia using Sysmex XE 2100/5000 CBC analyzers**
T. Ogilby, M. Roebuck, E. Leung

21. **Reticulocyte hemoglobin equivalent (RET-He) in the management of pre-operative anemia: is it useful information? A pilot study**
Touchie D, Henderson M, Goyette E, Padmore R, Giulivi A

22. **Implementation of a multiple myeloma high risk mini-FISH panel: implications for treatment management of patients in Eastern Ontario** ***Beaulieu Bergeron M,*** Yoshimoto M, Clifford B, Sinclair-Bourque E, Padmore R, McGowan-Jordan J, Tay J

WELCOME

RADIOLOGICAL AND PATHOLOGICAL FEATURES ASSOCIATED WITH EARLY DISEASE PROGRESSION AND DEATH IN NEWLY DIAGNOSED ANAPLASTIC ASTROCYTIC TUMOURS

Jason K. Wasserman MD PhD, Santanu Chakraborty MD, Garth Nicholas MD, Rebecca Yaworski, Gerard Jansen MD, John Woulfe MD PhD, and Thanh Nguyen MD

Objective: Patients with anaplastic astrocytic gliomas demonstrate a highly variable prognosis. The purpose of the present study was to identify radiological and pathological features associated with early progression and death.
Methods: Patients with a new diagnosis of anaplastic astrocytoma or oligoastrocytoma were included in this study. All patients underwent an MRI and had tissue available for pathological evaluation. IDH1 mutation status was determined by immunohistochemistry. The primary outcomes were progression or death within 1-year of diagnosis.
Results: Thirty-seven patients were included in this study; 18 (49%) were IDH1-positive. Patients with IDH1-positive tumours were younger (44 years vs 54 years, p=0.038) and were less likely to die within the first year (17% vs 47%, p=0.046). IDH1-positive tumours had a greater minimum ADC (1058 vs 853, p=0.016) and a lower Ki67 labelling index (0.13 vs 0.21, p=0.034). In patients with IDH1-positive tumours, age ≥ 50 years (p=0.029) and greater axial diameter of the contrast enhancing tumour (14.49 vs 2.00, p=0.002) were associated with an increased risk of progression while age ≥ 50 years (p=0.007) was associated with an increased risk of death. In contrast, chemotherapy (p=0.009) was associated with a decreased risk of death. In patients with IDH1-negative tumours, age ≥ 50 years (p=0.001) and subtotal resection (p=0.01) was associated with an increased risk of progression while peri-tumoral edema (p=0.046) was associated with an increased risk of death. Chemotherapy (p=0.02) and mitoses ≥ 5 / mm2 (p=0.009) were both associated with a decreased risk of death. In IDH1-positive patients, only age ≥ 50 years was independently associated with progression and death (OR 18.00; CI 1.19-271.5). In IDH1-negative patients, age ≥ 50 years was independently associated with an increased risk of progression (OR 60.00; CI 3.10-1160.0) while chemotherapy (OR 0.063; CI 0.005-0.760) and mitoses ≥ 5 / mm2 (OR 0.063; CI 0.005-0.760) were associated with a decreased risk of death.
Conclusions: Age is the most important prognostic factor for patients diagnosed with anaplastic astrocytic gliomas. Patients with IDH1-negative tumours and a high mitotic rate appear to respond better to chemotherapy and are associated with better outcome.

AN EXPLANATION FOR PATHOLOGICAL UTERO-PLACENTAL BLOOD FLOW IN PLACENTA CRETA

Christopher G. Ball[1], David Grynspan[1], Raymond W. Redline[2], Bojana Djordjevic[1], Julien Yockell-Lelievre[3], Andree Gruslin[3], Joseph de Nanassy[1]

Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, Ontario, Canada
Division of Anatomic Pathology, Case Western Reserve University, Cleveland, Ohio, USA
Department of Obstetrics and Gynecology, University of Ottawa, Ottawa, Ontario, Canada

Background: The geometry of maternal vasculature to the placenta is poorly defined due to placental separation from the uterus, and a dearth of post-partum hysterectomies. In normal pregnancy, the maternal placental blood has low flow and cannot be seen by routine Doppler. In contrast, high flow eddies in placenta accreta or increta (creta) have been published. Careful examination of the uterine vascular histology in creta and its differences from normal may shed light on maternal flow to the placenta.
Design: In addition to an archival study-set of hysterectomy specimens, 5 post-partum hysterectomies for creta placentas were retrieved from hospital archives; photomicrographs were taken. The study set includes 1 non-pregnant uterus, 2 cases of placenta creta, 2 hysterectomy cases with recently involuted placental implantation after pregnancy, 2 cases of hysterectomy for post-partum hemorrhage, and 1 autopsy specimen with maternal demise unrelated to pregnancy.
Results: In non-creta cases, vascular remodelling of the superficial myometrial vessels causes complete replacement of the muscularis by hyalinised material. The remodelled myometrial supply vessels adopt a mildly distended serpiginous pattern (~ 0.5 mm diameter). Furthermore, it appears that the remodeled vessels conduct flow to the placenta via thin-walled slit-like channels in the endo-myometrial region, oriented parallel to the utero-placental plane. Post-partum, these vessels become rounder and smaller; however, the hyaline material persists for some time. In creta cases, most of the normally remodeled superficial smaller myometrial vessels are obliterated by deep villous invasion into the myometrium, while the myometrial-placental interface is irregular. Large calibre vessels (~ 2.5 mm diameter) with negligible remodelling are seen adjacent to the placenta, communicating directly with the maternal placental space via irregular distended thin-walled channels.
Conclusion: In normal pregnancy, remodelled myometrial vessels conduct blood to the placenta via slitlike channels that likely act as flow diffusers. In creta cases, deep myometrial penetration by placental villi demands incomplete remodelling of larger vessels. In such a system, with the placenta supplied by large negligibly remodelled vessels and irregular distended channels, inadequate flow diffusion may fail to guard against pathological flow states.

“SEEING IS BELIEVING”: DIRECT AUTOFLUORESCENCE VISUALIZATION TO GUIDE BREAST SPECIMEN GROSSING - A PROOF-OF-CONCEPT

Soufiane El Hallani (1), Catherine Poh (2), Shaheed Hakim (1), Pierre Lane (2), Denis Gravel (1), Susan Robertson (1), Shahidul Islam (1)

(1) The Ottawa Hospital; (2) British Columbia Cancer Agency

Background: Traditional gross examination of breast specimen relies on naked eye visualization under white light in conjunction with palpation and radiograph correlation. In many situations; however, the lesion is occult and non-palpable due to the lack of surrounding desmoplastic response (i.e. DCIS; lobular carcinoma) or small tumor size (i.e. early cancer detection; area of invasion in DCIS). When practical, the specimen is extensively sampled and multiple cassettes are submitted in sequential fashion for histological examination. This practice affects the tech-time and significantly increases the laboratory cost and the work-load of microscopic examination. A simple method to identify such occult lesions is tested.
Methods: A hand-held device that delivers blue excitation light was employed to induce green fluorescence from endogenous fluophores (i.e. collagen) in the extracellular matrix of the mammary fibrous tissue. Breakdown of the fluorescent collagen is observed in cancer invasion and stromal inflammation and lead to loss of normal autofluorescence (LOA) (Figure 1). Ninety-five (N=95) breast tissue sections taken from fifteen fixed surgical Breast specimens during routine gross procedures were randomly selected and examined with the DAV device. Digital images of tissue white-light reflectance and fluorescence of the whole sections were saved and compared to the H&E slides of final pathology.
Results: Using histology as the gold standard, the device achieved a sensitivity of 100% when discriminating benign mammary conditions (n=49) from invasive ductal carcinoma (n=26) and lobular carcinoma (n=6). Comprehensive white light examination showed some invasive ductal carcinoma lesions; however, the addition of DAV was helpful in identifying the true extent of these lesions through the LOA. Biopsy site (n=5) showed also LOA in correlation with fat necrosis and surrounding stromal inflammation. Almost 85% of total DCIS lesions (n=9) appeared with a specific pattern of small round LOA areas that is different from the invasive pattern. We also present a representative case in which occult invasive lobular carcinoma would have been missed in the margin of a Breast lumpectomy specimen without extensive sampling whereas DAV clearly showed LOA.
Conclusion: We believe that DAV technology has a potential application in guiding the gross examiner to perform targeted sampling and precise extent assessment of cancerous Breast lesions including the occult ones, setting a new standard in gross examination. Ultimately, this will save the tech-time and reduce the financial laboratory cost.

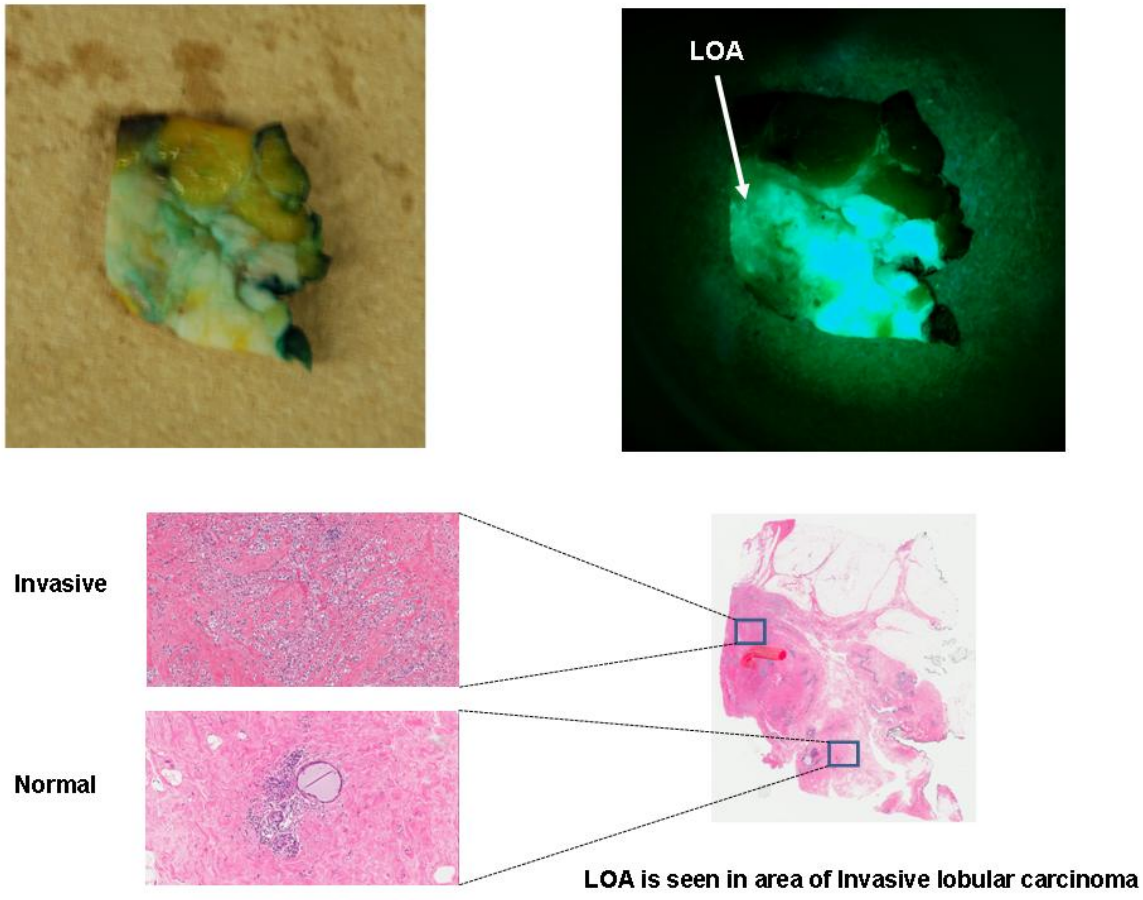

Figure 1: Illustration case of Direct Autofluorescence Visualization

PATHOLOGICAL PHENOTYPING OF UTERINE LEIOMYOMAS FROM PATIENTS WITH HEREDITARY LEIOMYOMATOSIS AND RENAL CELL CANCER (HLRCC) SYNDROME

S. Strickland, G. Graham, C. Gilpin, I. Teo, E. Belanger, B. Djordjevic

Background: HLRCC syndrome is an autosomal dominant disorder resulting from mutations in the fumarate hydratase gene which predisposes patients to cutaneous and uterine smooth muscle tumors as well as renal cell carcinoma. The latter is often aggressive and can present in young patients. Uterine leiomyomas (ULs) are more common, and their recognition may offer an opportunity to identify patients with HLRCC. Recently, the histological features of HLRCC ULs have been described, however, the specificity and sensitivity of these features is not known. The aim of this project was to compare the frequency of previously described HLRCC features in ULs of patients with known HLRCC syndrome to those in patients with sporadic ULs.

Design: 4 patients with known HLRCC and ULs, and 100 patients under the age of 41 with no personal or family history of HLRCC syndrome and ULs (multiple with at least one >3 cm or solitary > 10 cm) were identified. The following tumor pathological features were assessed: tumor number and size, cellularity, mitotic activity, nuclear enlargement, and presence of prominent nucleoli and perinucleolar clearing.

Results: The clinical and pathological features of 4 HLRCC ULs and 100 presumed sporadic ULs are summarized in Table 1. Increased cellularity (8%) and focal cells with prominent nucleoli and perinucleolar clearing (67%) were commonly found in sporadic ULs The most distinguishing characteristic of HRLCC ULs compared to sporadic ULs was the presence of diffusely distributed cells (5-10%) with a *combination of both* enlarged (3x) nuclei with irregular contours *and* eosinophilic nucleoli with perinucleolar clearing. These features were identified in 100% of HRLCC and 3% of presumed sporadic ULs.

Conclusion: Recognition of specific morphologic features in ULs may identify patients who can benefit from genetic testing for HLRCC syndrome. If subsequently diagnosed, these patients and their family members can be followed by renal cancer surveillance programs.

Table 1

Clinicopathologic Feature	**Known HLRCC Patients (n=4)**	**Presumed Sporadic Patients (n=100)**
Patient age (years)	Mean 36.7 Range 29-41	Mean 36.4 Range 24-41
Surgical Procedure:		
Hysterectomy	2	2
Myomectomy	50	50
Number of ULs:		
Single	0	17
Multiple	4	83
Size (cm)	Mean 11.0 Range 6.3-16.7	Mean 8.7 Range 3.0-19.2
Cellularity:		
Normal	2	92
Mixed	1	3
Increased	1	5
Mitoses:		
≤ 5 /10 HPF	4	100

< 5/10 HPF	0	0
Nuclear enlargement and irregular contour:		
Present	4	16
Absent	0	84
Prominent nucleoli and perinucleolar clearing:		
None	0	28
Focal	2	67
Diffuse	2	5

EPITHELIAL TO MESENCHYMAL TRANSITION IN MELANOMA CELLS

Lauren St-Germain, Sarah Schock, Bill Staines, Shahid Islam, John Bell, David Grynspan

Department of CMM, University of Ottawa, Department of Pathology, CHEO, OHRI, University of Ottawa

Introduction: Interferon-gamma (IFN-gamma) has been used as a form of immunotherapy in metastatic melanoma with equivocal results. While there is relatively solid evidence that IFN-gamma in the host shows anti-tumor response, the possibility that it also induces other biological pathways that offset this benefit has to be considered. In this respect we speculate that IFN-gamma may induce epithelial to mesenchymal transition (EMT) which is a mechanism well proven to increase tumor malignancy by making cells more discohesive, angioinvasive and motile. This hypothesis has never before been suggested or tested. In this project we assess markers of EMT (TWIST, vimentin) in vitro in melanoma cell lines with and without IFN-gamma treatment.
Materials and methods: Two different melanoma cell lines M14 and SK-MEL-28 were maintained in RPMI and DMEM in 96 well plates at 37 degrees in a humidified culture chamber. Treatment conditions were 0.1ng/ml, 2ng/ml and 10ng/ml IFN-gamma for 1, 5, or 24 hours prior to fixation in 4% PFA for 20min. The wells were stained by immunohistochemistry for TWIST and vimentin etc….
Results: There was a visible definite increase in vimentin and TWIST expression for both cell lines after 5 hours, most noticeably with the 2ng/ml dose. In addition there was a notable morphological shift from elongate to rounded cell contours.
Conclusion: For the first time we demonstrate that IFN-gamma upregulates markers of EMT in melanoma cell lines. Furthermore, there is a corresponding shift in cellular morphology. We recommend that immunotherapeutic approaches that use IFNs can be coupled with pharmacological approaches to block EMT in order to unmask the true benefit.

BREAK AND POSTER VIEWING (ATRIUM)

TUMOUR-INFILTRATING LYMPHOCYTES AS A PREDICTOR OF RESPONSE IN NEOADJUVANT ENDOCRINE THERAPY IN BREAST CANCER

Nina Chang[1] MD, Shaheed W Hakim[1] MD, Zuzana Kos[1] MD FRCPC, Angel Arnaout[2] MD FRCSC, Susan J Robertson[1] MD FRCPC

[1]Department of Laboratory Medicine, University of Ottawa, Ottawa, ON, Canada
[2]Department of General Surgery, University of Ottawa, Ottawa, ON, Canada

Background: The potential predictive value of tumour-infiltrating lymphocytes (TILs) in breast cancer is recognized in the literature. Studies have focused on TILs in neoadjuvant chemotherapy, which though controversial, have been associated with complete pathologic response and survival. By comparison, few studies exist on TILs in neoadjuvant endocrine therapy (NET). Only changes in T-cell subtypes have been studied in relation to NET response. Here we explore the response of ER+ tumours to short course estrogen blockade, and whether a raw TIL count, which is more practical than lymphocyte subtype analysis, can predict treatment response (TR).
Design: Patients with strongly ER+ invasive breast cancer on core biopsy were treated with an aromatase inhibitor (anastrozole 1mg daily) for 14-35 days (m=24.7, sd=6.7) prior to surgical resection. We evaluated TILs in biopsies and resection specimens from 19 NET patients (NETPs) and 23 retrospective controls closely matched for time (4-8 weeks) between biopsy and resection. TILs were scored as follows: 0 (absent), 1 (weak), 2 (moderate), 3 (dense). Ki67 and ER/PR status were reviewed for TR. Pearson correlation coefficients (r) and paired and independent T-tests were performed for statistical analysis.
Results: TILs correlated with Ki67 in biopsies from all cases ($r=0.488$, $p<0.001$), indicating higher raw TIL count is associated with more proliferative tumours. Ki67 significantly decreased between biopsy and resection in NETPs (m=-14.6, sd=17.8, $p=0.002$), but not in controls, indicating a rapid TR to estrogen blockade in NETPs. PR Allred scores also significantly decreased post-NET (Wilcoxon signed-rank test $Z=-3.41$, $p<0.001$). TILs correlated with Ki67 in resections from controls ($r=0.439$, $p<0.02$), as expected with no intervention, but not in NETPs. No correlation was found between post-NET change in Ki67 and change in TIL or TIL score, indicating TIL does not predict change in Ki67. No significant difference was found in TILs pre- and post-NET. When NETPs were subdivided into responders and non-responders based on ≥40% Ki67 drop from baseline, no significant difference was found in TILs pre- and post-NET in responders, or in TIL change in responders versus non-responders.
Conclusion: Although short course NET led to a rapid suppression of proliferation in strongly ER+ tumours, raw TIL count does not predict TR. The lack of change in TILs pre- and post-NET does not preclude alteration of lymphocyte subtypes, for which further study may be warranted.

TUMOR INFILTRATING LYMPHOCYTES ARE CORRELATED WITH RCBI AND KI67 IN POST NEOADJUVANT BREAST CANCER

Shaheed W. Hakim MD[1], Nina Chang MD[1], Mark Clemons MD FRCP(UK)[2], Angel Arnaout MD FRCSC[1], Denis H. Gravel MD FRCPC[1], Susan J Robertson MD FRCPC[1]

[1]Department of Pathology and Department of Surgery, University of Ottawa, Ottawa, ON, Canada
[2]The Ottawa Hospital Cancer Centre, Ottawa ON, Canada

Background: Neoadjuvant chemotherapy (NAC) in locally advanced breast cancers (LABC) has become a mainstay modality in treatment strategies. Tumor infiltrating lymphocytes (TIL) have been associated with complete pathologic response (pCR). Various predictive factors associated with TIL, albeit controversial, have been described. Correlation of Ki67 and residual cancer burden index (RCBI) to overall survival are reported in the literature. Also, TIL, although controversial, has been correlated with pCR. Until now TIL has not been correlated with RCBI or Ki67 post NAC. We explore the influence of TIL on these 2 variables.
Design: We reviewed 51 patients over a period of 3 years with LABC who received NAC. ER/PR/HER2 and nuclear grade from the pre-NAC biopsies and post-NAC resections as well as the RCBI and post NAC Ki67 scores were reviewed. The TIL were scored by a 4 tier grading system; 0 (absent), 1 (weak), 2 (moderate), and 3 (dense). Each case was scored in pre-NAC biopsies and post-NAC resections. A separate score (dTIL) represented the difference of the two. A Pearson correlation coefficient (r) was then computed to assess the relationship between these TIL scores and each variable mentioned above.
Results: Of the 51 patients (mean age = 49.7, sd = 9.2), 5 had pCR. The remaining 46 patients had RCBI scores and post-NAC ki67 indices. Pre-NAC TIL scores were: 11 = score 3, 24 = score 2, 13 = score 1 and 3 = score 0. Post-NAC TIL scores were: 9 = score 3, 20 = score 2, 13 = score 1, 9 = score 0. The pre TIL score negatively correlated with the RCBI score (r= -0.266, p=0.03), indicating that higher pre-TIL is associated with lower tumor burden on resection. Post TIL score showed direct correlation with the RCBI score (r=0.25, p=0.03) and with the post resection Ki67 (r=0.41, p=0.002), demonstrating that high levels of post-TIL were associated with an increased tumor burden/proliferation rate. The dTIL correlated with RCB (r=0.31, p=0.01) and post nuclear grade (r=0.31, p=0.01). HER2 showed a negative correlation both pre (r = -0.41, p = 0.002) and post (r = -0.38, p=0.005) NAC.
Conclusion: These results suggest that TIL post NAC correlates with post NAC Ki67 and RCBI and therefore may be used as independent predictors for therapeutic efficacy. Although this study was limited by its retrospective design, if validated prospectively, it could be incorporated into clinical patient care. Consequently, further studies to assess TIL during active NAC, when modifications can be made, are also likely warranted.

MECHANISM FOR IRON DELIVERY TO HEME: ENDOSOME-MITOCHONDRIA INTERACTIONS AUGMENT FOLLOWING ERYTHROID DIFFERENTIATION

Tariq Roshan[1,2,3], Daniel Garcia Santos[2], Anne Mason[4], Alex Sheftel[5], Prem Ponka[2,3]

[1]Division of Hematological Pathology, The Ottawa Hospital, General Campus, EORLA Lab Ottawa, ON, Canada; Experimental Medicine, [2]McGill University, Montreal, QC, Canada; [3]Lady Davis Institute for Medical Research, Jewish General Hospital and Department of Physiology, McGill University, Montreal, QC, Canada; [4]Department of Biochemistry, College of Medicine, University of Vermont, Burlington, VT; [5]University of Ottawa Heart Institute, Ottawa, Canada

Introduction: Iron (Fe) acquisition by cells requires the binding of diferric transferrin (Tf) to membrane transfserrin receptors, followed by the internalization of Tf-receptor complexes by endocytosis. After endosomal acidification and iron reduction, Fe^{2+} is released from endosomes *via* DMT1. In erythroid cells, more than 90% of iron enters mitochondria where ferrochelatase inserts Fe^{2+} into protoporphyrin IX. The path of Fe^{2+} from endosomes to ferrochelatase is not fully understood.

Methods: We have shown, using 2D and 3D live confocal imaging that there is a transient interaction of endosomes with mitochondria ("kiss-and-run") and that this interaction is required for efficient iron delivery to heme in reticulocytes. Moreover, we have demonstrated the interaction of these organelles by a novel method exploiting flow cytometry to analyze reticulocyte lysates labeled with Alexa Green Transferrin (AGTf) and MitoTracker Deep Red (MTDR). By using this new technique (flow subcytometry), we identified a double-labeled population representing endosomes interacting with mitochondria. The dynamic nature of this interaction was shown by chase experiments in which a time-dependent decrease of the double-labeled population was observed when reticulocytes were washed and re-incubated with unlabeled Fe_2-Tf. Furthermore, we have shown that the iron status of endosomes governs the efficacy of endosome-mediated iron delivery to mitochondria.

Results: In addition, we have used different 'locked' mutants of fluorescence-labeled, recombinant human Tf, which either remain permanently bound to iron (recombinant diferric-transferrin; L-Fe_2-hTf) or cannot bind to iron (recombinant apotransferrin; L-apo-hTf), in flow subcytometry studies. In these experiments, reticulocytes incubated with MTDR and L-apo-hTf failed to produce a double-labeled population. We also measured ^{59}Fe incorporation from ^{59}Fe-Tf into reticulocytes and their heme and showed that L-Fe_2-hTf, as compared to the wild-type hTf (WT-hTf), significantly decreased ^{59}Fe incorporation into both cells and heme. These results suggest that L-Fe_2-hTf (which cannot release iron) remains associated with mitochondria for a longer time than WT-hTf and thus blocks ^{59}Fe incorporation into heme. This probably slows the transferrin cycle, leading to decreased ^{59}Fe uptake by reticulocytes.

Conclusion: Experiments with murine erythroleukemia (MEL) and fetal liver (FL) cells show a significant increase in the magnitude of contacts between endosomes and mitochondria when cells are induced for differentiation. Hence, we conclude that the mitochondria-endosome interaction is universally involved in iron delivery for heme biosynthesis, but that this process is dramatically augmented in erythroid cells. Taken together our results show that endosomes come in contact with mitochondria to deliver iron for heme biosynthesis not only in erythroid cells but also in their progenitors.

TUMOUR REGRESSION IN MAMMARY HIGH GRADE DUCTAL CARCINOMA IN SITU IS ASSOCIATED WITH HORMONE STATUS BUT NOT INVASION

Jason K Wasserman MD PhD and Carlos Parra-Herran MD

Objective: Regression changes in high grade ductal carcinoma in situ (HG-DCIS) are associated with increased risk of concomitant or subsequent invasive carcinoma. Its prevalence and relationship with other relevant morphologic and molecular features has not been fully explored.
Methods: We reviewed breast biopsy cases with diagnosis of HG-DCIS followed by excision in a two – year period. We assessed features such as periductal inflammation, fibrosis and epithelial changes ranging from flattening to complete obliteration of the lesion. Degree of regression was documented in terms of intensity and distribution. Hormone receptor status and presence of invasive carcinoma on excision were recorded.
Results: 52 patients were included. Regression changes were frequently observed, including periductal fibrosis (49 cases, 94%), flattening of the intraductal neoplastic population (39 cases, 75%) and complete obliteration (20 cases, 38%). Invasive cancer was diagnosed on subsequent excision in 17 cases (32.6%). Regression in HG-DCIS was not associated with invasive outcome. Hormone receptor status was available in 48 patients: 28 and 22 patients were positive for estrogen and progesterone receptors, respectively; 20 patients were negative for both receptors. Hormone negative tumours were positively associated with regressive changes, specifically flattening of the intraductal neoplastic population ($p = 0.007$) and complete ductal obliteration ($p = 0.031$).
Conclusions: Regression changes are common in HG-DCIS. Regression appears to be related to distinct molecular subtypes of intraductal neoplasia, and may represent a targeted immunological response. Based on our results, regression does not portend an increased risk of invasive cancer on subsequent resection.

LYMPHATIC ENDOTHELIAL MIMICRY IN PAPILLARY THYROID CARCINOMA: HIDDEN EVIDENCE OF LYMPHATIC INVASION AND HISTOPATHOGENESIS OF CYSTIC METASTASIS IN CERVICAL LYMPH NODES

Christopher G. Ball, Soufiane El Hallani, Chi K Lai MD[1], Bibianna Purgina MD[1] MD[1]Kien T Mai MD[1. 1]Department of Anatomical Pathology

The Ottawa Hospital and University of Ottawa, Ottawa, Ontario, Canada

Introduction: We hypothesize that cystic structures in metastatic papillary thyroid carcinoma (PTC) develops along the framework of lymphatic channels in the lymph node.
Materials and Methods: Ten cases from each different categories of PTC with or without solid or cystic lymph node metastasis were immunostained for D2-40 and TTF1.
Results: TTF1 were reactive in the endothelial lining of lymphatic channels in lymph node. D2-40 displayed focal reactivity in TTF1-reactive follicular thyroid cells lining cysts and lymphatic -like channels. In addition, in solid and cystic primary and metastatic PTC, TTF1-reactive cells were identified lining focally in the lymphatic endothelium. These TTF1-follicular cells may appear as benign or atrophic thyroid follicular cells. We were able to identify the above changes in 10/10, 8/10, 4/10 and 0/10 PTC associated with cystic metastasis, with solid metastasis, classic PTC without metastasis and encapsulated PTC of follicular variant, respectively. For metastatic PTC with or without cystic architecture, the above changes were seen in 8/10 and 6/ 10 cases respectively
Conclusion: Evidence of endothelial mimicry is supported by thyroid follicular cells expressing both TTF1 and D2-40. The recognition of these changes is helpful in the identification of hidden lymphatic invasion often masquerading as benign or malignant follicles

NUCLEAR H&E STAINING PATTERN IN FLAT EPITHELIAL ATYPIA OF THE BREAST PREDICTS PRESENCE OF CARCINOMA ON EXCISION: A DIGITAL IMAGE BASED HISTOPATHOLOGIC ANALYSIS

Phillip A. Williams MD, Bojana Djordjevic MD, Yasmine Ayroud MD, Shahidul Islam MD, Denis Gravel MD, Susan Robertson MD and Carlos E. Parra-Herran MD

Department of Pathology and Laboratory Medicine, University of Ottawa and Eastern Ontario Regional Laboratory Association (EORLA), Critical Care Wing, The Ottawa Hospital, 501 Smyth Road, Ottawa, ON, Canada K1H 8L6.

Background: Diagnosis of flat epithelial atypia (FEA) on biopsy has prognostic and therapeutic relevance, given its documented association with breast cancer. However, rates of malignancy in subsequent excision are low in most studies, and to date, there are no morphologic or immunohistochemical features that can separate cases of FEA with concomitant breast carcinoma from those otherwise benign. We present a histopathologic study aiming to identify morphologic features unique to cases of FEA associated with cancer using digital image analysis.
Design: Resection specimens (lumpectomy, mastectomy) containing FEA were retrieved from our files. Diagnosis was confirmed by two breast pathologists and one senior resident using strict established morphologic criteria. Cases were divided in two groups: FEA associated with situ and/or invasive ductal and/or lobular carcinoma (FEA-C, n=30) and FEA without malignancy (FEA-0, n=28). Slides were digitally scanned (Aperio), TIFF images from two representative areas at 20x magnification were obtained and analyzed using digital software (Zen 2011, Carl Zeiss Microscopy, Germany). Several nuclear features were measured, including diameter, area, perimeter, convexity, ellipse angle, feret ratio, roundness, grey, blue, red and green levels. Collected data was analyzed using statistical software (SPSS v 17).
Results: Parameters related to nuclear shape and size (area, diameter) were similar in both groups. However, FEA-C cases had significantly higher densitometric green (p=.01), red (p=.04), blue (p=.03) and grey (p=.02) scale levels compared to FEA-0 cases, indicating that nuclei in FEA-C are less dense (more open) than nuclei in FEA-0. 13 randomly selected images from each group were examined by two additional breast pathologists, who blindly classified them based on nuclear staining alone as H (*Heterogeneous/open* chromatin pattern with inconspicuous nucleoli) or U (*Uniform* chromatin pattern without visible nucleoli). Neither pathologist labelled the 13 FEA-0 images as H, whereas 10/13 FEA-C images were labelled as H by one or both pathologists (100% specificity and positive predictive value of “H” staining pattern in diagnosis of FEA-C).
Conclusions: Based on digital image analysis and pathologist evaluation using routine microscopy, we conclude that nuclear staining pattern is the most important morphologic parameter of FEA in predicting concomitant carcinoma. Thus, FEA nuclear H&E staining pattern may potentially serve as a guide for clinical management (observation versus excision).

LUNCH AND POSTER VIEWING

(ATRIUM 2ND FLOOR, FACULTY OF MEDICINE)

GUEST SPEAKER

DR. WEDAD HANNA

SUNNYBROOK HEALTH SCIENCE CENTRE, UNIVERSITY OF TORONTO

TITLE: DUCTAL CARCINOMA-IN-SITU: AN UPDATE

THE PROGNOSTIC SIGNIFICANCE OF C-MET AND EGFR OVEREXPRESSION IN GASTRIC CARCINOMAS.

Aleksandra Paliga, Horia Marginean, Bibianna Purgina , Basile Tessier, Derek Jonker, Esmeralda C Marginean.

Background: ErbB-1 (EGFR) and c-MET are tyrosine kinase growth factor receptors implicated in numerous malignancies, including gastric carcinoma (GC). Recent literature suggests both pathways are highly interdependent and dual receptor inhibitors are in production. The aim of this study was to investigate the prognostic value of EGFR and c-MET proteins overexpression by immunohistochemistry (IHC) in a surgically resected North American GC cohort and correlate it with clinicopathologic characteristics.
Design: We constructed Tissue Microarray (TMA) blocks from 120 consecutive GC, between 2002 -2008, containing 4 cores/tumor to account for tumor heterogeneity. TMAs were stained by IHC with EGFR and c-MET and scored by 2 pathologists from 0-3+, based on membranous and cytoplasmic staining intensity respectively. Descriptive statistics, Kaplan Meyer and Cox regression were used for statistical analyses.
Results: Of 113 interpretable cases individual overexpression of EGFR and c-MET was noted in 17 (15%) and 65 (57%) respectively; co-expression of both EGFR and c-MET was observed in 12 (10%) tumors. We observed no correlation between T, N stage, age, histology, grade, and tumor location with EGFR or c-MET overexpression. Tumors with EGFR and c-MET overexpression showed inferior overall survival (OS): median 13 months vs. 30 months in EGFR +ve vs. -ve GC [HR=1.67, 95% CI 0.95-2.93, p=0.08]; 27 months vs. 49 months in c-MET +ve vs. -ve GC [HR=1.22, 95% CI 0.77-1.92, p=0.40], respectively. GC co-expressing both EGFR and c-MET was correlated with the poorest survival: 12 months vs. 29 months in double +ve vs. -ve tumors [HR=2.38, 95% CI 1.16-4.88, p=0.02].
Conclusion: This study describes the prevalence and prognostic value of EGFR and c-MET in a North American population of patients undergoing curative intent resection for GC. Both c-MET and EGFR status trended towards poor OS, however dual receptor positivity conferred the poorest survival. Larger studies to confirm our findings are warranted, since targeted therapy may provide a major therapeutic advance.

RESOLUTION OF MATERNAL D TYPING USING SEROLOGY AND GENOTYPING

P Berardi[1], J Hannon[2], G Clarke, T Alport[2], G Growe[2], D Lane[2], R Fallis[2],J Cote[2], G Ochoa[3], M Goldman[2]

[1]The Ottawa Hospital, Department of Pathology and Laboratory Medicine, Ottawa, ON, Canada
[2]Canadian Blood Services Prinatal Testing Laboratories in Edmonton, AB; Regina, SK; Vancouver, BC; and Winnipeg, MB; National Immunohematology Reference Laboratory,Ottawa,ON, Canada
[3]R&D Department, Progenika Inc, Medford, MA, USA

Background: All prenatal patients undergo serologic screening for RhD to determine if Rh immune globulin (RhIG) should be administered at 28 weeks gestation. For patients with low expression of D, an algorithm incorporating RBC genotyping can assist in the classification of D variants (weak-RhD or partial-RhD).
Methods: Samples that gave weak (≤1+) or inconclusive results using automated solid phase testing (Immucor Galileo™) with Series 4 (S4) and Series 5 (S5) monoclonal anti-D reagents, were subsequently tested on the manual bench using the S4, S5 reagents and the Immucor Novaclone anti-D. Samples with weakly positive macroscopic results on immediate spin or after a 5-minute RT incubation with one or more reagents were sent for genotyping using an automated platform (Progenika Bloodchip™).
Results: From April/2011 to April/2013, approximately 284,000 patients were tested; 37,127 classified as D negative by serology. Results of the 102 sent for genotyping are shown in the table, along with policy for RhIG administration. There were 6 samples that required subsequent interrogation by gene sequencing, 2 compound heterozygotes (RHD*DAR, RHD*weak D type1and RHD*DAR, RHD Psi) and 2 novel D alleles not listed in the Rhesus Base or dbRBC database at the time of detection; 1 variant found in 3 samples was localised to exon 9 by Bloodchip, while the other found in a patient with anti-D gave a "no call" result.

Genotype	n	RhIG
RHD-Positive	11	No
RHD-Negative	1	Yes
Weak D - Types 1, 2, 3	56	No
Weak D - Types 4.0, 4.1	6	No
Weak D - Type 42	2	Yes
Partial D - DAR	8	Yes
Partial D - Others	12	Yes
Compound Heterozygotes	2	Yes
New variant, exon 9	3	Yes
New variant, exon 1	1	No(anti-D present)
TOTAL	102	28

Conclusion: Use of the algorithm resulted in better classification of prenatal patients, with avoidance of RhIG administration in 72% of genotyped cases.

ANTIMICROBIAL RESISTANCE AND SEROTYPE DISTRIBUTION OF *STREPTOCOCCUS PNEUMONIAE* IN EASTERN ONTARIO

C. Ellis[1,3], R. Robinson[2], M. Desjardins[1,2,3], B. Toye[1,2,3]

[1]The Ottawa Hospital, Ottawa, ON, [2]Ottawa Hospital Research Institute, Ottawa, ON, [3]University of Ottawa, Ottawa, ON

Objective: We determined the prevalence of antimicrobial resistance and the mechanism of macrolide resistance in *Streptococcus pneumoniae* and the serotype distribution of invasive isolates in Eastern Ontario.
Methods: Single patient clinical isolates of *S. pneumoniae* were collected from all 14 acute hospitals and one community laboratory in Eastern Ontario during 2011. Oxacillin, norfloxacin, clindamycin and erythromycin susceptibility were determined by disk diffusion testing. PCR for both *erm(B)* and *mef* genes was done on all erythromycin resistant isolates. Penicillin and levofloxacin MICs were determined by E-test. Serotype data was collected for blood and CSF isolates from 2011-12.
Results: 411 isolates of *S. pneumoniae* (including 43% respiratory, 27% sterile sites) were collected. Of these, 83 (21%) were resistant to erythromycin: 37% due to *erm(B)*, and 57% due to *mef*; 5% were found to carry both genes. 48 isolates (11%) had penicillin MIC of 0.12-1 mg/L and only 3 isolates (0.7%) had penicillin MIC ≥ 2 mg/L. Resistance to erythromycin (24% vs 19%) and penicillin MIC >0.06 mg/L (17% vs 9%) were slightly higher in the tertiary care hospitals than in the community hospitals, respectively. 3/321 (0.9%) isolates tested were resistant to norfloxacin and had a levofloxacin MIC > 2 mg/L. 48% of the 212 invasive *S. pneumoniae* isolates had serotypes that are contained in the 13-valent conjugate vaccine (PCV-13). The most common serotype in both children and adults was 7F (24%). **Conclusion**: The prevalence of macrolide resistance emphasizes the need for continued surveillance, especially for isolates with a dual mechanism of erythromycin resistance which have been associated with multidrug resistance. The serotype distribution of invasive *S. pneumoniae* isolates supports the introduction of PCV-13 in Ontario in 2011.

PROSTATIC DUCTAL ADENOCARCINOMA (PDCA); AN AGGRESSIVE VARIANT THAT IS UNDERDIAGNOSED AND UNDERSAMPLED ON TRANSRECTAL ULTRASOUND (TRUS) GUIDED BIOPSY

Previn Gulavita[1], Nicola Scheida[2], Susan J Robertson[1], Kein T Mai[1], Eric Belanger[1], Trevor Flood[1]

Departments of Pathology and Laboratory Medicine[1] and Diagnostic Imaging[2], University of Ottawa, The Ottawa Hospital, Ottawa, Ontario, Canada.

Background: Prostatic ductal adenocarcinoma (PDCa) is the most common histologic subtype of prostatic carcinoma. It is associated with a poor prognosis and with high frequency of advanced pathologic stage on radical prostatectomy (RP). PDCa is clinically difficult to detect due to its' frequent central location (which limits detection with digital rectal examination) and lower levels of serum prostatic serum antigen compared to conventional adenocarcinomas. Furthermore, PDCa is occult on T2 weighted magnetic resonance imaging. The purpose of this study is to determine if PDCa is undersampled and/or underdiagnosed on transrectal ultrasound (TRUS) guided biopsy.

Materials and Methods: A search of our pathology database between the dates of 2007 and 2013 was conducted for RP specimens with a diagnosis of at least 10% PDCa. Forty-six patients were identified of which 18 had slides from TRUS biopsies available for review. The original pathology report was reviewed and the presence or absence of PDCa and the highest Gleason Scores reported were recorded. The TRUS biopsy slides were retrospectively examined and the presence or absence of PDCa was determined. Diagnostic accuracy was calculated with results from RP as the reference standard. Ductal morphology was defined as tall columnar cells with amphophilic cytoplasm arranged in papillary or cribriform structures with slit like lumen.

Results: From the original report, there were: three cases of 3+3=6, five cases of 3+4=7, three cases of 4+3=7, four cases of 4+4=8, and three cases of 4+5=9 Gleason Score tumors. Only 2 of the 18 original biopsy reports detected the presence of PDCa for a sensitivity of 11%. An additional five biopsies demonstrated PDCa upon retrospective review, with an increased sensitivity of 39%. The remaining eleven cases did not show PDCa on TRUS biopsies and consisted of three patients with 3+3=6, three patients with 3+4=7, one patient with 4+3=7 and four patients with 4+4=8 Gleason Score tumors.

Conclusions: PDCa is commonly undersampled on TRUS guided biopsy which is related to the tumors' more frequent central gland location; a site that is not routinely sampled with TRUS biopsy techniques. When PDCa is present on needle biopsies, it frequently goes unrecognized or under-reported possibly due to the relative rarity of the tumor, frequent admixture with conventional adenocarcinoma and confusion with microscopic mimickers such as cribriform high grade prostatic intraepithelial neoplasia. The undersampling and underdiagnosis of this aggressive variant could have major clinical implications resulting in under-treatment, particularly in patients being considered for active surveillance with otherwise low Gleason Score tumors at TRUS biopsy.

EVALUATION OF EFFICIENCY OF HISTOLOGICAL CRITERIA IN DETECTING LYNCH SYNDROME (HNPCC) IN COLORECTAL CARCINOMA.

PA Williams, E Tomiak, LE Bourns, F Halwani.

Department of Pathology and Laboratory Medicine, and Medical Genetics; EORLA, The Ottawa Hospital and University of Ottawa, Ontario, Canada

Introduction: Lynch syndrome (LS), or hereditary non-polyposis colorectal cancer, is the most common inherited colorectal carcinoma (CRC) syndrome accounting for 2-5% of all cases. Pathologists initiate IHC testing based on patient age and defined histological criteria. This study aimed to evaluate the efficiency of these criteria.
Design: All CRC cases diagnosed at The Ottawa Hospital (TOH) from January 2012-July 2013 were reviewed. Cases with MMR deficiency were reviewed with Medical Genetics for referral rates and outcomes.
Results: Of 480 cases of CRC reviewed, 90 (19%) cases had IHC performed for MMR status with 45 (9%) cases having intact MMR gene expression and 45 (9%) deficient (39 MLH1, 4 MSH2, 1 MSH6 and 1 PMS2). 15 patients were referred for genetic counseling with 5 patients declining, 9 patients tested and 1 pending. 3 germline pathogenic mutations were identified (1 each in MLH1, MSH2 and PMS2) with 4 cases pending. Histological criteria that were informative are: right colon cancer (82% vs 44%, p=0.0004), intraepithelial lymphocytes (IEL) (67% vs 31%, p=0.007), high histologic grade (60% vs 22%, p=0.0003) and any amount of mucinous differentiation (60% vs 27%, (p=0.0014). Other criteria without statistical significance were mucinous carcinoma (>70%), Crohn's-like lymphoid aggregate (CLLA), stage and tumor size.
Conclusions: At TOH the incidence of LS with current detection is 0.6% of total CRC cases. Standardization of histological criteria will increase detection rate. We recommend testing be performed on any CRC that fulfils any of the following single criterion: 1) age <70 years, 2) right colon, 3) IEL, 4) high grade and 5) any mucinous differentiation. Using this strategy, 62% of total CRC cases will be tested with a positive predictive value of 57% and negative predictive value of 92%. We also recommend better physician and patient awareness of the value of genetic testing to other family members.

BREAK AND POSTER VIEWING (ATRIUM)

POSTERS

MOLECULAR REGULATION OF EARLY MYOGENESIS

Hamood Alsudais and Qiao Li

Abstract: Skeletal muscle development undergoes complex and multi-regulatory molecular steps. It involves the commitment of myogenic procurers to the myogenic lineage, which is followed by the proliferation of myoblasts then the fusion, which results in the formation of myotubes. Among of the molecular regulatory groups is the Muscle Regulatory Factors (MRFs); a family of four genes that control myogenesis and the regeneration of damaged muscles. These MRFs are a family of basic helix-loop-helix (bHLH) transcription factors, which include: MyoD, Myf-5, Myogenin, and Mrf4. 1 These MRFs, as heterodimers with bHLH E proteins, bind directly to a specific DNA motif, E-box (CANNTG), at the targeted muscle genes promoters and enhancers, or one of them, in order to control their expression. 2 During the proliferation stage of myoblasts, Myf5 and MyoD are expressed, while the expression of Myogenin and MRF4 appears late during differentiation.3,4 Moreover, studies show the involvement of other essential genes that control myogenesis. This will open the door for other potential clinical approaches to enhance muscle regeneration in various diseased conditions. Recently, Tmem8c was identified as the first muscle specific protein that governs myoblasts fusion and it was named Myomaker.6

The availability of powerful tools like the next generation technologies gives us a chance to uncover the involvement of genes that contribute significantly to the myogenesis. This will lead to a better understanding of the myogenic differentiation.

MOLECULAR MECHANISM OF P300 ACTIVITY DURING EARLY MYOGENESIS

Munerah Hamed and Qiao Li

Departments of Pathology and Laboratory Medicine, and of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa.

Skeletal myogenesis is regulated by the myogenic regulatory factors (MRFs) that bind DNA to regulate gene expression. The MRF family consists of Myf5, MyoD, Myogenin and MRF4, which are all belong to a basic-Helix-Loop-Helix (bHLH) family of transcription factors. It has been demonstrated that Myf5 and MyoD induce early specification of muscle lineages. In epigenetics, chromatin modifications can provide a useful readout for enhancer activities. However, the mechanism by which p300 regulates myogenesis is still unclear. We have previously examined in details p300 association and histone acetylations at different regulatory regions of the Myod1 locus. Our studies demonstrate that the histone acetyltransferase (HAT) p300 is stepwise enriched at distinct Myod1 regulatory regions during myogenic differentiation. This enrichment of p300 is associated with increased histone acetylation in a distinct pattern. We showed that p300 is directly involved in the early Myod1 enhancer regulation, and provide molecular insights into how p300 HAT activity and histone acetylation are related to enhancer activation and, consequently, gene transcription. Our research aims to gain novel, in depth mechanistic insight into the role of p300 HAT activity in early skeletal myogenesis. Therefore, we are interested in investigating the role of p300 HAT activity in myogenic regulators expression. ChIP-seq was performed to globally identify p300 enrichment, in alignment with acetylation of H3 (K9, K18, K27) and tri-methylation of H3K27 during early myogenic differentiation. The aim is to identify and study in details potential myogenic genes regulated by p300. In addition, we will determine the potential regulators that contribute to p300 recruitment at the enhancer regions.

CHARACTERIZATION OF IMPAIRED INSULIN SIGNALLING IN ALZHEIMER'S DISEASE

Yuka Sai, Wandong Zhang, and Qiao Li

Department of Pathology and Laboratory Medicine, and Department of Cellular and Molecular Medicine, University of Ottawa

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by extracelullar deposits of beta-amyloid peptides (Aβ) and intracellular aggregates of hyperphosphorylated tau in the brain. While its risk and pathogenesis are currently accepted to be multifactorial, the pathogenic tau pathway is a major factor in Alzheimer's neurodegeneration. Tau is a microtubule-associated protein which stabilizes microtubules in axons of neurons. Recent studies suggest that there is insulin resistance and signaling dysregulation in AD brain. Insulin signalling is known to be essential for normal brain function. A disruption in normal insulin signalling, such as reduced cellular responsiveness to insulin, affects downstream effectors that regulate protein synthesis and phosphorylation in the cells, including tau among others. The mTOR (mammalian target of rapamycin) pathway is of particular interest in AD because its downstream effectors regulate the translation and phosphorylation of tau, which when present in excessive and hyperphosphorylated states, leads to neuronal cell death. The extent to which mTOR in the insulin signalling pathway affect each other in the context of insulin resistance must be elucidated to more effectively address future avenues of therapy. To this end, cell models were used to investigate the mechanisms of insulin resistance and its inte ractions with mTOR pathway in AD, including a parental mouse neuroblastoma 2a (N2a) line, and the N2a line transfected with hAPP gene which produces Aβ peptides (N2a-APP). Cellular responses to 0.1nm – 100nm concentrations of insulin were assessed based on the presence of phosphorylated proteins in the insulin signalling pathway. The results suggest that kinase activities in the signalling pathway differ in the two lines in response to insulin. IRS1 in N2a-APP cells shows a pronounced resistance to all concentrations of insulin, while decreased phosphorylation of Akt, GSK3, and mTOR was observed in N2a-APP cells at the physiological levels of insulin in comparison to parental N2a cells.

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF METABOLIC ENZYMES AND CO-FACTORS IN CALF MUSCLE OF RATS EXPOSED TO BROMINATED FLAME RETARDANT (BFR) HEXABROMOCYCLODODECANE (HBCD)

[1,2]Syed Aziz, [4]**Clarine Chan**, [1]Ivan Curan, [1]Virginia Liston, [3]Don Caldwell, [1]Kamla Kapal, [1]Rekha Mehta.

[1]Regulatory Toxicology Research Division, Food Directorate, Bureau of Chemical Safety, Health Products and Food Branch, Health Canada, Ottawa, Ontario; [2]Department of Pathology, University of Ottawa ; [3]Scientific Services Division and [4] Co-op Students Program, University of Ottawa.

Background: HBCD ($C_{12}H_{18}Br_6$) is identified as one of the 3 most widely used brominated flame retardants in North America. The additive-type flame retardant is found in a wide variety of products, mainly in electronic appliances, thermal insulation materials, plastics, and textiles. HBCD is proved to be a persistent organic pollutant where bio-monitoring studies have detected HBCD residues within the environment and biomagnifies in food chains. Studies show that dietary intake is a source of human exposure to HBCD, as well as various other exposure pathways such as inhalation of dust, and oral contact with consumer products containing HBCD. The objective of this study was to apply enzyme histochemistry and immunohistochemistry to determine HBCD-related effects in calf muscles in rats during a developmental study with technical HBCD.

Materials and Methods: Male and female Fisher F344 rats were exposed to HBCD via diet (0, 250, 1250, and 5000 mg/kg bw) for 28 days. At necropsy, the rat calf muscle samples were embedded in Gum Tragacanth, rapidly frozen in isopentane and cooled in liquid nitrogen at -80^{o}C until cryosectioning. Cross sections were stained histochemically using routine stain H&E, Gomeri trichrome to determine mitochondrial distribution, PAS and PAS-D for glycogenic activity, ATPase activity at pH 4.3, 4.6 and 9.4 to examine fiber types, and NADH for oxidative enzymes. Immunohistochemical staining of antibodies against Dystrophin forms 1, 2, and 3 was used to evaluate fiber connectivity. All data collected from HBCD treated rats were then analyzed using morphometric methods and compared with untreated controls.

Results: A dose dependant increase of moth-eaten and abnormal myopathy determined by different stains was prominent in both male and female calf muscles ($P \leq 0.001$). A decrease in the connectivity of the muscle fiber was seen in Dystrophin-2 with increasing HBCD dosage. A difference in the diameter of all three fiber types was noted in ATPase staining at different pHs in both sexes ($P \leq 0.001$). No significant differences were detected between males and females with respect to the morphometric parameters examined.

Conclusion: Results obtained by enzyme histochemical and immunohistochemical analysis demonstrate that rat calf muscles are affected by HBCD treatment. The present study suggests that HBCD exposure affects the calf muscle via changes in muscular structure and specifically the muscle protein dystrophin-2. Identification of enzymes and other properties using enzyme histochemical and immunohistochemical techniques have proven to be useful when assessing muscle myopathies, and the development of muscle fibers. Further biochemical and molecular analysis is required to confirm the present findings.

THE EFFECTS OF 3-METHYLFURAN ON THE CALF MUSCLE OF FISCHER 344 RATS

Syed Aziz [1, 3], **Sana Aman[4]**, Meghan Kavanagh[1], Michael Barker [2], Kamla Kapal[1], Wendy Cherry[1], Rekha Mehta[1] and Santokh Gill[1]

[1]Regulatory Toxicology Research Division, and [2]Scientific Services Division , Bureau of Chemical Safety, Food Directorate , Health Products and Food Branch, Health Canada, Ottawa, Ontario; [3]Department of Pathology, University of Ottawa, Ottawa, Ontario ; and [4] Honour's Student, Carleton University, Ottawa, Ontario..

Background: Furan and its alkyl derivatives (2- and 3-methylfuran) are a class of compounds which have been identified as by-products of thermal degradation in heat processed foods. A 28-day gavage toxicity study in male Fischer 344 rats with 3-methylfuran was conducted. This portion of the study focuses on the effects of 3-methylfuran on the muscle tissue.
Materials and Methods: Male Fischer F344 rats were exposed to 3-methylfuran by gavage at 0, 0.1, 0.3, 1.5, 3.0, 6.0, 12.0, and 25.0 mg/kg bw/day for 28 days. At necropsy, calf muscle samples were embedded in Gum Tragacanth, rapidly frozen in isopentane submerged in liquid nitrogen and stored at -80^{o}C until cryosectioning. Cross-sectional muscle tissues were examined using immunohistochemistry and both routine and special histological stains. Immunohistochemical staining with antibodies against Dystrophin forms 1, 2, and 3 was used to evaluate fiber connectivity. Routine and special histological stains included H&E, Gomori Trichrome, Succinic Dehydrogenase (SDH) and Cytochrome Oxidase (COX) to determine mitochondrial distribution, Periodic Acid-Schiff (PAS) and Periodic Acid Shift-Diastase (PAS-D) for glycogenic activity, Myosin ATPase (mATPase) activity at pH 4.3, 4.6 and 9.4 to examine fiber types and Nicotinamide Adenine Dinucleotide (NADH) for oxidative enzymes.
Results: Dose-dependent changes were observed in terms of centralized nuclei, pale necrotic fibers, devoid and isolated fibers, nemaline rods, central core disease, glycogen storage disease, both moth-eaten and lobulated fibers, abnormal mitochondria, and neuromuscular junctions. Changes in type 1 and 2A fibers using specific stains were also very prominent.
Conclusion: Exposure to 3-methylfuran affects thestructure of the calf muscle. Immunohistochemical, routine and special histological stains have proven to be useful when assessing muscular myopathies and the development of muscle fibers. Further biochemical and molecular analysis is required to confirm the present findings.

PSEUDO-OUTBREAK OF *MYCOBACTERIUM FORTUITUM* DUE TO CONTAMINATED ICE MACHINES

N. Sant[1-3]*, M. Desjardins[1-3], V. Chirip[1], R. Ettinger[1], I. Gorn[1], V. Roth[2-3]

[1]Eastern Ontario Regional Laboratories, Ottawa, ON, [2]The Ottawa Hospital, Ottawa ON, [3]The University of Ottawa, Ottawa, ON

Background: We observed a 10-fold increase in isolation rates of *Mycobacterium fortuitum* from respiratory specimens over a 3-month period. The majority of these isolates were from patients coming in contact with one of three wards at The Ottawa Hospital. This prompted an epidemiologic investigation to identify the source.
Methods: Environmental samples were collected from water/ice machines and tap water on affected and uninvolved wards as well as the Microbiology laboratory. All specimens were cultured with the use of a continuously monitored broth system for the isolation of mycobacteria. Environmental samples positive for mycobacteria were sent to the regional Public Health Laboratory for identification. Case and control isolates as well as environmental isolates were compared by genotyping using ERIC-PCR. A retrospective chart review was conducted on patients who had positive specimens to evaluate clinical impact.
Results: *M. fortuitum* was isolated from 20 respiratory samples from 17 patients. Surveillance cultures obtained from uninvolved areas of the hospital were negative for mycobacteria. *M. fortuitum* was isolated from 3 ice machines on affected wards but not from tap water or water in the Microbiology laboratory. ERIC-PCR based typing revealed that patient isolates were identical or closely related, with the exception of one isolate from a patient previously known to be colonized. Further, the isolates obtained from environmental cultures also were identical or closely related to the patient isolates. All but one case was thought to represent transient colonization and not infection. One patient was inappropriately changed from an anti-tuberculous treatment regimen to a regimen for treatment of *M. fortuitum*; this resulted in clinical progression of tuberculosis infection that was later microbiologically confirmed.
Conclusion: The *M. fortuitum* pseudo-outbreak was due to contaminated ice machines located on each of the affected units. A negative clinical impact was observed in one patient.

BASAL-LIKE VARIANT OF NON-INVASIVE UROTHELIAL CARCINOMA: A VARIANT OF UROTHELIAL CARCINOMA WITH IMMUNOHISTOCHEMICAL FEATURES OF BASAL-LIKE UROTHELIAL CELLS ASSOCIATED WITH HIGH RATE OF RECURRENCE AND INVASION

Christopher G. Ball, Trevor A. Flood, Eric C. Belanger, Kien T. Mai

Department of Pathology and Laboratory Medicine, University of Ottawa and the Ottawa Hospital, Ottawa, Ontario, Canada

Background: Low-grade urothelial carcinoma frequently displays positive CD44 reactivity for urothelial stem cells in the basal and suprabasal layers of neoplastic urothelium. We investigated the clinical and pathological significance of a subgroup of non-invasive urothelial carcinoma that displays full thickness reactivity for CD44.
Design: A series of 123 consecutive cases of non-invasive urothelial carcinoma with squamous differentiation was reviewed; these were immunostained with basal cell markers CD44, CK5, CK34bE12, bcl2, EP4, and p63. Those cases selected for study showed strongly positive CK5 immunoreactivity in more than 1/2 thickness of neoplastic urothelium and more than 1/2 the area of non-invasive tumor. We subsequently designated this subgroup as basal-like urothelial carcinoma (BUC). Ten cases of low-grade and high-grade non-BUC were selected as controls.
Results: There were 20 cases of non-invasive low-grade papillary BUC, and 14 of high-grade BUC; cases with strong CD44 but limited CK5 reactivity were excluded. Immunoreactivity to CK5 is stronger than that of CD44, which tends to be uniform and expansive. Cytokeratin 34bE12 was more sensitive but nonspecific; whereas p63, bcl2, and EP4 were neither sensitive nor specific in detecting BUC. Mild to moderate nuclear atypia was seen to be characteristic of BUC. Focal squamous and/or glandular differentiation were occasionally seen. Low-grade BUC was associated with multifocal disease, developing up to 10 concomitant tumors. All cases with at least 12 months' follow-up were associated with recurrence, up to 4 times per year. Progression of low-grade BUC to high-grade disease, or to development of superficial invasive carcinoma carcinoma, within a 3 to 42 month period, was seen in 6 and 19 cases respectively. Distant metastasis occurred in 5 cases during a 36 month period following diagnosis of low-grade or high-grade non-invasive BUC.
Conclusion: In analogy to basaloid carcinoma in other sites of the body, basal-like urothelial carcinoma likely represents the basal-like counterpart of common urothelial carcinomas. Identification of noninvasive basal-like urothelial carcinoma with CD44/CK5 immunoreactivity involving full or nearly full thickness is helpful to identify a subgroup of aggressive non-invasive urothelial carcinomas.

BOUNDARY CONDITIONS FOR THREE PLACENTAL FLOW FIELDS THAT MAY PREDICT AND CAUSE INTRA-UTERINE GROWTH RETARDATION

Christopher G. Ball[1], Andree Gruslin[2], and David Grynspan[3]

Department of Anatomical Pathology and Laboratory Medicine, The Ottawa Hospital and the University of Ottawa
Department of Obstetrics and Gynecology, & Cellular Molecular Medicine, The Ottawa Hospital and the University of Ottawa
Department of Anatomical Pathology and Laboratory Medicine, Children's Hospital of Eastern Ontario and the University of Ottawa

Introduction: Altered end-diastolic flow in the umbilical artery is a reliable predictor of intrauterine growth retardation (IUGR). It appears that an earlier and more sensitive prediction can be made when chorionic vessel flow patterns are characterised; we hypothesize that this results from remodelling of the villous circulatory bed. However, neither the true extent nor the causative mechanism of these altered flow patterns is known. In addition to the villous tree, the lacunar blood flows and the spiral arteries may also be prime suspects. Unfortunately, direct observation of these physical flows is severely restricted.
Aim: Our aim is to accurately determine the boundary conditions of each of these flows. These, along with geometry and permissible assumptions, form the basis for computational simulation or modelling of these flows.
Methods: Direct measurement of morphological features will permit construction of typical geometries for each flow system. Inlet and outlet conditions may be extrapolated from known nearby flows.
Discussion: Preliminary enquiry indicates that each system may be compared to a unique physical flow problem. The villous tree as a complicated flow-in-pipes system; the lacunar pools as a porous medium flow; the spiral arteries as a wall shear stress interactions problem.

NOVEL TECHNIQUE OF SAMPLING THE URINARY BLADDER IN RADICAL CYSTECTOMY FOR UROTHELIAL CARCINOMA

Christopher G. Ball, Joanne Swift, Trevor A. Flood, Eric C. Belanger, Kien T. Mai

Department of Pathology and Laboratory Medicine, University of Ottawa and the Ottawa Hospital, Ottawa, Ontario, Canada

Background: Sampling of the urinary bladder in radical cystectomy specimens is usually performed by obtaining three or more full thickness strips of bladder wall, in sagittal planes, extending from the bladder neck to the dome. While pathologic assessment of specimens is often sufficient for continuing clinical follow-up and therapy, it is hindered by the difficulty in identifying the anatomical relationship of the urothelial carcinoma to the remaining urinary bladder.
Design: Fifty radical cystectomy specimens (39 from men, 11 from women) were identified; these comprised 9 cases of superficial urothelial carcinoma, and 41 of muscle invasive urothelial carcinoma. Each of these was bisected in the transverse plane at the middle portion of the urinary bladder; these were then fixed without stretching in 10 % buffered formalin for at least 24 hours. After fixation, the urinary bladders (while still attached to the prostate or uterus) were serially sectioned in transverse planes, from urinary bladder neck to dome, in 5 mm thick rings. These sections were arranged in order, mapped, and photographed. At least one ring of tissue was submitted in toto, along with other interesting or representative sections.
Results: Our proposed method of transverse sections results in no real increase in the number of sections submitted for microscopic examination, with 12±5 sections for the transverse sections method versus 11±5 for the tissue strip method. The advantages of our method are: a) consistency and ease of sampling; b) suitability for determining depth of invasion by gross examination; c) improved identification of tumor size(s), topographic location, and multifocality; d) suitability for mapping neoplastic lesions; and e) increased tissue sampling that exceeds 50 % of bladder mucosa.
Conclusion: Transverse sectioning of the urinary bladder facilitates and maximises the pathological examination of the bladder wall in radical cystectomy specimens.

POROUS MEDIA FLOW MODELS FOR MATERNAL PLACENTAL CIRCULATION

Christopher G. Ball[1], David Grynspan[2], and Andree Gruslin[3]

Department of Anatomical Pathology and Laboratory Medicine, The Ottawa Hospital and the University of Ottawa
Department of Anatomical Pathology and Laboratory Medicine, Children's Hospital of Eastern Ontario and the University of Ottawa
Department of Obstetrics and Gynecology, & Cellular Molecular Medicine, The Ottawa Hospital and the University of Ottawa

Introduction: It is reasonable to believe that the presence of abnormal flow patterns in the chorionic vessels is an earlier and more sensitive predictor of IUGR than altered end diastolic flow in the umbilical artery. It furthermore appears that the prediction of IGUR becomes increasingly sensitive as predictive flow measurements are taken increasingly distal (to the fetus) in the umbilical cord. This, we hypothesise, results from remodelling of the villous circulatory bed – the most proximate correlate of the factors that lead to IUGR. The effect of lacunar blood flow, the intimately connected maternal side of the placental flow, cannot be ignored.
Aim: Our aim is to model the fluid mechanics of the normal maternal lacunar blood flows in order to assess its influence on flow within the measurable parts of the fetal arterial tree. In view of the placental structure, we aim to apply a model of flow through porous media to the maternal flow bed.
Methods: Preliminary inquiry has lead us to suspect that this flow problem may be analogous to the problem of laminar flow through a porous medium (such as in models of fuel cells, ground water, etc.). Here, we will illustrate how this canonical flow model as been applied to that of the maternal placenta.
Discussion: The problem of modelling EDFR provides an example where interdisciplinary collaboration between medicine and engineering hopefully will permit rapid progress through novel application to one field of established constructs from another.

ORBITAL IGG4-RELATED DISEASE WITH SUPERIMPOSED GRAVE'S OPHTALMOPATHY MIMICKING AN ORBITAL TUMOR

Soufiane El Hallani, Susane J. Robertson, Paula Blanco, James Farmer, Manisha Lamba

Department of Pathology and Laboratory Medicine
The Ottawa Hospital, Ottawa ON, Canada

Background:
Immunoglobulin (IgG) 4-related disease (IgG4-RD) is characterized by elevated serum IgG4 and tissue infiltration by IgG4-positive plasma cells. While IgG4-RD in association with Hashimoto's thyroiditis has been reported, the relationship between IgG4-RD and Graves' disease is not well known.
Case report:
We describe the case of a 56-year-old woman with chronic Grave's ophtalmopathy who presented with acute onset of eyelid swelling and conjunctival injection. CT scan demonstrated the presence of an infiltrative right orbital mass and lachrymal gland enlargement, mimicking a lymphoma. Biopsy samples from the orbital mass tissue showed non-neoplastic lymphoplasmacytic infiltration, increased number of IgG4-positive plasmacyte per high-power field and IgG4-positive/IgG-positive plasmacyte ratio of > 40%. The histopathological findings of orbital tissue matched the characteristic features of IgG4-RD. The patient responded well to corticosteroid therapy.
Discussion:
We discuss here the simulation of orbital tumour by IgG4-RD and the association with Graves's disease with questionable pathogenesis overlapping. Further data and reports are needed to gain more knowledge about a rare presentation.

TUMOR AND ENDOTHELIAL CELL HYBRIDS CONTRIBUTE TO GLIOBLASTOMA VASCULATURE: FACT OR ARTEFACT

Soufiane El Hallani[1], Carole Colin[2], Ahmed Idbaih[3], Karima Mokhtari[4], Jean-Yves Delattre[3], Marc Sanson[3]

1- UMR975, Hôpital de la Salpêtrière, Université Pierre et Marie Curie, Paris
2- UMR911-CRO2, Faculté de Médecine Timone, Université de la Méditerranée, Marseille
3- Service de Neurologie Mazarin, Hôpital de la Salpêtrière, Paris
4- Service de Neuropathologie Raymond Escourolle, Hôpital de la Salpêtrière, Paris

Abstract: Recently antiangiogenic therapy with bevacizumab has shown a high but transient efficacy in glioblastoma (GBM). Indeed, GBM is one of the most angiogenic human tumors and endothelial proliferation is a hallmark of the disease. We hypothesized that GBM endothelial cells may originate from tumor cells. In a subset of GBM tissues, we found that several tumor endothelial cells carry *EGFR* amplification, characteristic of GBM tumor cells. This observation was reproduced *in vitro*: when stem-like tumor cells derived from GBM were grown in the presence of human endothelial cells, a fraction of them acquired endothelial markers (CD31, CD105, VE-Cadherin and vWF). By transduction with GFP and DsRed expressing lentiviral vectors, we demonstrate that this phenomenon is due to cell fusion and not transdifferentiation. A fraction of GBM stem-like cells thus have the capacity to fuse with endothelial cells and the resulting hybrids may contribute to tumor microvascular proliferation and treatment resistance.

ROLE OF FINE-NEEDLE ASPIRATION IN THE SURGICAL MANAGEMENT OF PANCREATIC NEUROENDOCRINE TUMORS: UTILITY AND LIMITATIONS IN LIGHT OF THE NEW WORLD HEALTH ORGANIZATION CLASSIFICATION.

Shaheed W. Hakim, MD; Wayne S. Kendal, MD, PhD; Avijit Chatterjee, MD; Derek J. Jonker, MD; Jean A. Maroun, MD; Laval Grimard, MD; Wael Shabana, MD; Richard Mimeault, MD; Terence N. Moyana MD

Context: Pancreatic neuroendocrine tumors (Panc-NETs) are rare and tend to get overshadowed by their more prevalent and aggressive ductal adenocarcinoma counterparts. The biological behavior of PancNETs is unpredictable, and thus management is controversial. However, the new World Health Organization classification has significantly contributed to the prognostic stratification of these patients. Concurrently, there have been advances in surgical techniques for benign or lowgrade pancreatic tumors. These procedures include minimally invasive and parenchyma-sparing operations such as laparoscopy and enucleation.
Objective: To report on the utility and limitations of fine-needle aspiration in the preoperative evaluation and management of PancNETs.
Design: This was a retrospective review of our institutional tumor database from 2002 to 2012. There were 25 cases of PancNETs that were localized and staged by medical imaging and diagnosed by fine-needle aspiration.
Results: Fourteen patients underwent laparotomy, with some requiring only limited surgery; 4 had laparoscopic resections; 4 were serially observed without surgical intervention; and another 3 were inoperable. After a mean follow-up of 37 months, more than half of the patients had no evidence of disease, including most of those who underwent minimally invasive surgery.
Conclusions: Fine-needle aspiration is a useful diagnostic adjunct to medical imaging in the preoperative evaluation and management of PancNETs. However, there are limitations with regard to grading PancNETs using this technique.
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SUPERFICIAL INVASIVE UROTHELIAL CARCINOMA OF LARGE NESTED VARIANT WITH REGIONAL OR DISTANT METASTASES: A VARIANT OF UROTHELIAL CARCINOMA WITH HIDDEN EVIDENCE OF STROMAL INVASION

Shaheed W. Hakim, Christopher G. Ball, Joelle Levac, Fawaz Halwani, Trevor A. Flood, Eric C. Belanger, Kien T. Mai.

The Ottawa Hospital and University of Ottawa, Pathology and Laboratory Medicine, Ottawa, ON, Canada

Background: Superficial (non-muscle) invasive urothelial carcinoma (SINUC) is a well-known entity and is characterized by its features of disease recurrence and progression. Its potential of regional and distant metastases, however, has not been well investigated.
Method: We reviewed all cases of SINUC in our institution for a period of 4 years to identify SINUC with metastasis.
Results: Of 165 SINUC, there were 5 (3 ureter, 2 bladder) with regional lymph node metastasis (2/5) and distant metastasis (3/5 cases, in lung and/or bone). Male:female ratio was 3:2, and patient age ranged from 55-90. The SINUC were multifocal in 4 cases and measured up to 3 cm in diameter. Microscopically the SINUC predominantly formed solid and compact tumor masses with an endophytic pattern associated with subtle papillary growth. The neoplastic cells showed mild to moderate cytologic atypia, reaching high grade urothelial carcinoma (UC) in 3 cases. The invasive component consisted of medium to large nests of often slightly irregular contours extending beyond the basal contour of the neoplasm. Peri-tumoral edema, as evidenced by retraction artifact, was present in 4 cases. These 4 cases were originally diagnosed as non-invasive UC. The remaining case was a high grade SINUC associated predominantly with small invasive cell nests. Immunostaining revealed strong and diffuse CK5 and CD44 reactivity in 3 cases and variable reactivity for CK20.
Conclusion: SINUC with regional and distant metastases are often under-diagnosed as noninvasive UC due to the failure to recognize large invasive cell nests.

IMPROVING THE AUTOPSY SERVICE THROUGH A PATHOLOGY RESIDENT-LED EDUCATIONAL INITIATIVE FOR CLINICAL RESIDENTS

Dr. Sarah Strickland, Dr. Aleksandra Paliga, Dr. M. Gomes

Background: Rates of hospital autopsy across North America have been declining and currently sit at approximately 5% of in-hospital deaths. The reasons for this decline are multifactorial but may be in part due to lack of physician comfort with obtaining autopsy consent. The autopsy consent is unusual within medicine because it is obtained by a physician who will not be performing the procedure. Within a tertiary care or academic center, the task of obtaining consent often falls upon clinical residents who may not have received any training regarding autopsies and may not be familiar with the autopsy procedure and its logistics. This, in turn, may cause residents to be uncomfortable or unable to answer families' questions regarding the procedure. Subsequently, residents may be hesitant to discuss autopsies and this may contribute to declining autopsy rates.

Design: In order to determine the experiences, training and comfort level of clinical residents towards autopsy a needs assessment questionnaire was distributed to residents in various clinical specialties. Following the needs assessment, a resident-led educational campaign was initiated by anatomical pathology residents. The goals for these sessions were to improve the clinical residents' knowledge of the autopsy service as well as to increase resident comfort with obtaining autopsy consent. One hour interactive lectures were conducted during clinical specialty academic half days and covered the autopsy procedure, autopsy service logistics as well as how to properly complete a consent form. Prior to and immediately following the education sessions, residents completed an electronic quiz composed of scenarios relating to obtaining autopsy consent.

Results: The needs assessment questionnaire was distributed to residents from five different specialties including internal medicine, general surgery, cardiac surgery, neurology, and critical care (total of 77 residents). The results showed that the vast majority (97.4%) of residents had received no training regarding the hospital autopsy service but yet the majority (87%) had previously approached families to obtain autopsy consent. Furthermore, 83% of residents reported that they were not comfortable answering the family's questions regarding the autopsy service. The pre-session quiz prior to the presentation confirmed that the residents did poorly when trying to answer pertinent questions about the procedure and its logistics (average score 52%). Following the session, the residents showed significant improvement (average score 88%) demonstrating acquisition of knowledge.

Conclusion: Although clinical residents are often tasked with obtaining autopsy consent, they receive no training regarding the autopsy service and often feel uncomfortable answering families' questions. Pathology resident-led educational sessions can improve clinical resident knowledge of the autopsy procedure. Hospitals should consider initiating autopsy service training programs for clinical residents.

GATA3 EXPRESSION PROFILE IN INVASIVE BREAST CARCINOMA POST NEO-ADJUVANT SYSTEMIC CHEMOTHERAPY.

Phillip A. Williams MD MSc, Shahidul Islam MD PhD FRCPC

Department of Pathology and Laboratory Medicine, University of Ottawa and Eastern Ontario Regional Laboratory Association (EORLA), Critical Care Wing, The Ottawa Hospital, 501 Smyth Road, Ottawa, ON, Canada K1H 8L6.

Introduction: Immunohistochemistry for GATA3, a transcription factor, has become a new favourite for the diagnosis of metastatic breast carcinoma. Large studies have shown that 84.5-94% of breast carcinomas are positive for GATA3. GATA3 is also expressed in urothelial carcinoma, and up to 51% of salivary gland neoplasms (including 100% of all salivary duct carcinomas). A recent paper showed GATA3 also showed strong expression in many cutaneous tumours including basal cell carcinoma, squamous cell carcinoma and adnexal tumours, with expression in non-cutaneous squamous cell carcinomas, mesothelioma, pancreatic ductal carcinoma and chromophobe renal cell carcinoma. GATA3 has been previously demonstrated to be positive in all metastatic breast carcinomas that have GATA3 positive primaries. GATA3 expression is positively linked to estrogen receptor (ER) expression. ER positive breast carcinoma is well documented to potentially lose ER expression post neo-adjuvant systemic chemotherapy, and ER status is retested in the post-treatment resection specimen to guide hormonal therapy. To date, there have been no studies examining how systemic chemotherapy affects expression of GATA3.
Materials and Methods: All cases of post neo-adjuvant breast carcinoma surgically resected at The Ottawa Hospital between June 2010 and September 2013 were reviewed. A total of 77 cases were identified that had paraffin embedded tissue blocks available for both pre-treatment biopsies and post-treatment resections. All cases had ER status recorded previously. Each specimen was reacted with antibodies for GATA3 and reactivity was recorded for pre-treatment and post-treatment specimens. Expression profiles were created for each paired biopsy and resection for GATA3 and ER. ER and GATA3 expression was scored according to the Allred score and stratified weak (1+), moderate (2+) or strong (3+).
Results: 59 cases (77%) had strong (3+) ER reactivity both pre and post chemotherapy with all having strong (3+) GATA3 reactivity pre-chemotherapy and all having either moderate (10, 17%) or strong (49, 83%) GATA3 expression post-chemotherapy. ER expression was lost in ER positive biopsies in 5 cases (6.5%) with corresponding GATA3 loss in 2 cases (40% of ER loss cases) and weak (1+) reactivity in 1 case (20% of ER loss cases).
Conclusions: We conclude that GATA3 reactivity is potentially weak or absent in ER negative breast carcinomas post systemic neo-adjuvant chemotherapy. Immunohistochemistry for GATA3 will be reactive for the majority of cases post-chemotherapy. Immunohistochemistry for GATA3 is therefore still useful in identifying potential breast carcinoma metastases post systemic chemotherapy, but must be cautiously used when ER is negative or weak.

MEAN PLATELET VOLUME AND IMMATURE GRANULOCYTE COUNT IN ICU SEPSIS PATIENTS AND DISPOSITION AT 30 DAYS

Vito Sanci [1,2], Ruth Padmore[1,2]

[1]*The Ottawa Hospital - General Campus, Ottawa, ON, Canada,* [2]*University of Ottawa, Ottawa, ON, Canada*

Introduction: The mean platelet volume (MPV) and immature granulocyte count (IGC) have been higher in patients in whom local infection progressed to septicemia or death. Both measures have also been postulated to be early markers of outcome in ICU patients with sepsis. Since MPV and IGC are now easily accessible as novel automated CBC parameters, associations between them and patient progression could prove useful in directing patient care and in improving patient outcomes. Therefore, we hypothesize that MPV and/or IGC in ICU sepsis patients will be significantly different in those dead versus alive, 30 days post-admission.

Methods: After research ethics board approval, charts of patients in the ICU with sepsis between June 2, 201I and October 2, 2012 were retrospectively reviewed. MPV and IGC were measured using a Sysmex X E-5000 automated hematology analyzer. Statistical analyses were performed using SPSS® software (SPSS Inc., Chicago, IL) with a level of significance of 95% ($p<0.05$) and 2-tailed tests.

Results: N=641 patients were recruited: 350 (54.6%) male; 291 (45.4%) female. At 30 days post-admission, 229 (35.7%) were dead and 412 (64.3%) were alive. Data are presented as (mean±SD; 95% CI; p-value) in those dead versus alive at 30 days. The MPV (in fL) and age (in years) were significantly increased (10.9±1.01; 0.8-11.0 vs 10.7±1.1; 10.6-10.8; p=0.023) and (69.7±15.6; 67.8-71.6 vs 61.1±15.2; 59.6-62.6; p=0.000), respectively. No IGC ($xl0^9$/L) differences were found (0.11±0.15; 0.08-0.14 vs 0.12±0.18; 0.1-0.14; p=0.640). No interactions between M PV or IGC and gender or age were found.

Conclusions: Significantly increased MPV levels were found in ICU septic patients who were dead at 30 days post-admission. Increased M PV may prove to be a useful marker in identifying septic patients in the ICU at increased risk of dying.

THE PROGNOSTIC EFFECT OF MLH1 LOSS IN ENDOMETRIAL ENDOMETRIOID ADENOCARCINOMA

Bojana Djordjevic[1], Amanda Bruegl[2], Bryan Fellman[3],Su-Su Xie[4], Diana Urbauer[3] and Russell Broaddus[4]

[1]Department of Pathology, University of Ottawa, The Ottawa Hospital, Ottawa, ON, Canada
[2]Department of Gynecologic Oncology and Reproductive Medicine, [3]Division of Quantitative Sciences, [4] Department of Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, United States

Background: Microsatellite instability (MSI) arises due to loss of mismatch repair (MMR) protein function. 15-20% of sporadic endometrial carcinomas have MSI due to methylation of the *MLH1* gene promoter with subsequent transcriptional silencing. The value of MLH1 as a prognostic parameter is not well established. The aim of this study was to assess the effect of MLH1 loss on recurrence free survival (RFS) and overall survival (OS) in a large cohort of endometrial endometrioid adenocarcinomas.
Design: Immunohistochemistry for MMR proteins, MLH1, MSH2, MSH6 and PMS2 was performed in 106 early stage (I and II) and 106 advanced stage (III and IV) endometrial endometrioid carcinoma cases. The cases were closely matched by tumor grade and patient age. The patients had no known history of Lynch Syndrome. Tumors with negative nuclear immunohistochemical staining for any one of the four MMR proteins were classified as having MSI, with the remainder classified as microsatellite stable (MSS).
Results: MSI was identified in 30.7% of all patients (22.2% MLH1, 2.8% MSH2, 2.4% MSH6, 2.8% PMS2 and 0.5% MSH6/PMS2). MLH1 loss was detected in 16.0% of early stage and 28.3% of advanced stage patients (p=0.065). Cases with non-MLH1 MMR loss were excluded from subsequent analyses. Follow-up was censored at 5 years. By multivariate Cox proportional hazards regression model, MLH1 loss was not significantly associated with RFS (p=0.767) or OS (p=0.770) in early stage cases. However, in advanced stage cases, MLH1 loss conferred reduced RFS (HR 2.02 [95% CI, 1.08-3.78], p=0.028). This effect was independent of tumor grade and patient age. MLH1 loss was not significantly associated with OS in the advanced stage group (p=0.220).
Conclusion: MLH1 loss in advanced stage endometrial endometrioid adenocarcinomas is an independent negative prognostic factor of recurrence. As such, identification of MLH1 loss in these tumors in the preoperative setting may help guide patient management decisions.

CHALLENGES IN THE DIAGNOSIS OF THE ALEUKEMIC PRODROME OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

Elaine Leung, Mylene Bassal, Luke Shier.

Children's Hospital of Eastern Ontario and The Ottawa Hospital, Ottawa, Ontario, Canada.

Background: Pediatric acute lymphoblastic leukemia (ALL) may present with a myriad of clinical manifestations. In 1-2% of pediatric ALL, an aleukemic prodrome of transient profound peripheral pancytopenia (pre-ALL) precedes overt leukemia. This presentation is often challenging, both from a clinical and a laboratory perspective.
Objectives: To describe the challenges encountered in the clinical and laboratory diagnosis of pediatric ALL presenting as pre-ALL, focusing on morphology and flow immunophenotyping data.
Design/Method: All cases of pre-ALL were reviewed from 2006 – 2013 at a tertiary care pediatric hospital.
Results: Out of 119 new ALL cases, 2 had a pre-ALL presentation (1.7%). Both patients were female, one was 21 months and the other 10 years old at time of presentation. The younger presented with fevers, vulvar ulcers and disseminated pseudomonas bacteremia; the older with a several month history of fatigue and weight loss. Both demonstrated a profound peripheral pancytopenia and absence of, or rare, circulating blasts. Initial bone marrow evaluation in both showed small populations of cells morphologically suspicious for blasts, although benign hematogones with atypical features could not be excluded. Dyserythropoiesis was common to both. Flow immunophenotyping also showed subtle features suspicious for leukemia, but could not definitively identify the cells as lymphoblasts or hematogones. Within weeks, both patients' peripheral blood counts gradually improved. After repeated bone marrow evaluations over the subsequent months, evolution to frank precursor-B lymphoblastic leukemia was confirmed. The time from initial presentation to overt leukemia was 2 – 3 months.
Conclusion: Pre-ALL is an uncommon aleukemic presentation for pediatric ALL and can pose diagnostic challenges. Despite the presence of only a small population of abnormal cells, clues to the diagnosis can be found on both morphology and flow immunophenotyping. Recognition of this entity should prompt close follow-up of any patient presenting with peripheral pancytopenia and even minimal morphologic or flow immunophenotyping abnormalities, to assess for evolution of disease. An improvement in peripheral blood counts may be transient and does not exclude ALL. Consideration should be given to the possibility of pre-ALL in the spectrum of diagnostic investigations that one would order on a patient presenting with unexplained pancytopenia.

THE ABILITY TO DETECT PERIPHERAL BLASTS IN CHILDREN WITH NEWLY DIAGNOSED OR RELAPSED LEUKEMIA USING THE SYSMEX XE 2100/5000 CBC ANALYZERS

T. Ogilby, M. Roebuck and E. Leung.

Division of Haematology and Transfusion Medicine, Children's Hospital of Eastern Ontario.

Introduction: The Children's Hospital of Eastern Ontario is a 165-bed tertiary care pediatric hospital and has approximately 20-25 new diagnosis of acute leukemia annually. Patients who are ultimately diagnosed with leukemia present with a myriad of clinical and CBC perturbations, ranging from near normal counts to significant cytopenias or leukocytosis. It is impossible to review all peripheral blood (PB) smears, so that the detection of peripheral blasts relies on a combination of analyzer flags/info and PB review policies. We wanted to assess the ability of the Sysmex XE 2100/5000 CBC analyzers to detect the presence of peripheral blasts in children with leukemia.
Methods: Between September 1, 2012 and December 31, 2012 we had 9 cases of newly diagnosed or relapsed leukemia. We retrospectively reviewed the laboratory data available for each of these cases including: analyzer flagging and scatterplots, CBC (hemogram) results and PB morphologic findings.
Results: Of the 9 cases analyzed, 5 (56%) were easily diagnosed and had the presence of significant CBC abnormalities, as well as positive flagging on the Sysmex analyzers and/or abnormal scatterplot. These cases fulfilled our laboratory policy for PB review; circulating blasts were detected in all cases, with blast percentage ranging from 6 – 96%. Several of the cases that had a "blast" flag also triggered a "atypical lymphocyte" flag. Two cases had PB review because of CBC abnormalities, but did not have evidence of blasts on either the Sysmex analyzer or initial PB morphology. One case had absence of both Sysmex flagging or CBC abnormalities, but had PB review based on laboratory review criteria (previous diagnosis of leukemia); PB blasts (o.9 x 10^9/L) were detected. The last case had no abnormalities, but was shown on bone marrow aspirate to have relapsed disease.

	Group1	Group2	Group3	Group4	Group5
Abnormal CBC	+	+	+	-	-
Sysmex "Blast" Flag	+	+	-	-	-
Sysmex Flags (Other)/Scatterplot Abnormalities	+	-	+	-	-
PB Blasts (Morphology)	+	+	-	+	-
Number of Patients	4	1	2	1	1

Conclusions: The Sysmex XE analyzers provide useful information that can be incorporated into laboratory PB review policies. Familiarity with the scatterplots is useful to further augment the Sysmex flagging information. In paediatric populations, the higher frequency of lymphoblastic leukemia

can cause challenges to CBC analyzers, since these cells may not be classified as blasts, but as atypical lymphocytes. A proportion of individuals who are ultimately diagnosed with leukemia will either present without CBC abnormalities, or have no evidence of peripheral blasts based on either analyzer data or morphologic review.

RETICULOCYTE HEMOGLOBIN EQUIVALENT (RET-HE) IN THE MANAGEMENT OF PRE-OPERATIVE ANEMIA: IS IT USEFUL INFORMATION? A PILOT STUDY

Touchie D[1], Henderson M[2], Goyette E[1], Padmore R[1], Giulivi A[1].

Divisions of [1]Hematopathology and Transfusion Medicine and [2]Biochemistry, Department of Pathology and Laboratory Medicine, The Ottawa Hospital and Eastern Ontario Regional Laboratory Association (EORLA)

Purpose: A reticulocyte hemoglobin equivalent (RET-He) is a new CBC parameter available from modern CBC analyzers. The RET-He provides a direct estimate of the recent functional availability of iron into erythrocyte hemoglobin and has clinical utility in evaluating changes in iron status in renal dialysis patients. The clinical utility of RET-He in the diagnosis and management of preoperative (preop) iron deficiency has not been studied. The purpose of this study is to observe the relationship between the results of standard serum iron studies, hemoglobin (Hgb) levels and the RET-He in patients receiving preoperative intravenous (IV) iron therapy

Methods: A retrospective chart review was conducted on fifty consecutive preoperative patients treated for iron deficiency. The results of serum iron indices, Hgb, and RET-He were observed pre and post iron therapy.

Results: Inspection of the raw data was done using scatter and slope plots. Scatter plots suggested a possible relationship between Hgb and RET-He. Slope plots for Hgb and RET-He were not informative due to the wide variation in timing of the pre and post blood work relative to the date of the IV iron therapy. The raw data was standardized as percent change and rate of change for the Hgb and RET-He after the 1st IV iron infusion. The only time interval measured was between the 1st IV iron infusion and the 1st IV iron infusion blood work. The greatest percent and rate of increase in RET-He was observed in the samples obtained soonest (3 days) after the IV infusion and declined steadily thereafter, and with the most rapid increase seen with lower pre-infusion RET-He levels. The Hgb level did not show a positive percent and rate of increase until approximately 7 days after the IV iron infusion. A multiple linear regression model for percent change and percent rate of change for Hgb and RET-He suggest that low pre-infusion concentrations of RET-He result in a greater rate of change in RET-He post iron infusion compared to rate of change of hemoglobin.

Conclusions: RET-He appears to be an early maker of iron sufficiency. Based on these findings future prospective studies are needed to further investigate pre RET-He as a predictor for the rate of hemoglobin increment post IV iron therapy.

IMPLEMENTATION OF A MULTIPLE MYELOMA HIGH RISK MINI-FISH PANEL: IMPLICATIONS FOR TREATMENT MANAGEMENT OF PATIENTS IN EASTERN ONTARIO

M. Beaulieu Bergeron[1,2], M. Yoshimoto[1], B. Clifford[1], E. Sinclair-Bourque[1], R. Padmore[1,2,3], J. McGowan-Jordan[1,2], J. Tay[4].

[1]Children's Hospital of Eastern Ontario; [2]Department of Pathology and Laboratory Medicine, University of Ottawa; [3]Eastern Ontario Region Laboratory Association; [4]Department of Medicine, The Ottawa Hospital.

Multiple myeloma (MM) is characterised by accumulation of clonal plasma cells in the bone marrow and is the second most common hematological malignancy after lymphoma. Cytogenetic abnormalities are among the most important prognostic parameters for patients with MM, *IGH* rearrangements such as t(4;14) or t(14;16), loss of the *TP53* locus and amplifications of 1q21 being associated with an adverse prognosis while deletions 13q are often associated with an intermediate prognosis. Autologous stem cell transplantation remains an integral part of the management of patients with MM; however in patients with "poor" cytogenetic abnormalities such as *TP53* deletion or t(4;14), its value may be debatable. Located in Ottawa, Canada, Children's Hospital of Eastern Ontario (CHEO) hosts the Eastern Ontario Regional Genetics Program and provides genetics laboratory services for its population of over 1 million individuals, as well as to patients living in the western part of the adjacent province of Quebec. In order to provide risk stratification and assist physicians in advocating for the best possible therapy for MM patients, the CHEO Cytogenetics Laboratory, in collaboration with The Ottawa Hospital, recently began offering interphase FISH analysis on selected plasma cells using probes for the detection of t(4;14) and loss of *TP53*. This assay, which is performed on whole bone marrow aspirates following enrichment for CD138+ plasma cells, offers a flexible testing platform as other probes can be added to the FISH panel to target other regions of prognostic significance as literature evolves. This MM mini-FISH panel is offered to patients 60 years or younger with no significant co-morbidities and in whom the plasma cells count in the bone marrow is at least 10% before CD138 selection. We present the results obtained so far and discuss implications on treatment management of MM patients.

AWARDS TO BE ANNOUNCED